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Margaret F. Docker *Editor*

Lampreys: Biology, Conservation and Control

Volume 1

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Volume 37

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In memory of my father, Sandy Docker

Foreword

Welcome to the Lamprey Inn! I feel some attachment to lampreys, recognized at least in part by the photograph I took in Gloucester, England. That was purely an academic interest, of course. By the authority vested in me as Editor of this series, it seems that I have defined lampreys as "fishes" and resolved a question that my students in ichthyology courses frequently posed to me. This monumental work must surely bring these remarkable creatures to the attention of a much broader audience. How could anyone not be interested in lampreys after reading this compilation? There is a room for everyone at the Lamprey Inn. They offer incredible opportunities for research on virtually any aspect of biology you might imagine. Perhaps the most striking feature yet to be resolved in lampreys is the so-called "paired species" phenomenon. Anyone interested in speciation must study these lampreys, particularly this question of the origin of parasitic and non-parasitic species. The life history strategies and tactics, plasticity, and epigenetics of lampreys cry out for attention. Have there really been multiple evolutionary origins of these different species, or are they just life history forms? What could determine the developmental pathway of either form or species? You could study almost any aspect of their physiology, from their striking metamorphosis to the demands of anadromous spawning migrations. They have incredible shifts in feeding ecology, digestion, and metabolism during very complex life cycles. We already have a detailed understanding of the role of pheromones in lampreys, and the potential applications of that knowledge for management are quite astounding. Think about the feeding stages of anadromous lampreys, presumably being carried over distances by their hosts. How do they find their way back to spawning streams for their spawning migrations? Those of us preoccupied with management and conservation of salmonid fishes are only just beginning to appreciate the potential importance of lampreys as alternate prey to salmonids. Is zoogeography more of interest to you? Consider the antipodal distributions of the Northern and Southern Hemisphere lampreys! Conservation, management, and restoration of native species?—lampreys have those features as well. We would not likely consider lampreys as charismatic megafauna, but that itself seems like a wonderful challenge. How can you convince a skeptical public to devote conservation efforts to what most consider a writhing, blood-sucking vampire?

I have a rather long and detailed personal history with lampreys, which is greatly influenced by geography. For a number of years I collaborated with the Great Lakes Fishery Commission in and around the Laurentian Great Lakes in North America. We carried out a major study to investigate the installation and operation of low-head barrier dams as an alternative control for the invasive sea lamprey *Petromyzon marinus* in tributary streams to the Great Lakes. Everything about those dams was designed to prevent the passage of lampreys on their spawning migrations. Our study showed that the design of those dams was indeed effective in blocking spawning migrations of parasitic sea lamprey. Now I am in the Pacific Northwest, and we take pride in our claim that the fish ladder at the Oregon Hatchery Research Center is the first "lamprey friendly" fish ladder in Oregon. My efforts are now directed to ensure that as many lampreys as possible can pass upstream to complete their spawning migrations. One of my post-graduate students studied the lamprey ammocoetes in the Great Lakes basin in an attempt to define any characters that could be used to identify those larvae, and particularly to discriminate between the native species and the invasive parasitic sea lamprey. He found that existing morphological keys were of limited use, mostly because there are so few characters to study. The absence of characters, however, does not mean the absence of species. Genetic information was more useful for species recognition, but in turn that approach led to suggestions of intriguing patterns of speciation and life history patterns. Of course there is a very different and very serious interest in lampreys from the indigenous peoples who have had such an important social and cultural connection with them for the longest time. They have seen lamprey populations decline precipitously as a consequence of the construction and operation of dams, changes in watershed management, and habitat alterations. They are the people who are taking some of the immediate actions to rear lampreys in hatcheries, transfer adults to former spawning areas, and restore early rearing habitats.

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The Lamprey Inn, Gloucester, England (photo by David L. G. Noakes)

Acknowledgments

First and foremost, I would like to express my deep gratitude to series editor David L. G. Noakes for wisely recognizing that there was a need for an updated book on the biology of lampreys and for trusting (not foolishly, I hope) that I was the person for the job. I would also like to acknowledge the support I received from Martine van Bezooijen at Springer when I first proposed and embarked on this project, and thank Alexandrine Cheronet and Judith Terpos for taking up the torch after Martine's untimely passing. Alex and Judith have been exceedingly patient with me as a novice editor, allowing me considerably more time than I had originally promised so that I could complete this book to my satisfaction. I am also very grateful to the University of Manitoba, particularly the Faculty of Science and Department of Biological Sciences, for allowing me the opportunity to pursue this book project (and for providing me with such a collegial academic home).

I must also thank, of course, the many authors who have contributed to this volume. Without their considerable expertise and hard work, this book would not have been possible. I would also like to acknowledge the many peer reviewers who likewise contributed their time and expertise: Pedro R. Almeida, Howard S. Gill, John B. Hume, Scott I. Kavanaugh, Nicholas E. Mandrak, Richard G. Manzon, Neal D. Mundahl, Ian C. Potter, Stewart B. Reid, Stacia A. Sower, Trent M. Sutton, Eric B. Taylor, Andrew J. Treble, and Michael P. Wilkie. The additional insights they provided are greatly appreciated.

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Finally, on a personal note, I would like to express my heartfelt gratitude to my family: my parents (Sandy and Hilda) and siblings (Elizabeth, Susan, and Ian) who have always showed their support and enthusiasm for my chosen career (even before it was "cool" to work on lampreys), and the rest of the extended Docker family (Jason, Michael, Benjamin, Nicholas, Jonathan, Stuart, Henry, and Josephine). A very special thanks is reserved for my daughter, Sylvia, who has joyously embraced the life that I have chosen for us, and who has enriched mine beyond words.

Male pouched lamprey *Geotria australis* from the Okuti River in the South Island of New Zealand. The function of the gular pouch, which develops during sexual maturation, is unknown. (Photo: © www.rodmorris.co.nz)

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Chapter 1
Introduction: A Surfeit of Lampreys

Margaret F. Docker, John B. Hume and Benjamin J. Clemens

Abstract Lampreys have long been the food of kings. They have been highly appreciated by the English monarchy and upper classes since medieval times, and long before that, by the ancient Romans, the Māori, and Native Americans. Historically, lampreys have also received attention from at least a small group of anatomists and other scientists (including Sigmund Freud), given their "lofty" status at the base of the vertebrate family tree (and their wonderfully large reticulospinal neurons that are so amenable to experimental manipulation). Research related to lamprey biology increased in the 1950s in support of sea lamprey control in the Laurentian Great Lakes, and these efforts considerably advanced our understanding of lamprey ecology, behavior, and chemical communication. Recently, lampreys have started getting more widespread attention. Research related to lamprey endocrinology (particularly the pivotal hypothalamic-pituitary axis and gonadotropin-releasing hormones), the ecology and conservation of native lampreys, and the use of lampreys in evolutionary developmental (evo-devo) and biomedical studies has raised the profile of this group of ancient fishes. Lampreys are providing important and promising model systems in our quest to better understand the early evolutionary history of the vertebrates—particularly given the recent publication of the complete sea lamprey genome—and their increasing use in biomedical research is providing insights into treatment for people suffering from blood coagulation disorders, biliary atresia, hemochromatosis, and spinal cord injuries. In this introduction to Vols. 1 and 2 of *Lampreys: Biology, Conservation and Control*, we provide a broad perspective on the cultural, ecological, and scientific importance of lampreys, outline some historical trends in lamprey research, and celebrate the growing interest—among scientists and laypeople—in this previously underappreciated group of fishes.

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1.1 Introduction

Lampreys have long been the food of kings. They were highly appreciated by the ancient Romans 2,000 years ago and by the English monarchy and upper classes since medieval times. King Henry I of England is famously said to have died after eating a surfeit of lampreys (although the lampreys themselves were perhaps not to blame for his overindulgence). The Māori in New Zealand and Native Americans in the Columbia River basin have also long valued lampreys for human consumption and ceremonial purposes. Historically, lampreys have also received attention from at least a small group of anatomists and other scientists, often in an attempt to understand the origin of the vertebrate body plan.

Research related to lamprey biology increased in the 1950s in support of control of sea lamprey *Petromyzon marinus* in the Laurentian Great Lakes and continues today. These efforts have considerably advanced our understanding of lamprey ecology, behavior, and chemical communication. Recently, however, lampreys are receiving more widespread attention. For example, the use of lampreys in evolutionary developmental (evo-devo) and biomedical studies has further raised the profile of this group of ancient fishes, and the sequencing of the complete sea lamprey genome (Smith et al. 2013) was a major milestone. Research related to the ecology and conservation of native lampreys throughout many parts of the world has also increased, along with a greater appreciation for lampreys among laypeople. For example, "Neunaugen" (the German word for lampreys, deriving from the impression that they have "nine eyes" on each side of the body—the actual eye, the pineal or "third eye," and the seven lateral gill openings; Fig. 1.1) were named "Fish of the Year" in 2012 by the German Sport Fishermen Association, the Ministry for Nature Conservation, the German Angler Association, and the German Sport Divers Association (European Fly Angler 2012).

In this introduction to Vols. 1 and 2 of *Lampreys: Biology, Conservation and Control*, we provide a broad perspective on the historical, ecological, and scientific importance of lampreys, outline some interesting trends in lamprey research (e.g., in terms of topics and species covered), and celebrate the growing interest in this previously underappreciated group of fishes. This chapter will also outline the intended scope of the book and explain the nomenclatural conventions followed within. Subsequent chapters focus on detailed aspects of lamprey biology, but this introduction—which attempts to provide breadth rather than depth—is intended to place lamprey research into a broader context and demonstrate its relevance across a range of disciplines. Given their status as one of the oldest living groups of vertebrates, lampreys are finally starting to receive the attention they deserve.

Fig. 1.1 European river lamprey *Lampetra fluviatilis* captured during its upstream migration (at the end of February 2009) from the River Sorraia in the Tagus River basin, Portugal (total length c. 25 cm). All extant lamprey species possess seven pairs of gills; these plus the eyes and the single median pineal gland (the so-called third eye, located behind the nostril) have earned lampreys the German name "Neunaugen" ("nine eyes"). This species, although still abundant throughout its northern European range, is extremely rare in the Iberian Peninsula. (Photo: © Bernardo R Quintella)

1.1.1 Are Lampreys the Oldest Living Group of Vertebrates or One of the Oldest Living Groups of Vertebrates?

Whether the so-called cyclostomes—the extant jawless fishes, the hagfishes and lampreys—form a monophyletic group (i.e., are each other's closest relative) has been considered "one of the most vexing problems in vertebrate phylogenetics" (Near 2009) and a "taxonomic dispute that has troubled scientists for more than a century" (Nicholls 2009). Providing a detailed review of this taxonomic dispute is beyond the scope of this chapter (see Janvier 2007, 2010; Near 2009; see Chap. 2), but its resolution is important in order to understand the earliest events in vertebrate evolution (see Sect. 1.2.3.2). In brief, morphological characters typically suggest cyclostome paraphyly, that is, that lampreys are the sister group to the jawed vertebrates (superclass Gnathostomata) and that hagfishes represent an earlier offshoot from the vertebrate family tree. Lampreys were thought to share many derived traits or synapomorphies with the jawed vertebrates, such as rudimentary vertebrae, a closed circulatory system, neural control of heart rate, and the ability to osmoregulate. Lampreys were therefore generally considered the oldest extant vertebrates, whereas hagfishes were considered craniates but not vertebrates (Nelson 2006). Nelson (2006) therefore placed extant hagfishes and lampreys into separate superclasses (Myxinomorphi and Petromyzontomorphi) rather than the paraphyletic superclass Agnatha. Recent and compelling molecular evidence, however, strongly supports extant agnathans as a monophyletic group (e.g., Kuraku et al. 1999; Delarbre et al. 2002; Takezaki et al. 2003; Heimberg et al. 2010; Fig. 1.2a) and, in fact, suggests that hagfishes have "long overlooked vertebral elements"

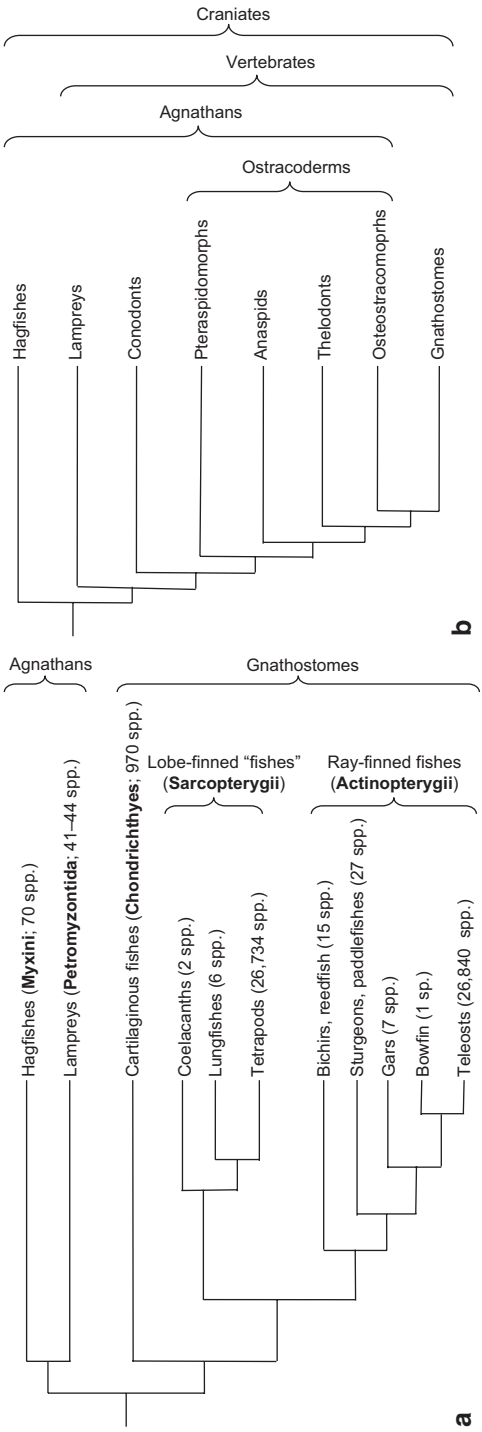

Fig. 1.2 Schematic representation showing presumed phylogenetic relationship between lampreys and other vertebrates. **a** Current understanding of the relationship among extant vertebrates (i.e., the "cyclostome monophyly" hypothesis), which holds that the extant jawless fishes (hagfishes and lampreys) shared an ancestor more recently with each other than either group did with the jawed vertebrates (the gnathostomes). This hypothesis is supported by considerable molecular evidence (e.g., Heimberg et al. 2010; see Sect. 1.1.1); under this hypothesis, both hagfishes and lampreys would be considered vertebrates. Interrelationships among the jawed vertebrates follow Nelson (2006); names of the five classes of extant fishes are given in bold; the numbers of extant species in each lineage are from Nelson (2006) or, for lampreys, from this volume (see Chaps. 2 and 8). Note that "fishes" is not a monophyletic group, even when the jawless fishes are omitted, nor is the former taxonomic group Osteichthyes (the "bony fishes," which traditionally included the actinopterygian and sarcopterygian fishes) monophyletic when the tetrapods are excluded. **b** Relationship among the agnathans and gnathostomes according to the "cyclostome paraphyly" hypothesis and when extinct agnathans are included. This hypothesis is generally supported by morphological data (see Sect. 1.1.1); under this scheme, hagfishes are often considered craniates but not vertebrates (Nelson 2006). Note that, regardless of whether the cyclostomes (the extant jawless fishes) form a monophyletic group, agnathans are paraphyletic when extinct jawless fishes are included

(Janvier 2011; Ota et al. 2011, 2013). Thus, throughout this book, lampreys are considered one of the (two) oldest living groups of vertebrates and, given the evidence for cyclostome monophyly, lampreys and hagfishes are equally distant from—or equally related to—the jawed vertebrates.

Cyclostome monophyly, however, means only that hagfishes and lampreys are each other's closest living relative; it does not necessarily mean that these lineages are closely related. Near (2009) suggested that the long-standing difficulties in resolving the relationship among hagfishes, lampreys, and gnathostomes are likely the result of trying to resolve events that occurred over a very short timescale relative to the hundreds of millions of years that have passed since. Recognizable hagfish and lamprey fossils have been found that date back at least 300–360 million years (Bardack 1991, 1998; Gess et al. 2006; see Chap. 2), indicating long independent evolutionary histories for these two lineages. It is important to recognize the significant differences between these two cyclostome lineages. Furthermore, it must be remembered that the lampreys and hagfishes (with approximately 40 and 70 extant species, respectively) are but a small representation of the once diverse jawless fishes, which included the now-extinct conodonts and ostracoderms (Nelson 2006). Despite compelling evidence for monophyly of the extant agnathans, there is little dispute that the agnathans represent a paraphyetic group when the extinct jawless fishes are included (Fig. 1.2b). Hence, although "agnathan" is a useful term for describing jawless vertebrates, it is not used in this book as a formal taxonomic term. Given, however, that lampreys and hagfishes are the sole survivors of this once diverse assemblage, these "living fossils" (sensu Janvier 2007) are absolutely invaluable for helping to piece together the evolutionary history of the vertebrates (Sect. 1.2.3.2).

1.1.2 Are Lampreys Fishes?

Some biologists who work on jawed fishes are often adamant that lampreys are not "true" fishes. This was indeed once the case (e.g., when the senior author of this chapter was a graduate student preparing for her PhD comprehensive exam). In the second edition of his authoritative *Fishes of the World*, Nelson (1984) recognized Pisces (including only the cartilaginous and bony fishes) as a formal taxon (Fig. 1.2a). With the third edition, however, Nelson (1994) adopted a cladistics classification and Pisces or "fishes" was no longer given taxonomic rank since it constitutes a paraphyletic group (i.e., the tetrapods are also descended from the common ancestor of all jawed fishes; Fig. 1.2a). Even the bony fishes—commonly, but no longer formally, known as the Osteicthyes, and including the ray-finned fishes (Actinopterygii) and the sarcopterygian fishes (i.e., lungfishes and coelacanths)—are paraphyletic. Nelson (2006) covered this nicely in the Introduction to the fourth edition of *Fishes of the World*. Fishes therefore no longer has formal taxonomic meaning, and we follow Nelson (2006), who simply, but artificially, defined fishes as "aquatic vertebrates that have gills throughout their life and limbs, if any, in the shape of fins" and include hagfishes and lampreys in this group. Lampreys are fishes; after all, they are being covered in Springer's *Fish and Fisheries* series!

1.2 The Cultural, Ecological, and General Scientific Value of Lampreys

As introduced above, lampreys—as one of the two surviving lineages of ancient jawless vertebrates—are not (or should not be) of interest to only lamprey biologists. The following sections provide overviews of the broader cultural, ecological, and scientific significance of this fascinating group of animals.

1.2.1 Historical and Cultural Significance of Lampreys

In some cultures, lampreys have long been valued for food and ceremonial purposes. Pacific lamprey *Entosphenus tridentatus* have been fished by Native Americans in the Columbia and Klamath river basins of the western United States for thousands of years (Close et al. 2002; Petersen Lewis 2009). In addition to being an important subsistence food (in part due to their high caloric value), Pacific lamprey also have medicinal and ceremonial value (Close et al. 2002). Called "ksuyas" or "asum" in the native tongue of the mid-Columbia Plateau tribes, Pacific lamprey are regarded as one of their cultural icons (Close et al. 2002). It is this long-held appreciation for Pacific lamprey that has been the impetus for many of the conservation efforts recently initiated for this species (see Chap. 8). The Māori in New Zealand have likewise used pouched lamprey *Geotria australis* for human consumption and ceremonial purposes (McDowall 1990), and native people in Alaska traditionally consumed the Arctic lamprey *Lethenteron camtschaticum* and used its rendered oil as fuel for lamps. In Japan, the Arctic lamprey is also highly valued as a medicine against night blindness (Renaud 2011).

Lampreys have also been appreciated as food in Europe, and references to lampreys have appeared in popular texts for close to 2,000 years. Romans of the first and second centuries considered them to be "regal food" (Renaud 2011), rearing them in ponds for such (and perhaps other) purposes. Pliny the Elder, in his *Naturalis Historia* from 77 AD, provides accounts of one Roman who became so excessively fond of a lamprey that, when it was dead, "he could not hold but weepe for love of it" (Holland 1601). When a Roman noblewoman later inherited this lamprey pond, she took such a liking to another lamprey that she reportedly adorned its gills with golden earrings. Pliny, however, painted a less favorable picture of lampreys when he recounted that the orator Vedius Pollio "kept in ponds huge lampreys that had been trained to eat men, and he was accustomed to throw to them such of his slaves that he desired put to death." This misunderstanding of—but apparent fascination with—the nature of lampreys persisted well into the twelfth century, as this excerpt from the Aberdeen Bestiary (1200) indicates: "Lampreys, it is said, are of the female sex only and conceive from intercourse with snakes; as a result, fishermen catch it by calling it with a snake's hiss."

In medieval Europe, lampreys were regularly captured and consumed by kings and commoners alike. They were especially appreciated during fasting periods

because their taste was considered much meatier than that of most other fishes. The ruling monarchs of England were particularly fond of lampreys (both sea lamprey and European river lamprey *Lampetra fluviatilis*), which they would obtain from the fisheries of Gloucester on the River Severn. King Henry I (1068–1135) is said to have died following an overindulgence of lampreys while on a military campaign in northern France, although it is disingenuous to suggest that it was a direct result of the meal itself (Dickens 1852; Deshpande 2002). In 1200, King John fined the city of Gloucester 40 marks (approximately £ 362,000 or $ 578,000 today) for forgetting to send him a lamprey pie at Christmas. In 1242, King Henry III was reported to have paid 12 pounds, seven shillings, and three pence for 188 lamprey, equivalent to approximately £ 168,000 ($ 268,000) today (Skinner 2012). A baked lamprey pie continues to be presented to the ruling monarch of England on special occasions; Queen Elizabeth II received one on the occasion of her coronation in 1952 and for her Silver Jubilee in 1977 (Renaud 2011; The Telegraph 2012). For the monarch's Diamond Jubilee in 2012, however, the city of Gloucester had to use Great Lakes sea lamprey because none were to be found in the River Severn (The Telegraph 2012). However, given the concern for the "surfeit" of mercury in Great Lakes sea lamprey (see Chap. 8), it is not known if the lamprey pie was eaten by the Queen. Fans of the television series *Game of Thrones* (which is based on the fantasy novels, collectively entitled *A Song of Ice and Fire*, by George R. R. Martin) will be familiar with lamprey pie.

In the eighteenth century, however, lampreys (particularly the European river lamprey) came to be exploited more and more efficiently in England and, given their apparent abundance, declined in value—culturally and monetarily. In the River Thames, for example, European river lamprey were captured by the hundreds of thousands (Wheeler 1979), and sold (for only £ 2–5 per 1000 lamprey; Hardisty 2006) to European cod fishermen for use as bait (see Chap. 8). According to Lanzing (1959), live lamprey would be held on board ship in large holding tanks and "every ship's crew included a 'lamprey biter' who killed the animal by a bite to the head thus destroying the brain. The paralyzed lamprey was then placed on an angling hook." The only commercial lamprey fishery currently operating in the U.K. (in the River Ouse) again supplies European river lamprey as bait for angling (although, until 2011, the lamprey were technically captured as "by-catch" in a licensed eel *Anguilla anguilla* fishery; Masters et al. 2006; Foulds and Lucas 2014).

Exploitation of European river lamprey for food, however, continues throughout much of northern Europe (e.g., in Finland, Sweden, Latvia, Estonia, Lithuania, Poland, and Russia; Sjöberg 2011; Lajus et al. 2013), and sea lamprey is fished commercially in France (Beaulaton et al. 2008), Spain (Gradín 2010), and Portugal (Quintella 2006; Mateus et al. 2012). Both species are still regarded as local delicacies, particularly in recent decades when—due largely to the effects of industrialization and urbanization—they have become scarcer (see Chap. 8). In the 1960s, for example, gourmets in Poland and Lithuania were reported to wait with great anticipation for the by-then infrequent appearance of lamprey in the fish markets or a sign (e.g., the sound of a rifleshot or the sight of a red flag over a beach snack bar) "proclaiming that fresh, roasted lampreys were available" (Sterba

1962). The purchase of these river lamprey, however, depended on "a well-filled purse" (Sterba 1962). Sea lamprey fetch even higher prices; a single sea lamprey in Portugal can cost € 45–50 (over $ 60) during the peak of the season (and, unfortunately, makes them a popular target for poachers; Quintella 2006; Andrade et al. 2007). In the 1990s, the idea of marketing sea lamprey from the North American Great Lakes (given their surfeit there) in Portugal and Spain was explored, but Great Lakes lamprey have mercury levels that are too high to meet European Union standards (MacEachen et al. 2000; Jeffrey L. Gunderson, Minnesota Sea Grant, Duluth, MN, personal communication, 2014). Lampreys figure prominently on the coats of arms in at least two European municipalities—Arbo in northwestern Spain and Nakkila in southwestern Finland (Municipality of Arbo 2010; Radio UusJussi 2013)—and lamprey festivals are held annually in Arbo and in villages in Latvia (e.g., Carnikava) and Portugal (e.g., Montemor-o-Velho).

Commercial fisheries for other lamprey species have been more limited, but nevertheless indicate the historical local significance of these species. There were important fisheries for the Caspian lamprey *Caspiomyzon wagneri* in Russia and Azerbaijan into the twentieth century, but these fisheries are no longer viable (Holčík 1986). Pacific lamprey were fished commercially in the Columbia River basin in Oregon and Washington state in the early twentieth century, but catches of this species were largely used in fishmeal (e.g., for hatchery salmon) or as teaching material in comparative vertebrate anatomy classes (Close et al. 2002; Renaud 2011). The Arctic lamprey is harvested commercially in Japan, and a small commercial fishery commenced for this species in Alaska in 2003 (Hayes and Salomone 2004).

1.2.2 Ecological Significance of Lampreys

Regardless of their direct value to humans as food, lampreys are also known to play important ecological roles at all stages of their life cycle. Larval lampreys are key components at the base of the food chain, and they can represent a large portion of the biomass in streams where they are abundant. Beamish and Youson (1987), for example, showed that the North American river lamprey *Lampetra ayresii* is the dominant organism by weight in the bottom sediments of the Fraser River in British Columbia. Larval lampreys are important in nutrient cycling, facilitating the conversion of nutrients derived from detritus and algae into stored biomass (see Chap. 3). Experimental removal of larval Pacific lamprey from the South Fork Eel River in north-central California appeared to impact the detrital processing of the river (Timothy Wootton, University of Chicago, Chicago, IL, personal communication, 2014). In anadromous species, the carcasses of spawned out lampreys are thought to provide a significant amount of marine-derived nutrients to freshwater aquatic ecosystems, in the same way that Pacific salmon do (e.g., Naiman et al. 2002). Lampreys are also ecosystem engineers; the burrowing and feeding activities of larval lampreys significantly increase substrate oxygen levels (Shirakawa et al. 2013) and the nest-building activity of spawning lampreys increases streambed complexity in ways that appear to benefit other fishes and stream invertebrates (Sousa et al. 2012; Hogg et al. 2014).

Lampreys are a food source for other animals (Cochran 2009), both during the larval stage (e.g., during emergence from their nests or during scouring events that dislodge larvae from their burrows) and then again—and particularly—during downstream migration, following metamorphosis. Outmigrating lampreys can significantly contribute to the diet of predatory fishes, birds (e.g., gulls and terms), and pinnipeds (see Close et al. 2002). Furthermore, in most anadromous species, outmigration appears to occur in pulses (correlated with abrupt increases in discharge; see Chap. 3), and this glut of young adult lampreys may buffer predation on commercially valuable juvenile salmonids, during their downstream migration and as they enter the ocean. Roffe and Mate (1984), for example, found that Pacific lamprey were the principal prey of pinnipeds in the lower reaches and estuary of the Rogue River in Oregon (constituting a higher proportion of their diet, by both weight and frequency, than salmonids). Similarly, in Scotland, sawbill ducks *Mergus merganser* have been reported to be "stuffed" with young lampreys rather than with commercially valuable salmon smolts (Ayrshire Rivers Trust, Ayr, U.K., personal communication, 2013). The extent to which feeding-phase lampreys are preyed upon, particularly at sea, is less well known. Predation is thought to be lower during this stage (Nursall and Buchwald 1972; Scott and Crossman 1973), since the adults are well dispersed. However, Cochran (2009) suggests that predation on lampreys will often go undetected, given their lack of bone and other hard structures (with the exception of their keratinized teeth; Roffe and Mate 1984) that would be resistant to digestion. Adult lampreys are again concentrated, and thus vulnerable to predation (human and other), during their upstream migration and spawning. For example, Steller sea lions *Eumetopias jubatus* at the mouth of the Klamath River feed largely on upstream migrating Pacific lamprey (Beamish 1980). During and after spawning—which occurs during daylight hours in shallow streams—they are fed on by a variety of aquatic, aerial, and terrestrial predators (Scott and Crossman 1973).

Parasitic lampreys are also important predators in aquatic ecosystems, thus constituting key components at both the base and top of the food chain. Parasitic lampreys are generally not viewed very favorably by commercial fishers or anglers since, as noted by Cochran (1994), prey species used by lampreys often coincide with the commercially and recreationally important fishes preferred by humans (see Renaud and Cochran in press). For example, the large (e.g., Pacific and sea lampreys) and even the smaller (e.g., North American river lamprey) anadromous lampreys, when abundant, provide competition to humans interested in Pacific salmon *Oncorhynchus* spp., Atlantic salmon *Salmo salar*, or cod *Gadus* spp. (Beamish and Neville 1995; Orlov et al. 2008; Renaud and Cochran in press). However, lampreys feed on a variety of fish species and even marine mammals (Silva et al. 2014; Renaud and Cochran in press), suggesting that they may be less likely than other predators (including humans) to significantly deplete one or a few prey species. A notable exception is the Great Lakes sea lamprey; the devastating effect of this invasive species on commercial fish stocks in the Laurentian Great Lakes is well documented (see SLIS 1980; Marsden and Siefkes in press). However, there is no evidence that native lampreys are detrimental to the ecosystems in which they occur (Heard 1966; Beamish 1980; Renaud 1997; Close et al. 2002).

1.2.3 Scientific Significance of Lampreys

Lampreys are one of the oldest living groups of vertebrates, and have survived at least four of the five mass extinction events documented since the Cambrian explosion. Given their importance, therefore, as "living fossils," lampreys provide important insight into the evolution of the vertebrates. It is no coincidence, of course, that university students everywhere have long been made to dissect lampreys during comparative vertebrate anatomy classes. Research related to general lamprey biology (particularly in support of sea lamprey control and conservation of native lampreys) is detailed in subsequent chapters of this book. The following sections, in contrast, demonstrate the relevance of lampreys and lamprey research across a wide range of disciplines.

1.2.3.1 Research Trends

Well over 20,000 scientific manuscripts have been published that directly or indirectly use lampreys as a study organism. Searching the database Web of Science (Thompson Reuters) using the term "lamprey*" generated 22,239 records from 1864 until the end of 2013, and many influential papers [e.g., Schultze (1856) and Müller (1856) on lamprey development; see Richardson et al. (2010)] predate this time period. For comparison, the search term "fish*" located 2,348,556 papers from 1864 to 2013. Granted, not all records for fishes will have been retrieved with this broad search term but, considering that extant lampreys comprise only 0.14% of the almost 28,000 recognized species of living fishes (Nelson 2006), the observation that they represent as much as 0.95% of the papers written on fishes is a testament to their scientific importance. Other numerically larger (e.g., cartilaginous fishes with almost 1,000 described species) and more commercially valuable (e.g., salmonids) taxa certainly receive more attention in the scientific literature. A total of 53,977 (2.30% of the total for fishes) and 147,404 (6.38%) records were retrieved using "chondrichthy*" and "salmon," respectively; we acknowledge that the search term "salmon" will not include all papers on salmonids, but the search term "salmon*" recovered a very large number of papers on salmonella. Compared to lampreys, other species-poor but evolutionarily important groups of fishes (Fig. 1.2a) are not as well represented: only 5,036 records (0.21% of the total for fishes) were retrieved for hagfishes (search term "hagfish*"); 1,871 records (0.08%) were retrieved for lungfishes (search term "lungfish*"); and 18,091 records (0.77%) were retrieved for sturgeons (search term "sturgeon*").

Furthermore, there has been a clear increase in the number of papers written on lampreys in recent decades (Table 1.1). From 1864 until 1943, an average of only 30 papers that directly or indirectly used lampreys as a study organism were published per decade. Since 1984, between 1,739 and 8,264 lamprey papers were published per decade (i.e., almost 70 per month in 2004–2013). Even accounting for the dramatic increase in the number of scientific papers published in all disciplines over this time period, we still see a proportionally greater increase in lamprey papers; from 1864 until 1943, papers that directly or indirectly dealt with lampreys represented 0.4% of all papers on fishes versus 1.1% and 0.9% in the last two decades (Table 1.1).

Table 1.1 Number of papers published between 1864 and 2013 that employed lampreys as direct or indirect study organisms, that is, that were retrieved in the database Web of Science (Thompson Reuters) using "lamprey*" as the search term. Number of papers is given per decade for each time interval indicated; number of papers is approximate. Number of papers retrieved using "fish*" as the search term is also given, as is the number of lamprey papers as a percentage of all fish papers. Total number of lamprey papers retrieved for 1864–2013 using research areas defined by Web of Science (and including "Control" as an additional search term of interest) is listed (note that research areas are not mutually exclusive, i.e., a manuscript classified as "Cell Biology" may simultaneously be defined as "Immunology"). For each time interval, the percentage of lamprey papers retrieved for each research area is given, the maximum percentage for each research area is in bold, and the trend over time for that research area (i.e., increasing ↑ or decreasing ↓ in proportion) is noted

	Trend	2004–2013	1994–2003	1984–1993	1974–1983	1964–1973	1954–1963	1944–1953	1934–1943	1864–1933	Total 1864–2013
Lamprey*		8,264	5,620	1,739	1,174	813	316	115	74	34	18,352
Fish*		958,685	489,141	267,130	335,783	168,934	48,195	21,274	18,687	8,161	
Percent Lamprey*	↑	0.9	1.1	0.7	0.3	0.5	0.7	0.5	0.4	0.4	
Zoology	↓	13.6	13.2	13.1	15.7	17.6	26.6	35.8	29.8	**51.9**	5,455
Neuroscience/Neurology	↑	7.1	**11.3**	**11.5**	7.2	5.4	5.4	2.6	3.2	1.1	3,380
Biochemistry/Molecular Biology	↑	**9.2**	**9.5**	8.7	7.8	8.9	5.6	0.7	1.1	1.6	3,335
Physiology		4.1	6.2	**7.8**	6.8	5.4	1.2	1.3	4.3	2.7	2,201
Cell Biology		2.9	5.3	**8.5**	6.2	5.2	2.1	0.0	0.0	0.0	1,982
Environmental Science/ Ecology	↑	**6.7**	4.7	3.6	4.9	5.9	2.1	2.0	1.1	1.6	1,941
Endocrinology/Metabolism		2.7	4.7	6.7	**7.2**	6.2	4.5	3.3	6.4	0.5	1,844
Anatomy/Morphology	↓	2.5	3.4	6.3	8.2	6.6	6.4	4.6	**10.6**	7.0	1,764
Developmental Biology	↓	3.8	3.8	4.6	5.5	5.4	7.8	**11.9**	8.5	9.2	1,674
Marine/Freshwater Biology	↑	**5.8**	3.9	2.3	3.5	2.2	0.4	0.7	0.0	2.2	1,473
Life Sciences/Biomedicine	↓	4.6	3.4	3.0	3.8	5.3	14.4	11.9	**24.5**	17.3	1,570
Genetics/Heredity	↑	**6.6**	4.0	1.8	1.4	1.2	0.4	0.0	0.0	0.0	1,407
Control	↑	4.5	**5.2**	2.2	2.1	2.5	3.9	4.0	3.2	0.5	1,402
Evolutionary Biology	↑	**4.7**	3.4	2.1	1.7	2.2	0.6	2.0	1.1	1.1	1,201
Behavioral Science	↑	**4.5**	3.6	2.4	1.2	0.8	0.8	0.0	0.0	0.5	1,157

Table 1.1 (continued)

	Trend	2004–2013	1994–2003	1984–1993	1974–1983	1964–1973	1954–1963	1944–1953	1934–1943	1864–1933	Total 1864–2013
Biodiversity/Conservation	↓	2.4	1.9	1.4	1.3	3.5	9.5	**15.2**	3.2	0.0	792
Immunology	↑	**2.4**	1.8	2.3	2.2	2.0	0.4	0.0	0.0	0.0	804
Reproductive Biology		1.8	1.6	1.8	**2.2**	**2.2**	1.4	0.7	0.0	0.5	688
Cardiovascular System/ Cardiology		0.7	0.9	2.1	3.2	**4.7**	2.7	2.6	2.1	1.6	637
Pharmacology		1.9	2.1	**2.2**	2.0	1.6	0.6	0.0	0.0	0.0	754
Fisheries	↑	**2.5**	1.3	0.9	1.7	1.6	1.6	0.0	0.0	0.0	625
Nutrition/Dietetics	↑	**1.2**	0.9	1.0	1.2	0.6	0.4	0.0	1.1	0.5	383
Biophysics	↑	**1.2**	1.4	1.0	0.8	0.5	0.6	0.0	0.0	0.0	413
Respiratory System		0.7	0.8	1.1	**1.2**	1.0	0.0	0.7	0.0	0.0	327
Toxicology		0.7	0.7	0.8	0.8	**0.9**	0.4	0.0	0.0	0.0	275
Computational Biology	↑	**1.0**	0.6	0.6	0.3	0.2	0.0	0.0	0.0	0.0	236
Palaeontology	↑	**0.3**	0.2	0.1	0.1	0.2	0.0	0.0	0.0	0.0	80

It is also of considerable interest to see how the focus of lamprey research has shifted since 1864. Using the research areas defined by the database Web of Science (and including "Control" as an additional search term of interest) shows a shift in research focus from more basic descriptions of lamprey biology to their use as model systems in the fields of evolutionary development, biomedical research, and bioengineering (Table 1.1). Whereas such research areas as "Zoology," "Developmental biology," and "Anatomy/morphology" represented as much as 51.9, 11.9, and 10.6%, respectively, of all lamprey records retrieved in decades prior to 1953, they made up only 13.6, 3.8, and 2.5% of retrieved papers in 2004–2013. In contrast, research areas such as "Neuroscience/neurology," "Biochemistry/molecular biology," and "Genetics/heredity" not surprisingly increased in prominence from <2% of all lamprey papers prior to 1943 to 6.6–9.2% in 2004–2013. Exciting discoveries in some of these research areas will be discussed in Sect. 1.5 and in subsequent chapters (e.g., see Chaps. 2, 4 and 7; Lee and McCauley in press). Not all increases depended on novel technologies; publications related to "Ecology/environmental science," "Marine/freshwater biology," and "Behavioral science" represented 0.5–2.2% of all publications prior to 1943 and 4.5–6.7% in 2004–2013. Much of this research directly or indirectly (e.g., through a better understanding of the basic biology and ecological requirements of these species) relates to the control of invasive sea lamprey or the conservation and management of native species (see Chaps. 3, 5, 6 and 8; Marsden and Siefkes in press).

The majority of research conducted on lampreys has focused on relatively few species. Perhaps not surprisingly, over 60% of all records retrieved through Web of Science for the 1864–2013 interval dealt directly or indirectly with sea lamprey (Table 1.2). Research on European river lamprey and European brook lamprey *Lampetra planeri* comprised 14% and 6% of the total, respectively, and records retrieved for Arctic lamprey represented 4.9% of the total. Despite increased interest in native lampreys in recent decades (e.g., anadromous Pacific lamprey and the many freshwater parasitic and non-parasitic lampreys with more restricted distributions), research on these species is still relatively limited. It is our hope that this is changing. Of necessity, we must often extrapolate among lamprey species and such extrapolations appear to be justified in many respects (e.g., given similarities in the ecology of larval lampreys: see Chap. 3). However, it is also becoming clear that there are, in many cases, pronounced species-specific differences (e.g., with respect to passage abilities: see Chap. 5; mating systems: see Chap. 6; and variability of life history type: Docker and Potter in press). These differences have significant management implications (Clemens et al. 2010; see Chaps. 5 and 8). This book has therefore attempted to include broader coverage of these other species (see Sect. 1.3.1), and we look forward to a greater research emphasis on these species.

1.2.3.2 Evolutionary Significance of Lampreys

Lampreys have received considerable attention in evolutionary studies, given their important phylogenetic position. They are the extant representatives of a lineage that diverged from the ancestor to the jawed vertebrates approximately 500 million

Table 1.2 Number of papers published between 1864 and 2013 that were retrieved in the database Web of Science (Thompson Reuters) on each of the following species or groups of lampreys (i.e., using the search terms indicated), and percentage of total. For some species (e.g., Pacific lamprey, Arctic lamprey, American brook lamprey) search terms were chosen to maximize the number of papers retrieved, given changes in classification over time. (see Chap. 2)

Species or group	Search term	Number	Percent of total
Sea lamprey *Petromyzon marinus*	Petromyzon marinus	7,165	60.7
European river lamprey *Lampetra fluviatilis*	Lampetra fluviatilis	1,657	14.0
European brook lamprey *Lampetra planeri*	Lampetra planeri	710	6.0
Arctic lamprey *Lethenteron camtschaticum*	Lamprey* and japonic*	559	4.9
	Lamprey* and camtschatic*	21	
Ichthyomyzon spp.	Ichthyomyzon	536	4.5
Pouched lamprey *Geotria australis*	Geotria	303	2.6
Pacific lamprey *Entosphenus tridentatus*	Lamprey* and tridentat*	257	2.2
Eudontomyzon spp.	Eudontomyzon	152	1.3
Mordacia spp.	Mordacia	102	0.9
Far Eastern brook lamprey *Lethenteron reissneri*	Lamprey* and reissneri	96	0.8
American brook lamprey *Lethenteron appendix*	American brook lamprey*	68	0.6
Western brook lamprey *Lampetra richardsoni*	Lampetra richardsoni	61	0.5
Caspian lamprey *Caspiomyzon wagneri*	Caspiomyzon	44	0.4
North American river lamprey *Lampetra ayresii*	Lampetra ayresi*	40	0.3
Tetrapleurodon spp.	Tetrapleurodon or lamprey* and spadice* or lamprey* and gemin*	25	0.2
		11,796	

years ago (Janvier 2007). Due to their relatively conserved morphology over the past 360 million years (Gess et al. 2006), these "living fossils" (a term first coined by Charles Darwin in his *On the Origin of Species*) are providing invaluable insight into the events that occurred at the dawn of vertebrate evolution and during the subsequent evolution of the gnathostomes (Janvier 1996; Kawauchi and Sower 2006; Osório and Rétaux 2008).

The origin of vertebrates represents one of the major jumps in evolution (Griffith 1994), and there is a large gulf between the non-vertebrate chordates—the lancelets (Cephalochordata) and tunicates (Urochordata), all of which are mostly-sessile marine filter feeders—and the active and morphologically complex Vertebrata. Given the relative paucity of the fossil record prior to the origin of mineralized tissue—and, of course, even well-preserved fossils provide little or no information on the physiology, development, or genomics of the organism—study of the extant jawless

vertebrates is helping to piece together the early evolutionary history of our group (Nicholls 2009; Shimeld and Donoghue 2012).

The major vertebrate advancements include: a cranium and pronounced cephalization; a set of highly specialized paired sense organs (i.e., image-forming eyes, olfactory organs, the lateral line, and various structures derived from the lateral line that are found in the inner ear); a large brain to integrate sensory information; an axial skeleton and muscle segmentation that permits effective swimming; neural crest cells (which give rise to the craniofacial skeleton and other derivatives); more complex circulatory, respiratory, and digestive systems; a glomerular kidney; and a complex endocrine system with pituitary, pineal, thyroid, and adrenal glands (Griffith 1994; Shimeld and Donoghue 2012). These advancements permitted "the active, sentient life" that distinguishes the vertebrates from the non-vertebrate chordates (Griffith 1994). Cyclostome paraphyly (i.e., with hagfishes representing an earlier offshoot from the craniate lineage) implied a more "gradual assembly of vertebrate characters" (Shimeld and Donoghue 2012). The strong support now available for cyclostome monophyly (see Sect. 1.1.1) suggests an even more phenotypically complex ancestral vertebrate, and further widens the gulf between the vertebrate and non-vertebrate chordates (Shimeld and Donoghue 2012).

Studies in lampreys have been instrumental in furthering our understanding of the evolution of vertebrate locomotion (e.g., Bicanski et al. 2013; Hsu et al. 2013), the vertebrate eye (e.g., Collin 2010; see Collin and Potter in press), and the neuroendocrine system (e.g., Kawauchi and Sower 2006; see Chap. 7). Up until the late 1970s, for example, it was thought that the agnathan vertebrates did not have the same neuroendocrine control of reproduction that is seen in the gnathostomes, again suggesting a more gradual evolution of complexity in the vertebrates. More than 30 years of research by Stacia Sower and colleagues, however, has firmly established that lampreys do have a complex hypothalamic-pituitary-gonadal axis and thus shows this to also be a vertebrate innovation and seminal event that emerged prior to or during the differentiation of the ancestral agnathans. Much has now been learned about the evolution of the key neuroendocrine hormones, especially the pivotal gonadotropin-releasing hormones, in vertebrates through this research on lampreys (see Chap. 7). Evidence for neuroendocrine control of reproduction in hagfishes is more recent and far less extensive, but it likewise suggests that hagfishes possess a hypothalamic-pituitary system (Sower et al. 1995; Uchida et al. 2010, 2013).

Comparisons between lampreys and jawed vertebrates have also been critical for shedding light on the evolution in the gnathostome lineage of articulated jaws and paired fins and appendages. Lampreys are being increasingly used in evo-devo studies, exploring the evolution and development, for example, of the neural crest and skeletal muscle of vertebrates, and the hinged jaw and paired appendages of gnathostomes (see Kuratani 2005, 2012; Shigetani et al. 2005; Osório and Rétaux 2008; Shimeld and Donoghue 2012; Lee and McCauley in press).

Lampreys and hagfishes have also been key to elucidating the evolution of adaptive immunity in vertebrates. Adaptive immunity, also known as acquired immunity, is the ability to recall previous encounters with a "nearly unlimited variety of antigens" (Minton 2009), thus leading to a more rapid and efficient response

to subsequent encounters with the same pathogen. This antigen-specific memory, which is the basis of vaccination, leads to a rapid and efficient secondary immune response but requires "extraordinarily diverse repertoires of somatically assembled antigen receptors" (Boehm et al. 2012). Adaptive immunity is considered another hallmark of the vertebrates but, unlike the jawed vertebrates that recognize antigens using immunoglobulin-based B-cell and T-cell receptors, extant agnathans use variable lymphocyte receptors (VLRs). Both jawless and jawed vertebrates create the necessary diversity of antigen receptor genes through gene rearrangement, but the mechanisms are different in the two vertebrate lineages. In lampreys and hagfishes, antigen receptor diversity is generated through the somatic assembly of variable leucine-rich repeat (LRR) modules, whose product is expressed clonally on lymphocytes (Kasahara 2013; Kishishita and Nagawa 2014). However, despite the independent evolution of alternative antigen receptor systems in jawless and jawed vertebrates, it also appears that their adaptive immune systems share some fundamental similarities. In particular, recent evidence suggests that both agnathan and gnathostome vertebrates have three major lymphocyte lineages (i.e., that lampreys and hagfishes have one B-cell-like lineage and two T-cell-like lineages; Hirano et al. 2013; Li et al. 2013), suggesting that these cell lineages were present in the common vertebrate ancestor before the advent of the different antigen receptor gene rearrangement systems (Kasahara 2013; Kishishita and Nagawa 2014). In both agnathans and gnathostomes, only one type of antigen receptor is expressed per lymphocyte, and the three different lineages of lymphocytes express three different antigen receptors (Kishishita and Nagawa 2014). Given the intriguing similarities and differences between the agnathan and gnathostome systems, further study of the lamprey (and hagfish) adaptive immune system will improve our fundamental understanding of "this elegant system" (Kishishita and Nagawa 2014), and can potentially improve treatment for people with faulty immune systems (Boehm et al. 2012).

Evidence has recently emerged suggesting that "hemoglobin" has also evolved independently in agnathan and gnathostome vertebrates. Phylogenetic analysis of the vertebrate globin gene superfamily suggests that a specialized oxygen transport function was acquired independently in paralogous globin genes (i.e., genes that diverged after a gene or genome duplication event) in jawless and jawed vertebrates (Hoffmann et al. 2010). These results indicate that lamprey and hagfish hemoglobin is most closely related to the gnathostome cytoglobin protein. The two vertebrate lineages therefore appear to make use of functionally similar (but "independently invented") respiratory pigments to increase the oxygen carrying capabilities of the blood, a key physiological innovation that permitted larger body size and "opened up new opportunities for the evolution of aerobic metabolism" in vertebrates (Hoffmann et al. 2010).

The recent publication of the sea lamprey genome assembly (Smith et al. 2013)—and other advances in developmental biology and molecular genetics (e.g., McCauley and Bronner-Fraser 2006; Nikitina et al. 2009; Heath et al. 2014; see Lee and McCauley in press)—now promise to lead to further advances in many of the research areas described above (and countless others). Freely available as a

public resource, sequencing of the sea lamprey genome is already providing valuable information on genes related, for example, to olfaction (Libants et al. 2009), neuron regeneration in the central nervous system (Smith et al. 2011), and the neuroendocrine control of reproduction (Decatur et al. 2013). By virtue of its phylogenetic position, the sea lamprey genome is "uniquely poised to provide insight into the ancestry of vertebrate genomes and the underlying principles of vertebrate biology" (Smith et al. 2013). One major finding that emerged from sequencing of this genome is confirmation that two rounds of genome duplication likely occurred prior to the agnathan-gnathostome divergence (Barucchi et al. 2013; Decatur et al. 2013; Smith et al. 2013; see Chap. 7). Although it has long been thought that two large-scale genome duplications occurred during the evolution of early vertebrates (Ohno 1970), there has been continuing controversy over whether the second duplication occurred in the lineage leading to all extant vertebrates or whether there was one round of duplication prior to and one round of duplication after divergence of the jawless vertebrates (see Kuraku et al. 2009; Shimeld and Donoghue 2012). Gene and especially genome duplication events are thought to provide genetic raw material for evolution; two rounds of whole genome duplication at the base of the vertebrate family tree (i.e., on either side of the large gulf that exists between the non-vertebrate chordates and even the earliest extant vertebrates) would have enabled the large number of vertebrate-specific innovations (Ohno 1970; Shimeld and Holland 2000).

Also of interest in this regard is the recent report of potential horizontal gene transfer (HGT) between lampreys and their teleost hosts (Kuraku et al. 2012). Highly similar sequences of a DNA transposon were discovered in multiple fishes that are phylogenetically disparate, but almost all of these fishes serve as hosts to parasitic lampreys, suggesting that these elements were transferred through parasite-host interactions (Kuraku et al. 2012). HGT has been well documented in prokaryotes (e.g., Gogarten et al. 2002)—and increasingly so in eukaryotes (through viral infection, phagocytosis, symbiosis, and parasitism)—and has been speculated to enhance the evolutionary potential of the recipient lineage (Koonin 2009; Wijayawardena et al. 2013).

Another feature of the sea lamprey genome that has generated interest is the observation of programmed genome rearrangement (PGR); during embryogenesis, approximately 20% of the germline genome (hundreds of millions of base pairs) is lost from somatic cell lineages (Smith et al. 2009). Although a small number of programmed local rearrangements is typical during development in vertebrates (and more extensive genomic reorganizations have been noted in some invertebrate species; e.g., Goday and Esteban 2001; Bachmann-Waldmann et al. 2004; McKinnon and Drouin 2013), lampreys and hagfishes (Kubota et al. 1997; Kojima et al. 2010) are the only vertebrates known to undergo broad-scale PGR. Programmed genome rearrangement and loss may represent an ancient biological strategy to ensure that pluripotential functions are segregated to the germline, thereby preventing the potential for somatic misexpression (Smith et al. 2009, 2012). Understanding the mechanisms by which agnathan vertebrates regulate programmed changes to their genomes has the potential to help understand the dysregulated changes that give rise to cancers and other genomic disorders (Smith et al. 2009).

Lampreys (and hagfishes) are thus providing important and promising model systems in our quest to better understand the evolutionary history of the vertebrates. Neither lampreys nor hagfishes are directly representative of the common vertebrate ancestor (specializations unique to each lineage appear to have arisen early in phylogeny and then been retained), but comparisons among lampreys, hagfishes, and gnathostomes are providing great insights into the morphology, physiology, development, and genomics of the common ancestor to all vertebrates (Janvier 2007; Shimeld and Donoghue 2012). This exciting topic is reviewed very briefly here; interested readers are directed to the many excellent reviews on this topic (e.g., Shigetani et al. 2005; Osório and Rétaux 2008; Kuratani 2012; Shimeld and Donoghue 2012; Lee and McCauley in press) and are encouraged to follow what are sure to be exciting advancements in the next decade and beyond.

1.2.3.3 Use of Lampreys in Biomedical and Biomimetic Studies

Lampreys have long been used as models in biomedical research (Table 1.1). For example, Sigmund Freud, while a young medical student, studied the spinal ganglia and spinal cord in the lamprey (Freud 1877, 1878). Such biomedical research continues today and, recently, a biorobotic system inspired by lampreys has been developed as an investigative tool for studying high level motor tasks. A brief overview of some areas in which lampreys may provide insights into the treatment of human health problems and as a prototype for studies on vertebrate locomotion is provided below.

Anti-coagulants The salivary glands of blood-feeding organisms have long been of interest to pharmacologists and biochemists, due to the bioactive substances that they contain (Odani et al. 2012). The European medicinal leech *Hirudo medicinalis*, for example, was used therapeutically as far back as 2,500 years ago (e.g., with Hippocrates advocating bloodletting as a means of balancing "the four humors"). Its saliva contains approximately 30 biologically active substances, including the peptide hirudin to keep the blood flowing and enzymes to anesthetize the host and reduce inflammation at the site of the bite (Nature 2012). Hirudin was isolated in the 1950s, and recombinant techniques are now used to produce hirudin for treatment of blood coagulation disorders (Rydel et al. 1991). The salivary gland secretions of many other hematophagous animals have been studied for their possible biomedical applications; these include insects such as mosquitoes, ticks, and the kissing bugs *Rhodnius prolixus* and *Triatoma infestans*, as well as vampire bats *Desmodus rotundus*, with their colorfully named anti-coagulant "draculin" (see Basanova et al. 2002; Odani et al. 2012). The anti-coagulating action of the buccal gland secretions in parasitic lampreys was identified by Gage and Gage-Day in 1927, but the biochemical nature of these secretions has received little attention until recently. The diverse bioactive proteins (termed "lamphredin" by Lennon in 1954) are being investigated in the Arctic lamprey, and have been shown to have fibrinogenolytic and vasodilatory properties (Ito et al. 2007; Xiao et al. 2007, 2012). In addition to these proteinaceous components, Odani et al. (2012) discov-

ered L-3-hydroxykynurenine *O*-sulphate in the buccal glands of this species; this molecule, remarkably, was found in *Rhodnius prolixus* and *Triatoma infestans* in the 1960s (see Odani et al. 2012). The buccal gland secretions of parasitic lampreys are thus another potential source for the development of novel anti-coagulants, local anesthetics, immunosuppressants, and thrombolytic agents (Xiao et al. 2012).

Biliary Atresia Research on lampreys may also improve our understanding of a medical condition known as cholestasis, whereby bile is unable to flow from the liver to the duodenum and, in particular, biliary atresia, the most common cause of cholestasis during infancy (Youson 1993; Morii et al. 2012; Suchy 2013). In the congenital form of biliary atresia, babies are born lacking a common bile duct between the liver and small intestine, resulting in jaundice, malabsorption of nutrients and growth retardation, fat-soluble vitamin deficiencies, and eventually cirrhosis. While biliary atresia is rare, it is fatal if a liver transplant is not possible (Youson 1993; Morii et al. 2012). Lampreys are an excellent—and again unparalleled—vertebrate model system in which to study cholestasis. Larval lampreys possess an intrahepatic gallbladder and a biliary tree that is well equipped for the storage, transport, and elimination of bile into the intestine; at metamorphosis, however, lampreys undergo a programmed loss of the gall bladder and biliary tree (see Chap. 4) and yet are able to survive without these structures—for several years in some parasitic species (Youson 1993). As expected, bile pigments (bilirubin and biliverdin) are not detected in the serum of larval lampreys, but become detectable after biliary atresia (Makos and Youson 1987). However, serum concentrations following metamorphosis are lower than expected (e.g., compared to humans suffering from jaundice), suggesting either storage of bile in the liver or another organ or an alternate mechanism for the transport and elimination of these potentially toxic pigments (Makos and Youson 1987; Youson 1993). Bilirubin concentration has been shown to increase dramatically in the liver and caudal intestine of sea lamprey following loss of the larval bile ducts (Langille and Youson 1983; Makos and Youson 1988), leading to suggestions that bilirubin in the liver may be mobilized and transported (via the blood) to the caudal intestine for subsequent elimination (Langille and Youson 1983; Youson 1993). Bilirubin and biliverdin may be bound for transport and detoxified by lamprey-specific serum proteins (Filosa et al. 1982). Serum bilirubin and biliverdin concentrations were higher in upstream migrants, but were only slightly above normal values observed in humans (Makos and Youson 1987). What is more remarkable are reports of one population of American brook lamprey *Lethenteron appendix* larvae with serum biliverdin concentrations ranging from 142 to 305 μmol/L (Eng and Youson 1991). In this population, the bile ducts are infested with larval nematodes (Pybus et al. 1978), causing bile pigment regurgitation into the blood. The highest value recorded in a human with biliverdinemia is 46 μmol/L (Greenberg et al. 1971), and yet there is no evidence of deleterious effects in these larvae (Eng and Youson 1991). Thus, juvenile and adult lampreys apparently respond to the normal programmed loss of the gall bladder and biliary tree by using alternate mechanisms for the transport and elimination of bile pigments whereas, under conditions of abnormal cholestasis, larvae appear able to cope with very high levels of these otherwise toxic bile

pigments. Recent studies have identified apoptosis as an early event in bile duct loss in induced (Boomer et al. 2010) and spontaneous (Morii et al. 2010) metamorphosis, and have investigated the way in which lampreys are able to deal with cytotoxic bile salts (i.e., in addition to bile pigments) following biliary atresia (Yeh et al. 2012; Cai et al. 2013).

Iron Loading Lampreys could provide insights into treatment for people suffering from hemochromatosis, a genetic condition in which the body absorbs an excessive amount of iron from the diet. In individuals with hemochromatosis, iron continues to be absorbed even after the body's daily requirements are met, and this excess is stored in different organs and tissues (e.g., liver, heart, pituitary gland; Nichols and Bacon 1989). Hereditary hemochromatosis is the most common single-gene disease in western populations, affecting 1 out of every 200–300 people (American Diabetes Association 2013). Once diagnosed, it can be managed by the regular removal of blood but, if untreated, it can result in chronic fatigue, arthritis, diabetes, heart and liver disease, and may eventually lead to death (Nichols and Bacon 1989). Given their sanguivorous foraging strategy, parasitic lampreys ingest large amounts of iron, and have a unique capacity to store and tolerate high concentrations of iron in various body tissues (e.g., liver and adipose tissue; Tsioros et al. 1996; Tsioros and Youson 1997). Another sanguivorous vertebrate, the vampire bat—despite a daily iron intake 800-fold greater than that of humans—controls iron content by controlling rate of absorption (Morton and Wimsatt 1980; Morton and Janning 1982). Furthermore, exceptional iron concentrations in lampreys are not just observed in blood-feeding adults; larvae also show very high iron concentrations, apparently as the result of maternal transfer (probably during vitellogenesis) and uptake from the substrate (Tsioros et al. 1996). Larval lampreys accumulate iron in their blood and nephric fold at levels that would be toxic to other vertebrates: compared with 127 μg/100 mL in the serum of a normal average man (Underwood 1977), levels ranging from 5,119 to 26,773 μg/100 mL have been reported in larval lampreys (e.g., Macey et al. 1982a, 1985; Macey and Potter 1986; Youson et al. 1987). Plasma iron concentrations decline markedly at metamorphosis (e.g., Macey et al. 1982b), but iron concentration in the liver shows a dramatic increase (Harris et al. 1990); by the end of metamorphosis, lamprey hepatocytes resemble the iron-loaded hepatocytes seen in humans suffering from hemochromatosis (Youson et al. 1983). Since neither larvae nor adults show any deleterious effects as a result of this excess iron, lampreys are an excellent model system to elucidate the biochemical mechanisms by which they counteract the problems associated with iron loading in other vertebrates (Youson et al. 1983; Tsioros and Youson 1997; Macey et al. 1988). The activity of detoxifying enzymes responsible for minimizing the production of hydroxyl radicals (e.g., superoxide dismutase in the liver; Macey et al. 1988; Harris et al. 1990, 1995) and the nature of the iron-binding proteins in the plasma (e.g., ferritin; Macey et al. 1982b; Andersen et al. 1998) have received some attention (see Chap. 4), but beg further study.

Spinal Cord Regeneration The lamprey central nervous system (CNS) shares its basic organization and structure with other vertebrates (Rovainen 1979;

Grillner and Jessell 2009), but is characterized by two features in particular that have led to its extensive use as a model system in neurological studies. Not only might the comparatively simple brain and neural networks reflect the structure of early vertebrate ancestors, but lampreys are endowed with both unusually large ("giant") reticulospinal (RS) neurons and the ability to recover nearly full function after complete spinal cord transection (Rovainen 1976). The large size of both the somata and axons of the giant RS neurons allows for microinjection of substances (e.g., tracers, antibodies, recombinant proteins) for experimental manipulation, and allows detailed examination of their responses to injury and regeneration (Smith et al. 2011). In most vertebrates, including humans, severe spinal cord injury results in permanent loss of voluntary motor control below the lesion site due to the low regenerative capacity of injured RS neurons (Bradbury and McMahon 2006). In contrast, lampreys are capable of spontaneous functional recovery due to the regeneration of RS axons, even following complete spinal cord transections (Cohen 1988; Cohen et al. 1988, 1989; Rodicio and Barreiro-Iglesias 2012). In fact, lampreys are the only vertebrates for which sufficient experimental data exist to satisfy the criteria for functional spinal cord regeneration after injury, as defined by the National Institutes of Neurological Disorders and Stroke (Cohen et al. 1988, 1989). With lampreys as a model, we may be able to better understand what factors promote or inhibit regeneration (e.g., Smith et al. 2011; Lau et al. 2011, 2013; Pale et al. 2013; Zhang et al. 2011, 2014) and develop novel therapies for people suffering from motor neuron disease and injury (Cornide-Petronio et al. 2011).

Biomimetics Lampreys have also been the inspiration for biorobotic research, representing an exciting intersection between neuroscience and robotics (Ijspeert et al. 2013). Their relatively simple and well-studied neural networks and their highly efficient swimming abilities—requiring coordination between the nervous system, the musculoskeletal system, and the environment—have led to their selection as simple animal models in which to study the general principles of locomotion (e.g., Kozlov et al. 2009; Stefanini et al. 2012; Manfredi et al. 2013). Lamprey-like bio-inspired robots are the basis of the European LAMPETRA (Life-like Artifacts for Motor-Postural Experiments and development of new control Technologies inspired by Rapid Animal locomotion) project (Ijspeert et al. 2013). Lamprey spinal central pattern generator networks have been explored through large-scale computer simulations (Kozlov et al. 2009); validation of these biological models is now possible with robots (Stefanini et al. 2012; Manfredi et al. 2013). Recently, a lamprey was used as the basis for a computer-simulated animal model that was able to move around an environment containing visually detectable objects. This model was able to respond to multiple, sometimes conflicting, stimuli and provided accurate predictions of how even an animal with neural lesions would subsequently interact with its environment (Kamali Sarvestani et al. 2013). In addition to providing new insights into functioning of the vertebrate central nervous system, these lamprey-inspired robots may also lead to new engineering solutions for high-performance artificial locomotion (Ijspeert et al. 2013).

1.3 Introduction to *Lampreys: Biology, Conservation and Control*

1.3.1 Focus of the Book

This book is intended to provide a comprehensive review of the phylogeny, evolution, ecology, and general biology of lampreys, including coverage of the conservation of native lampreys, control of the invasive sea lamprey in the Laurentian Great Lakes, and the use of lampreys as vertebrate model organisms. It is meant to provide an update to influential previous compilations, particularly Hardisty and Potter's five-volume *The Biology of Lampreys* (Hardisty and Potter 1971a, 1972, 1981, 1982a, b) and the *Proceedings of the 1979 Sea Lamprey International Symposium*, published in the Canadian Journal of Fisheries and Aquatic Sciences (SLIS 1980). The advent of new technologies (e.g., improved electrofishing gear, miniaturized active and passive transmitters, molecular genetic markers)—and a continuing or renewed interest, respectively, in the control and conservation of lampreys (see Chap. 8; Marsden and Siefkes in press)—have led to many advances in our knowledge of lamprey ecology and behavior (see Chaps. 3, 5 and 6; Renaud and Cochran in press). Studies on the endogenous control of metamorphosis (see Chap. 4), lamprey pheromones (see Chaps. 5 and 6), reproductive endocrinology (see Chap. 7; Docker et al. in press), molecular phylogenetics (see Chap. 2; Docker and Potter in press), and genomics (Lee and McCauley in press) were in their infancy or unknown three decades ago.

A conscious effort has been made to include coverage of the less well-known lamprey species, if for no other reason than to highlight the gaps in our knowledge regarding them, and to include topics of interest to lamprey researchers worldwide and coverage of international conservation and management efforts. It is hoped that this book will be used as a reference for researchers working on any aspect of lamprey biology—the already-dedicated lamprey biologist (for whom there is no such thing as a surfeit of lampreys), those just starting to use lampreys as model organisms (but who appreciate the need to better understand the biology of their model), and fishery managers whose mandate is to control or conserve lamprey populations.

1.3.2 Nomenclatural Conventions

As is the case in any discipline, there exists some disagreement and confusion regarding how we name and discuss lampreys (e.g., regarding the names of the different stages in their complex life cycle and accepted common and scientific names for each species). The conventions adopted for this book (and the rationale for doing so) are outlined below.

What Are the Appropriate Names of Each Stage in the Lamprey Life Cycle? Terms used to describe the stages of a lamprey life cycle are diverse and may include the following: ammocoetes (or larvae) → transformers (or metamorphosing lampreys) → juveniles (metamorphosed but sexually-immature lampreys, including macrophthalmia, downstream migrants, and feeding-phase "adults") → upstream migrants → sexually-mature adults. There is not universal agreement, however, on the use of these terms and not all apply to all lamprey species. The term "ammocoetes" is a holdover from times when larval and post-metamorphic lampreys were considered separate genera of cyclostome fishes (with hagfishes comprising the third cyclostome genus; Duméril 1806). "Ammocoetes" literally (in Greek) means "burrower of sand" (Renaud 2011; cf. sand lances *Ammodytes* spp.). Decades later, Müller (1856) recognized that the ammocoete was in fact an immature developmental stage of a lamprey, yet the term was still used to refer to larval lampreys. In this book, either term is used (at the discretion of the contributing authors), although we prefer to use "larval lamprey" when writing for more general audiences as the term "ammocoetes," in our opinion, conveys no more additional information than the more universally-understood term "larva." The term "transformer" is more of a colloquial term, generally referring to lampreys that are undergoing metamorphosis (transformation). "Transformer" is often used in place of the less concise term "metamorphosing lamprey." Although it is rarely used by those describing the process of metamorphosis (e.g., Youson and Potter 1979; see Chap. 4), where precision is preferred to concision—and Applegate (1950) used the less concise but more informative terms "transforming lampreys," "lampreys in advanced stages of transformation," "recently-transformed lampreys," or "newly-transformed lampreys"—it is a useful term when knowledge of the specific stages of metamorphosis is either not available or not essential. Lampreys that have completed metamorphosis but are not yet sexually mature are generally referred to as "juveniles" (see Chap. 3), although this term is sometimes (but should not be) confused with the larval stage. The juvenile stage may include a "macrophthalmia" stage, the immediate post-metamorphic stage so named because of its conspicuous eyes when compared to the blind larvae. This term was apparently first applied to this stage by Maskell (1929), referring to juvenile pouched lamprey (Fig. 1.3), but the term originated in 1897 when the Chilean form of this species was described as *Macrophthalmia chilensis* (Plate 1897). Although the eyes are smaller in non-parasitic species (see Fig. 4.1), the term has also—but less frequently—been applied to immediately post-metamorphic brook lampreys (e.g., Hardisty et al. 1970). In parasitic species, the macrophthalmia stage ends with the onset of feeding (Hardisty and Potter 1971b). After observing that feeding in Pacific lamprey commenced (in either fresh or salt water) almost immediately after the completion of metamorphosis, Beamish (1980) suggested that this species does not have a macrophthalmia stage or it is very short. The term "macrophthalmia" is still used (e.g., Moser et al. 2007; Streif 2009), but reference simply to "recently-transformed lampreys" (or "recently-transformed juveniles") and "downstream migrants" (or "downstream-migrating juveniles") is more common. The parasitic feeding phase is technically still part of the juvenile stage since the lampreys are sexually immature; sexual maturation (and hence "adulthood")

Fig. 1.3 Pouched lamprey *Geotria australis* "macrophthalmia" from the Okuti River in the South Island of New Zealand. Although the large eyes for which this stage is named are evident in all species (particularly parasitic species), the beautiful iridescent blue coloration seen here is unique to pouched lamprey. (Photo: © http://www.rodmorris.co.nz)

occurs sometime during or upon completion of upstream migration (see Chap. 5; Docker et al. in press). In non-parasitic species, the juvenile stage is truncated or essentially non-existent (Hardisty 2006; Docker 2009); since the "sexual products are almost ripe on the eve of metamorphosis" (Berg 1948), the adult stage in brook lampreys is generally said to commence on completion of metamorphosis.

What Is the Correct Plural of "Lamprey"? This book will follow the convention used by Nelson (2006) regarding the use of "fish" versus "fishes" and refer to individuals of more than one species as "lampreys" (e.g., lampreys have long been valued for food and ceremonial purposes, 12 sea and European river lampreys were captured) and one or more individuals of a single species as "lamprey" (e.g., 12 sea lamprey were captured, the Pacific lamprey were tracked for six months).

How Many Lamprey Species Are There? Several years ago, the senior author of this chapter wrote in the introduction of a manuscript on lamprey phylogeny that there were "approximately 40 species of lampreys worldwide." One of the peer reviewers asked that the *exact* number of lamprey species be given, but there is no exact, universally agreed upon, objectively definable number. Although most biologists (lamprey and otherwise) agree that species are evolutionarily independent units that are isolated by a lack of gene flow, there is a lack of consensus on how—in practice—these evolutionarily independent units are recognized (Mayden 1997; de Queiroz 2007). Do two groups of organisms, for example, need to exhibit diagnostic morphological differences to be recognized as distinct species or are diagnostic molecular differences, even in the absence of clear morphological differences, sufficient indication of evolutionary independence? At what point are differences among populations considered species-level differences rather than, say, differences among subspecies? Are obvious morphological differences (e.g., between parasitic and non-parasitic "paired" lamprey species as adults) necessarily indicative of a lack of gene flow? Debating the relative merits of the three most commonly applied species concepts (i.e., the morphological, biological, and phylogenetic species concepts) is far beyond the scope of this chapter (see Mayden 1997; Docker 2009), but readers should be aware that inferring the boundaries between species (and hence the number of distinct species) can be subjective. Whereas

Renaud (2011) recognized 40 species in his *Lampreys of the World*, Potter et al. (see Chap. 2) recognize 41 species and Maitland et al. (see Chap. 8)—recognizing three recently-described "cryptic" brook lamprey species (Mateus et al. 2013) as distinct from European brook lamprey—list 44 species. It is also important to acknowledge that these numbers are for formally described species; a number of putative lamprey species (e.g., Yamazaki et al. 2003; Boguski et al. 2012) have not yet been described (see Chap. 2). Thus, a succinct answer to the question "How many lamprey species are there?" still remains "approximately 40."

What Are the Conventions Used for Common and Scientific Names in this Book? Within each chapter, both scientific and common names are given on first use, and common names are used exclusively thereafter. The only exception is in the chapter dealing with lamprey taxonomy (see Chap. 2), where the scientific names are more informative in that context (i.e., with placement in the same genus implying closer relationship). In Chap. 2, where genus names that start with the same letter are abbreviated, the first two letters are used (e.g., *Le.* and *La.* to distinguish *Lethenteron* from *Lampetra*). The scientific names follow the American Fisheries Society's (AFS) seventh edition of *Common and Scientific Names of Fishes from the United States, Canada, and Mexico* (Page et al. 2013a) for North American species and, with one exception (the Po brook lamprey *Lampetra zanandreai*; see Chap. 2), FishBase (Froese and Pauly 2014) for other species. The describing authorities and date of authorship are given in Appendix 2.1; note that, in the case of changed genus and species combinations (i.e., where a species has been reassigned to a different genus than that in which it was originally described), the authorship and date are set in parentheses. Common names used in the book generally agree with the standard common names recommended in the AFS Names of Fishes list (Page et al. 2013a) or those used in FishBase, but authors were not restricted to using these names only. In some cases, common names selected by the AFS are not yet well known (particularly elsewhere in the world) or their stabilities have yet to be proven (see Kendall 2002); in other cases, experts working on these species prefer other common names (see Table 2.1). In no case have more than two common names been used for a single species and, of course, the species in question has been clearly identified on first mention by its scientific name. Thus, it was felt that the use of alternate (i.e., other common) common names would reduce (and not lead to) confusion among readers, particularly given the intended international audience for this book.

Why Are Common Names Not Capitalized in this Book? In 2002, an ad hoc committee of the American Society of Ichthyologists and Herpetologists (ASIH) advocated capitalizing the common names of fish species (Nelson et al. 2002). Two compelling arguments for capitalization included elimination of ambiguity (i.e., because "treating common names as proper nouns ensures that adjectives are recognized as part of the names rather than as a descriptive adjective") and giving emphasis to the name (letting it "stand out and be easier to spot in scientific publications"). In December 2003, the editorial board of *Copeia*, the journal of the ASIH, started capitalizing species common names in this journal. The seventh edition of *Common and Scientific Names of Fishes from the United States, Canada, and Mexico* (Page et al. 2013a) included capitalization of English (but not

French or Spanish) common names and, as of January 2013, AFS publications (e.g., *Transactions of the American Fisheries Society*, *North American Journal of Fisheries Management*, *Fisheries*, *Journal of Aquatic Animal Health*) likewise required that English common names be capitalized. Some organizations [e.g., the International Union for the Conservation of Nature (IUCN) and the Committee on the Status of Endangered Wildlife in Canada (COSEWIC)] have followed suit. Despite this recent trend toward capitalization of (English) common names, however, common names are not capitalized in this book. It was the editor's feeling that, despite being passionately embraced by many ichthyologists and fish biologists, the preference for capitalized common names is far from universal. Even among North American ichthyologists, there seems to be dissent (e.g., Kendall 2002) and the majority of "fishy" journals in which many of us publish (e.g., *Journal of Fish Biology*, *Canadian Journal of Fisheries and Aquatic Sciences*, *Environmental Biology of Fishes*, *Journal of Applied Ichthyology*, *Ecology of Freshwater Fish*, *Fish and Fisheries*) do not capitalize common names. Furthermore, given that there are subtle rules associated with the capitalization of common names (e.g., individual species names are capitalized but not the common portions of names shared by two or more species, common names of fish species are capitalized but not the names of non-fish species, common names of subspecies are capitalized but not the names of life history variants; see Page et al. 2013b), the editor was concerned that this will create a divide between ichthyologists and other biologists interested in lampreys. Having *fewer* rules for common names is more likely to *improve* communication among those interested in lampreys. The use, on first mention, of both common names and scientific names (with all the latter's rules; International Commission on Zoological Nomenclature 1999) should prevent any ambiguity. Thus, despite the many arguments given for capitalization—and our willingness, of course, to capitalize common names when required—we felt it was both unnecessary and premature to do so in this book. Let's see first if this preference for capitalized common names has, like lampreys, a broad global distribution and the ability to stand the test of time.

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References

Aberdeen Bestiary (1200) University of Aberdeen MS 24

American Diabetes Association (2013) Hemochromatosis. http://www.diabetes.org/living-with-diabetes/complications/related-conditions/hemochromatosis.html. Accessed 12 April 2014

Andersen O, Pantopoulos K, Kao HT et al (1998) Regulation of iron metabolism in the sanguivore lamprey *Lampetra fluviatilis*. Molecular cloning of two ferritin subunits and two iron-regulatory proteins (IRP) reveals evolutionary conservation of the iron-regulatory element (IRE)/IRP regulatory system. Eur J Biochem 254:223–229

Andrade NO, Quintella BR, Ferreira J et al (2007) Sea lamprey (*Petromyzon marinus* L.) spawning migration in the Vouga river basin (Portugal): poaching impact, preferential resting sites and spawning grounds. Hydrobiologia 582:121–132

Applegate VC (1950) Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. US Fish and Wildlife Service Special Scientific Report 55, Washington, DC

Bachmann-Waldmann C, Jentsch S, Tobler H, Müller F (2004) Chromatin diminution leads to rapid evolutionary changes in the organization of the germline genomes of the parasitic nematodes *A. suum* and *P. univalens*. Mol Biochem Parasitol 134:53–64

Bardack D (1991) First fossil hagfish (Myxinoidea): a record from the Pennsylvanian of Illinois. Science 254:701–703

Bardack D (1998) Relationships of living and fossil hagfishes. In: Jørgensen JM, Lomholt JP, Weber RE, Malte H (eds) The biology of hagfishes. Chapman and Hall, London, pp 3–14

Barucchi V, Giovannotti M, Cerioni P, Splendiani A (2013) Genome duplication in early vertebrates: insights from agnathan cytogenetics. Cytogenet Genome Res 141:80–89

Basanova AV, Bakova I, Zavalova L (2002) Vascular-platelet and plasma hemostasis regulators from bloodsucking animals. Biochemistry 67:167–176

Beamish RJ (1980) Adult biology of the river lamprey (*Lampetra ayresi*) and the Pacific lamprey (*Lampetra tridentata*) from the Pacific coast of Canada. Can J Fish Aquat Sci 37:1906–1923

Beamish RJ, Neville CM (1995) Pacific salmon and Pacific herring mortalities in the Fraser River plume caused by river lamprey (*Lampetra ayresi*). Can J Fish Aquat Sci 52:644–650

Beamish RJ, Youson JH (1987) Life history and abundance of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the Strait of Georgia. Can J Fish Aquat Sci 44:525–537

Beaulaton L, Taverny C, Castelnaud G (2008) Fishing, abundance and life history traits of the anadromous sea lamprey (*Petromyzon marinus*) in. Europe Fish Res 92:90–101

Berg LS (1948) Freshwater fishes of the USSR and adjacent countries. Guide to the fauna of the USSR no 27, vol 1. Israel Program for Scientific Translations, Jerusalem

Bicanski A, Ryczko D, Cabelguen JM, Ijspeert AJ (2013) From lamprey to salamander: an exploratory modeling study on the architecture of the spinal locomotor networks in the salamander. Biol Cybern 107:565–587

Boehm T, Iwanami N, Hess I (2012) Evolution of the immune system in the lower vertebrates. Annu Rev Genomics Hum Genet 13:127–149

Boguski DA, Reid SB, Goodman DH, Docker MF (2012) Genetic diversity, endemism and phylogeny of lampreys within the genus *Lampetra sensu stricto* (Petromyzontiformes: Petromyzontidae) in western North America. J Fish Biol 81:1891–1914

Boomer LA, Bellister SA, Stephenson LL et al (2010) Cholangiocyte apoptosis is an early event during induced metamorphosis in the sea lamprey, *Petromyzon marinus* L. J Pediatr Surg 45:114–120

Bradbury EJ, McMahon SB (2006) Opinion. Spinal cord repair strategies: why do they work? Nat Rev Neurosci 7:644–653

Cai SY, Lionarons DA, Hagey L et al (2013) Adult sea lamprey tolerates biliary atresia by altering bile salt composition and renal excretion. Hepatology 57:2418–2426

Clemens BJ, Binder TR, Docker MF, Moser ML, Sower SA (2010) Similarities, differences, and unknowns in biology and management of three parasitic lampreys of North America. Fisheries 35:580–596

Close DA, Fitzpatrick MS, Li HW (2002) The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries 27:19–25

Cochran PA (1994) Why lampreys and humans "compete" (and when they don't): toward a theory of host species selection by parasitic lampreys. In: Stouder DJ, Fresh KL, Feller RJ (eds) Theory and application in fish feeding ecology. Belle W Baruch Library in Marine Science, No 18, University of South Carolina Press, Columbia, pp. 329–345

Cochran PA (2009) Predation on lampreys. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 139–151

Cohen AH (1988) Regenerated fibers of the lamprey spinal cord can coordinate fictive swimming in the presence of curare. J Neurobiol 19:193–198

Cohen AH, Mackler SA, Selzer ME (1988) Behavioral recovery following spinal transection: functional regeneration in the lamprey CNS. Trends Neurosci 11:227–231

Cohen AH, Baker MT, Dobrov TA (1989) Evidence for functional regeneration in the adult lamprey spinal cord following transection. Brain Res 496:368–372
Collin SP (2010) Evolution and ecology of retinal photoreception in early vertebrates. Brain Behav Evol 75:174–185
Collin SP, Potter IC (in press) Sensory ecology and behavior in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Cornide-Petronio ME, Ruiz MS, Barreiro-Iglesias A, Rodicio MC (2011) Spontaneous regeneration of the serotonergic descending innervation in the sea lamprey after spinal cord injury. J Neurotrauma 28:2535–2540
Decatur WA, Hall JA, Smith JJ, Li W, Sower SA (2013) Insight from the lamprey genome: glimpsing early vertebrate development via neuroendocrine-associated genes and shared synteny of gonadotropin-releasing hormone (GnRH). Gen Comp Endocrinol 192:237–245
Delarbre C, Gallut C, Barriel V, Janvier P, Gachelin G (2002) Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. Mol Phylogen Evol 22:184–192
de Queiroz K (2007) Species concepts and species delimitation. Syst Biol 56:879–886
Deshpande SS (2002) Handbook of food toxicology. CRC, Boca Raton
Dickens C (1852) A child's history of England. Bradbury & Evans, London
Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp. 71–114
Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Docker MF, Beamish FWH, Bryan MB, Spice EK (in press) The lamprey gonad. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Duméril AMC (1806) Zoologie analytique, ou méthode naturelle de classification des animaux; rendue plus facile à l'aide de tableaux synoptiques. Allais, Paris
Eng F, Youson JH (1991) Biliverdin in the serum of ammocoetes of *Lampetra lamottenii* (Le Sueur). Can J Zool 69:1126–1129
European Fly Anger (2012) Lamprey: Fish of the Year 2012. http://www.euroflyangler.com/html/articles/lamprey/lamprey.php. Accessed 15 April 2014
Filosa MF, Sargent PA, Fisher MM, Youson JH (1982) An electrophoretic and immunoelectrophoretic characterization of the serum proteins of the adult lamprey, *Petromyzon marinus* L. Comp Biochem Physiol B Biochem Mol Biol 72:521–530
Foulds WL, Lucas MC (2014) Paradoxical exploitation of protected fishes as bait for anglers: evaluating the lamprey bait market in Europe and developing sustainable and ethical solutions. PLoS One 96:e99617
Freud S (1877) Über den Ursprung der hinteren Nervenwurzeln im Rückenmarke von Ammocoetes (Petromyzon Planeri). S B Akad Wiss Wien Abt III 75:15–27
Freud S (1878) Über ber Spinalganglien und Rückenmark des Petromyzon. S B Akad Wiss Wien Abt III 78:81–167
Froese R, Pauly D (2014) FishBase (eds). http://www.fishbase.org, version 04/2014. Accessed 9 June 2014
Gage SH, Gage-Day M (1927) The anticoagulant action of the secretion of the buccal glands of the lampreys (*Petromyzon*, *Lampetra* and *Entosphenus*). Science 66:282–284
Gess RW, Coates MI, Rubidge BS (2006) A lamprey from the Devonian period of South Africa. Nature 443:981–984
Goday C, Esteban MR (2001) Chromosome elimination in sciarid flies. Bioessays 23:242–250
Gogarten JP, Doolittle WF, Lawrence JG (2002) Prokaryotic evolution in light of gene transfer. Mol Biol Evol 19:2226–2238
Gradín C (2010) Estado de conservación da lamprea mariña (*Petromyzon marinus* Linnaeus 1758) en ríos de Galicia. Xunta de Galicia. Cansellería do Media Rural. Dirección Xeral de Conservación da Natureza, Santiago de Compostela, Spain

Greenberg AJ, Bossenmaier I, Schwartz BA, Schwartz S (1971) Green jaundice: study of serum biliverdin, mesobiliverdin and other green pigments. Am J Dig Dis 16:873–880
Griffith RW (1994) The Life of the first vertebrates. Bioscience 44:408–417
Grillner S, Jessell TM (2009) Measured motion: searching for simplicity in spinal locomotor networks. Curr Opin Neurobiol 19:572–586
Hardisty MW (2006) Lampreys. Life without jaws. Forrest Text, Tresaith
Hardisty MW, Potter IC (1971a) The biology of lampreys, vol 1. Academic, London
Hardisty MW, Potter IC (1971b) The general biology of adult lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 127–205
Hardisty MW, Potter IC (1972) The biology of lampreys, vol 2. Academic Press, London
Hardisty MW, Potter IC (1981) The biology of lampreys, vol 3. Academic Press, London
Hardisty MW, Potter IC (1982a) The biology of lampreys, vol 4A. Academic Press, London
Hardisty MW, Potter IC (1982b) The biology of lampreys, vol 4B. Academic Press, London
Hardisty MW, Potter IC, Sturge R (1970) A comparison of the metamorphosing and macrophthalmia stages of the lampreys *Lampetra fluviatilis* and *L. planeri*. J Zool 162:383–400
Harris LR, Cake MH, Macey DJ, Potter IC (1990) An increase in the concentration of hepatic iron during the metamorphosis of the lamprey *Geotria australis* is accompanied by increased superoxide dismutase activity. Fish Physiol Biochem 8:451–457
Harris LR, Macey DJ, Potter IC, Cake MH (1995) Hepatic molecular conversion and detoxification of ferritin iron in adult lampreys (*Geotria australis*), following natural and induced iron loading. Biochem J 305:975–980
Hayes S, Salomone P (2004) Informational letter: 2003 Yukon River arctic lamprey fishery summary. Alaska Department of Fish and Game Commercial Fisheries Division, Anchorage
Heard WR (1966) Observations on lampreys in the Naknek River system of southwest Alaska. Copeia 1966:332–339
Heath G, Childs D, Docker MF, McCauley DW, Whyard S (2014) RNA interference technology to control pest sea lampreys-a proof-of-concept. PLoS ONE 9:e88387
Heimberg AM, Cowper-Sal·lari R, Sémon M, Donoghue PCJ, Peterson KJ (2010) microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. Proc Natl Acad Sci U S A 107:19379–19383
Hirano M, Guo P, McCurley N et al (2013) Evolutionary implications of a third lymphocyte lineage in lampreys. Nature 501:435–438
Hoffmann FG, Opazo JC, Storz JF (2010) Gene cooption and convergent evolution of oxygen transport hemoglobins in jawed and jawless vertebrates. Proc Natl Acad Sci U S A 107:14274–14279
Hogg RS, Coghlan SM, Zydlewski J, Simon KS (2014) Anadromous sea lampreys (Petromyzon marinus) are ecosystem engineers in a spawning tributary. Freshw Biol 59:1294–1307
Holčík J (1986) *Caspiomyzon wagneri* (Kessler, 1870). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part I, Petromyzontiformes). Aula-Verlag, Wiesbaden, pp 119–142
Holland P (1601) The naturall historie of C. Plinius Secundus, London
Hsu LJ, Zelenin PV, Grillner S, Orlovsky GN, Deliagina TG (2013) Intraspinal stretch receptor neurons mediate different motor responses along the body in lamprey. J Comp Neurol 521:3847–3863
Ijspeert AJ, Grillner S, Dario P (2013) Foreword for the special issue on lamprey and salamander robots and the central nervous system. Biol Cybern 107:495–496
International Commission for Zoological Nomenclature (1999) International code for zoological nomenclature, 4th edn. International Trust for Zoological Nomenclature, London
Ito N, Mita M, Takahashi Y et al (2007) Novel cysteine-rich secretory protein in the buccal gland secretion of the parasitic lamprey, *Lethehteron japonicum*. Biochem Biophys Res Commun 358:35–40
Janvier P (1996) The dawn of the vertebrates: characters versus common ascent in the rise of current vertebrate phylogenies. Paleontology 39:259–287
Janvier P (2007) Living primitive fishes and fishes from deep time. In: McKenzie DJ, Farrell AP, Brauner CJ (eds) Primitive fishes, vol 26, Fish physiology. Academic Press, San Diego, pp 1–51

Janvier P (2010) microRNAs revive old views about jawless vertebrate divergence and evolution. Proc Natl Acad Sci U S A 107:19137–19138
Janvier P (2011) Comparative anatomy: all vertebrates do have vertebrae. Curr Biol 21:R661–R663
Kamali Sarvestani I, Kozlov A, Harischandra N, Grillner S, Ekeberg O (2013) A computational model of visually guided locomotion in lamprey. Biol Cybern 107:497–512
Kasahara M (2013) Evolution of lymphocyte lineages, Déjà vu: the identity of a third lineage of lymphocytes in lampreys. Immunol Cell Biol 91:599–600
Kawauchi H, Sower SA (2006) The dawn and evolution of hormones in the adenohypophysis. Gen Comp Endocrinol 148:3–14
Kendall RL (2002) Capital punishment. Fisheries 27:33–34
Kishishita N, Nagawa F (2014) Evolution of adaptive immunity: implications of a third lymphocyte lineage in lampreys. Bioessays 36:244–250
Kojima NF, Kojima KK, Kobayakawa S et al (2010) Whole chromosome elimination and chromosome terminus elimination both contribute to somatic differentiation in Taiwanese hagfish *Paramyxine sheni*. Chromosome Res 18:383–400
Koonin EV (2009) Darwinian evolution in the light of genomics. Nucleic Acids Res 37:1011–1034
Kozlov A, Huss M, Lansner A, Kotaleski JH, Grillner S (2009) Simple cellular and network control principles govern complex patterns of motor behavior. Proc Natl Acad Sci U S A 106:20027–20032
Kubota S, Ishibashi T, Kohno S (1997) A germ line-restricted, highly repetitive DNA sequences: an interspecifically conserved, but somatically eliminated, element. Mol Gen Genet 256:252–256
Kuraku S, Hoshiyama D, Katoh K, Suga H, Miyata T (1999) Monophyly of lampreys and hagfishes supported by nuclear DNA-coded genes. J Mol Evol 49:729–735
Kuraku S, Meyer A, Kuratani S (2009) Timing of genome duplications relative to the origin of the vertebrates: did cyclostomes diverge before or after? Mol Biol Evol 26:47–59
Kuraku S, Qiu H, Meyer A (2012) Horizontal transfers of Tc1 elements between teleost fishes and their vertebrate parasites, lampreys. Genome Biol Evol 4:817–824
Kuratani S (2005) Developmental studies of the lamprey and hierarchical evolutionary steps towards the acquisition of the jaw. J Anat 207:489–499
Kuratani S (2012) Evolution of the vertebrate jaw from developmental perspectives. Evol Dev 14:76–92
Lajus J, Kraikovski A, Lajus D (2013) Coastal fisheries in the eastern Baltic Sea (Gulf of Finland) and its basin from the 15 to the early 20th centuries. PLoS ONE 8:e77059
Langille RM, Youson JH (1983) Biliverdin accumulation in the caudal intestinal segment of juvenile adult lampreys, *Petromyzon marinus* L. Can J Zool 61:1824–1834
Lanzing WJR (1959) Studies on the river lamprey, *Lampetra fluviatilis*, during its anadromous migration. Uitgeversmaatschappij, Utrecht
Lau BY, Foldes AE, Alieva NO et al (2011) Increased synapsin expression and neurite sprouting in lamprey brain after spinal cord injury. Exp Neurol 228:283–293
Lau BY, Fogerson SM, Walsh RB, Morgan JR (2013) Cyclic AMP promotes axon regeneration, lesion repair and neuronal survival in lampreys after spinal cord injury. Exp Neurol 250:31–42
Lee M-J, McCauley DW (in press) Lampreys as model vertebrates in evolutionary developmental biology. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Lennon RE (1954) Feeding mechanism of the sea lamprey and its effect on host fishes. US Fish Wildl Serv Fish Bull 56:247–293
Li J, Das S, Herrin BR, Hirano M, Cooper MD (2013) Definition of a third VLR gene in hagfish. Proc Natl Acad Sci U S A 110:15013–15018
Libants S, Carr K, Wu H et al (2009) The sea lamprey *Petromyzon marinus* genome reveals the early origin of several chemosensory receptor families in the vertebrate lineage. BMC Evol Biol 9:180
MacEachen DC, Russell RW, Whittle DM (2000) Spatial distribution of mercury and organochlorine contaminants in Great Lakes sea lamprey (*Petromyzon marinus*). J Great Lakes Res 26:112–119

Macey DJ, Potter IC (1986) Concentrations of non-heme iron in ammocoetes of species representing the three extant lamprey families. Comp Biochem Physiol A Physiol 84:77–79
Macey DJ, Webb J, Potter IC (1982a) Distribution of iron-containing granules in lampreys, with particular reference to the southern hemisphere species *Geotria australis* Gray. Acta Zool 63:91–99
Macey DJ, Webb J, Potter IC (1982b) Iron levels and major iron-binding proteins in the plasma of ammocoetes and adults of the southern hemisphere lamprey *Geotria australis* Gray. Comp Biochem Physiol A Physiol 72:307–312
Macey DJ, Smalley SR, Potter IC, Cake MH (1985) The relationship between total nonheme, ferritin and hemosiderin iron in larvae of southern hemisphere lampreys (*Geotria australis* and *Mordacia mordax*). J Comp Physiol B Biochem Syst Environ Physiol 156:269–276
Macey DJ, Cake MH, Potter IC (1988) Exceptional iron concentrations in larval lampreys (*Geotria australis*) and the activities of superoxide radical detoxifying enzymes. Biochem J 252:167–172
Makos BK, Youson JH (1987) Serum levels of bilirubin and biliverdin in the sea lamprey, *Petromyzon marinus* L., before and after their biliary atresia. Comp Biochem Physiol A Physiol 87:761–764
Makos BK, Youson JH (1988) Tissue levels of bilirubin and biliverdin in the sea lamprey, *Petromyzon marinus* L, before and after biliary atresia. Comp Biochem Physiol A Physiol 91:701–710
Manfredi L, Assaf T, Mintchev S et al (2013) Sea lamprey control in the Great Lakes, Finger Lakes, and Lake Champlain. Biol Cybern 107:513–527
Marsden JE, Siefkes MJ (in press) Sea lamprey control in the Great Lakes, Finger Lakes, and Lake Champlain. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Maskell FG (1929) On the New Zealand lamprey, *Geotria australis* Gray. Trans N Z Inst 60:167–210
Masters JEG, Jang MH, Ha K et al (2006) The commercial exploitation of a protected anadromous species, the river lamprey (*Lampetra fluviatilis* (L.)), in the tidal River Ouse, north-east England. Aquat Conserv Mar Freshw Ecosyst 16:77–92
Mateus CS, Rodríguez-Muñoz R, Quintella BR, Alves MJ, Almeida PR (2012) Lampreys of the Iberian Peninsula: distribution, population status and conservation. Endang Species Res 16:183–198
Mateus CS, Alves MJ, Quintella BR, Almeida PR (2013) Three new cryptic species of the lamprey genus *Lampetra* Bonnaterre, 1788 (Petromyzontiformes: Petromyzontidae) from the Iberian Peninsula. Contrib Zool 82:37–53
Mayden RL (1997) A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge MF, Dawah HA, Wilson MR (eds) Species: the units of biodiversity. Chapman and Hall, New York, pp 381–424
McCauley DW, Bronner-Fraser M (2006) Importance of SoxE in neural crest development and the evolution of the pharynx. Nature 441:750–752
McDowall RM (1990) New Zealand freshwater fishes-a natural history and guide. Heinemann Reed, Auckland
McKinnon C, Drouin G (2013) Chromatin diminution in the copepod *Mesocyclops edax*: elimination of both highly repetitive and nonhighly repetitive DNA. Genome 56:1–8
Minton K (2009) Evolution: a common solution to an age-old problem. Nat Rev Immunol 9:464
Morii M, Mezaki Y, Yamaguchi N et al (2010) Onset of apoptosis in the cystic duct during metamorphosis of a Japanese lamprey, *Lethenteron reissneri*. Anat Rec 293:1155–1166
Morii M, Mezaki Y, Yoshikawa K et al (2012) How do lampreys avoid cholestasis after bile duct degeneration? In: Tripodi V (ed) Cholestasis. InTech, Shanghai, pp 81–98
Morton D, Janning JT (1982) Iron balance in the common vampire bat *Desmodus rotundus*. Comp Biochem Physiol A Physiol 73:421–425
Morton D, Wimsatt WA (1980) Distribution of iron in the gastrointestinal tract of the common vampire bat: evidence for macrophage-linked iron clearance. Anat Rec 198:183–192
Moser ML, Butzerin JM, Dey DB (2007) Capture and collection of lampreys: the state of the science. Rev Fish Biol Fish 17:45–56

Müller A (1856) Ueber die Entwickelung der Neunaugen. Ein vorläufiger Bericht. Arch Anat Physiol Wiss Med 323–339
Municipality of Arbo (2010) Municipality of Arbo (Pontevedra Province, Galicia, Spain). http://www.crwflags.com/fotw/flags/es-po-ar.html. Accessed 26 May 2014
Naiman RJ, Bilby RE, Schindler DE, Helfield JM (2002) Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5:399–417
Nature (2012) Suckers for success. Nature 484:416
Near TJ (2009) Conflict and resolution between phylogenies inferred from molecular and phenotypic data sets for hagfish, lampreys, and gnathostomes. J Exp Zool (Mol Dev Evol) 312B:749–761
Nelson JS (1984) Fishes of the world, 2nd edn. Wiley, Hoboken
Nelson JS (1994) Fishes of the world, 3rd edn. Wiley, Hoboken
Nelson JS (2006) Fishes of the world, 4th edn. Wiley, Hoboken
Nelson JS, Starnes WC, Warren ML (2002) A capital case for common names of species of fishes: a white crappie or a White Crappie? Fisheries 27:31–33
Nicholls H (2009) Mouth to mouth. Nature 461:164–166
Nichols GM, Bacon BR (1989) Hereditary hemochromatosis: pathogenesis and clinical features of a common disease. Am J Gastroenterol 84:851–862
Nikitina N, Bronner-Fraser M, Sauka-Spengler T (2009) Microinjection of RNA and morpholino oligos into lamprey embryos. Cold Spring Harb Protoc 2009:pdb.emo113
Nursall JR, Buchwald D (1972) Life history and distribution of the arctic lamprey (*Lethenteron japonicum* (Martens)) of Great Slave Lake, N.W.T. Fish Res Board Can, Tech Rep 304
Odani S, Ito N, Hasegawa M, Uchiumi T, Hase S (2012) Identification of L-3-hydroxykynurenine O-sulfate in the buccal gland secretion of the parasitic lamprey, *Lethenteron japonicum*. Amino Acids 43:2505–2512
Ohno S (1970) Evolution by gene duplication. Allen and Unwin Springer, New York
Orlov AM, Savinyh VF Pelenev DV (2008) Features of the spatial distribution and size structure of Pacific lamprey *Lampetra tridentata* in the North Pacific. Rus J Mar Biol 34:276–287
Osório J, Rétaux S (2008) The lamprey in evolutionary studies. Dev Genes Evol 218:221–235
Ota KG, Fujimoto S, Oisi Y, Kiratani S (2011) Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. Nat Commun 2:1–6
Ota KG, Fujimoto S, Oisi Y, Kuratani S (2013) Late development of hagfish vertebral elements. J Exp Zool B Mol Dev E 320:129–139
Page LM, Espinosa-Pérez H, Findley LT et al (2013a) Common and scientific names of fishes from the United States, Canada, and Mexico, 7th edn. American Fisheries Society, Special Publication 34, Bethesda
Page LM, Espinosa-Pérez H, Findley LT et al (2013b) New seventh edition of common and scientific names of fishes changes include capitalization of common names. Fisheries 38:188–189
Pale T, Frisch EB, McClellan AD (2013) Cyclic AMP stimulates neurite outgrowth of lamprey reticulospinal neurons without substantially altering their biophysical properties. Neuroscience 245:74–89
Petersen Lewis RS (2009) Yurok and Karuk traditional ecological knowledge: Insights into Pacific lamprey populations of the Lower Klamath Basin. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 1–39
Plate L (1897) Ein neuer Cyclostome mit grossen, normal entwickelten Augen, *Macropthalmia chilensis*, n.g., n.sp. Stizber Ges Naturf Fr, Berlin
Pybus MJ, Uhazy S, Anderson RC (1978) Life cycle of *Truttaedacnitis stelmoides* (Vessichelli, 1910) (Nematoda: cucullanidae) in American brook lamprey (*Lampetra lamottenii*). Can J Zool 56:1420–1429
Quintella BR (2006) Biology and conservation of sea lamprey (*Petromyzon marinus* L.) in Portugal. PhD thesis, University of Lisbon, Lisbon
Radio UusJussi (2013) The coat of arms of Karhuseutu. http://www.radiouusjussi.com/?p=106. Accessed 26 May 2014

Renaud CB (1997) Conservation status of Northern Hemisphere lampreys (Petromyzontidae). J Appl Ichthyol 13:143–148
Renaud CB (2011) Lampreys of the world. An annotated and illustrated catalogue of lamprey species known to date. FAO Species Cat Fish Purp 5, FAO, Rome
Renaud CB, Cochran PA (in press) Post-metamorphic feeding in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Richardson MK, Admiraal J, Wright GM (2010) Developmental anatomy of lampreys. Biol Rev 85:1–33
Rodicio MC, Barreiro-Iglesias A (2012) Lampreys as an animal model in regeneration studies after spinal cord injury. Revista Neurol 55:157–166
Roffe TJ, Mate BR (1984) Abundances and feeding habits of pinnipeds in the Rogue River, Oregon. J Wildl Manag 48:1262–1274
Rovainen CM (1976) Regeneration of Muller and Mauthner axons after spinal transection in larval lampreys. J Comp Neurol 168:545–554
Rovainen CM (1979) Neurobiology of lampreys. Physiol Rev 59:1007–1077
Rydel TJ, Tulinsky A, Bode W, Huber R (1991) Refined structure of the hirudin-thrombin complex. J Mol Biol 221:583–601
Schultze MS (1856) Die Entwickelungs-Geschichte von *Petromyzon planeri*. Haarlem, Loosjes
Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Bulletin Fisheries Research Board of Canada 184, Ottawa
Shigetani Y, Sugahara F, Kuratani S (2005) A new evolutionary scenario for the vertebrate jaw. Bioessays 27:331–338
Shimeld SM, Donoghue PCJ (2012) Evolutionary crossroads in developmental biology: cyclostomes (lamprey and hagfish). Development 139:2091–2099
Shimeld SM, Holland PWH (2000) Vertebrate innovations. Proc Natl Acad Sci U S A 97:4449–4452
Shirakawa H, Yanai S, Goto A (2013) Lamprey larvae as ecosystem engineers: physical and geochemical impact on the streambed by their burrowing behavior. Hydrobiologia 701:313–322
Silva S, Araújo M, Bao M, Mucientes G, Cobo F (2014) The haematophagous feeding stage of anadromous populations of sea lamprey *Petromyzon marinus*: low host selectivity and wide range of habitats. Hydrobiologia (in press)
Sjöberg K (2011) River lamprey *Lampetra fluviatilis* (L.) fishing in the area around the Baltic Sea. J North Stud 5:51–86
Skinner J (2012) A taste of Gloucestershire and the Cotswalds: regional recipes from Gloucestershire and the Cotswalds. The Francis Frith Collection, Salisbury
SLIS (1980) Proceedings of the 1979 Sea Lamprey International Symposium (SLIS). Can J Fish Aquat Sci 37:1585–2214
Smith JJ, Antonacci F, Eichler EE, Amemiya CT (2009) Programmed loss of millions of base pairs from a vertebrate genome. Proc Natl Acad Sci U S A 106:11212–11217
Smith J, Morgan JR, Zottoli SJ et al (2011) Regeneration in the era of functional genomics and gene network analysis. Biol Bull 221:18–34
Smith JJ, Baker C, Eichler EE, Amemiya CT (2012) Genetic consequences of programmed genome rearrangement. Curr Biol 22:1524–1529
Smith JJ, Kuraku S, Holt C et al (2013) Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. Nat Genet 45:415–421
Sousa R, Araújo M, Antunes C (2012) Habitat modifications by sea lampreys (*Petromyzon marinus*) during the spawning season: effects on sediments. J Appl Ichthyol 28:766–771
Sower SA, Nozaki M, Knox CJ, Gorbman A (1995) The occurrence and distribution of GnRH in the brain of Atlantic hagfish, an agnatha, determined by chromatography and immunocytochemistry. Gen Comp Endocrinol 97:300–307
Stefanini C, Orofino S, Manfredi L et al (2012) A novel autonomous, bioinspired swimming robot developed by neuroscientists and bioengineers. Bioinspir Biomim 7:025001
Sterba G (1962) Die Neunaugen (Petromyzonidae). In: Handbuch der Binnenfischerei Mitteleuropas, vol 3. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart

Streif B (2009) Considering Pacific lampreys when implementing instream activities. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 255–268

Suchy FJ (2013) Biliary atresia in sea lampreys. What can it tell us about the disorder in human infants? Hepatology 57:2114–2116

Takezaki N, Figueroa F, Zaleska-Rutczynska Z, Klein J (2003) Molecular phylogeny of early vertebrates: monophyly of the agnathans as revealed by sequences of 35 genes. Mol Biol Evol 20:287–292

The Telegraph (2012) Traditional lamprey pie for the Queen has to come from Canada for the first time in centuries. http://www.telegraph.co.uk/news/uknews/queen-elizabeth-II/9221440/Traditional-lamprey-pie-for-the-Queen-has-to-come-from-Canada-for-first-time-in-centuries.html. Accessed 12 April 2014

Tsioros KK, Youson JH (1997) Distribution of iron during embryogenesis and early larval life in sea lampreys (*Petromyzon marinus*). Can J Zool 75:137–147

Tsioros KK, Holmes JA, Youson JH (1996) Origin of iron in sea lamprey, *Petromyzon marinus*, tissues during embryogenesis and early larval life. Can J Zool 74:2206–2210

Uchida K, Moriyama S, Chiba H et al (2010) Evolutionary origin of a functional gonadotropin in the pituitary of the most primitive vertebrate, hagfish. Proc Natl Acad Sci U S A 107:15832–15837

Uchida K, Moriyama S, Sower SA, Nozaki M (2013) Glycoprotein hormone in the pituitary of hagfish and its evolutionary implications. Fish Physiol Biochem 39:75–83

Underwood EJ (1977) Trace elements in human and animal nutrition, 4th edn. Academic Press, New York

Wheeler A (1979) The tidal Thames. Routledge/Keegan Paul, London

Wijayawardena BK, Minchella DJ, DeWoody J (2013) Hosts, parasites, and horizontal gene transfer. Trends Parasitol 29:329–338

Xiao R, Li QW, Perrett S, He RQ (2007) Characterisation of the fibrinogenolytic properties of the buccal gland secretion from *Lampetra japonica*. Biochimie 89:383–392

Xiao R, Pang Y, Li QW (2012) The buccal gland of *Lampetra japonica* is a source of diverse bioactive proteins. Biochimie 94:1075–1079

Yamazaki Y, Goto A, Nishida M (2003) Mitochondrial DNA sequence divergence between two cryptic species of *Lethenteron*, with reference to an improved identification technique. J Fish Biol 62:591–609

Yeh CY, Chung-Davidson YW, Wang H, Li K, Li W (2012) Intestinal synthesis and secretion of bile salts as an adaptation to developmental biliary atresia in the sea lamprey. Proc Natl Acad Sci U S A 109:11419–11424

Youson JH (1993) Biliary atresia in lampreys. In: Cornelius CE (ed) Advances in veterinary science and comparative medicine, vol 37, animal models in liver research. Academic Press, San Diego, pp 197–255

Youson JH, Potter IC (1979) A description of the stages in the metamorphosis of the anadromous sea lamprey, *Petromyzon marinus* L. Can J Zool 57:1808–1817

Youson JH, Sargent PA, Sidon EW (1983) Iron loading in the livers of metamorphosing lampreys, *Petromyzon marinus* L. Cell Tissue Res 234:109–124

Youson JH, Sargent PA, Barrett A (1987) Serum iron concentration and other blood parameters during the life cycle of the sea lamprey, *Petromyzon marinus* L. Comp Biochem Physiol A Physiol 88:325–330

Zhang G, Jin L, Selzer ME (2011) Assembly properties of lamprey neurofilament subunits and their expression after spinal cord transection. J Comp Neurol 519:3657–3671

Zhang G, Pizarro IV, Swain GP, Kang SH, Selzer ME (2014) Neurogenesis in the lamprey central nervous system following spinal cord transection. J Comp Neurol 522:1316–1332

Chapter 2
The Taxonomy, Phylogeny, and Distribution of Lampreys

Ian C. Potter, Howard S. Gill, Claude B. Renaud and Dalal Haoucher

Abstract The lampreys (Petromyzontiformes), one of the two surviving groups of agnathan (jawless) vertebrates, currently consist of 41 recognized species. This group has an antitropical distribution, with the 37 species of Northern Hemisphere lampreys assigned to the Petromyzontidae, whereas the four species of Southern Hemisphere lampreys are separated into either the Geotriidae (one species) or Mordaciidae (three species). All lamprey species have a blind and microphagous, burrowing larva (ammocoete), which spends a number of years in the soft sediment of creeks and rivers, after which it undergoes a radical metamorphosis. Eighteen lamprey species then embark on an adult parasitic phase (nine at sea and nine in fresh water) during which they increase markedly in size, whereas the other 23 species do not feed as adults and remain in fresh water. On the basis of morphology, 17 of the 23 non-parasitic species each evolved from a particular parasitic species whose descendants are still represented in the contemporary fauna. The remaining six non-parasitic species, the so-called "southern relict" species, have no obvious potential ancestral parasitic species, implying they have diverged markedly from their parasitic ancestor or that the parasitic ancestor is now extinct. Many of the main taxonomic characteristics reside in features that are associated with parasitic feeding, for example, the type and arrangement of the teeth on the suctorial disc and tongue-like piston. The phylogenetic relationships, derived by maximum parsimony analyses of morphological and anatomical data for the 18 parasitic species, were similar in most respects to those obtained by subjecting molecular data (cytochrome *b* mitochondrial DNA sequence data) for those species to Bayesian analyses. However, in contrast to the results of morphological analyses, the genera

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Eudontomyzon and *Lampetra* were not monophyletic when using molecular analyses. When non-parasitic species were included in the molecular analyses, some of the six relict non-parasitic species formed clades with parasitic species which, from their morphology, had been allocated by taxonomists to different genera. More genes, and particularly nuclear genes, should be used to help resolve the basis for these differences between the morphological and molecular phylogenies.

Keywords Evolution · Geotriidae · Mordaciidae · Morphological and molecular analyses · Paired species · Petromyzontidae

2.1 Introduction

The lampreys, together with the hagfishes, are the two sole surviving groups of agnathan (jawless) vertebrates (Janvier 1981; Hardisty 2006; see Chap. 1). The possession by these two groups of "round mouths" led to them being termed by Duméril (1806), collectively, as the Cyclostomata, a term retained as a class by Holly (1933) in his important taxonomic treatise on these animals. The implication that lampreys and hagfishes formed a monophyletic group was accepted for many years. However, detailed comparisons of their anatomy, morphology, and physiology, in conjunction with comparisons to the morphology of extinct agnathans, led to an alternative viewpoint (Hardisty 1979, 1982; Janvier 1981). The latter authors came independently to the conclusion that lampreys were more closely related to the gnathostomatous (jawed) vertebrates than to the hagfishes. Since that time, however, the majority of the numerous molecular studies undertaken on the two surviving groups of agnathans have supported the monophyly of lampreys and hagfishes (e.g., Stock and Whitt 1992; Mallatt and Sullivan 1998; Kuraku et al. 1999; Delarbre et al. 2002; Takezaki et al. 2003; Blair and Hedges 2005; Kuraku and Kuratani 2006). The question of whether or not cyclostomes are considered to constitute a monophyletic group was subsequently shown by Near (2009) to be influenced by the characters used and the types of analyses employed. A subsequent study, however, by Heimberg et al. (2010), employing microRNAs and a reanalysis of morphological characters, provided such overwhelming evidence for cyclostome monophyly that it convinced Janvier (2010) that this was indeed the case.

The first fossil lamprey to be described was the beautifully-preserved *Mayomyzon pieckoensis* from the upper Carboniferous (c. 280 million years ago, mya) deposits of Mazon Creek in Illinois (Bardack and Zangerl 1968, 1971). This fossil clearly possessed many of the morphological and anatomical characters of the adults of extant lampreys, such as an annular cartilage, which maintains the structural integrity of the suctorial disc, a piston cartilage, dorsolateral eyes, and seven gill apertures on either side of the body. Since the landmark discovery of *M. pieckoensis*, a further three definitive fossil lampreys have been found. The youngest of these is *Mesomyzon mengae* from the lower Cretaceous of China c. 125 mya (Chang et al. 2006), followed in age by *Hardistiella montanensis* from lower Carboniferous deposits in Montana c. 320 mya (Janvier and Lund 1983), and then *Priscomyzon riniensis*

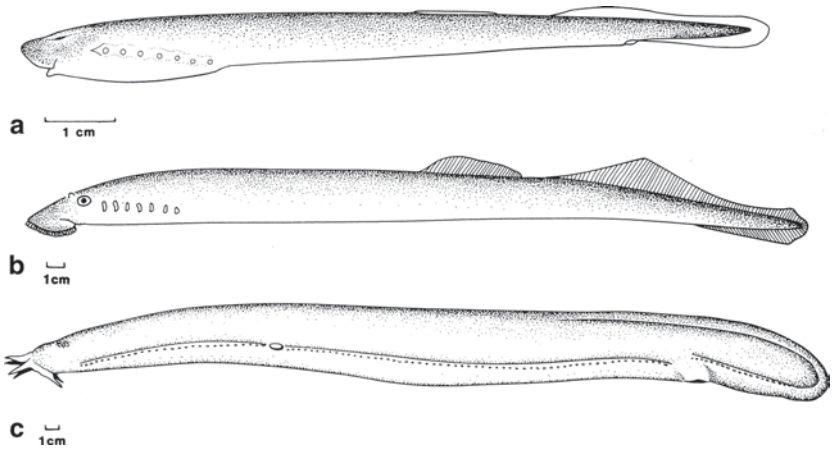

Fig. 2.1 Lateral views of **a** a larval lamprey (ammocoete), **b** an adult lamprey, and **c** a hagfish. This figure was originally published in Hardisty et al. (1989). (Reproduced by permission of The Royal Society of Edinburgh from Transactions of the Royal Society of Edinburgh: Earth Sciences volume 80 (1989), pp. 241–254)

from upper Devonian deposits in South Africa c. 360 mya (Gess et al. 2006). The first indisputable fossil hagfish to be discovered was *Myxinikela siroka,* which was found in the same geological horizon and general locality as the lamprey *M. pieckoensis*, and thus likewise dates back c. 300 mya (Bardack 1991, 1998). More recently, another hagfish fossil, *Myxineidus gononorum*, was discovered in upper Carboniferous deposits in France and is therefore also of approximately the same age as the above two fossils (Poplin et al. 2001). Germain et al. (2014) have cast doubt, however, on whether *M. gononorum* is a hagfish and provide evidence that it could be a lamprey.

Both groups of extant cyclostomes possess a similar body shape (Fig. 2.1) and typically have an antitropical distribution (Hubbs and Potter 1971; Hardisty 1979). Although lampreys are thereby essentially confined to temperate regions of the world, two species (genus *Tetrapleurodon*) are found in elevated cooler waters in a restricted sub-tropical area (Álvarez del Villar 1966). The living lampreys are represented by three families (Mordaciidae, Geotriidae, and Petromyzontidae) and 41 species (Table 2.1; Potter et al. 2014) and the hagfishes by two subfamilies (Eptatretinae and Myxininae) and approximately 60 species (Fernholm 1998). However, whereas the Mordaciidae and Geotriidae are confined to the Southern Hemisphere and the Petromyzontidae to the Northern Hemisphere, the two subfamilies of hagfishes are represented in both hemispheres.

The aim of this chapter is to provide a comprehensive list of the species, genera, subfamilies, and families of extant lampreys, providing details of the types of morphological characters used in taxonomic studies and the distributions of each species. Emphasis is also placed on outlining the schemes that have been proposed for the interrelationships of the various species, based on morphological and molecular criteria, and discussing the implications of any differences between those schemes.

Table 2.1 Classification, common names, life cycle types, and distributions of the 41 extant species of lampreys, following Potter et al. (2014) in all respects except that *hubbsi* is assigned to *Lampetra* rather than *Entosphenus*. Authorities for each taxon are given in Appendix 2.1. In the life cycle column, attention is drawn to those non-parasitic species that can unambiguously be paired with a particular parasitic species. Drainage refers to a river and its tributaries. Other frequently used common names, for example, those adopted by the American Fisheries Society (AFS; Page et al. 2013a) or Food and Agriculture Organization (FAO; see FishBase: Froese and Pauly 2013) or explanations regarding alternative or previous classifications are also provided

Classification	Common name	Life cycle type	Distribution	Comments
Family Mordaciidae (southern top-eyed lampreys)				
Genus *Mordacia* (3 species)				
Mordacia mordax	Short-headed lamprey	Anadromous; parasitic	Drainages and coastal waters of southeastern Australia, including Tasmania	Also known as Australian lamprey, but not recommended as it is imprecise
Mordacia praecox	Precocious lamprey	Freshwater; non-parasitic derivative of *M. mordax*	Drainages of southeastern Australia	Also known as Australian brook lamprey, but not recommended as it is imprecise
Mordacia lapicida	Chilean lamprey	Anadromous; parasitic	Drainages and coastal waters of Chile	
Family Geotriidae (southern striped lamprey)				
Genus *Geotria* (1 species)				
Geotria australis	Pouched lamprey	Anadromous; parasitic	Drainages of southern Australia, New Zealand, Chile, Argentina and widespread in intervening oceans	
Family Petromyzontidae (Northern Hemisphere lampreys)				
Subfamily Petromyzontinae				Nelson (2006) excluded *Caspiomyzon* from this subfamily (see Sect. 2.4)
Genus *Caspiomyzon* (1 species)				
Caspiomyzon wagneri	Caspian lamprey	Anadromous; parasitic	Caspian Sea and its drainages	
Genus *Petromyzon* (1 species)				
Petromyzon marinus	Sea lamprey	Anadromous and freshwater; parasitic	Atlantic drainages in North America from Newfoundland southwards to Florida and into the Gulf of Mexico and in Europe from Varanger Fjord southwards into the Mediterranean Sea. Widespread in the North Atlantic Ocean	

Table 2.1 (continued)

Classification	Common name	Life cycle type	Distribution	Comments
Genus *Ichthyomyzon* (6 species)				
Ichthyomyzon unicuspis	Silver lamprey	Freshwater; parasitic	Hudson Bay, Great Lakes, St. Lawrence River, and Mississippi River drainages	
Ichthyomyzon fossor	Northern brook lamprey	Freshwater; non-parasitic derivative of *I. unicuspis*	As for *I. unicuspis*	
Ichthyomyzon castaneus	Chestnut lamprey	Freshwater; parasitic	Hudson Bay, Great Lakes, St. Lawrence River, and Gulf of Mexico drainages	
Ichthyomyzon gagei	Southern brook lamprey	Freshwater; non-parasitic derivative of *I. castaneus*	Gulf of Mexico drainages	
Ichthyomyzon bdellium	Ohio lamprey	Freshwater; parasitic	Ohio River drainage	
Ichthyomyzon greeleyi	Mountain brook lamprey	Freshwater; non-parasitic derivative of *I. bdellium*	As for *I. bdellium*	
Subfamily Lampetrinae				Nelson (2006) included *Caspiomyzon* in this subfamily; Vladykov (1972) separated these five genera into subfamilies Entospheninae (*Entosphenus* and *Tetrapleurodon*), and Lampetrinae (*Lampetra*, *Lethenteron*, and *Eudontomyzon*)
Genus *Tetrapleurodon* (2 species)				Formerly synonymized with *Lampetra* (see Sect. 2.4)
Tetrapleurodon spadiceus	Mexican lamprey	Freshwater; parasitic	Celio, Duero, Zula, and Lerma rivers, and Lake Chapala, Mexico	Also known as Chapala lamprey
Tetrapleurodon geminis	Mexican brook lamprey	Freshwater; non-parasitic derivative of *T. spadiceus*	Celio and Duero rivers, and Rio Grande de Morelia drainage, Mexico	Also known as Jacona lamprey

Table 2.1 (continued)

Classification	Common name	Life cycle type	Distribution	Comments
Genus *Entosphenus* (6 species)				Formerly synonymized with *Lampetra* (see Sect. 2.4)
Entosphenus tridentatus	Pacific lamprey	Anadromous and freshwater; parasitic	Drainages of western Canada, USA and Mexico, and Japan. Widespread in the North Pacific Ocean	
Entosphenus minimus	Miller Lake lamprey	Freshwater; parasitic	Upper Klamath River drainage, Oregon	
Entosphenus similis	Klamath lamprey	Freshwater; parasitic	Klamath River drainage, Oregon and California	
Entosphenus macrostomus	Vancouver lamprey	Freshwater; parasitic	Lake Cowichan drainage, Vancouver Island, British Columbia	Cowichan lamprey more precise than Vancouver lamprey (see Beamish and Wade 2008)
Entosphenus folletti	Northern California brook lamprey	Freshwater; non-parasitic	Klamath River drainage, California	Formerly considered by AFS to be synonymous with *En. lethophagus* (Robins et al. 1980), but recognized as distinct on recent list (Page et al. 2013a) Appears to be recent non-parasitic derivative but not clear whether *En. tridentatus* or *En. similis* is the ancestor (see Sect. 2.2)
Entosphenus lethophagus	Pit-Klamath brook lamprey	Freshwater; non-parasitic	Klamath River drainage, Oregon, and Pit River, California	Appears to be recent non-parasitic derivative but not clear whether *En. tridentatus* or *En. similis* is the ancestor (see Sect. 2.2)
Genus *Lethenteron* (6 species)				Formerly synonymized with *Lampetra* (see Sect. 2.4)
Lethenteron camtschaticum	Arctic lamprey	Anadromous and freshwater; parasitic	Drainages of Arctic and North Pacific oceans	Formerly known as *Lethenteron japonicum* (see Renaud et al. 2009b)
Lethenteron alaskense	Alaskan brook lamprey	Freshwater; non-parasitic derivative of *Le. camtschaticum*	Drainages of Brooks and Chatanika rivers, Alaska, and Mackenzie River, Canada	

Table 2.1 (continued)

Classification	Common name	Life cycle type	Distribution	Comments
Lethenteron appendix	American brook lamprey	Freshwater; non-parasitic derivative of *Le. camtschaticum*	Great Lakes drainages and eastern USA, St. Lawrence, and Mississippi river drainages	
Lethenteron reissneri	Far Eastern brook lamprey	Freshwater; non-parasitic derivative of *Le. camtschaticum*	Drainages of Amur River, Sakhalin Island and Kamchatka Peninsula, Russia and in South Korea and Japan	
Lethenteron kessleri	Siberian brook lamprey	Freshwater; non-parasitic derivative of *Le. camtschaticum*	Drainages between Ob and Anadyr rivers, and of Sakhalin Island, Russia and Hokkaido Island, Japan	Also known as Siberian lamprey, but "brook" lamprey makes clear that this species is non-parasitic
Lethenteron ninae	Western Transcaucasian brook lamprey	Freshwater; non-parasitic	Drainages of the Black Sea	
Genus *Eudontomyzon* (6 species)				Formerly synonymized with *Lampetra* (see Sect. 2.4)
Eudontomyzon danfordi	Carpathian lamprey	Freshwater; parasitic	Danube River drainage	
Eudontomyzon mariae	Ukrainian brook lamprey	Freshwater; non-parasitic derivative of *Eu. danfordi*	Drainages of Baltic, Azov, Black, Adriatic, and Aegean seas	
Eudontomyzon stankokaramani	Drin brook lamprey	Freshwater; non-parasitic derivative of *Eu. danfordi*	Drainages of Adriatic Sea	Synonymized with *Eu. mariae* in Renaud (2011)
Eudontomyzon morii	Korean lamprey	Freshwater; parasitic	Yalu River drainage, China and North Korea	
Eudontomyzon *hellenicus*	Macedonia brook lamprey	Freshwater; non-parasitic	Strymon River drainage, Greece	Also known as Greek brook lamprey, but Macedonia brook lamprey distinguishes this species from the more recently-described *Eu. graecus*
Eudontomyzon graecus	Epirus brook lamprey	Freshwater; non-parasitic	Loúros River drainage, Greece	

Table 2.1 (continued)

Classification	Common name	Life cycle type	Distribution	Comments
Genus *Lampetra* (9 species)				
Lampetra ayresii	Western river lamprey	Anadromous and possibly freshwater; parasitic	Drainages of North American Pacific Coast	Western river lamprey proposed as common name by Renaud et al. (2009b) and adopted by AFS, but also commonly referred to as river lamprey
Lampetra pacifica	Pacific brook lamprey	Freshwater; non-parasitic derivative of *La. ayresii*	Drainages of Columbia River, Oregon and Sacramento-San Joaquin rivers, California (but see Sect. 2.5)	Formerly considered by AFS to be synonymous with *La. richardsoni* (Robins et al. 1991), but recognized as distinct on recent list (Page et al. 2013a)
Lampetra richardsoni	Western brook lamprey	Freshwater; non-parasitic derivative of *La. ayresii*	Drainages of Pacific Ocean, British Columbia, Washington, Oregon and Alaska	
Lampetra hubbsi	Kern brook lamprey	Freshwater; non-parasitic	Friant-Kern Canal and Merced River, California	Formerly referred to *Entosphenus* (see Sect. 2.4.2)
Lampetra aepyptera	Least brook lamprey	Freshwater; non-parasitic	Drainages of northwestern Atlantic Ocean and Gulf of Mexico, USA	
Lampetra fluviatilis	European river lamprey	Anadromous and freshwater; parasitic	Drainages of northeastern Atlantic Ocean	
Lampetra planeri	European brook lamprey	Freshwater; non-parasitic derivative of *La. fluviatilis*	As for *La. fluviatilis,* plus Danube and Volga river drainages	
Lampetra lanceolata	Turkish brook lamprey	Freshwater; non-parasitic derivative of *La. fluviatilis*	Iyidere River, Turkey	
Lampetra zanandreai	Po brook lamprey	Freshwater; non-parasitic	Drainages of the Adriatic Sea	Also known as Lombardy brook lamprey Sometimes referred to *Lethenteron* (see Sect. 2.4.2)

2.2 Life Cycles and "Paired Species"

The ability to describe accurately a species of lamprey and thereby facilitate its allocation to the appropriate genus and family requires both a thorough understanding of the features that characterize the divergent larval and adult stages and recognition that, in some species, the morphology changes markedly during adult life. It should also be recognized that the types of life cycle vary amongst lampreys, with some containing a parasitic adult phase whereas others do not feed after the completion of larval life (see later).

The life cycle of all lamprey species contains a protracted larval phase that is spent in fresh water (Hardisty and Potter 1971a; Potter 1980a; see Chap. 3). The larva, termed an ammocoete, has a worm-like body shape and is blind and toothless (Fig. 2.1a). The ammocoete spends most of its time burrowed in the soft substrata in the slower-flowing regions of streams and rivers, feeding on the detritus and microorganisms (e.g., diatoms) that it extracts from the water overlying its burrow (Moore and Mallatt 1980; Yap and Bowen 2003). After typically between 3 and 7 years, the ammocoete undergoes a radical metamorphosis, which leads to the development of functional eyes, a suctorial disc and protrusible tongue-like piston (both of which are armed with teeth), and enlargement of the dorsal fins (Figs. 2.1b, 2.2, and 2.3; Hardisty and Potter 1971b; Potter 1980a; Youson 1980; see Chap. 4), with metamorphosis typically occurring at body lengths of 80–200 mm (Hardisty and Potter 1971a).

Following the completion of the larval phase, the life cycle of the lamprey diverges in one of two main directions. One course leads to the development, during metamorphosis, of a sexually immature young adult (Fig. 2.1b) that embarks on a parasitic feeding phase (Renaud et al. 2009a; Renaud and Cochran in press). The young adults of nine of these eighteen parasitic species feed at sea following a downstream migration. When fully grown they cease feeding and return to rivers, but not necessarily their natal systems, where they become sexually mature, spawn and die (Table 2.1; Hardisty and Potter 1971b; Potter et al. 2014; see Chap. 5). Five of these nine anadromous species have given rise to freshwater-resident or landlocked forms, whose immature adults feed in lakes or in the wider regions of large rivers (Table 2.1; Applegate 1950; Nursall and Buchwald 1972; see Docker and Potter in press). The remaining nine parasitic species are confined to fresh water and have essentially the same life cycle as the landlocked forms of anadromous species (Table 2.1; Hubbs and Trautman 1937; Chappuis 1939; Álvarez del Villar 1966; Renaud and Cochran in press). The maximum total length attained by parasitic species varies markedly, ranging from 145 mm in the freshwater Miller Lake lamprey *Entosphenus minimus* to 310–490 mm in small anadromous species, such as the western and European river lampreys (*Lampetra ayresii* and *La. fluviatilis*, respectively) to between 780 and 1,200 mm in the large anadromous pouched lamprey *Geotria australis*, Pacific lamprey *Entosphenus tridentatus*, and sea lamprey *Petromyzon marinus* (Oliva 1953; Vladykov and Follett 1958; Hardisty and Potter 1971b; Potter et al. 1983; Hardisty 1986; Lorion et al. 2000).

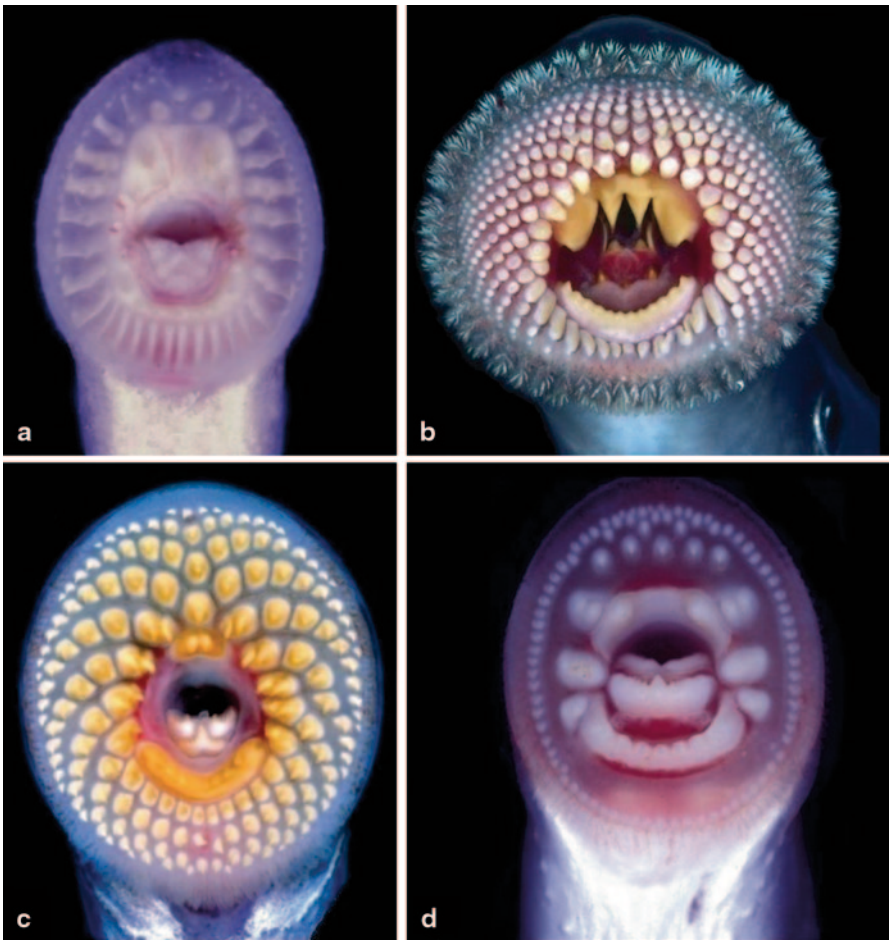

Fig. 2.2 The suctorial disc and dentition of **a** a fully-metamorphosed *Mordacia mordax*, **b** an early upstream migrant of *Geotria australis*, **c** a young feeding adult of anadromous *Petromyzon marinus*, and **d** a recently-metamorphosed *Lampetra fluviatilis*. (Photos b–d: David Bird)

The second main direction exhibited by the lamprey life cycle involves a shifting in the timing of sexual maturation relative to metamorphosis, such that it commences during the transition from the ammocoete to the adult rather than after the completion of a parasitic phase as with the species above. The parasitic phase thus becomes eliminated and spawning takes place soon after the completion of metamorphosis (Hardisty 2006; Docker 2009). Consequently, these non-parasitic species breed at a length no greater than that of their longest ammocoetes. As most of these non-parasitic species are morphologically similar to a particular parasitic species in all aspects other than body size, it has been assumed that each evolved from that parasitic species (Potter 1980b; Docker 2009). On this basis, 15 of the 23 non-parasitic species listed in Table 2.1 can be "paired" with a congeneric parasitic species (in some cases, with a single parasitic "stem" species giving rise

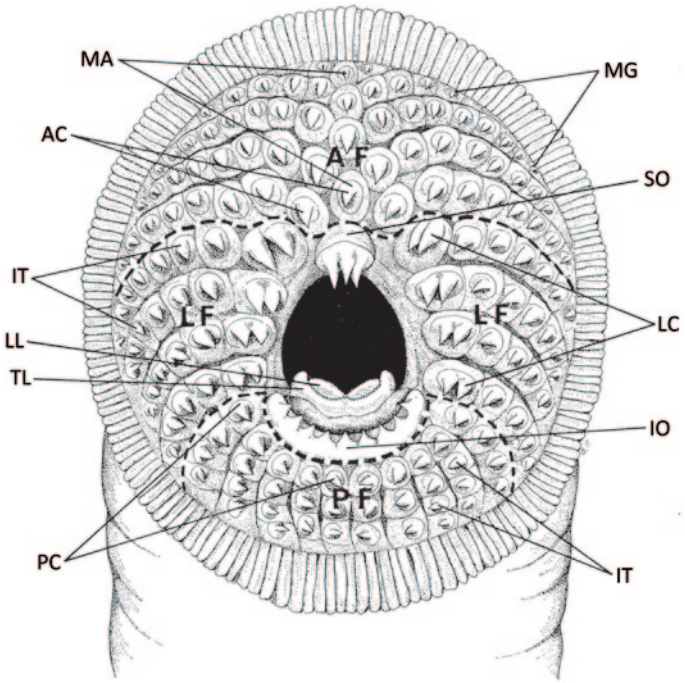

Fig. 2.3 Oral disc of *Ichthyomyzon bdellium*, showing the different fields and types of teeth and laminae and their nomenclature. Note that alate rows comprise an inner circumoral and an outer marginal, and the intervening intermediate rows of disc teeth: median anterior tooth row (*MA*), marginal teeth (*MG*), anterior field (*AF*), anterior circumoral teeth (*AC*), supraoral lamina (*SO*), lateral field (*LF*), intermediate disc teeth (*IT*), lateral circumoral teeth (*LC*), longitudinal lingual lamina (*LL*), transverse lingual lamina (*TL*), infraoral lamina (*IO*), posterior circumoral teeth (*PC*), and posterior field (*PF*). (This figure was originally published in Hubbs and Potter (1971) and reproduced with permission of Elsevier)

to more than one non-parasitic "satellite" species; Vladykov and Kott 1979a). An additional two species (Northern California brook lamprey *Entosphenus folletti* and Pit-Klamath brook lamprey *En. lethophagus*) also appear to be recent non-parasitic derivatives but, in these cases, it is not clear whether *En. tridentatus* or *En. similis* is the ancestor (Potter et al. 2014). The reader is referred to the reviews by Hardisty (2006), Docker (2009), Renaud et al. (2009b), and Docker and Potter (in press) for a comprehensive discussion of the issues surrounding the relationships between non-parasitic and parasitic species. We focus below on the taxonomic status of these species.

Despite the morphological similarities that link these non-parasitic derivative species with their presumed parasitic ancestor, a number of studies have revealed significant anatomical differences between species in at least some pairs. The differences between the non-parasitic and parasitic members of one or more species pairs include, in the non-parasitic member of the pair, a lower prevalence of pigmentation on the tongue precursor and usually fewer oocytes in the ovaries of the ammocoetes and, following metamorphosis, a less well-developed gut, a

relatively smaller eye and suctorial disc, less well-developed teeth and velar tentacles, and fewer trunk myomeres (Hughes and Potter 1969; Hardisty and Potter 1971c; Potter and Osborne 1975; Vladykov and Kott 1976a, 1979a; Potter 1980b; Beamish and Thomas 1983). In exceptional cases, however, a particular trait can go in one direction in some species pairs and in the opposite direction in other species pairs. Thus, while the number of teeth in the anterior field and in the lateral and posterior fields were greater in the non-parasitic European brook lamprey *Lampetra planeri* than in its parasitic ancestor *Lampetra fluviatilis* (Hardisty et al. 1970), the number of posterial teeth in the non-parasitic *Entosphenus folletti* and *En. lethophagus* were less than in parasitic *En. tridentatus* and *En. similis* (Vladykov and Kott 1976b, 1979b).

The above differences between non-parasitic species and corresponding parasitic species indicate that there are genetic differences between such pairs. Yet, as pointed out by Docker (2009) in her extensive review, the use of molecular techniques for analyzing the genetic compositions of a number of species pairs has generally not been able to detect differences between the members of such pairs. An inability to distinguish genetically between an ancestral parasitic and derivative non-parasitic species is widespread, encompassing species in different genera and from different geographical regions (e.g., Docker et al. 1999; Docker 2006; Yamazaki et al. 2006; Espanhol et al. 2007; Blank et al. 2008; April et al. 2011). The techniques used, however, may not have provided sufficient resolution to determine whether the lack of genetic distinction merely reflects a recent divergence of a non-parasitic species from a parasitic species or lack of genetic divergence in the particular markers used (Docker 2009). In their study of the Arctic lamprey *Lethenteron camtschaticum*—Far Eastern brook lamprey *Le. reissneri* species pair, Yamazaki et al. (2006) noted that results, based on analyses of nuclear and mitochondrial genomes, were incongruent and suggested that the failure of a mitochondrial-based phylogeny to distinguish between members of a species pair may have been due to incomplete lineage sorting.

In an attempt to resolve this issue, Docker et al. (2012) examined over 10,000 base pairs of the mitochondrial genome in adults of the freshwater parasitic silver lamprey *Ichthyomyzon unicuspis* and its non-parasitic derivative northern brook lamprey *I. fossor* in populations across the Laurentian Great Lakes, and concluded that the two taxa were not reciprocally monophyletic. Where *I. unicuspis* and *I. fossor* occurred sympatrically in the Lake Huron basin, these authors further found no significant differences in mitochondrial haplotype or microsatellite allele frequencies, suggesting that, at least in this locality, there was gene flow between these species. A recent exciting study by Mateus et al. (2013a), however, has taken analyses of whether there are genetic distinctions between the members of paired species a step further. The results obtained by these authors, using restriction site-associated DNA sequencing, provided incontrovertible evidence of genome-wide divergence between *La. fluviatilis* and *La. planeri*. The validity of these conclusions is supported by the fact that the individuals of the two species used for these analyses were obtained from the same spawning site. It is particularly relevant that, in the latter study, most of the genes showing fixed allelic differences between the two

species are related to functions implicated in adaptations to a freshwater-resident life style, as with *La. planeri,* as opposed to a migratory and anadromous mode of life, as with *La. fluviatilis*. The differences between the outcomes of the above studies may be due to the markers used (i.e., a small number of presumably neutral loci versus a large number of potentially functional loci) or to the species pairs examined (e.g., *I. unicuspis* and *I. fossor* are both freshwater residents). However, as these discrepancies reinforce previous suggestions that the taxonomic status of each pair should be determined individually (e.g., Docker 2009; Renaud et al. 2009b), we have adopted a conservative approach in this chapter that taxonomic changes should not be made hastily. We thus consider it appropriate to follow Renaud et al. (2009b) in continuing to regard, as distinct species, each of the non-parasitic species and its presumed parasitic ancestor that are listed in Table 2.1, recognizing that these species are separable on the basis of morphological criteria, particularly body size, and also by life style.

Although mitochondrial DNA sequence data have been unable to differentiate between parasitic and non-parasitic members of many species pairs, such data have provided sufficient resolution to distinguish among brook lamprey populations from different geographic locations, at least in some widespread species such as *La. planeri* and the western brook lamprey *Lampetra richardsoni* (e.g., Espanhol et al. 2007; Mateus et al. 2011; Boguski et al. 2012). This poses the question of whether different populations of the same species have originated independently, that is, at different times or different locations (see Docker 2009). The notion that some recognized brook lamprey species may be polyphyletic was suggested by Hubbs (1925) and Hubbs and Trautman (1937). In the absence of distinct morphological differences among such populations, however, we continue to consider these populations (despite molecular synapomorphies) to constitute a single species (see below). In this context, we have decided not to recognize three cryptic "species" which were recently described by Mateus et al. (2013b) from Portugal and belong to the *Lampetra planeri* complex. At present, we do not recognize these populations as specifically distinct from *La. planeri* for two reasons: (1) the authors did not compare the putative new species with material of *La. planeri* from its type locality (i.e., brooks of Thuringia, Germany; Bloch 1784); and (2) none of the putative species is morphologically diagnosable from either of the others at better than 78 %, when using a stepwise discriminant function analysis.

In addition to the 17 recent non-parasitic derivatives discussed above, the contemporary fauna also contains six non-parasitic species for which there is no obvious potential ancestral parasitic species, implying either that these species have diverged markedly from their parasitic ancestor or that the parasitic ancestor is now extinct. These so-called "southern relict" species (non-parasitic lampreys that occur at or near the extreme southern limits of distribution of the Northern Hemisphere lampreys; Hubbs and Potter 1971) are the: Western Transcaucasian brook lamprey *Lethenteron ninae*; Macedonia brook lamprey *Eudontomyzon hellenicus*; Epirus brook lamprey *Eu. graecus*; Kern brook lamprey *Lampetra hubbsi*; least brook lamprey *Lampetra aepyptera*; and Po brook lamprey *Lampetra zanandreai* (see Sect. 2.4.2).

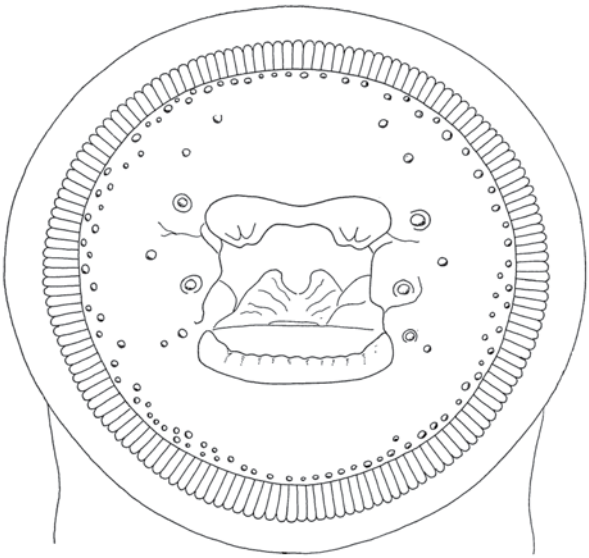

Fig. 2.4 Oral disc of the least brook lamprey *Lampetra aepyptera,* showing the highly degenerate dentition of this non-parasitic species. (This figure was originally published in Hubbs and Potter (1971) and reproduced with permission of Elsevier)

2.3 Taxonomic Characters

A comprehensive list of the morphological characters used in the taxonomy of lampreys has been provided by Holčík (1986a) and Renaud (2011), while a list of the synapomorphies for genera and families are given in Gill et al. (2003). It should be recognized, however, that whatever characters are used, it is far more difficult to distinguish between the ammocoetes than the adults of the various species. Indeed, the ammocoetes of some species belonging to the same genus, and especially of those representing the particular parasitic and non-parasitic species that constitute a species pair, have frequently been unable to be unequivocally separated using morphological criteria (see Sect. 2.2). For example, this is the case with the Mexican lamprey *Tetrapleurodon spadiceus* and Mexican brook lamprey *T. geminis* (Álvarez del Villar 1966) and with the short-headed lamprey *Mordacia mordax* and precocious lamprey *M. praecox* (Potter 1968; Potter et al. 1968).

The main morphological characters used to describe the ammocoetes of the various species are the number of trunk myomeres, the shape of the caudal fin, and the patterns of pigmentation on various parts of their body surface and tongue precursor (Vladykov 1950; Potter and Osborne 1975; Neira et al. 1988). In contrast, the most important characters for describing the adults of the various species are those involving the dentition on the suctorial disc and piston (Figs. 2.2 and 2.3). Although this disc and dentition are not fully developed until late in metamorphosis (Bird and Potter 1979; see Chap. 4) and the dentition of one species, *Lampetra aepyptera*, is extremely degenerate (Fig. 2.4), the number of teeth in the various tooth series and the arrangement and shape of those teeth are very useful diagnostic tools for identifying the adults of different species (Hubbs and Trautman

1937; Vladykov and Follett 1967; Potter and Strahan 1968). The number and arrangement of the velar tentacles of adult lampreys (Vladykov and Kott 1976a), structures which guard the entrance to the water tube that leads into the branchial chamber and thus prevent large particles from entering that chamber and potentially clogging the gills (Renaud et al. 2009a), also represent valuable taxonomic tools. As with ammocoetes, the number of trunk myomeres is also often useful for identifying the adults of certain species (Hubbs and Trautman 1937; Zanandrea 1957; Iwata et al. 1985; Renaud and Economidis 2010). Although Bond and Kan (1986) suggested that myomere counts in *La. richardsoni* and the Pacific brook lamprey *La. pacifica* followed Jordan's rule, that is, increasing in number with increasing latitude and thus decreasing temperature, Reid et al. (2011) found no such latitudinal cline in either species. Likewise, Creaser and Hubbs (1922) proposed that the Pacific lamprey comprised a northern subspecies *Entosphenus tridentatus tridentatus* with 68–74 trunk myomeres and a southern subspecies *En. tridentatus ciliatus* with 57–67 trunk myomeres, but this proposal was later dismissed as untenable (Hubbs and Potter 1971). Beamish (2010) has shown that the number, size, shape and arrangement of the papillae on the posterior rim of the gill pores of adult lampreys vary among certain species and that the central process, which lies just inside this rim in some species, varies in shape. As this latter suite of characters was capable of distinguishing between even the individuals of closely-related non-parasitic species, it clearly has considerable potential for refining the descriptions of lamprey species.

In the case of Southern Hemisphere lampreys, a suite of characters can readily be used to distinguish the sole species of *Geotria* (i.e., *G. australis*) from those of *Mordacia*, the only other genus of lamprey in the Southern Hemisphere and with which it co-occurs in the rivers and coastal waters of southeastern Australia (including Tasmania) and Chile (Potter and Strahan 1968; Potter 1986). Thus, as *Geotria* is monotypic, the differences between *G. australis* and the three *Mordacia* species also apply at the generic, and indeed family, levels. In the case of ammocoetes, these characters include differences in body pigmentation, the position of the cloaca relative to the second dorsal fin, and the number of lobes and internal structure of their intestinal diverticula (Neira et al. 1988; Bartels and Potter 1995). The differences between the adults of *G. australis* and the *Mordacia* species are even more pronounced, and particularly so in the case of the structure of their teeth and the arrangement of their dentition (Fig. 2.2). Thus, the divergence between the two genera of Southern Hemisphere lampreys, which collectively contain only four species, is far greater than that among Northern Hemisphere lampreys, even though the latter comprise a far greater number of genera (eight) and species (37; Table 2.1). This difference in the extent of divergence is consistent with the separation of the Southern Hemisphere lampreys into two families, Mordaciidae and Geotriidae, and to the Northern Hemisphere lampreys being assigned to a single family, Petromyzontidae (Gill et al. 2003; Potter et al. 2014).

Among Northern Hemisphere lampreys, only the species of *Ichthyomyzon* possess a single rather than two dorsal fins (Hubbs and Trautman 1937). The ability to readily distinguish the ammocoetes of the six *Ichthyomyzon* species from those of

other genera is particularly useful as *Ichthyomyzon* has a wide distribution in North America and one or more of its species are often found in the same river system as those of *Petromyzon*, *Lampetra*, and *Lethenteron* (Table 2.1). There are no other characters that are clearly unique to any particular Northern Hemisphere genus.

2.4 Current Taxonomic Schemes

The taxonomic scheme employed in this chapter, at the family and generic level, is based predominantly on the results of a detailed cladistic study that employed morphological characters for all parasitic species of lampreys (Gill et al. 2003). This scheme was subsequently adopted by Nelson (2006) in his fourth edition of *Fishes of the World*, and by Renaud (2011) in his *Lampreys of the World*. All authorities have recognized, for some time, that the lampreys consisted of three groups, one comprising all Northern Hemisphere species and the other two representing the two Southern Hemisphere genera (e.g., Potter and Strahan 1968; Hubbs and Potter 1971; Bailey 1980; Gill et al. 2003; Renaud 2011). Based on the large number of unique morphological characters that define each of these three groups, we still consider that they are best represented by three families, that is, Petromyzontidae for Northern Hemisphere lampreys and Geotriidae and Mordaciidae for the two Southern Hemisphere genera (Table 2.1; Gill et al. 2003). It should be noted that the common name southern striped lamprey is now used for the Geotriidae following Potter et al. (2014), rather than southern lampreys as in Nelson (2006), in order to avoid confusion with the other family of Southern Hemisphere lampreys, Mordaciidae, the common name for which is southern top-eyed lampreys. The separation of genera in the Petromyzontidae into the subfamilies Petromyzontinae and Lampetrinae follows that of Nelson (2006) in all respects, except that *Caspiomyzon* is placed in Petromyzontinae rather than Lampetrinae (see Potter et al. 2014 and subsequent text for rationale). The common and scientific names of all parasitic and non-parasitic species and their generic allocations follow those given in Potter et al. (2014), except in the case of *Lampetra hubbsi,* which was formerly referred to *Entosphenus* (Vladykov and Kott 1976c; see Docker et al. 1999; Goodman et al. 2009; Boguski et al. 2012). *Lampetra hubbsi* has now been reconfirmed by the American Fisheries Society (Page et al. 2013a) as the official species name. Other frequently used common names, for example, those adopted by the American Fisheries Society (Page et al. 2013a) or Food and Agriculture Organization (see FishBase; Froese and Pauly 2013), but not used here, are provided in Table 2.1. Renaud (2011) lists additional common names and provides synonyms for each species. A list of the authorities for each lamprey family, genus, and species is given in Appendix 2.1.

Note that, as discussed in relevant parts of the subsequent text, the results of a reanalysis of the molecular data for parasitic species, which was used by Lang et al. (2009) and employed a single gene, sometimes did not match those of the morphological analyses (Fig. 2.5a, b). Although certain implications of the molecu-

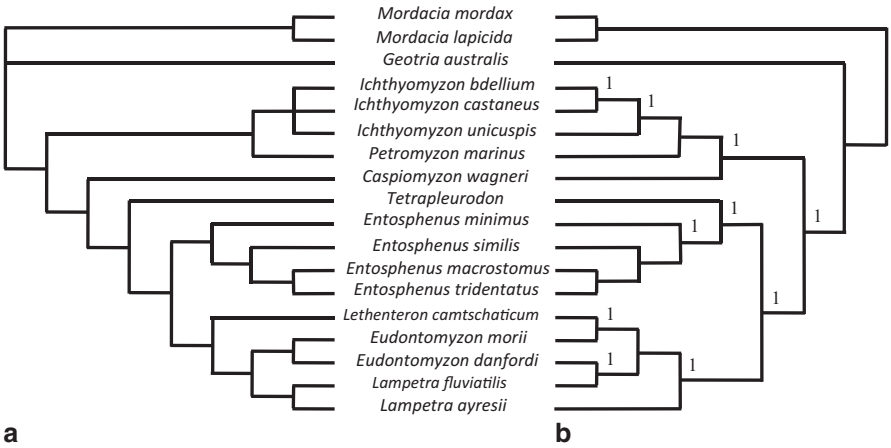

Fig. 2.5 Phylogenetic relationships among the parasitic species of the three lamprey families, derived from **a** morphological data using maximum parsimony analyses, and **b** cytochrome *b* sequence data using Bayesian analyses. As no molecular data were available for the parasitic *Tetrapleurodon spadiceus*, the cytochrome *b* data for *Tetrapleurodon geminis*, its non-parasitic derivative, were used instead. Bayesian posterior probabilities are given for those nodes where values are greater than 0.95

lar analyses may turn out to be valid, it was decided not to change the and mainly acrocentric generic allocation of any species until more comprehensive genetic analyses have been undertaken. The key differences, as well as similarities, in the implications of cladistic analyses of the morphological and molecular data sets are discussed in the following text.

The taxonomy of the Southern Hemisphere lampreys was in a state of disarray until the late 1960s. There was wide disagreement regarding, not only the number of species present in Australia, New Zealand, and South America, but also the number of genera and even families that they represent (Potter and Strahan 1968). The taxonomic problems posed by Southern Hemisphere species were shown by the latter authors to have arisen largely from taxonomists not having recognized that, during its spawning run, each of these species undergoes far more extreme morphological and other alterations than any of their Northern Hemisphere counterparts. Such pronounced alterations include very marked changes in the structure and arrangement of the teeth and in the body coloration and, depending on the species, the development by males of an exceptionally large gular pouch (Potter and Strahan 1968; Potter and Welsch 1997; see Chap. 6). As a consequence, the species now designated as *Geotria australis*, for example, was demonstrated by Potter and Strahan (1968) to have previously been considered to constitute a total of 11 species and to represent eight genera! At the family level, there had also been disagreement, for example, as to whether *G. australis* should be allocated to a family on its own or included with that comprising all Northern Hemisphere species (Potter and Strahan 1968). Eventually, the Southern Hemisphere lampreys were considered to be represented

by just four species, *Mordacia mordax, M. praecox*, Chilean lamprey *M. lapicida*, and *G. australis* (Table 2.1). As there are, however, some obvious morphological differences between the ammocoetes of *G. australis* from Australia, Argentina, and Chile (Neira et al. 1988), it is important that further studies be undertaken to ascertain whether *Geotria* comprises two or more closely-related species rather than a single species.

The two genera of Southern Hemisphere lampreys were shown by Potter and Strahan (1968) to each possess highly distinctive characteristics and that these differed from those of the group comprising Northern Hemisphere lampreys. Thus, these authors assigned these three groups to the subfamilies Mordaciinae, Geotriinae, and Petromyzoninae, which were later elevated to family level, that is, Mordaciidae, Geotriidae, and Petromyzontidae (Hubbs and Potter 1971), an arrangement that remains widely accepted (Nelson 2006). The morphological differences between the three families are paralleled by differences in their karyotypes. Thus, *Mordacia* species possess 76 predominantly metacentric or submetacentric chromosomes, whereas *G. australis* has approximately 180 small and mainly acrocentric chromosomes and the Northern Hemisphere lampreys possess 164–168 largely acrocentric chromosomes (see Potter et al. 2014).

The taxonomy of Northern Hemisphere lampreys was the subject of a number of sound studies during the first half of the last century. Such studies included a remarkably detailed and quantitative analysis by Hubbs and Trautman (1937) of the interrelationships between the various species of the exclusively freshwater genus *Ichthyomyzon*. These were supplemented, between 1955 and 1982, by the detailed descriptions provided by particularly Vladykov and his co-workers for species belonging to various other genera of holarctic lampreys (see Vladykov and Kott 1979c). The full list of the 37 species of Northern Hemisphere lampreys recognized here is given in Table 2.1. This list includes the 34 Northern Hemisphere species recognized in previous oft-cited reviews (e.g., Renaud 1997), plus the Drin brook lamprey *Eudontomyzon stankokaramani,* which was subsequently recognized as a valid species (rather than as a synonym of the Ukrainian brook lamprey *Eu. mariae*) by Holčík and Šorić (2004), and two recently-described species, *Lethenteron ninae* and *Eudontomyzon graecus* (Naseka et al. 2009; Renaud and Economidis 2010). In his *Lampreys of the World*, Renaud (2011) included 36 of these species, preferring to leave *Eu. stankokaramani* as a synonym of *Eu. mariae* until a more comprehensive study of the variation in the velar tentacle morphology of the wide-ranging *Eu. mariae* had been undertaken. As discussed above (Sect. 2.2), we consider the three cryptic brook lamprey "species" proposed by Mateus et al. (2013b) as synonyms of *La. planeri*.

Most species of Northern Hemisphere lampreys have long been recognized as distinct entities on the basis of clear morphological criteria, with the result that only two new species have been described since 1982 (i.e., Naseka et al. 2009; Renaud and Economidis 2010; Appendix 2.1). Furthermore, the monotypic *Petromyzon* and *Caspiomyzon*, and also *Ichthyomyzon* with its six species, have each long been regarded as generically discrete. The taxonomy of *Lampetra* has had a rather more checkered history (reviewed by Docker et al. 1999). Thus, some workers have

considered this genus to contain not only the species that are almost invariably listed for *Lampetra*, but also those in *Lethenteron* and *Entosphenus,* which were regarded by Hubbs and Potter (1971) as subgenera of *Lampetra*, and also even *Tetrapleurodon* and *Eudontomyzon* (Bailey 1980). Following the latter author, the American Fisheries Society Committee on Names of Fishes supported synonymizing *Entosphenus* and *Lethenteron* with *Lampetra* in the fourth and fifth editions of their *Common and Scientific Names of Fishes* lists (Robins et al. 1980, 1991), and added *Tetrapleurodon* as another synonym in the sixth edition that was expanded to include the fishes of Mexico (Nelson et al. 2004). The results of cladistic studies using morphological characters supported, however, the separate generic designation of *Lethenteron*, *Entosphenus*, *Tetrapleurodon*, and *Eudontomyzon* (Gill et al. 2003; Potter et al. 2014), and the seventh edition of the *Common and Scientific Names of Fishes* recognizes *Entosphenus*, *Lethenteron*, and *Tetrapleurodon* as genera (Page et al. 2013a). Although we follow Docker et al. (1999) and Potter et al. (2014) in also using *Lampetra* to include *aepyptera*, we recognize that its dentition, which is the most important of lamprey taxonomic characters (see Sect. 2.3), is highly degenerate and that the arrangement of the few remaining teeth and of other characters do not readily fall under the compass of those of other genera (Fig. 2.4). Indeed, Hubbs and Potter (1971) suggested that this species be allocated to a genus of its own, *Okkelbergia*, which was originally created as a subgenus of *Lampetra* by Creaser and Hubbs (1922).

Additionally, a number of putative lamprey species remain undescribed. For example, two non-parasitic species in Japan, which have been referred to as *Lethenteron* sp. N and *Le*. sp. S, are morphologically indistinguishable from each other (Yamazaki and Goto 1997) but, on the basis of molecular studies, are clearly distinct (Yamazaki and Goto 1996, 1998; Yamazaki et al. 2003, 2006). Furthermore, Bogusski et al. (2012) found four morphologically cryptic, but molecularly-distinct populations of *Lampetra* spp. in Oregon and California. However, until these putative species have been formally described, taxonomists are not in a position to accept their validity.

2.4.1 Interrelationships Among Parasitic Taxa

A phylogeny of the lampreys was constructed in the early 2000s by subjecting, to maximum parsimony analyses, data for mainly the morphological characteristics of the parasitic species of Southern and Northern hemisphere lampreys (Fig. 2.5a; Gill et al. 2003). The analyses were restricted to the 18 parasitic species, which represent each of the currently recognized genera of lampreys, because only 20 phylogenetically-informative characters were available for analysis, which is far less than the total number of lamprey species (41). Furthermore, apart from body size, the morphological characteristics of the species comprising each pair of parasitic and non-parasitic species are often indistinguishable (see Sect. 2.2). Of the six currently recognized non-parasitic species that are morphologically distinct from extant parasitic species (i.e., the relict species; Sect. 2.2), two had not been

described as of 2003 (*Eu. graecus* and *Le. ninae*) and one (*La. aepyptera*) is characterized by extremely degenerate dentition (Sect. 2.3). The outgroups employed for these analyses were three species of fossil from Carboniferous deposits, that is, the lampreys *Mayomyzon pieckoensis* and *Hardistiella montanensis* (Sect. 2.1) and the putative lamprey *Pipiscius zangerli,* and a composite fossil. It was considered inappropriate to use extant hagfishes or gnathostomes as outgroups since these groups share virtually no morphological features that can be used to establish relationships among the living lamprey species.

The above analyses revealed that there was a well-defined clade that contained all Northern Hemisphere parasitic species, which is consistent with the allocation of all Northern Hemisphere lampreys to the single family Petromyzontidae (Fig. 2.5a). Within the clade comprising Northern Hemisphere lampreys, the genera formed two major groups, the first represented by *Ichthyomyzon* and *Petromyzon* and the second by the other six genera, that is, *Caspiomyzon, Tetrapleurodon, Entosphenus*, *Lethenteron*, *Eudontomyzon*, and *Lampetra* (Fig. 2.5a). The analyses failed to resolve, however, the precise relationships between those parasitic species and the two *Mordacia* species and the monotypic *Geotria*. It is highly relevant, however, that many of the characteristics of the Northern Hemisphere species differ markedly from those of *Mordacia* and *Geotria,* which, in many respects, are also often very different (Potter and Strahan 1968; Hubbs and Potter 1971; Potter and Gill 2003; Renaud et al. 2009a). For this reason we reiterate that it is considered appropriate to continue to regard *Geotria* and *Mordacia* as representing separate families, i.e. Geotriidae and Mordaciidae (see Sect. 2.4).

The cytochrome *b* gene sequences (1,133 base pairs), derived by Lang et al. (2009) from samples for the parasitic species of lampreys, have been re-subjected to Bayesian analyses (Fig. 2.5b). The outgroups used for these molecular analyses represent the two subfamilies of the other extant agnathan group (i.e., the hagfishes *Myxine glutinosa* and *Eptatretus burgeri*), a gnathostome (*Chimaera monstrosa*) and, as in the study of Lang et al. (2009), the more distantly-related cephalochordate *Branchiostoma belcheri*. In the following account of the results of molecular analyses, the generic names for each species, which have been traditionally recognized on the basis of morphological criteria, have been retained (Gill et al. 2003; Docker 2009; Renaud et al. 2009a, b). Furthermore, as no molecular data were available for one of the parasitic species, *Tetrapleurodon spadiceus*, those for its non-parasitic derivative, *Tetrapleurodon geminis*, were used instead when employing molecular data to analyze the relationships of the parasitic species. It should be noted that a cladogram produced using Maximum Likelihood analysis of the cytochrome *b* data was essentially the same as that shown in Fig. 2.5b using Bayesian analysis.

Although the number of appropriate morphological characters available for analyses was limited and the molecular analyses were based on data for a single gene, the cladograms produced from both data sets for the parasitic species were similar in several respects (Fig. 2.5a, b). Thus, the molecular analyses also produced very strong support for a clade that comprised all Northern Hemisphere parasitic species and that, within that clade, one group that likewise contained all *Ichthyomyzon* species and *Petromyzon,* another with all *Entosphenus* species, and yet another the

species of *Lethenteron*, *Eudontomyzon*, and *Lampetra* (Fig. 2.5b). The molecular analyses placed *Geotria australis* as the sister to the Northern Hemisphere species, albeit with very low posterior probability or bootstrap support.

The molecular analyses resulted in the "shift" of *Caspiomyzon* from within a clade that comprises *Tetrapleurodon*, *Entosphenus*, *Lethenteron*, *Eudontomyzon*, and *Lampetra*, as in the analyses conducted using morphological data, to the clade that contains *Petromyzon* and *Ichthyomyzon* (Fig. 2.5a, b and see above). Furthermore, the relationships of the species within the clade comprising *Lethenteron*, *Eudontomyzon*, and *Lampetra* differ from those traditionally assigned on the basis of morphology, with, for example, *La. fluviatilis* now being more closely related to *Eu. danfordi* (Carpathian lamprey) than to *La. ayresii*, and *Eu. morii* (Korean lamprey) being more closely related to *Le. camtschaticum* than to *Eu. danfordi.* It should be noted, however, that the specimen of *Eu. morii* used in Lang et al. (2009) was a metamorphosing individual with developing dentition, and thus possibly represents a misidentification since members of *Lethenteron* from the same broad geographical area are known, in some cases, to possess one or a few exolaterals.

Unlike the trends exhibited by the analyses performed by Gill et al. (2003) using morphological data (Fig. 2.5a), those involving cytochrome *b* provided overwhelming support for *Caspiomyzon wagneri* (Caspian lamprey) belonging to the clade that contained *Petromyzon marinus* and the *Ichthyomyzon* species and for *Tetrapleurodon* species being sister to the species of *Entosphenus* (Fig. 2.5b). The inference that *Caspiomyzon* is related to *Petromyzon* is consistent with an earlier proposal that the former species was derived from a *Petromyzon*-like species that became isolated in the Caspian Sea in probably the pre-Pleistocene (Hubbs and Potter 1971). Moreover, a closer alignment of *Tetrapleurodon* with *Entosphenus* is also consistent with an earlier taxonomic scheme in which, on the basis of similarities in their dentitions and geographical distributions, these two genera were placed in the subfamily Entospheninae (Vladykov 1972; Vladykov and Kott 1979c). For the above reasons, it is tentatively proposed that the relationships derived for the above five genera using molecular data, which are consistent with those given in the above much earlier morphological studies, are likely to be valid.

The conflicting results regarding the interrelationships among *Lethenteron*, *Eudontomyzon*, and *Lampetra* are more difficult to reconcile. At the morphological level, the characteristics of the species are consistent within each genus and differ between genera. Indeed, within *Lampetra*, the morphological characteristics of *La. ayresii* are so similar to those of *La. fluviatilis* that they were not regarded as distinct species until comprehensive and careful comparisons were undertaken by Vladykov and Follett (1958), yet several molecular studies (albeit always using mitochondrial DNA sequences; e.g., Docker et al. 1999; Lang et al. 2009) consistently place these two species in separate clades. Lang et al. (2009) were the first to suggest, after using molecular data, that *Eu. morii* is more closely related to *Le. camtschaticum* than it is to other *Eudontomyzon* species. This finding is interesting, particularly since Berg (1931) suggested that *Eu. morii* may have evolved from *Le. camtschaticum* but, as noted above, this conclusion was based on a single metamorphosing individual (and single, mitochondrial gene) and requires independent confirmation with

other specimens and other (nuclear) genes. Thus, in view of the conflict between the phylogenetic implications of the morphological and molecular analyses regarding the above species/genera, we follow our earlier intention of retaining the original generic allocation of these species until more definitive evidence becomes available. As pointed out by Page et al. (2013b), making changes that are short-lived has the effect of confusing rather than improving the situation.

2.4.2 Relationships of Non-Parasitic Species

The inclusion in the molecular analysis of DNA sequence data for cytochrome *b* for non-parasitic species had essentially no influence on the interrelationships of the genera of lampreys (Fig. 2.6). Furthermore, this analysis resulted in most non-parasitic species being grouped with the parasitic species which, on the basis of morphology, is their presumed ancestor, as, for example with the three pairings within *Ichthyomyzon*, as originally proposed by Hubbs and Trautman (1937). Indeed, all 13 of the 17 recently-derived non-parasitic species for which molecular data were available for both parasitic and non-parasitic species (see Table 2.1, Sect. 2.2) grouped with their presumed parasitic ancestor. Additionally, *Eu. stankokaramani* grouped with *Eu. danfordi* (Lang et al. 2009). Two non-parasitic species (*La. pacifica* and *En. folletti*) and one parasitic species (*T. spadiceus*) were not included in Lang et al. (2009), but other studies support some of the presumed pairings (e.g., *La. pacifica* with *La. ayresii*: Boguski et al. 2012; *En. folletti* with *En. tridentatus* and other parasitic species in this genus: Docker and Reid unpublished data).

Surprisingly, however, certain non-parasitic and parasitic species, which, from their morphology, had been allocated by taxonomists to different genera, were grouped together by this analysis. For example, analyses using cytochrome *b* data led to the non-parasitic species classically designated as *Eudontomyzon hellenicus* being aligned with *Caspiomyzon wagneri* (Fig. 2.6). Although *Eu. hellenicus* and *C. wagneri* both occur in Europe, there is a substantial gap between their present-day distributions (Table 2.1) and their morphological features differ in a number of conspicuous respects (Vladykov et al. 1982; Gill et al. 2003). Note that the *Eu. hellenicus* from the Ionian Sea basin in the cladogram by Lang et al. (2009) has now been identified as *Eu. graecus* and that, together with *Eu. hellenicus* from the Aegean Sea basin, constitute a clade that is the sister group to *C. wagneri*. However, although *Eu. hellenicus* and *Eu. graecus* were shown to form a clade with *C. wagneri*, they are still genetically very distinct from *C. wagneri* (i.e., differing by 10.5–10.7 % in their cytochrome *b* sequences, compared to the above species pairs that differed by 0–3 %; see Docker and Potter in press). Furthermore, the presence of two synapomorphies in the two brook lampreys from Greece, namely, a wide supraoral lamina and a very large median tooth on the transverse lingual lamina (Renaud and Economidis 2010), as well as in the parasitic members of the genus (i.e., *Eu. danfordi* and *Eu. morii*), and their absence in *C. wagneri* (Gill et al. 2003) emphasize the importance of using more than just a single genetic marker in the future to resolve the relationships among the above taxa.

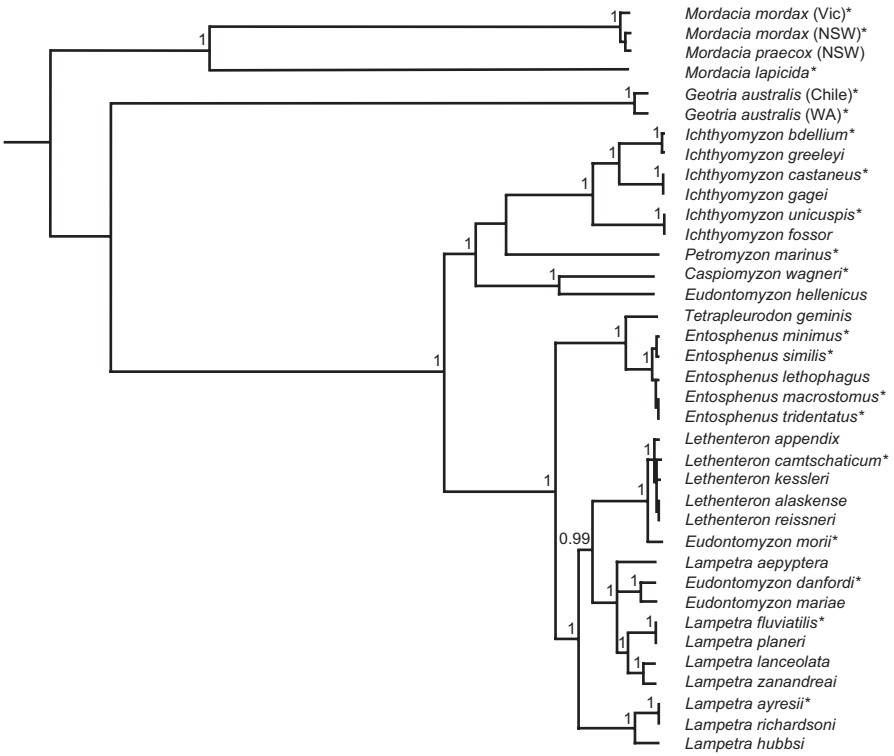

Fig. 2.6 Phylogenetic relationships of the parasitic and non-parasitic species of the three lamprey families, derived from cytochrome *b* sequence data using Bayesian analyses: *asterisks* designate parasitic species. The data were derived from those employed by Lang et al. (2009) together with additional data for *Mordacia mordax* from New South Wales, Australia (*NSW*); other abbreviations Victoria (*VIC*), Western Australia (*WA*). Bayesian posterior probabilities are given for those nodes where values are greater than 0.95. The clades that included: 1 *Lethenteron camtschaticum, Le. kessleri, Le. alaskense,* and *Le. reissneri;* and 2 *Le. kessleri, Le. alaskense*, and *Le. reissneri* each had a posterior support of 1

Holčík (1986b) and Bianco (1986) placed *Lampetra zanandreai* in the genus *Lethenteron* because its lateral circumorals (endolaterals) are usually bicuspid and because posterior circumorals (posterials) are present in most specimens. This arrangement was followed by Renaud (1997), Potter and Gill (2003), and Renaud (2011). However, Kottelat and Freyhof (2009) argued that, while these two characters may be useful in diagnosing species, they are not useful in defining lineages. We have therefore reverted to the original generic assignment, which is consistent with the molecular-based cladogram that shows *Lampetra zanandreai* within a Eurasian *Lampetra* clade (Fig. 2.6).

The Kern brook lamprey was originally assigned by Vladykov and Kott (1976c) to the genus *Entosphenus* on the basis of its dentition (reviewed in Docker et al. 1999). The molecular analyses of Lang et al. (2009) place this species in a clade together with *La. ayresii–La. richardsoni* and, as mentioned above (Sect. 2.4), this species is

now recognized as *Lampetra hubbsi* (Page et al. 2013a). As mentioned previously, the very pronounced degeneration of the dentition of *La. aepyptera* has hindered an unequivocal generic assignment of this species. Molecular analyses suggested that this species, which is confined to eastern North America, resembles more closely *La. fluviatilis,* which is restricted to Europe, than *La. ayresii*, which occurs along the western seaboard of North America (Lang et al. 2009; Fig. 2.6). This is consistent with the results of Docker et al. (1999), who used neighbor-joining analysis of cytochrome *b* and NADH dehydrogenase subunit 3 (ND3) DNA sequences. However, the analyses of Lang et al. (2009) indicate that *La. aepyptera* is also related to two species of *Eudontomyzon*, which, like *La. fluviatilis*, are confined to European waters. It is thus noteworthy that, in the cladogram produced from molecular data, two clades (Fig. 2.6; node with 0.99 posterior probability) tended to comprise species from either the Atlantic Ocean basin (*La. aepyptera*+*Eu. danfordi*+*Eu. mariae*+*La. fluviatilis*+*La. planeri*+*La. lanceolata*+*La. zanandreai*) or the Pacific Ocean basin (*Le. camtschaticum*+*Le. kessleri*+*Le. alaskense*+*Le. reissneri*+*Eu. morii*), with the notable exception of *Le. appendix,* which has an Atlantic distribution, being grouped with the Pacific clade.

2.5 Distribution

The antitropical distribution of all three families of lampreys within river systems is related to the inability of ammocoetes to tolerate high temperatures. This conclusion is based on the fact that the ultimate incipient lethal temperatures for the three species for which there are such data, that is, *Petromyzon marinus* from North America, *Lampetra planeri* from Europe, and *Geotria australis* from Australia, are only 31.4 °C, 29.4 °C, and 28.3 °C, respectively (Potter and Beamish 1975; Macey and Potter 1978).

Mordacia is represented by an anadromous species in rivers and coastal marine waters of southeastern mainland Australia and Tasmania (i.e., *M. mordax*) and by another (*M. lapicida*) in those of Chile (Table 2.1; Fig. 2.7). The single non-parasitic species in this genus (*M. praecox*) occurs within creeks and rivers in the same geographical region as its presumed ancestor *M. mordax* (Potter 1980b). Since preparing this review, we have become aware of isolated pockets of ammocoetes of *Mordacia* in Queensland over 1,000 km to the north of the previously recorded distribution of this genus. Work is currently in progress to provide details of these populations (Moffat et al. unpublished data). In contrast to *Mordacia, Geotria,* which is represented solely by the large anadromous parasitic species *G. australis,* is found in rivers throughout temperate Australasia and southern South America and ranges widely in marine waters (Table 2.1; Fig. 2.7; Potter et al. 1979).

The Northern Hemisphere genus *Ichthyomyzon,* which belongs to the subfamily Petromyzontinae, and comprises three parasitic species and their three respective non-parasitic derivatives (Table 2.1), is confined to river systems and lakes in central and eastern North America (Fig. 2.8). Several lines of evidence indicate that this

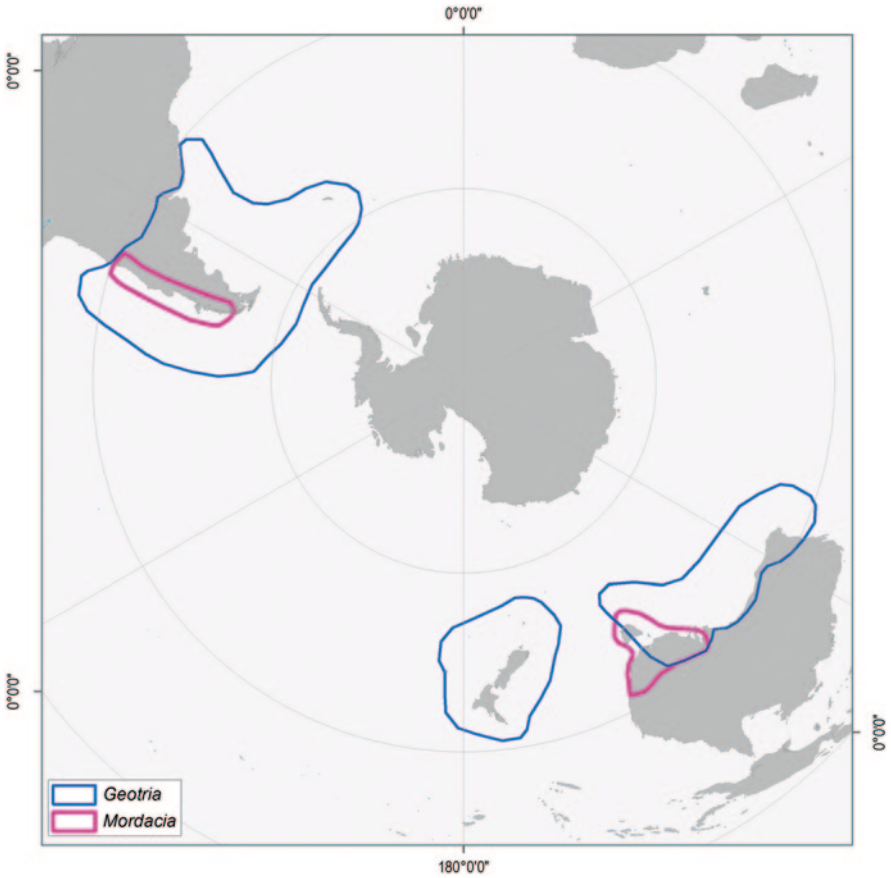

Fig. 2.7 Distributions of the Southern Hemisphere genera of lampreys (*Mordacia* and *Geotria*) by polar projection. (Modified from Hubbs and Potter 1971)

genus either evolved in fresh water or has been confined to fresh water for a very long period (see Bartels et al. 2012). The anadromous and monotypic *Petromyzon* is found along the eastern and western seaboards of the North Atlantic Ocean and throughout the Mediterranean Sea and is represented by a landlocked form in North America (Fig. 2.9; Hubbs and Potter 1971; Çevik et al. 2010). Like *G. australis*, the large anadromous form of *P. marinus* ranges widely in the marine environment (Halliday 1991). *Caspiomyzon*, the remaining genus of the subfamily Petromyzontinae (see Sect. 2.4), and which contains only the anadromous parasitic *C. wagneri*, is restricted to the Caspian Sea basin (Fig. 2.8).

The second subfamily, Lampetrinae, contains five genera (Table 2.1). Although *Tetrapleurodon* is unique among lampreys in that its distribution is entirely restricted to a sub-tropical area, this apparent anomaly is explained by the fact that the single parasitic and derivative non-parasitic species that comprise this genus occur only in high altitude lakes and rivers, in which the waters are relatively cool

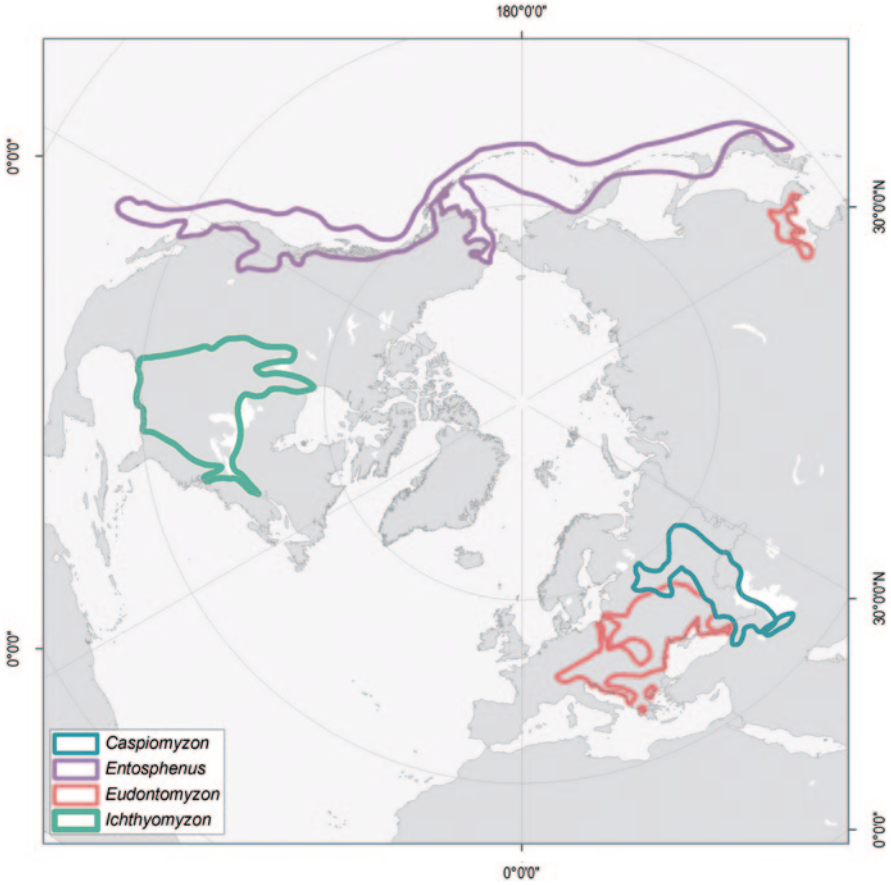

Fig. 2.8 Distributions of four of the eight Northern Hemisphere genera of lampreys (*Caspiomyzon*, *Entosphenus*, *Eudontomyzon*, *Ichthyomyzon*) by polar projection. (Updated from Hubbs and Potter 1971)

(Table 2.1; Fig. 2.10; Álvarez del Villar 1966; Cochran et al. 1996). The four parasitic and two non-parasitic species of *Entosphenus* are all found in drainages along the west coast of North America (Table 2.1; Fig. 2.8). *Entosphenus tridentatus*, the large and sole anadromous species in this genus, ranges widely throughout the North Pacific Ocean during its parasitic phase (Hubbs and Potter 1971; Fukutomi et al. 2002; Renaud 2008, 2011). While a few freshwater-resident populations of *En. tridentatus* have been reported along the western coast of North America, there is some uncertainty regarding their taxonomic status (e.g., Moyle et al. 2009; Taylor et al. 2012; see Docker and Potter in press). The single parasitic species of *Lethenteron*, *Le. camtschaticum,* which comprises both anadromous and landlocked forms (Heard 1966; Nursall and Buchwald 1972; Kucheryavyi et al. 2007a, 2007b), is found to the northern tip of Alaska at about 72 °N (McPhail and Lindsey 1970), which is further north than any other lamprey species. This species has a wide

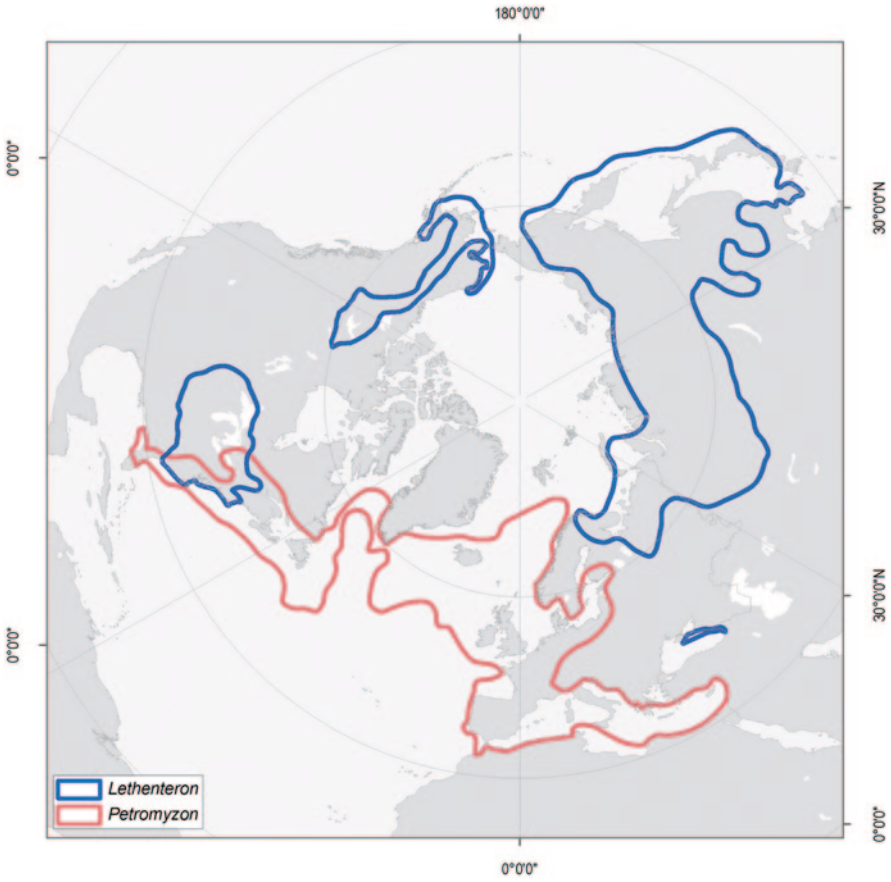

Fig. 2.9 Distributions of two of the eight Northern Hemisphere genera of lampreys (*Lethenteron*, *Petromyzon*) by polar projection. (Updated from Hubbs and Potter 1971)

distribution in the Arctic Ocean, extending from the White Sea in Russia to the Beaufort Sea in Canada and southwards to Japan in the western North Pacific Ocean (Table 2.1; Fig. 2.9). Although this range encompasses those of three of its non-parasitic derivatives (i.e., *Le. alaskense*, *Le. reissneri*, and *Le. kessleri*), the fourth, *Le. appendix*, occupies drainages in middle and eastern North America and is thus separated from its presumed ancestor by nearly 2,500 km (Table 2.1; Fig. 2.9; Renaud et al. 2009b). The remaining non-parasitic species of *Lethenteron*, *Le. ninae*, whose affinity is unclear, is found in the drainage of the Black Sea (Table 2.1; Fig. 2.9).

As with *Ichthyomyzon* in North America, *Eudontomyzon*, which is confined to Eurasia, is an exclusively freshwater genus (Table 2.1; Fig. 2.8). Note that we do not recognize *Eudontomyzon* sp. nov. "migratory," listed as extinct by the International Union for the Conservation of Nature (IUCN), because it was never formally described (see Kottelat et al. 2005). In Europe, the parasitic *Eu. danfordi* occurs in tributaries of the Danube River. One of its non-parasitic derivatives, *Eu. mariae*, has

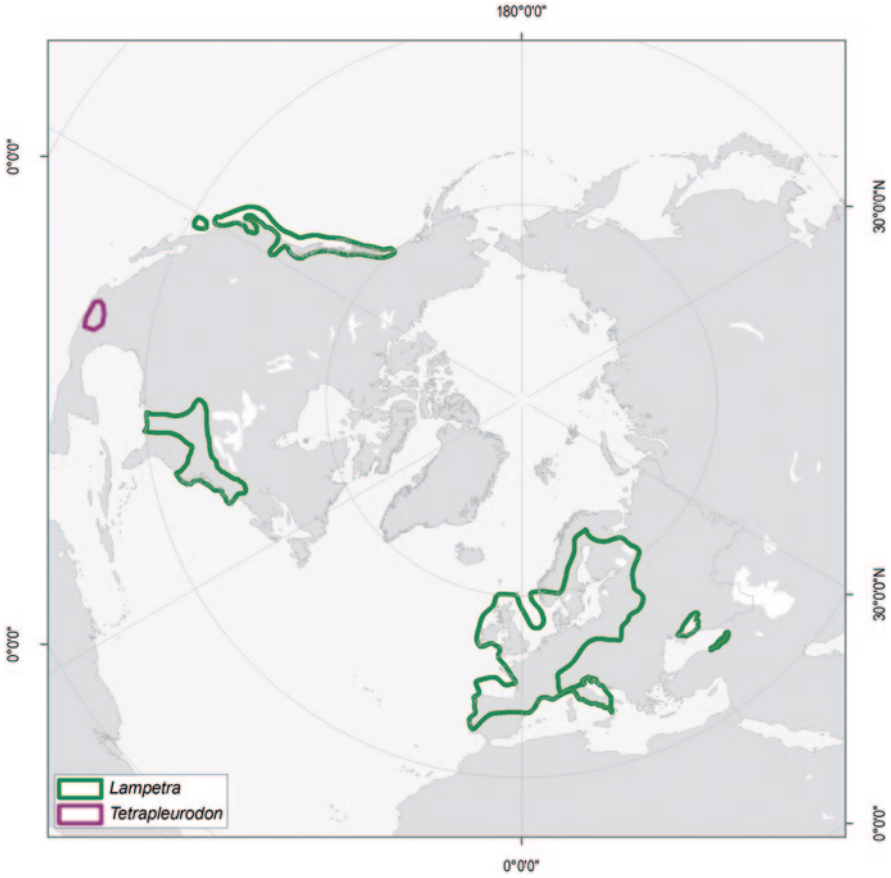

Fig. 2.10 Distributions of two of the eight Northern Hemisphere genera of lampreys (*Lampetra*, *Tetrapleurodon*) by polar projection. (Updated from Hubbs and Potter 1971)

a wide-ranging distribution that includes drainages from the Baltic Sea in the north to the Aegean Sea in the south, whereas the other, *Eu. stankokaramani*, is restricted to drainages of the Adriatic Sea (Table 2.1). Two other non-parasitic species, *Eu. hellenicus* and *Eu. graecus*, whose parasitic ancestries are unclear (see Sect. 2.4.2), each occur in a single drainage on the east and west side, respectively, of the Pindus Mountain range in Greece (Table 2.1). The parasitic species *Eu. morii* is confined to a single drainage that traverses China and North Korea (Table 2.1).

Within the genus *Lampetra*, the anadromous parasitic species *La. ayresii* and its non-parasitic derivative *La. richardsoni*, co-occur along an extensive strip of the western seaboard of North America, while its analogs, the anadromous parasitic *La. fluviatilis* and the non-parasitic *La. planeri*, co-occur and are widely distributed throughout Europe (Table 2.1; Fig. 2.10). In contrast to the above two non-parasitic species, *La. pacifica* (a second derivative of *La. ayresii*) and *La. lanceolata* (a second derivative of *La. fluviatilis*) both have very restricted distributions. Vladykov

(1973) suggested that *La. pacifica* was distributed in the Columbia River drainage in Oregon and also in the Sacramento and San Joaquin river systems in California, but Reid et al. (2011) recommend restriction of *La. pacifica* to the Columbia River basin, at least until further systematic information (e.g., regarding unresolved populations of *Lampetra* brook lampreys; see Sect. 2.4) is available. Nevertheless, while *La. pacifica* is found within the distribution of its presumed ancestor, that of *La. lanceolata* is far removed from that of its presumed ancestral species. Although an anadromous lamprey recently discovered in the Sea of Azov and referable to the genus *Lampetra* might be *La. fluviatilis*, Naseka and Diripasko (2008) concluded that they were not conspecific because they differed, in admittedly minor morphological respects, and were widely separated geographically. The remaining non-parasitic species of *Lampetra*, *La. zanandreai* and *La. hubbsi*, whose parasitic ancestry have not been established, are both considered southern relicts (see Sect. 2.2). The former species is found in the drainage of the Adriatic Sea and the latter in the Friant-Kern Canal and Merced River, California (Vladykov and Kott 1984), but Boguski et al. (2012) suggest that *La. hubbsi* may also occur in the upper Sacramento River (Table 2.1; Fig. 2.10).

Trees derived from molecular data (Fig. 2.6; Docker et al. 1999) suggest that *La. aepyptera*, which has normally been assigned to *Lampetra*, is more closely related to European species than to any extant North American species. Furthermore, the region where *La. aepyptera* is found in eastern North America is widely separated from the west coast of this continent where *La. ayresii*, the only North American parasitic representative of *Lampetra,* occurs (Docker et al. 1999; Potter et al. 2014). As emphasized previously, future studies should address the question of the ancestry of *La. aepyptera* and therefore the basis for the geographical distribution.

It is clear from comparisons of the distributions of the various lamprey species that the largest species, *P. marinus, G. australis*, and *En. tridentatus*, have the widest distributions and that these can extend well out into oceanic waters. During their parasitic phase, the smaller anadromous species, such as *M. mordax, La. fluviatilis*, and *La. ayresii*, occupy coastal waters and those of freshwater species each tend to occur in a restricted number of river systems.

The data compiled for this review emphasize that the lamprey fauna in the Northern Hemisphere, with 37 species and eight genera, is far more diverse than that in the Southern Hemisphere, which contains only four species and two genera. This reflects the presence of a greater number and diversity of rivers in temperate regions of the Northern Hemisphere than in corresponding regions of the Southern Hemisphere.

2.6 Conclusions and Future Directions

The aforegoing accounts and discussion demonstrate that progress is being made in understanding the phylogenetic relationships among extant lampreys (Petromyzontiformes). There is now widespread recognition, for example, that the

extant lampreys comprise three families, that is, Geotriidae, Mordaciidae, and Petromyzontidae. However, the precise relationships among the three families remain unresolved. Although there is not a complete consensus at the lower levels of classification, a clearer picture is emerging. Within the Petromyzontidae, the eight genera have either been separated into: (1) three subfamilies by Vladykov and co-workers (e.g., Vladykov 1972; Vladykov and Kott 1979c), namely, Petromyzontinae (*Petromyzon*, *Caspiomyzon*, and *Ichthyomyzon*), Entospheninae (*Entosphenus* and *Tetrapleurodon*), and Lampetrinae (*Lampetra*, *Lethenteron*, and *Eudontomyzon*); or (2) two subfamilies by Nelson (2006), namely, Petromyzontinae (*Petromyzon* and *Ichthyomyzon*) and Lampetrinae (*Caspiomyzon*, *Lampetra*, *Lethenteron*, *Eudontomyzon*, *Entosphenus*, and *Tetrapleurodon*). Although the three subfamilies proposed by Vladykov and co-workers may be most appropriate (see Sect. 2.4.1), we have adopted a conservative approach in this chapter, placing *Caspiomyzon* within Petromyzontinae but proposing that other taxonomic changes not be made prematurely.

At the generic level, morphological and molecular data support most of the existing classifications. While some uncertainties remain regarding the relationships among *Lampetra*, *Lethenteron*, and *Eudontomyzon*, we emphasize that taxonomic changes should not be made until the results of more comprehensive studies become available. In particular, the basis for the differences between the phylogenetic schemes produced using morphological and molecular data for *Lethenteron, Eudontomyzon*, and *Lampetra* needs to be clarified. This includes determining: (1) whether the parasitic and non-parasitic species designated as *Eudontomyzon*, which are represented in three different clades on the basis of the molecular data, are appropriately assigned to that genus according to morphological criteria; and (2) whether *Lampetra fluviatilis* and *La. ayresii* belong to the same clade, as suggested by their great morphological similarity or to different clades, as suggested by cytochrome *b* DNA sequence data. The resolution of these questions will require the use of a wider range of genes and particularly of nuclear genes.

Another remaining uncertainty is the phylogenetic relationship between the parasitic and non-parasitic members of species pairs. We recommend that no new non-parasitic species is erected until there has been a thorough morphological and molecular analysis aimed at elucidating the extent of the relationship between the putative new species and its presumed ancestor and comparisons with appropriate type specimens. This is particularly pertinent because the individuals in different populations of non-parasitic species may be genetically divergent but, at present, are morphologically indistinguishable. Furthermore, the phylogenetic positions of the six non-parasitic southern relict species for which there are no obvious ancestors (e.g., *La. aepyptera*, *Le. ninae*) need to be investigated using a wide range of independent genetic loci.

Acknowledgments We gratefully acknowledge Dr Halina Kobryn for generating the maps of the distribution of lampreys, Dr David Bird for kindly supplying Figs. 2.2b–d and Dr James Tweedley for helping to prepare the figures.

Appendix 2.1 List of Lamprey Families, Genera and Species and Their Authorities

- Mordaciidae Gill 1893
  - *Mordacia* Gray 1851
    - *Mordacia mordax* (Richardson 1846)
    - *Mordacia praecox* Potter 1968
    - *Mordacia lapicida* (Gray 1851)
- Geotriidae Jordan 1923
  - *Geotria* Gray 1851
    - *Geotria australis* Gray 1851
- Petromyzontidae Bonaparte 1832
  - *Caspiomyzon* Berg 1906
    - *Caspiomyzon wagneri* (Kessler 1870)
  - *Petromyzon* Linnaeus 1758
    - *Petromyzon marinus* Linnaeus 1758
  - *Ichthyomyzon* Girard 1858
    - *Ichthyomyzon unicuspis* Hubbs and Trautman 1937
    - *Ichthyomyzon fossor* Reighard and Cummins 1916
    - *Ichthyomyzon castaneus* Girard 1858
    - *Ichthyomyzon gagei* Hubbs and Trautman 1937
    - *Ichthyomyzon bdellium* (Jordan 1885)
    - *Ichthyomyzon greeleyi* Hubbs and Trautman 1937
  - *Tetrapleurodon* Creaser and Hubbs 1922
    - *Tetrapleurodon spadiceus* (Bean 1887)
    - *Tetrapleurodon geminis* Álvarez del Villar 1966
  - *Entosphenus* Gill 1862
    - *Entosphenus tridentatus* (Gairdner in Richardson 1836)
    - *Entosphenus minimus* (Bond and Kan 1973)
    - *Entosphenus similis* Vladykov and Kott 1979c
    - *Entosphenus macrostomus* (Beamish 1982)
    - *Entosphenus folletti* Vladykov and Kott 1976b
    - *Entosphenus lethophagus* (Hubbs 1971)
  - *Lethenteron* Creaser and Hubbs 1922
    - *Lethenteron camtschaticum* (Tilesius 1811)
    - *Lethenteron alaskense* Vladykov and Kott 1978
    - *Lethenteron appendix* (DeKay 1842)
    - *Lethenteron reissneri* (Dybowski 1869)
    - *Lethenteron kessleri* (Anikin 1905)
    - *Lethenteron ninae* Naseka et al. 2009
  - *Eudontomyzon* Regan 1911
    - *Eudontomyzon danfordi* Regan 1911
    - *Eudontomyzon mariae* (Berg 1931)
    - *Eudontomyzon stankokaramani* Karaman 1974
    - *Eudontomyzon morii* (Berg 1931)
    - *Eudontomyzon hellenicus* Vladykov et al. 1982
    - *Eudontomyzon graecus* Renaud and Economidis 2010

Lampetra Bonnaterre 1788
Lampetra ayresii (Günther 1870)
Lampetra pacifica Vladykov 1973
Lampetra richardsoni Vladykov and Follett 1965
Lampetra hubbsi (Vladykov and Kott 1976c)
Lampetra aepyptera (Abbott 1860)
Lampetra fluviatilis (Linnaeus 1758)
Lampetra planeri (Bloch 1784)
Lampetra lanceolata Kux and Steiner 1972
Lampetra zanandreai Vladykov 1955

References

Abbott CC (1860) Descriptions of new species of American fresh-water fishes. Proc Acad Natl Sci Phila 12:325–328
Álvarez del Villar J (1966) Ictiología michoacana, IV. Contribución al conocimiento biológico y sistemático de las lampreas de Jacona, Mich., México. An Esc Nac Cienc Biol Méx 13:107–144
Anikin VP (1905) Opisanie novÿkh aziatskikh vidov rÿb [Description of new Asian species of fish]. Izv Tomsk Univ 1905:1–18
Applegate VC (1950) Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. US Fish Wildl Serv Spec Sci Rep Fish 55:1–237
April J, Mayden RL, Hanner RH, Bernatchez L (2011) Genetic calibration of species diversity among North America's freshwater fishes. Proc Natl Acad Sci U S A 108:10602–10607
Bailey RM (1980) Comments on the classification and nomenclature of lampreys-an alternative view. Can J Fish Aquat Sci 37:1626–1629
Bardack D (1991) First fossil hagfish (Myxinoidea): a record from the Pennsylvanian of Illinois. Science 254:701–703
Bardack D (1998) Relationships of living and fossil hagfishes. In: Jørgensen JM, Lomholt JP, Weber RE, Malte H (eds) The biology of hagfishes. Chapman and Hall, London, pp 3–14
Bardack D, Zangerl R (1968) First fossil lamprey: a record from the Pennsylvanian of Illinois. Science 162:1265–1267
Bardack D, Zangerl R (1971) Lampreys in the fossil record. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 67–84
Bartels H, Potter IC (1995) Structural organization and epithelial cell types of the intestinal diverticula (protopancreas) of ammocoetes of southern hemisphere lampreys: functional and phylogenetic implications. Cell Tissue Res 280:313–324
Bartels H, Docker MF, Fazekas U, Potter IC (2012) Functional and evolutionary implications of the cellular composition of the gill epithelium of feeding adults of a freshwater parasitic species of lamprey, *Ichthyomyzon unicuspis*. Can J Zool 90: 1278–1283
Beamish FWH, Thomas EJ (1983) Potential and actual fecundity of the "paired" lampreys, *Ichthyomyzon gagei* and *I. castaneus*. Copeia 1983:367–374
Beamish RJ (1982) *Lampetra macrostoma*, a new species of freshwater parasitic lamprey from the west coast of Canada. Can J Fish Aquat Sci 39:736–747
Beamish RJ (2010) The use of gill pore papillae in the taxonomy of lampreys. Copeia 2010:618–628
Beamish RJ, Wade J (2008) Critical habitat and the conservation ecology of the freshwater parasitic lamprey, *Lampetra macrostoma*. Can Field-Nat 122:327–337
Bean TH (1887) Descriptions of five new species of fishes sent by Prof. A Dugès from the province of Guanajuato, Mexico. Proc U S Natl Mus 10:370–375 + 1 pl
Berg LS (1906) Ubersicht der Marsipobranchii des Russischen Reiches. Bull Acad Imp Sci 24:169–183

Berg LS (1931) A review of the lampreys of the northern hemisphere. Ann Mus Zool Acad Sci URSS 32:87–116 + 8 pls

Bianco PG (1986) *Lethenteron zanandreai* (Vladykov, 1955). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 237–246

Bird DJ, Potter IC (1979) Metamorphosis in the paired species of lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). 1. A description of the timing and stages. Zool J Linn Soc 65:127–143

Blair JE, Hedges SB (2005) Molecular phylogeny and divergence times of deuterostome animals. Mol Biol Evol 22:2275–2284

Blank M, Jürss K, Bastrop R (2008) A mitochondrial multigene approach contributing to the systematics of the brook and river lampreys and the phylogenetic position of *Eudontomyzon mariae*. Can J Fish Aquat Sci 65:2780–2790

Bloch ME (1784) M. Marcus Elieser Bloch's …, ausübenden Arztes zu Berlin, Oeconomische Naturgeschichte der Fische Deutschlands, vol 3. Aus Kosten des Verfassers, und in Commission in der Buchlandlung der Realschule, Berlin, viii + 234 + pls 73–108

Boguski DA, Reid SB, Goodman DH, Docker MF (2012) Genetic diversity, endemism and phylogeny of lampreys within the genus *Lampetra sensu stricto* (Petromyzontiformes: Petromyzontidae) in western North America. J Fish Biol 81:1891–1914

Bonaparte CL (1832) Saggio d'una distribuzione metodica degli animali vertebrati a sangue freddo. Giorn Arcadico 52:129–189

Bond CE, Kan TT (1973) *Lampetra* (*Entosphenus*) *minima* n. sp., a dwarfed parasitic lamprey from Oregon. Copeia 1973:568–574

Bond CE, Kan TT (1986) Systematics and evolution of the lampreys of Oregon. In: Uyeno T, Arai R, Taniuchi T, Matsuura K (eds) Indo-Pacific fish biology, Proc 2nd Int Conf Indo-Pacific Fish. Ichthyol Soc Jpn, Tokyo, p 919

Bonnaterre JP (1788) Tableau encyclopédique et méthodique des trois règnes de la nature. Ichthyologie, Paris, lvi (there is no p iii) + 215 + pls A and B + 100 pls

Çevik C, Ergüden D, Tekelioğlu N (2010) Confirmation of the presence of the sea lamprey, *Petromyzon marinus* Linnaeus, 1758 in the Levantine Sea (Petromyzoniformes: Petromyzonidae). Zool Middle East 49:107–108

Chang M-m, Zhang J, Miao D (2006) A lamprey from the Cretaceous Jehol biota of China. Nature 441:972–974

Chappuis PA (1939) Über die Lebensweise von *Eudontomyzon danfordi* Regan. Arch Hydrobiol 34:645–658 + 1 pl

Cochran PA, Lyons J, Merino-Nambo E (1996) Notes on the biology of the Mexican lampreys *Lampetra spadicea* and *L. geminis* (Agnatha: Petromyzontidae). Ichthyol Explor Freshw 7:173–180

Creaser CW, Hubbs CL (1922) A revision of the Holarctic lampreys. Occas Pap Mus Zool Univ Mich 120:1–14 + 1 pl

DeKay JE (1842) Zoology of New-York, or the New-York fauna; comprising detailed descriptions of all the animals hitherto observed within the state of New-York, with brief notices of those occasionally found near its borders, and accompanied by appropriate illustrations, part IV, Fishes. W & A White & J Visscher, Albany, xv + 415 + 79 pls

Delarbre C, Gallut C, Barriel V, Janvier P, Gachelin G (2002) Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. Mol Phylogen Evol 22:184–192

Docker MF (2006) Bill Beamish's contributions to lamprey research and recent advances in the field. Guelph Ichthyol Rev 7:1–52

Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 71–114

Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Docker MF, Youson JH, Beamish RJ, Devlin RH (1999) Phylogeny of the lamprey genus *Lampetra* inferred from mitochondrial cytochrome *b* and ND3 gene sequences. Can J Fish Aquat Sci 56:2340–2349

Docker MF, Mandrak NE, Heath DD (2012) Contemporary gene flow between "paired" silver (*Ichthyomyzon unicuspis*) and northern brook (*I. fossor*) lampreys: implications for conservation. Conserv Genet 13:823–835

Duméril AMC (1806) Zoologie analytique, ou méthode naturelle de classification des animaux; rendue plus facile à l'aide de tableaux synoptiques. Allais, Paris, pp xxxiii + 344

Dybowski BN (1869) Vorläufige Mittheilungen über die Fischfauna des Ononflusses und des Ingoda in Transbaikalien. Verh k-k Zool Bot Ges 19:945–958 + 1 table + pls 14–18

Espanhol R, Almeida PR, Alves MJ (2007) Evolutionary history of lamprey paired species *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) as inferred from mitochondrial DNA variation. Mol Ecol 16:1909–1924

Fernholm B (1998) Hagfish systematics. In: Jørgensen JM, Lomholt JP, Weber RE, Malte H (eds) The biology of hagfishes. Chapman and Hall, London, pp 33–44

Froese R, Pauly D (eds) (2013) FishBase. http://www.fishbase.org, version 04/2013. Accessed 31 May 2013

Fukutomi N, Nakamura T, Doi T, Takeda K, Oda N (2002) Records of *Entosphenus tridentatus* from the Naka River system, central Japan; physical characteristics of possible spawning redds and spawning behavior in the aquarium. Jpn J Ichthyol 49:53–58

Germain D, Sanchez S, Janvier P, Tafforeau P (2014) The presumed hagfish *Myxineidus gononorum* from the Upper Carboniferous of Montceau-les-Mines (Saône-et-Loire, France: new data by means of propagation phase contrast X-ray synchroton microtomography. Ann Paléontol 100:131–135

Gess RW, Coates MI, Rubidge BS (2006) A lamprey from the Devonian period of South Africa. Nature 443:981–984

Gill TN (1862) Notes on some genera of fishes of western North America. Proc Acad Natl Sci U S A 14:329–332

Gill TN (1893) Families and subfamilies of fishes. Mem Natl Acad Sci 6:127–138

Gill HS, Renaud CB, Chapleau F, Mayden RL, Potter IC (2003) Phylogeny of living parasitic lampreys (Petromyzontiformes) based on morphological data. Copeia 2003:687–703

Girard CF (1858) Fishes. General report on the zoology of the several Pacific railroad routes. United States Pacific Railroad Route Explorations and Surveys, vol 10, part 4, War Department, Washington, DC, pp 400 + 21 pls

Goodman DH, Kinziger AP, Reid SB, Docker MF (2009) Morphological diagnosis of *Entosphenus* and *Lampetra* ammocoetes (Petromyzontidae) in Washington, Oregon, and California. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 223–232

Gray JE (1851) List of the specimens of fish in the collection of the British Museum, part 1. Chondropterygii. British Museum (Natural History), London, pp xi + 160 + 2 pls

Günther A (1870) Catalogue of the fishes in the British Museum, vol 8, catalogue of the Physostomi, containing the families Gymnotidae, Symbranchidae, Muraenidae, Pegasidae, and of the Lophobranchii, Plectognathi, Dipnoi, Ganoidei, Chondropterygii, Cyclostomata, Leptocardii in the British Museum. Taylor and Francis, London, pp xxv + 549

Halliday RG (1991) Marine distribution of the sea lamprey (*Petromyzon marinus*) in the northwest Atlantic. Can J Fish Aquat Sci 48:832–842

Hardisty MW (1979) Biology of the cyclostomes. Chapman and Hall, London, pp 428

Hardisty MW (1982) Lampreys and hagfishes: analysis of cyclostome relationships. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 4B. Academic Press, London, pp 165–260

Hardisty MW (1986) *Lampetra fluviatilis* (Linnaeus, 1758). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 249–278

Hardisty MW (2006) Lampreys. Life without jaws. Forrest Text, Tresaith, pp 272

Hardisty MW, Potter IC (1971a) The behaviour, ecology and growth of larval lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 85–125
Hardisty MW, Potter IC (1971b) The general biology of adult lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 127–206
Hardisty MW, Potter IC (1971c) Paired species. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London. pp 249–277
Hardisty MW, Potter IC, Sturge R (1970) A comparison of the metamorphosing and macrophthalmia stages of the lampreys *Lampetra fluviatilis* and *L. planeri*. J Zool 162:383–400
Hardisty MW, Potter IC, Hilliard RW (1989) Physiological adaptations of the living agnathans. Trans R Soc Edinb Earth Sci 80:241–254
Heard WR (1966) Observations on lampreys in the Naknek River system of southwest Alaska. Copeia 1966:332–339
Heimberg AM, Cowper-Sal·lari R, Sémon M, Donoghue PCJ, Peterson KJ (2010) MicroRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. Proc Natl Acad Sci U S A 107:19379–19383
Holčík J (1986a) Determination criteria. In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 24–32
Holčík J (1986b) *Lethenteron* Creaser and Hubbs, 1922. In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 196–197
Holčík J, Šorić V (2004) Redescription of *Eudontomyzon stankokaramani* (Petromyzontes, Petromyzontidae)-a little known lamprey from the Drin River drainage, Adriatic Sea basin. Folia Zool 53:399–410
Holly M (1933) Cyclostomata. Das Tierreich 59:1–62
Hubbs CL (1925) The life-cycle and growth of lampreys. Pap Mich Acad Sci Arts Lett 4:587–603
Hubbs CL (1971) *Lampetra* (*Entosphenus*) *lethophaga*, new species, the nonparasitic derivative of the Pacific lamprey. Trans S Diego Soc Natl Hist 16:125–164
Hubbs CL, Potter IC (1971) Distribution, phylogeny and taxonomy. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 1–65
Hubbs CL, Trautman MB (1937) A revision of the lamprey genus *Ichthyomyzon*. Misc Publ Mus Zool Univ Mich 35:7–109 + 2 pls
Hughes RL, Potter IC (1969) Studies on gametogenesis and fecundity in the lampreys *Mordacia praecox* and *M. mordax* (Petromyzonidae). Austr J Zool 17:447–464
Iwata A, Goto A, Hamada K (1985) A review of the Siberian lamprey, *Lethenteron kessleri*, in Hokkaido, Japan. Bull Fac Fish Hokkaido Univ 36:182–190
Janvier P (1981) The phylogeny of the Craniata, with particular reference to the significance of fossil "agnathans". J Vert Paleontol 1:121–159
Janvier P (2010) MicroRNAs revive old views about jawless vertebrate divergence and evolution. Proc Natl Acad Sci U S A 107:19137–19138
Janvier P, Lund R (1983) *Hardistiella montanensis* n. gen. et sp. (Petromyzontida) from the lower Carboniferous of Montana, with remarks on the affinities of the lampreys. J Vert Paleontol 2:407–413
Jordan DS (1885) A catalogue of the fishes known to inhabit the waters of North America, north of the tropic of Cancer, with notes on the species discovered in 1883 and 1884. Rep U S Comm Fish 13:789–973
Jordan DS (1923) A classification of fishes including families and genera as far as known. Stanford Univ Publ Univ Ser Biol Sci 3:77-243 + i–x
Karaman MS (1974) *Eudontomyzon vladykovi stankokaramani* n. ssp., a new subspecies of lamprey from tributaries of the Ohrid-Drim-Skadar System in west Balkan Peninsula. Folia Balc 3:1–10 + 6 tables + 2 figs
Kessler K (1870) Volzhskaya minoga (*Petromyzon Wagneri* n. sp.) [Volga lamprey (*Petromyzon Wagneri* n. sp.)]. Trud St-Peterbg Obshchestva Estetvoznaniya 1:207–214
Kottelat M, Freyhof J (2009) Notes on the taxonomy and nomenclature of some European freshwater fishes. Ichthyol Explor Freshw 20:75–90

Kottelat M, Bogutskaya NG, Freyhof J (2005) On the migratory Black Sea lamprey and the nomenclature of the ludoga, Peipsi and ripus whitefishes (Agnatha: Petromyzontidae; Teleostei: Coregonidae). Zoosyst Rossica 14:181–186

Kucheryavyi AV, Savvaitova KA, Pavlov DS et al (2007a) Variations of life history strategy of the Arctic lamprey *Lethenteron camtschaticum* from the Utkholok River (western Kamchatka). J Ichthyol 47:37–52

Kucheryavyi AV, Savvaitova KA, Gruzdeva MA, Pavlov DS (2007b) Sexual dimorphism and some special traits of spawning behavior of the Arctic lamprey *Lethenteron camtschaticum*. J Ichthyol 47:481–485

Kuraku S, Kuratani S (2006) Time scale for cyclostome evolution inferred with a phylogenetic diagnosis of hagfish and lamprey cDNA sequences. Zool Sci 23:1053–1064

Kuraku S, Hoshiyama D, Katoh K, Suga H, Miyata T (1999) Monophyly of lampreys and hagfishes supported by nuclear DNA-coded genes. J Mol Evol 49:729–735

Kux Z, Steiner HM (1972) *Lampetra lanceolata*, eine neue Neunaugenart aus dem Einzugsgebiet des Schwarzen Meeres in der nordöstlichen Türkei. Čas Morav Mus Acta Mus Morav 56-57:375–384 + 10 figs

Lang NJ, Roe KJ, Renaud CB et al (2009) Novel relationships among lampreys (Petromyzontiformes) revealed by a taxonomically comprehensive molecular dataset. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 41–55

Linnaeus C (1758) Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis, vol 1. Laurentii Salvii, Stockholm

Lorion CM, Markle DF, Reid SB, Docker MF (2000) Redescription of the presumed-extinct Miller Lake lamprey, *Lampetra minima*. Copeia 2000:1019–1028

Macey DJ, Potter IC (1978) Lethal temperatures of ammocoetes of the Southern Hemisphere lamprey, *Geotria australis* Gray. Environ Biol Fish 3:241–243

Mallatt J, Sullivan J (1998) 28S and 18S rDNA sequences support the monophyly of lampreys and hagfishes. Mol Biol Evol 15:1706–1718

Mateus CS, Almeida PR, Quintella BR, Alves MJ (2011) MtDNA markers reveal the existence of allopatric evolutionary lineages in the threatened lampreys *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) in the Iberian glacial refugium. Conserv Genet 12:1061–1074

Mateus CS, Stange M, Berner D et al (2013a) Strong genome-wide divergence between sympatric European river and brook lampreys. Curr Biol 23:R649–R650

Mateus CS, Alves MJ, Quintella BR, Almeida PR (2013b) Three new cryptic species of the lamprey genus *Lampetra* Bonnaterre, 1788 (Petromyzontiformes: Petromyzontidae) from the Iberian Peninsula. Contrib Zool 82:37–53

McPhail JD, Lindsey CC (1970) Freshwater fishes of northwestern Canada and Alaska. Fish Res Board Can Bull 173:1–381

Moore JW, Mallatt JM (1980) Feeding of larval lamprey. Can J Fish Aquat Sci 37:1658–1664

Moyle, PB, Brown LR, Chase SD, Quiñones RM (2009) Status and conservation of lampreys in California. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 279–292

Naseka AM, Diripasko OA (2008) A recent record of an anadromous lamprey (Agnatha: Petromyzontidae) from the Sea of Azov. Ichthyol Explor Freshw 19:283–287

Naseka AM, Tuniyev SB, Renaud CB (2009) *Lethenteron ninae*, a new nonparasitic lamprey species from the north-eastern Black Sea basin (Petromyzontiformes: Petromyzontidae). Zootaxa 2198:16–26

Near TJ (2009) Conflict and resolution between phylogenies inferred from molecular and phenotypic data sets for hagfish, lampreys, and gnathostomes. J Exp Zool (Mol Dev Evol) 312B:749–761

Neira FJ, Bradley JS, Potter IC, Hilliard RW (1988) Morphological variation among widely dispersed larval populations of anadromous southern hemisphere lampreys (Geotriidae and Mordaciidae). Zool J Linn Soc 92:383–408

Nelson JS (2006) Fishes of the world, 4th edn. Wiley, Hoboken, pp 601
Nelson JS, Crossman EJ, Espinosa-Pérez H et al (2004) Common and scientific names of fishes from the United States, Canada, and Mexico, 6th edn. American Fisheries Society Special Publication 29, Bethesda
Nursall JR, Buchwald D (1972) Life history and distribution of the Arctic lamprey (*Lethenteron japonicum* (Martens)) of Great Slave Lake, N.W.T. Fish Res Board Can Tech Rep 304:1–28
Oliva O (1953) Příspěvek k přehledu našich mihulí (Petromyzones Berg 1940). Věstn Král Česk Spol Nauk 9:1-19 + 2 pls
Page LM, Espinosa-Pérez H, Findley LT et al (2013a) Common and scientific names of fishes from the United States, Canada, and Mexico, 7th edn. American Fisheries Society Special Publication 34, Bethesda
Page LM, Espinosa-Pérez H, Findley LT et al (2013b) New seventh edition of common and scientific names of fishes changes include capitalization of common names. Fisheries 38:188–189
Poplin C, Sotty D, Janvier P (2001) A hagfish (Craniata, Hyperotreti) from the Late Carboniferous Konservat-Lagerstätte of Montceau-les-Mines (Allier, France). C R Acad Sci Earth Planet Sci 332:345–350
Potter IC (1968) *Mordacia praecox*, n. sp., a nonparasitic lamprey (Petromyzonidae), from New South Wales, Australia. Proc Linn Soc N S W 92:254–261 + pl XIV
Potter IC (1980a) Ecology of larval and metamorphosing lampreys. Can J Fish Aquat Sci 37:1641–1657
Potter IC (1980b) The Petromyzoniformes with particular reference to paired species. Can J Fish Aquat Sci 37:1595–1615
Potter IC (1986) The distinctive characters of southern hemisphere lampreys (Geotriidae and Mordaciidae). In: Uyeno T, Arai R, Taniuchi T, Matsuura K (eds) Indo-Pacific fish biology. Proc 2nd Int Conf Indo-Pacific Fish. Ichthyological Society of Japan, Tokyo, pp 9–19
Potter IC, Beamish FWH (1975) Lethal temperatures in ammocoetes of four species of lampreys. Acta Zool 56:85–91
Potter IC, Gill HS (2003) Adaptive radiation of lampreys. J Great Lakes Res 29(Suppl 1):95–112
Potter IC, Osborne TS (1975) The systematics of British larval lampreys. J Zool 176:311–329
Potter IC, Strahan R (1968) The taxonomy of the lamprey *Geotria* and *Mordacia* and their distribution in Australia. Proc Linn Soc Lond 179:229–240 + 1 pl
Potter IC, Welsch U (1997) The structure of the gular pouch of mature males of the lamprey *Geotria australis*. Acta Zool 78:97–106
Potter IC, Lanzing WJR, Strahan R (1968) Morphometric and meristic studies on populations of Australian lampreys of the genus *Mordacia*. Zool J Linn Soc 47:533–546
Potter IC, Prince PA, Croxall JP (1979) Data on the adult marine and migratory phases in the life cycle of the southern hemisphere lamprey, *Geotria australis* Gray. Environ Biol Fish 4:65–69
Potter IC, Hilliard RW, Bird DJ, Macey DJ (1983) Quantitative data on morphology and organ weights during the protracted spawning-run period of the Southern Hemisphere lamprey *Geotria australis*. J Zool 200:1–20
Potter IC, Gill HS, Renaud CB (2014) Petromyzontidae: lampreys. In: Burr BM, Warren ML Jr (eds) North American freshwater fishes: natural history, ecology, behavior, and conservation. Johns Hopkins University Press, Baltimore, pp 106–139
Regan CT (1911) A synopsis of the marsipobranchs of the order Hyperoartii. Ann Mag Natl Hist 7:193–204
Reid SB, Boguski DA, Goodman DH, Docker MF (2011) Validity of *Lampetra pacifica* (Petromyzontiformes: Petromyzontidae), a brook lamprey described from the lower Columbia River basin. Zootaxa 3091:42–50
Reighard J, Cummins H (1916) Description of a new species of lamprey of the genus *Ichthyomyzon*. Occas Pap Mus Zool Univ Mich 31:1–12 + 2 pls
Renaud CB (1997) Conservation status of Northern Hemisphere lampreys (Petromyzontidae). J Appl Ichthyol 13:143–148
Renaud CB (2008) Petromyzontidae, *Entosphenus tridentatus*: southern distribution record, Isla Clarión, Revillagigedo Archipelago, Mexico. Check List 4:82–85

Renaud CB (2011) Lampreys of the world. An annotated and illustrated catalogue of lamprey species known to date. FAO Species Cat Fish Purposes 5, FAO, Rome

Renaud CB, Cochran PA (in press) Post-metamorphic feeding in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Renaud CB, Economidis PS (2010) *Eudontomyzon graecus*, a new nonparasitic lamprey species from Greece (Petromyzontiformes: Petromyzontidae). Zootaxa 2477:37–48

Renaud CB, Gill HS, Potter IC (2009a) Relationships between the diets and characteristics of the dentition, buccal glands and velar tentacles of the adults of the parasitic species of lamprey. J Zool 278:231–242

Renaud CB, Docker MF, Mandrak NE (2009b) Taxonomy, distribution, and conservation of lampreys in Canada. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 293–309

Richardson J (1836) Fauna Boreali-Americana; or the zoology of the northern parts of British America: containing descriptions of the objects of natural history collected on the late northern land expeditions under the command of Captain Sir John Franklin, R.N., part 3, the fish. J Murray, London, pp xv + 327 + pls 74–97

Richardson J (1846) Ichthyology of the voyage of H.M.S. Erebus & Terror. In: Richardson J, Gray JE (eds) The zoology of the voyage of the H.M.S. Erebus & Terror, under the command of Captain Sir James Clark Ross, R.N., F.R.S., during the years 1839 to 1843, vol 2. EW Janson, London, pp 53-74 + pls 31–44

Robins CR, Bailey RM, Bond CE et al (1980) A list of common and scientific names of fishes from the United States and Canada, 4th edn. American Fisheries Society Special Publication 12, Bethesda

Robins CR, Bailey RM, Bond CE et al (1991) Common and scientific names of fishes from the United States and Canada, 5th edn. American Fisheries Society Special Publication 20, Bethesda

Stock DW, Whitt GS (1992) Evidence from 18S ribosomal RNA sequences that lampreys and hagfishes form a natural group. Science 257:787–789

Takezaki N, Figueroa F, Zaleska-Rutczynska Z, Klein J (2003) Molecular phylogeny of early vertebrates: monophyly of the agnathans as revealed by sequences of 35 genes. Mol Biol Evol 20:287–292

Taylor EB, Harris LN, Spice EK, Docker MF (2012) Microsatellite DNA analysis of parapatric lamprey (*Entosphenus* spp.) populations: implications for evolution, taxonomy, and conservation of a Canadian endemic. Can J Zool 90:291–303

Tilesius von Tilenau WG (1811) *Piscium camtschaticorum* descriptions et icones. Mém Acad Imp Sci St-Pétersbg 3:225–285 + pls 8–13

Vladykov VD (1950) Larvae of eastern American lampreys (Petromyzonidae). 1. Species with two dorsal fins. Nat Can 77:73–95

Vladykov VD (1955) *Lampetra zanandreai*, a new species of lamprey from northern Italy. Copeia 1955:215–223 + 4 pls

Vladykov VD (1972) Sous-division en trois sous-familles des lamproies de l'hémisphère-nord de la famille Petromyzonidae. ACFAS 39:148

Vladykov VD (1973) *Lampetra pacifica*, a new nonparasitic species of lamprey (Petromyzontidae) from Oregon and California. J Fish Res Board Can 30:205–213 + 9 figs

Vladykov VD, Follett WI (1958) Redescription of *Lampetra ayresii* (Günther) of western North America, a species of lamprey (Petromyzontidae) distinct from *Lampetra fluviatilis* (Linnaeus) of Europe. J Fish Res Board Can 15:47–77

Vladykov VD, Follett WI (1965) *Lampetra richardsoni*, a new nonparasitic species of lamprey (Petromyzonidae) from western North America. J Fish Res Board Can 22:139–158 + 9 figs

Vladykov VD, Follett WI (1967) The teeth of lampreys (Petromyzonidae): their terminology and use in a key to the Holarctic genera. J Fish Res Board Can 24:1067–1075

Vladykov VD, Kott EE [sic] (1976a) The taxonomic significance of velar tentacles in Holarctic lampreys (Petromyzonidae). Rev Trav Inst Pêches Marit 40:787–789

Vladykov VD, Kott E (1976b) A second nonparasitic species of *Entosphenus* Gill, 1862 (Petromyzonidae) from Klamath River system, California. Can J Zool 54:974–989
Vladykov VD, Kott E (1976c) A new nonparasitic species of lamprey of the genus *Entosphenus* Gill, 1862, (Petromyzonidae) from south central California. Bull South Calif Acad Sci 75:60–67
Vladykov VD, Kott E (1978) A new nonparasitic species of the holarctic lamprey genus *Lethenteron* Creaser and Hubbs, 1922, (Petromyzonidae) from northwestern North America with notes on other species of the same genus. Biol Pap Univ Alaska 19:1–74
Vladykov VD, Kott E (1979a) Satellite species among the holarctic lampreys (Petromyzonidae). Can J Zool 57:860–867
Vladykov VD, Kott E (1979b) A new parasitic species of the holarctic lamprey genus *Entosphenus* Gill, 1862 (Petromyzonidae) from Klamath River, in California and Oregon. Can J Zool 57:808–823
Vladykov VD, Kott E (1979c) List of northern hemisphere lampreys (Petromyzonidae) and their distribution. Dep Fish Ocean Misc Spec Publ 42:1–30
Vladykov VD, Kott E (1984) A second record for California and additional morphological information on *Entosphenus hubbsi* Vladykov and Kott 1976 (Petromyzontidae). Calif Fish Game 70:121–127
Vladykov VD, Renaud CB, Kott E, Economidis PS (1982) A new nonparasitic species of Holarctic lamprey, genus *Eudontomyzon* Regan 1911 (Petromyzontidae), from Greece. Can J Zool 60:2897–2915
Yamazaki Y, Goto A (1996) Genetic differentiation of *Lethenteron reissneri* populations, with reference to the existence of discrete taxonomic entities. Ichthyol Res 43:283–299
Yamazaki Y, Goto A (1997) Morphometric and meristic characteristics of two groups of *Lethenteron reissneri*. Ichthyol Res 44:15–25
Yamazaki Y, Goto A (1998) Genetic structure and differentiation of four *Lethenteron* taxa from the Far East, deduced from allozyme analysis. Environ Biol Fish 52:149–161
Yamazaki Y, Goto A, Nishida M (2003) Mitochondrial DNA sequence divergence between two cryptic species of *Lethenteron*, with reference to an improved identification technique. J Fish Biol 62:591–609
Yamazaki Y, Yokoyama R, Nishida M, Goto A (2006) Taxonomy and molecular phylogeny of *Lethenteron* lampreys in eastern Eurasia. J Fish Biol 68(Suppl B):251–269
Yap MR, Bowen SH (2003) Feeding by northern brook lamprey (*Ichthyomyzon fossor*) on sestonic biofilm fragments: habitat selection results in ingestion of a higher quality diet. J Great Lakes Res 29(Suppl 1):15–25
Youson JH (1980) Morphology and physiology of lamprey metamorphosis. Can J Fish Aquat Sci 37:1687–1710
Zanandrea G (1957) Esame critico e comparativo delle lamprede catturate in Italia. Arch Zool Ital 42:249–307 + 3 pls

Chapter 3
The Ecology of Larval and Metamorphosing Lampreys

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Abstract The life cycle of lampreys typically begins in streams where fertilized eggs hatch into small, wormlike larvae (ammocoetes) which burrow into soft stream bottoms where they filter feed on organic matter until the onset of metamorphosis. The relative importance of habitat variables can change with ammocoete size (and depending on the spatial scale measured), but habitat must provide adequate substrate for burrowing and a regular supply of the suspended organic matter upon which larval lampreys feed. Larval movement occurs significantly more often at higher densities and in warmer temperatures, and typically occurs in a downstream direction at night. Sex ratio of some lamprey species is often related to differences in larval density, with the proportion of males increasing with relative density. Larval mortality is thought to be high in the egg phase, immediately following hatching, and at metamorphosis. The duration of the larval period in the life cycle of lampreys has been found to vary among and within species, but generally ranges

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from 3 to 7 years. However, analyses of larval growth and duration of larval life have been hampered by the unreliability of age assessment methods for larval lampreys. Metamorphosis begins during the summer months, when water temperatures are the most favorable, and is completed by winter or early spring.

Keywords Age at metamorphosis · Feeding · Growth · Habitat · Larval density · Macrohabitat · Microhabitat · Movement · Sex ratio · Statolith

3.1 Introduction

Understanding the ecology and life history of larval and metamorphosing lampreys is important for the management of threatened or endangered lamprey species, as well as for control of the invasive sea lamprey *Petromyzon marinus* in the Laurentian Great Lakes. The decline of many anadromous lamprey species (e.g., European river lamprey *Lampetra fluviatilis*, Pacific lamprey *Entosphenus tridentatus*, and sea lamprey in Europe) is often attributed to overharvest of adults and obstacles to their spawning migration (e.g., Almeida et al. 2002a, b; Mateus et al. 2012), but disruptions to larval habitat (e.g., pollution, irrigation and municipal water diversions, and other forms of habitat degradation) have also contributed to the decline of several species (e.g., Almeida et al. 2000; Luzier et al. 2011; see Chap. 8). Identifying—and protecting—critical larval habitat is thus necessary for species of conservation concern, whereas identifying—and targeting—critical larval habitat is necessary for sea lamprey control (see Marsden and Siefkes in press). Effective sea lamprey control also depends on accurately predicting larval growth and metamorphic rates in order to kill sea lamprey larvae in their natal streams before they become parasitic-phase juveniles (Christie et al. 2003; Hansen et al. 2003). Natural mortality is thought to be highest in early life and at metamorphosis, and studies of these life stages provide important information for both conservation and control.

In this chapter, we review ecological information regarding the larval stage of more than 20 of the approximately 40 recognized lamprey species worldwide (see Chaps. 1, 2 and 8). Following metamorphosis, 18 species are parasitic (in the ocean or in rivers or lakes) and the remaining species (the "brook lampreys") are nonparasitic (i.e., they do not feed at all following metamorphosis and become sexually mature, in their natal streams, within 6–10 months of metamorphosis; see Docker 2009). The ecology of the larval life stage, in contrast, seems more consistent among species.

A review of the ecology of the larval and metamorphosing stages of lampreys was provided by Hardisty and Potter (1971a, b), and subsequently updated by Potter (1980a). In recent years, however, targeted sampling for lampreys with improved electrofishing gear (especially in concert with efforts to monitor larval sea lamprey populations in the Great Lakes), statolith aging techniques, and the use of molecular markers have led to advances in our knowledge regarding larval

abundance, distribution, habitat requirements, feeding, growth rates, duration of larval life, and possible compensatory mechanisms affecting growth, survival, and recruitment dynamics. This chapter therefore provides an updated synthesis of the ecology of the largely sedentary, stream-resident stage of the life cycle terminating with the outmigration of juveniles. The process of metamorphosis (e.g., the morphological and physiological changes that occur during metamorphosis, and the intrinsic and extrinsic factors triggering it) is covered in depth in another chapter (see Chap. 4). In our discussion, we will refer to larval lampreys (beginning after hatching and ending prior to metamorphosis) as larvae or ammocoetes. Lampreys that are in the process of metamorphosis are referred to as transformers, while lampreys that have completed metamorphosis but are not yet sexually mature will be referred to as juveniles. Lampreys that have reached sexual maturity will be referred to as adults. The chapter will focus on what we have learned about the ecology of larval and metamorphosing lampreys since 1980, including recently emerging topics and techniques which have advanced our knowledge.

3.2 Habitat

Although it has long been possible for "an experienced observer" to predict "with some accuracy" the location of larval lamprey populations within a river system (Hardisty and Potter 1971a), considerable efforts continue to be made to characterize, in precise physico-chemical terms, the essential features of larval lamprey habitat. The international control program for the invasive sea lamprey in the Laurentian Great Lakes ranks streams for lampricide treatment in part on electrofishing catches in the best available lamprey habitat; thus, the ability to define larval lamprey habitat is critical for successful sea lamprey control (Jones 2007). The need for conservation of many other lamprey species has resulted in calls for status assessment using standardized sampling methods, with the first step in assessing ammocoete abundance being the classification and quantification of habitat within the study area (Kirchhofer 1995; Harvey and Cowx 2003; Moser and Close 2003).

Larval lamprey distribution and abundance has long been studied at the microhabitat scale, and recent studies further quantify these factors and test the generality of previous observations in a range of lamprey species. These small-scale studies of larval lamprey habitat have been useful for developing a general understanding of the biology of lampreys. However, since the conservation and management of lamprey populations requires the ability to predict spatial patterns in larval abundance at several scales (Torgersen and Close 2004), other recent studies are evaluating lamprey abundance at multiple scales. Furthermore, examining how abundance is affected by factors on only one scale ignores the modifying effects of factors operating on other scales (Goodwin et al. 2008). Therefore, both microenvironmental and macroenvironmental factors are discussed below.

3.2.1 *Microenvironmental Factors as Indicators of Ammocoete Habitat*

Lamprey larvae generally occur in soft, burrowable substrates of fine sands, low current velocity, and some amount of organic detritus (e.g., Applegate 1950; Hardisty and Potter 1971a), and these qualitative observations form the basis of the habitat type classification used by sea lamprey control personnel and other lamprey biologists: Type I (preferred) habitat is located primarily in depositional zones and consists primarily of a mixture of sand and fine organic matter; Type II habitat (which is sometimes inhabited by larvae but at much lower densities) generally consists of shifting sand that may contain some gravel; and Type III (unacceptable) habitat consists of such substrates as hard packed gravel, hardpan clay, and bedrock (Slade et al. 2003). Mullett (1997) found that 93 % of sea lamprey larvae in Great Lakes tributaries were found in Type I habitat, and this qualitative classification system has become an effective tool in the assessment of larval lamprey habitat (e.g., Torgersen and Close 2004; Zerrenner and Marsden 2005; Neeson et al. 2007). Research continues, however, to quantify the microhabitat factors affecting lamprey distribution and abundance. Although multiple factors affect lamprey distribution and abundance in concert, those deemed to be most important are reviewed individually below. Water depth, water chemistry, and thermal and oxygen requirements are reviewed with macroenvironmental factors (Sect. 3.2.2).

3.2.1.1 Substrate Size and Depth

The availability of optimal substrate particle size is one of the most important factors limiting the distribution of larval lampreys (Hardisty 1979; Kainua and Valtonen 1980; Malmqvist 1980; Morman et al. 1980; Potter 1980a; Lee 1989; Young et al. 1990a, b; Todd and Kelso 1993; Beamish and Jebbink 1994; Ojutkangas et al. 1995; Beamish and Lowartz 1996; Sugiyama and Goto 2002; Goodwin et al. 2008; Table 3.1). As reviewed by Potter (1980a), Manion and McLain (1971) found sea lamprey larvae to be most abundant where 90 % of the substrate consisted of sand particles less than 0.5 mm in diameter. In the European brook lamprey *Lampetra planeri*, Malmqvist (1980) likewise observed only a small number of larvae where particle diameter exceeded 0.5 mm. More recent studies, in other species, have made similar observations. Potter et al. (1986) found density of larval pouched lamprey *Geotria australis* to be greatest in medium sand (0.2–0.6 mm diameter); in American brook lamprey *Lethenteron appendix*, larval density was related to the presence of medium-fine sand (0.15–0.25 mm: Beamish and Lowartz 1996). Beamish and Jebbink (1994) reported that abundance of southern brook lamprey *Ichthyomyzon gagei* was greatest when particles smaller than 0.15 and 1.0–2.0 mm in diameter represented at least 40 and 8 % dry weight of the substrate, respectively, and was lowest when the small particles represented less than 10 % dry weight of the substrate. Taverny et al. (2012) reported that sea lamprey and European river/brook

lamprey larvae were found most frequently in fine/medium sand (0.05–0.6 mm diameter) and medium/coarse sand (0.2–2 mm diameter), respectively. In their study, 95 % of all ammocoetes (of both species) were collected in sandy substrate, and silt was generally absent or very low at these locations. In a laboratory study, larval least brook lamprey *Lampetra aepyptera* were given a choice of six equally-available substrate types; they selected fine sand (0.125–0.5 mm diameter) 52.2 % of the time (i.e., disproportionately to its availability; Smith et al. 2011). Although there may be subtle differences in preferred substrate particle size among species—for example, Goodwin et al. (2008) and Taverny et al. (2012) suggested that European river and brook lamprey larvae might be associated with somewhat coarser sands than other species—substrate requirements appear similar among species and, in fact, multiple species are often found at the same sites (Hardisty and Potter 1971a; Dawson and Jones 2009).

An appropriate river substrate is an essential environmental characteristic for the development of larval lampreys, not only because it allows burrow construction, but also because it helps to maintain a vital water flux. The detritivorous larvae depend on a unidirectional flow of water through their branchial chamber for the provision of food and exchange of respiratory gases and metabolic wastes (Hardisty and Potter 1971a). Fine sand (or the combination of particle sizes observed by Beamish and Jebbink 1994) appears to be optimal for burrow construction and water flow. Finer particles (e.g., clay and silt) are more compact and difficult to burrow into, and could potentially smother existing burrows or clog the gill lamellae of the ammocoetes. In contrast, large particles (e.g., coarse sand or gravel) could be too heavy for larvae to move or too large to be adequately held together by mucous secretions (Beamish and Jebbink 1994; Beamish and Lowartz 1996; Smith et al. 2011).

Protection from predators will also depend on the speed with which ammocoetes can burrow and on burrow depth, which will be affected by substrate composition and depth. Smith et al. (2012) demonstrated, in experimental trials, that depredation by yellow bullhead *Ameiurus natalis* was lowest where least brook lamprey larvae were able to burrow into fine sand; survival in fine sand averaged 80 % whereas survival averaged 58 % in coarse sand and only 4 % in silt/clay. Ammocoetes showed the slowest burrowing times in the silt/clay substrate and were observed swimming outside their burrows when provided only with this substrate. Burrowing times in larval sea and least brook lampreys are faster in fine sand compared to coarse sand (Quintella et al. 2007; Smith et al. 2012). Deeper burrows would also be expected to offer greater protection from predators, and the depth to which ammocoetes can burrow might be limited in silt/clay or coarse sand substrates and will, of course, be limited in more shallow substrates. European river and brook lamprey larvae in Northern Ireland were more abundant when sediment depth was >11.5 cm (Goodwin et al. 2008). Sugiyama and Goto (2002) found that Far Eastern brook lamprey larvae *Lethenteron reissneri* were more likely to be found where substrate depth was >2 cm, but noted in an experimental trial that only large larvae showed a significant preference for deeper substrate (see Sect. 3.2.3). Presumably, larger ammocoetes require deeper burrows to completely hide from predators.

3.2.1.2 Water Velocity

In most studies that have defined optimal ammocoete habitat on small spatial scales, substrate grain size and water velocity were the most important indicators of larval lamprey abundance (Malmqvist 1980; Beamish and Jebbink 1994; Beamish and Lowartz 1996; Almeida and Quintella 2002; Sugiyama and Goto 2002; Table 3.1), and the two factors, of course, are interrelated. The size of substrate particles that are eroded, transported, or deposited at a specific location depends upon local hydraulic conditions, primarily boundary shear stress (Allen 1984). Typical ammocoete habitat is an area protected from major fluctuations in water levels or stream flow, and where current velocity is usually slow. Such conditions are commonly found in eddies, backwaters, or at bends in a river, where accumulations of silt and sand provide suitable substrate for burrowing ammocoetes.

Areas of high abundances of pouched and landlocked sea lamprey larvae are normally found in regions of the river where current velocity is less than 0.03 m/s (Thomas 1962; Potter et al. 1986), and Thomas (1962) concluded that flow rates of 0.6–0.8 m/s represented an upper limit for sea lamprey larvae. The maximum near-bottom velocity where anadromous sea lamprey larvae occurred in the Gironde-Dordogne River basin did not exceed 0.3 m/s (Taverny et al. 2012). Optimal velocity for *Lampetra* larvae (i.e., European river or brook lamprey larvae) in the same river system was up to 0.05 m/s, but larvae occurred in sites where velocity was as high as 0.5 m/s (Taverny et al. 2012). These rates of flow are highly consistent with those from previous studies of European river lamprey in Finland, where larval habitat was characterized by water velocities ranging from 0.01–0.05 m/s to about 0.5 m/s (Kainua and Valtonen 1980). Small larvae were proportionally more numerous in habitats where the flow was rapid and, where larvae were found in shallow water, the rate of flow was almost constantly below 0.1 m/s (Kainua and Valtonen 1980). Stone and Barndt (2005) reported optimal velocities of 0–0.1 m/s for Pacific lamprey ammocoetes in a Washington stream. Torgersen and Close (2004) indicated that larval Pacific lamprey require habitat containing adequate flow that is sufficient to provide a stable food supply, but slow enough to allow sediment deposition required for burrowing. Habitats that supported larval Arctic lamprey *Lethenteron camtschaticum* were characterized as having predominantly silt or sand substrates with woody debris and slow flow (0.0–0.1 m/s) (Trent M. Sutton, University of Alaska, Fairbanks, AK, personal communication, 2011). Far Eastern brook lamprey larvae were positively associated with areas where water velocity was less than 0.1 m/s (Sugiyama and Goto 2002).

3.2.1.3 Organic Matter in the Sediment

Whereas substrate particle size and water velocity are consistently considered two of the most important fine-scale predictors of larval lamprey abundance, the importance of organic matter in the sediment is less clear. Organic detritus is generally deposited in areas of slow flow where accumulations of silt and sand provide suit-

Table 3.1 Summary of past research on larval lamprey habitat selection. Variables listed are those considered more important for adequate larval lamprey habitat at different spatial scales. (Adapted from Smith 2009)

Species	Variables	Study type	Reference
Entosphenus tridentatus, Pacific lamprey	Water depth/open riparian canopy/current (slow), pool habitats/substrate type	Field study	Torgersen and Close (2004)
Entosphenus tridentatus	Large scale: conductivity/dissolved oxygen/gradient Small scale: wetted width/percent fine sediment/canopy density/water velocity	Field study	Stone and Barndt (2005)
Entosphenus tridentatus	Substrate (fine-medium)/riparian shade	Field study	Claire et al. (2007)
Entosphenus macrostomus, Vancouver lamprey	Substrate (fine sediment on top of fine sand and small gravel, where overlaying silt < 10 cm)	Field study	Beamish and Wade (2008)[a]
Geotria australis, pouched lamprey	Substrate (particle size, depth)/organic material/chlorophyll α/shade/macrophyte roots/eddies, water velocity	Field study	Potter et al. (1986)
Geotria australis	Substrate/water depth	Field study	Kelso and Todd (1993)
Geotria australis	Substrate (fine sand)/shade/run habitat	Field study	Jellyman and Glova (2002)
Ichthyomyzon fossor, northern brook lamprey	Substrate (silt-sand)/current (slow)	Field study	Reighard and Cummins (1916)
Ichthyomyzon fossor	Substrate (fine sand)/organic debris	Field study	Leach (1940)
Ichthyomyzon fossor	Organic sediment	Field study	Yap and Bowen (2003)
Ichthyomyzon gagei, southern brook lamprey	Organic debris	Field study	Dendy and Scott (1953)
Ichthyomyzon gagei	Substrate (medium-fine sand)	Field study	Beamish and Jebbink (1994)
Lampetra aepyptera, least brook lamprey	Clay/silt/fine sand	Field study	Seversmith (1953)
Lampetra aepyptera	Substrate (fine sand)	Lab experiment	Smith et al. (2011)
Lampetra fluviatilis/*Lampetra planeri*, European river and brook lampreys	Substrate (coarse sand)/pH (≥ 8.2)	Field study	Goodwin et al. (2008)
Lampetra fluviatilis/*Lampetra planeri*	Substrate (coarse-medium sand)/water depth/current (slow)/macrophyte roots	Field study	Taverny et al. (2012)
Lampetra fluviatilis/*Lampetra planeri*	Altitude (< 170 m)/distance to coast (< 150 km)/substrate (> 70 % sand)/ maximum temperature of warmest month/precipitation of driest month	Field study	Ferreira et al. (2013)

Table 3.1 (continued)

Species	Variables	Study type	Reference
Lampetra planeri	Current (slow)/water depth (low)/ substrate/chlorophyll α content (low)	Field study	Malmqvist (1980)
Larval lampreys in general	Stream gradient/substrate (silt-sand)/current (slow)/organic debris	Review	Hardisty and Potter (1971a)
Larval lampreys in general	Current (0.4–0.5 m/s)/substrate (silt and sand)	Field study	Hardisty (1979)
Lethenteron appendix, American brook lamprey	Substrate (medium-fine sand/organic content)	Field study	Beamish and Lowartz (1996)
Lethenteron appendix	Substrate (medium-fine sand)	Lab experiment	Lee (1989)
Lethenteron appendix	Substrate/distance from alluvial fans (close)/thermocline (above)/ water depth (low)	Field study	Lee and Weise (1989)[a]
Lethenteron appendix	Substrate (medium-fine sand)	Field study	Mundahl et al. (2006)
Lethenteron camtschaticum, Arctic lamprey	50–150 mm TL: substrate hardness/ DOM 10–50 mm TL: soft substrate/ velocity	Field study	Shirakawa et al. (2009)
Lethenteron reissneri, Far Eastern brook lamprey	Substrate (fine sand-silt)/current (slow)/depth (shallow)	Field study, lab experiment	Sugiyama and Goto (2002)
Lethenteron reissneri	Substrate (medium-fine sand)	Field study	Yamazaki (2007)
Petromyzon marinus, sea lamprey	Substrate (medium-fine sand)	Lab experiment	Lee (1989)
Petromyzon marinus	Substrate (sand)	Field study	Young et al. (1990a)
Petromyzon marinus	Substrate (silt-sand)	Field study	Young et al. (1990b)
Petromyzon marinus	Substrate (sand)	Field study	Almeida and Quintella (2002)
Petromyzon marinus	Substrate/distance from stream mouth/slope of the lake	Field study	Fodale et al. (2003)[a]
Petromyzon marinus	Substrate (sand/fine organic matter)	Field study	Slade et al. (2003)
Petromyzon marinus	Geomorphic features (river slope, radius of curvature)	Field study	Neeson et al. (2007)
Petromyzon marinus	Substrate (fine-medium sand)/water depth (>2 m)/current (slow)/ macrophyte roots	Field study	Taverny et al. (2012)

[a] lentic habitat

able substrate for burrowing ammocoetes and, in several studies (e.g., Shirakawa et al. 2009), larval abundance is related to substrate composition, water velocity, and dissolved organic material as predicted. Certainly, the presence of fine organic matter is one of the attributes of Type I (preferred) larval lamprey habitat (see Sect. 3.2.1). Potter et al. (1986) likewise found the presence of organic material in

the substrate to be an important environmental variable predicting the density of larval pouched lamprey in three of their four seasonal models (i.e., in all seasons but winter). In their habitat selection study, Smith et al. (2011) found that—after their preference for fine sand—least brook lamprey larvae exhibited a secondary preference for an organic substrate (consisting of approximately 70% decomposing leaves/stems and organic sediment particles and 30% silt/fine sand). In contrast, Malmqvist (1980) found that water current, water depth, substrate size, and chlorophyll α content explained a large part of the variation in distribution of larval European brook lamprey, but found that organic content in the sediment did not improve his discriminant model. Organic content was not even correlated with larval density when simple linear regression was applied. Malmqvist (1980) thus suggested that the presence of organic material in the sediment is not a prerequisite for the larvae since they can ingest their food directly from the water column above the sediment. Sugiyama and Goto (2002) likewise found that habitat use by Far Eastern brook lamprey larvae was not influenced by the amount of fallen leaves. Rather than interpreting these findings to mean, however, that the presence of organic material in the sediment is not a prerequisite for larval lampreys, these authors suggested that the amount consumed by larvae is low relative to the amount generally present in larval streams (i.e., that organic detritus may exceed the necessary threshold to sustain larval growth in all but the most oligotrophic streams; see Sects. 3.2.1.4 and 3.3).

Although less well studied, there is also a lack of consensus on the relationship between chlorophyll α content of the sediment and larval abundance. Potter et al. (1986) found that substrate chlorophyll α content (presumably reflecting the relative amounts of diatoms and other microalgae in the sediment) contributed to their model in a positive manner in spring, summer, and autumn. This finding was not surprising to these authors, as diatoms and other microalgae form an important component of the diet of larval lampreys (see Sect. 3.3). In contrast, however, Malmqvist (1980) found a negative correlation between chlorophyll α content and larval abundance, and suggested that sites where chlorophyll α content is high may be subject to lowered oxygen levels at night as the result of increased algal respiration.

3.2.1.4 Patchiness at Small Spatial Scales

As expected, given the patchy distribution of the above features within and among river systems, ammocoetes are patchily distributed at both small and large spatial scales. At a small spatial scale, for example, Torgersen and Close (2004) found considerable variation in the occurrence of Pacific lamprey larvae among 1-m^2 quadrat samples distributed throughout a 55-km section of the Middle Fork John Day River, Oregon. At this scale, patchiness was associated with low water velocity and substrate type, as expected, and with channel morphology. Larval abundance was heterogenous across the stream channel, with over 80% of the larvae being found along the stream margins. Several other studies have also reported ammocoetes aggregated at stream margin areas of fine silt and detritus (Farlinger and Beamish

1984; Brown and Moyle 1993; Roni 2002; Torgersen and Close 2004; Gunckel et al. 2009).

Patchiness at even finer scales has also been reported within habitat types. As expected, northern brook lamprey *Ichthyomyzon fossor* larvae in three oligotrophic Great Lakes tributaries were more abundant in Type I than Type II habitats (see Sect. 3.2.1), but they also showed a high degree of aggregation within habitat types (Yap and Bowen 2003). Within Type I habitats, larvae were generally aggregated at sites where obstacles to flow (e.g., stones or woody debris) caused localized accumulation of flocculent material (Yap and Bowen 2003); the authors referred to these sites as "Type Ia" habitat. These authors found that assimilation efficiency for organic matter and amino acids was higher in Type Ia habitat and that these larvae have higher condition factors (weight per length). Although the benefit of Type Ia habitats may not be as great in eutrophic streams (Yap and Bowen 2003), potential differences in habitat quality at such a fine scale are very interesting.

3.2.2 Macroenvironmental Factors as Indicators of Ammocoete Habitat

Patchiness is also evident at larger scales. Sea lamprey larvae, for example, have been detected in only 449 of the 5,747 (less than 8%) Great Lakes tributaries (Morman et al. 1980; Heinrich et al. 2003; Larson et al. 2003; Lavis et al. 2003; Morse et al. 2003; Sullivan 2003), despite the widespread distribution of adults throughout the Great Lakes. In Portugal, Ferreira et al. (2013) found *Lampetra* sp. larvae in 8 of the 15 basins (53%) and 60/401 sites (15%) surveyed. Lamprey distribution is ultimately influenced, of course, by the interaction between small-scale and large-scale habitat variables, and more studies are beginning to examine the effect of such variables over a range of spatial scales. The existence of suitable microhabitats for ammocoete colonization is dependent on larger-scale processes (e.g., stream gradients determine the overall velocity of the current, the type of substrate particles that are deposited, and the accumulation of organic debris; Hardisty and Potter 1971a). Climatic factors (e.g., temperature) may limit lamprey distribution in some cases (particularly at the northern or southern limit of the species' distribution). Other variables such as water depth, proximity to adult spawning areas, and riparian canopy can also be important indicators of larval lamprey abundance on moderate to large spatial scales (Almeida and Quintella 2002; Torgersen and Close 2004).

3.2.2.1 Gradient and Other Geomorphic Variables

Broad-scale distribution patterns of larval lampreys have long been attributed to variation in channel gradient within and among streams (e.g., Baxter 1957), and Young et al. (1990a) suggested that gradient could serve as a useful "surrogate variable" for the suite of environmental factors that describe optimal larval habitat.

However, whereas measurements of substrate particle size and water velocity seem to be rather consistent among studies (see Sects. 3.2.1.1 and 3.2.1.2), stream gradients reported for different lamprey streams and species appear more variable. For example, stream gradient for sea lamprey streams in England was found to range between 5.0 and 14.5 m/km, which allowed for good spawning habitat in the upper reaches and depositional areas for larval habitats downstream (Baxter 1954). Stream gradients measured by Dawson and Jones (2009) in four sea lamprey-producing Great Lakes tributaries were found to fall within this range. Similarly, Hardisty (1986) reported average stream gradients of 17.7 and 8.3 m/km, respectively, for European brook lamprey in the upper reaches of the Jeziorka River in Poland and Ukrainian brook lamprey *Eudontomyzon mariae* in the lower reaches. However, there are also many reports of lampreys occurring in lower-gradient streams. For example, *Lampetra* larvae have been found in streams in Finland with average gradients of about 2 m/km (Hardisty 1986), and European brook lamprey in the River Yeo in England occur where the stream gradient is 1.9–3.8 m/km (Hardisty 1961a). In a western Washington stream, Stone and Barndt (2005) found that Pacific lamprey abundance per reach was negatively associated with stream gradient. There may, of course, be differences among species—Gunckel et al. (2009), for example, found that Pacific lamprey were more likely to occur in relatively wider, lower-elevation streams than were western brook lamprey *Lampetra richardsoni*—and it is becoming clear that the relationship between stream gradient and lamprey distribution will depend on the scale at which gradient is measured (Torgersen and Close 2004).

Neeson et al. (2007) attempted to predict the distribution of suitable larval sea lamprey habitat at several spatial scales using water surface slope (as measured in the field) and GIS-derived geomorphic values within the East Branch of the Chagrin River, Ohio. Of the five geomorphic variables tested, field-measured slope and GIS-derived radius of curvature influenced the probability that a stream segment would contain suitable ammocoete habitat (i.e., Type I or Type II habitat; see Sect. 3.2.1) at a stream segment length of 50 m. Organic content was significantly higher in low-slope (≥ 0.005 or 5 m/km) areas compared to high-slope (>0.005) areas. GIS-estimated slopes were not sufficiently accurate at this scale, so the final model included only radius of curvature (to allow habitat categorization using only GIS). At longer stream segment lengths (100, 200, 300 m), however, no relationships between geomorphic variables and presence of ammocoete habitat were observed (Neeson et al. 2007). Fine sediments accumulate immediately downstream of sharp bends, which is consistent with radius of curvature affecting habitat characteristics only on a local scale. In terms of slope, Neeson et al. (2007) suggested that, at coarse scales, alternating channel units of varying slope (e.g., pools and riffles) would obscure a finer-scale association between slope and habitat. In contrast, Torgersen and Close (2004) suggested that the relative influence of channel gradient as a predictor of Pacific lamprey larval abundance might increase at larger spatial scales because of changes in bedform morphology at the stream segment or network scale. These authors found that channel gradient corresponded with large-scale larval abundance patterns, but was not an important predictor of adundance

after accounting for water depth and extent of the riparian canopy (see Sects. 3.2.2.1 and 3.2.2.3).

Ferreira et al. (2013) likewise found that GIS-derived slope did not improve the performance of their model predicting the distribution of European river and brook lamprey larvae in Portugal at a macrospatial scale. However, in this study, altitude was the strongest predictor of larval distribution, and altitude is generally correlated with gradient or slope. Predicted occurrence of larvae peaked at low altitudes (<170 m), before tailing off at higher elevations. The two other geomorphological variables that explained most of the variation in the distribution of these species was distance to coast and percentage of sand (at a resolution of 1 km^2). The distance upriver at which larval abundance peaked varied, depending on the size of the river basins, but was within 150 km from the coast. Microhabitat characteristics that are favorable for larval lampreys (e.g., low current velocity, preferred substrate) are more prevalent in the downstream reaches of the rivers. Neeson et al. (2012a) similarly found that distance to the river mouth was an important geomorphic predictor of the prevalence of preferred substrate habitat of Great Lakes sea lamprey in the Lower Peninsula of Michigan. In most cases, distance to the river mouth was negatively related to preferred substrate habitat, reflecting the increasing gradient in particle size from a river's mouth to its headwaters. These authors also found that distance to the nearest upstream dam or lake was likewise negatively related to preferred subtrate habitat, reflecting the general lack of fine sediments found below impounded lakes and, to a lesser degree, natural lakes (Neeson et al. 2012a).

Barriers to migration are also more prevalent with increasing distance from the river mouth. Goodwin et al. (2008) found that abundance of European river and brook lamprey larvae was inversely related to the distance upstream and the number of potential barriers, at least at the catchment level. At smaller (microhabitat) scales, larval abundance was most associated with substrate particle size and depth and, at the larger (regional) scale, abundance was associated with stream pH (see Sect. 3.2.2.4).

3.2.2.2 Water Depth

The association between water depth and larval abundance may also differ depending on the spatial scale considered. Previous studies suggest that the most favorable habitat conditions are usually found in shallow water near the edge of the river (Hardisty and Potter 1971a; Malmqvist 1980). Several recent studies likewise report maximum larval abundance or occupancy in shallower waters: Sugiyama and Goto (2002), for example, found more Far Eastern brook lamprey larvae where water depth was <30 cm; Stone and Barndt (2005) generally found Pacific lamprey ammocoetes in areas where water depth was 70 cm deep; and Taverny et al. (2012) found that European river and/or European brook lamprey larvae preferentially used shallow waters (<50 cm deep) and were rarely found above a depth of 150 cm. At a larger scale, however, Torgersen and Close (2004) found that larval abundance increased with water depth, likely because reaches containing large numbers of deep

pools were structurally complex and more likely to protect larvae from scouring and other forms of flow-induced stress. In addition to possible differences due to scale, other discrepancies may be related to larval size (e.g., with larger larvae being found in deeper waters; see Sect. 3.2.3) or species. Taverny et al. (2012), for example, found maximal occurrence of sea lamprey larvae in the Gironde-Dordogne River basin at depths >2 m, whereas *Lampetra* larvae generally occurred at much shallower depths. There may also be seasonal effects; Potter et al. (1986) found that pouched lamprey larval density was greater in shallower waters in the summer, but was greater in deeper waters in the winter. These authors explained this anomaly by suggesting that the geomorphology of this stream (which lacked a flood plain and was instead contained within a steep-sided U-shaped gutter) resulted in desirable substrate being deposited in deeper water in the winter when water levels and flow rates are high. Notably though, the water depth in the winter (2–71 cm) was still relatively shallow.

Furthermore, although larvae are most frequently captured in shallow ("wadeable") waters, they have also been found in relatively deep water. Sea lamprey ammocoetes have been documented in deepwater habitats in tributaries of the Great Lakes and in proximity to river mouths (Hansen and Hayne 1962; Wagner and Stauffer 1962; Lee and Weise 1989; Bergstedt and Genovese 1994). In Lake Superior, for example, it is thought that periodic floods scour the lower portions of the tributaries and flush sea lamprey larvae into the lake (Fodale et al. 2003). Although the majority of larvae located in lentic environments are likely due to downstream drift, Vancouver lamprey *Entosphenus macrostomus* (and occasionally other species) have been observed spawning in these environments (R. J. Beamish 1982; Russell et al. 1987; see Chap. 6). Use of deepwater sampling equipment such as suction dredges (e.g., Beamish and Youson 1987; Taverny et al. 2012) and deepwater electrofishers (e.g., Bergstedt and Genovese 1994; Jolley et al. 2012) are increasingly detecting larval and metamorphosing lampreys in deep water in large river systems as well. Metamorphosing North American river lamprey *Lampetra ayresii* are frequently recovered during dredging operations in the Fraser River, British Columbia (Beamish and Youson 1987), and, as noted above, sea lamprey larvae have been captured in the Gironde-Dordogne River basin in France at depths >2 m (Taverny et al. 2012). Recent studies of occupancy and habitat use by Pacific lamprey and *Lampetra* spp. in deepwater areas of the mainstem Willamette and Columbia rivers of the Pacific Northwest, found larval lampreys were widespread in a variety of habitats in depths up to 16 m (Silver et al. 2008; Jolley et al. 2012, 2013). Larvae were of a variety of sizes, suggesting multiple age classes and the ability of ammocoetes to disperse considerable distances. Other anecdotal observations exist regarding larval Pacific lamprey occurrence in large river mainstem habitats, mainly at hydropower facilities or in downstream juvenile bypass reaches (Moursund et al. 2003; Columbia River Inter-Tribal Fish Commission 2008), impinged on juvenile bypass screens, or through observation during dewatering events (Hammond 1979; Moursund et al. 2003; Dauble et al. 2006; Columbia River Inter-Tribal Fish Commission 2008). Occurrences of larvae at hydropower facilities are generally thought to be associated with their downstream movement (see Sect. 3.5). References to other species occurring in deepwater or lacustrine habitats are scarce but examples

may include the silver lamprey *Ichthyomyzon unicuspis* (Cochran and Lyons 2004) and Miller Lake lamprey *Entosphenus minimus* (Lorion et al. 2000).

As the collecting effectiveness of backpack electrofishing gear significantly declines as water depth increases (Steeves et al. 2003), estimates of larval populations in deepwater and lentic areas have been made using deepwater electroshockers equipped with a pump to move emerging larvae to the surface for collection (Bergstedt and Genovese 1994). For lampreys of conservation concern, a deepwater electroshocker was developed by Mueller et al. (2012) to reduce handling of larvae while determining their presence-absence with an optical camera in habitats up to 8 m deep. A remote seabed classification device has been used to identify potential larval sea lamprey habitat in a deepwater lentic area of Batchawana Bay, Ontario, which was then sampled with a deepwater electrofisher (Fodale et al. 2003). The authors found that, in this lentic environment, the presence of larvae was significantly related to substrate type, distance from the stream mouth, and slope of the lake bottom (Fodale et al. 2003). Ammocoetes were not found at depths greater than 15 m, however, and this absence was not explained by either substrate particle size distribution or thermal acclimation (Lee and Weise 1989). Lee and Weise (1989) therefore suggested that gross lentic habitat selection revolves around the nearshore distribution of food particles and the interdiction of the thermocline. Preliminary data collected on Great Lakes larval sea lamprey inhabiting three lentic areas indicate that growth and transformation rates of larvae in lentic areas are comparable to stream resident larvae (Nicholas S. Johnson, U.S. Geological Survey, Hammond Bay Biological Station, Millersburg, MI, personal communication, 2014). Given the potential importance of deepwater and lacustrine larval rearing habitat in some systems, the dredging of large rivers and river mouths for navigation may represent a significant but underappreciated loss of larval lamprey habitat for species of conservation concern (see Chap. 8) whereas in the invasive sea lamprey, these areas may contribute substantially to the production of parasitic juveniles (Fodale et al. 2003). The relative importance of these deepwater habitats should be studied further.

3.2.2.3 Riparian Canopy

The importance of riparian canopy as an indicator of larval lamprey abundance may also vary depending on the spatial scale examined (Almeida and Quintella 2002; Torgersen and Close 2004). At smaller spatial scales, larval abundance appears to increase with the presence of riparian cover. Removal of riparian vegetation is thought to have contributed to the declines observed in some lamprey species (e.g., northern brook lamprey; Fortin et al. 2007). This may be due to a loss of shade, since evidence suggests that ammocoetes are photophobic (Potter and Rogers 1972). Although Malmqvist (1980) did not find a significant relationship between amount of shade and abundance of European brook lamprey, Potter et al. (1986) found significant relationships between the density of larval pouched lamprey and the degree of low-angle shading (positive) and light intensity (negative)

in at least two of their seasonal models. Where burrowable habitat is found, often partially shaded by trees, diatoms may also form an incrustation on the interface between the silt and the water, probably contributing to the stability of larval microenvironments (Hardisty 1979). At larger spatial scales, Neeson et al. (2012a) found the amount of preferred sea lamprey habitat to be positively associated with the amount of forest in the riparian corridor. A negative correlation is expected between the amount of preferred larval substrate habitat and the sediment transport rate (Neeson et al. 2012a), and empirical studies describe the negative correlation between forested landscapes and sediment transport rates (Milliman et al. 1987). However, Torgersen and Close (2004) found that larval Pacific lamprey abundance was positively associated with an open riparian canopy. They observed exceptionally high larval density (>100 larvae/m^2) in the most exposed sites. Qualitative observations by Kan (1975) also indicated a negative association between abundance of this species and a closed riparian canopy, and suggested that this effect might be related to decreased primary productivity. Autumnal thinning of the riparian canopy was concluded to be the cause of increased primary algal productivity observed by Sutton and Bowen (1994), which coincided with a September peak in diet ash-free dry mass, and in assimilation efficiency for both ash-free dry mass and amino acids for northern brook lamprey in three oligotrophic Great Lakes streams. However, Arctic lamprey feeding on fallen leaf material had greater increases in mass than those on a diet of algae (Shirakawa et al. 2009), and the relative importance of such nutrient input from the canopy is not known.

3.2.2.4 Water Chemistry

Relatively few studies have found that water chemistry (e.g., conductivity, pH) is important in limiting larval lamprey distribution (Hardisty and Potter 1971a) although, as pointed out by these authors, this does not imply that such factors are not important, but only that they likely do not reach limiting values within the type of habitats where ammocoetes generally occur (i.e., where other factors are favorable). More recently, Goodwin et al. (2008) found that European river lamprey and European brook lamprey ammocoete abundance at a regional scale (i.e., across Northern Ireland), was associated with pH (Goodwin et al. 2008); sites with pH > 8.16 yielded more ammocoetes (9.5 per site) compared to those with lower pH (2.6 ammocoetes per site). These authors caution, however, that this relationship may be partly the result of differences in climate, bedrock type, land use, and watershed capacity, which in turn may influence pH. Nevertheless, water chemistry has been observed to affect larval growth. Young et al. (1990a) found that streams with higher conductivity (i.e., hardwater streams) were associated with larger sea lamprey larvae at age 2+ and found that conductivity was correlated with total phosphorus and alkalinity. Hardwater streams generally have greater productivity than softwater streams, and presumably provide a greater amount of food to larval lampreys (see Sect. 3.3). Interestingly, water chemistry differences among streams or sets of streams (i.e., in the elemental composition of the water) may provide stream- or watershed-specific

statolith signatures that allow stream of origin to be determined in Great Lakes sea or other lampreys (see Sect. 3.2.4).

3.2.2.5 Thermal Requirements

On a global scale, temperature is the environmental variable that best explains lamprey distribution (Ferreira et al. 2013). Lampreys are generally found north and south of the 20° isotherm, with average lethal temperatures around 28 °C (Potter 1980a). In their study evaluating the influence of 11 macrohabitat variables on the distribution of European river and brook lampreys in Portugal, Ferreira et al. (2013) found that lampreys were found in areas where the average maximum temperature of the warmest month did not exceed 30 °C. Morman et al. (1980) reported that high water temperatures may be responsible for the absence of lamprey ammocoetes from the lower sections of Lake Ontario streams during the late summer.

Temperature is not usually identified as an important factor affecting lamprey distribution at small spatial scales, but likely also has some localized effects. Where sea lamprey larvae are present in Great Lakes tributaries characterized by high summer temperatures, they are often limited to areas of groundwater inflow (Morman et al. 1980). Conversely, ammocoetes in cooler streams have been noted to avoid habitats influenced by cold springs and seeps (Applegate 1950).

These findings correspond with experimental studies on the temperature preferenda of lampreys. In a laboratory experiment, the preferred thermal niche of sea lamprey larvae was 20.8 °C, with maximum scope of activity occurring at approximately 19 °C (Holmes and Lin 1994). Rodríguez-Muñoz et al. (2003) assessed the influence of thermal regime on the development, survival rates, and early growth of embryos of sea lamprey incubated at five constant temperatures (7, 11, 15, 19 and 23 °C). Survival from fertilization to hatching was 61, 89, 91 and 89% at 11, 15, 19 and 23 °C, decreasing to 58, 70 and 70% from hatching to burrowing at 15, 19 and 23 °C, respectively. The authors also observed body length at the burrowing stage was longest for embryos incubated at 19 °C, but body mass increased in the interval 15–23 °C. Meeuwig et al. (2005) assessed the influence of thermal regime on development and survival rates of Pacific lamprey and western brook lamprey incubated at four temperatures (10, 14, 18 and 22 °C). Survival was greatest at 10, 14, and 18 °C, while survival at 22 °C was significantly lower than at other temperatures. An increased incidence of developmental abnormalities was also observed at 22 °C. The incipient lethal temperature of pouched lamprey is 28.3 °C, which almost certainly accounts for the restriction of this species to the southernmost rivers in Western Australia (Macey and Potter 1978).

3.2.2.6 Oxygen Requirements

Ammocoetes are sensitive to low oxygen tensions and unable to survive in very low concentrations (Potter et al. 1970). However, the rate of oxygen consumption,

and, thus the oxygen requirements of ammocoetes of the mountain brook lamprey *Ichthyomyzon greeleyi* is lower than the values given by Winberg (1956) for several teleost fishes of similar weight (Hill and Potter 1970). Ammocoetes can tolerate, for at least 4 days, oxygen tensions as low as 7–10 mmHg at 5 °C, 12–16 mmHg at 15.5 °C, and 13–21 mmHg at 22.5 °C (Potter et al. 1970). The very low oxygen consumption of ammocoetes may well be a major factor in enabling the animals to colonize the silt banks in slow-flowing areas where oxygen tensions must often be low (Hill and Potter 1970). In contrast, metamorphosing lampreys are generally found in water with higher dissolved oxygen content (Richards and Beamish 1981; see Sect. 3.2.3), consistent with their higher rate of oxygen consumption (see Chap. 4). Interestingly, oxygen levels in the streambed have been found to be significantly increased by the burrowing and feeding activities of ammocoetes (Shirakawa et al. 2013).

3.2.2.7 Proximity to Spawning Habitat

As demonstrated above, larval abundance is directly linked to environmental variables, but the spatial context of biological factors, such as the spawning distribution of adults, also plays an important role in larval distribution (Torgersen and Close 2004). The distribution of sea lamprey ammocoetes along the river is strongly associated with the spawning areas, with larval density being inversely related to the distance downstream from the spawning areas (Morman et al. 1980; Almeida and Quintella 2002; Quintella et al. 2003). Dawson and Jones (2009) studied four Great Lakes streams, and found that streams with higher sea lamprey survival-to-age-1 had distributions of spawning and larval habitats that were most favorable to ammocoete production (i.e., the largest amount of spawning habitat in the upper reaches and the largest amount of preferred larval habitat in the lower reaches). Distribution of ammocoetes of migratory species (e.g., European river lamprey and sea lamprey) is also related to adult access to spawning habitats from their feeding grounds, with distance from a large water body (e.g., sea, estuary, or lake) and presence of potential migration barriers influencing ammocoete abundance (Goodwin et al. 2008; Ferreira et al. 2013).

Furthermore, given that adult lampreys during the migratory season increase their upstream movements and are attracted to a pheromone cue released by larvae in the system (Yun et al. 2011; Meckley et al. 2012; see Chap. 5), Neeson et al. (2011, 2012b) suggest that there will be feedback loops between the number and distribution of spawners and the number and distribution of ammocoetes. These feedback loops will be influenced by the river's network structure (i.e., its branching pattern), since the downstream propagation of pheromone will be affected by the river's discharge and the number and size of confluent tributaries. In addition, each larval cohort will in turn contribute to the river's "pheromonal landscape," thus creating interannual feedback between adult migration and larval habitation. In streams where trapping has proven effective, catch rates of adults are often low or variable in years following removal of the larval population with lampricide treatments in

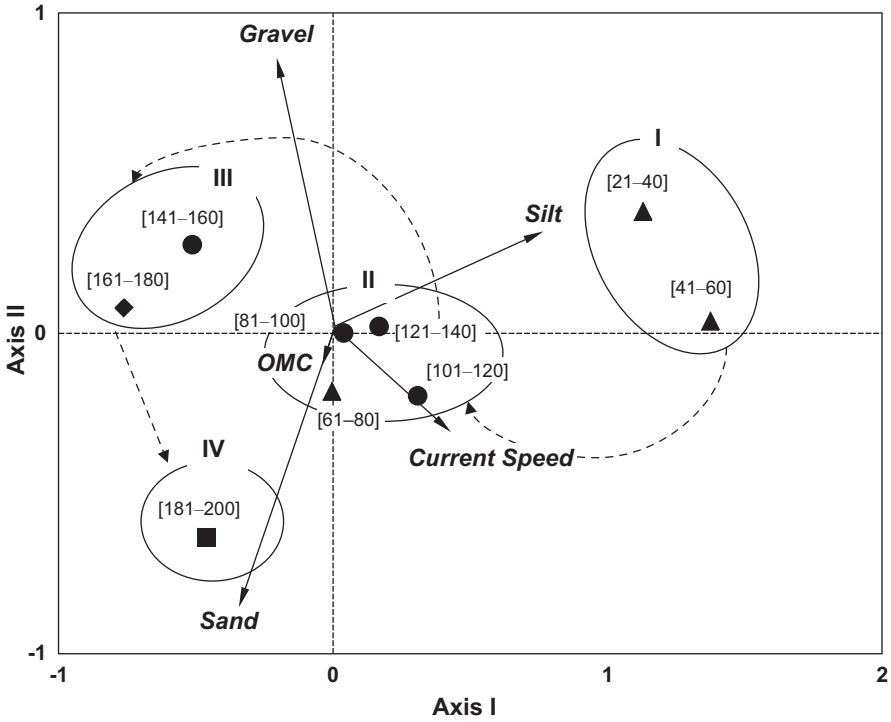

Fig. 3.1 Sea lamprey ammocoete length-class distribution according to sediment particle size, organic matter content (OMC) and velocity (current speed). Detrended Canonical Correspondence Analysis ordination diagram, with symbols corresponding to the nine length classes (from 20–40 mm to 180–200 mm), and *arrows* representing the environmental variables. The *roman numerals* identify the groups with similar preferences. The *dash arrows* represent the colonization sequence of the different sediment type. Age classes are also represented (▲, 1st year class; ●, 2nd year class; ♦, 3rd year class; ■, 3rd and 4th year class). (This figure was originally published in Almeida and Quintella (2002) and reproduced with permission of John Wiley & Sons, Inc.)

the Great Lakes basin (Moore and Schleen 1980). Thus, there is evidence that lampricide treatments in the Great Lakes basin alter the feedback between adults and larvae and affects subsequent patterns of larval habitation, regardless of the small- and large-scale environmental factors discussed in previous sections.

3.2.3 Habitat Preferences Related to Larval Size and Metamorphosis

The relative importance of habitat variables can change with ammocoete size (Young et al. 1990a; Almeida and Quintella 2002; Sugiyama and Goto 2002). Several studies, for example, have demonstrated that larger larvae appear to show preference toward larger particle sizes. In the anadromous sea lamprey, smaller

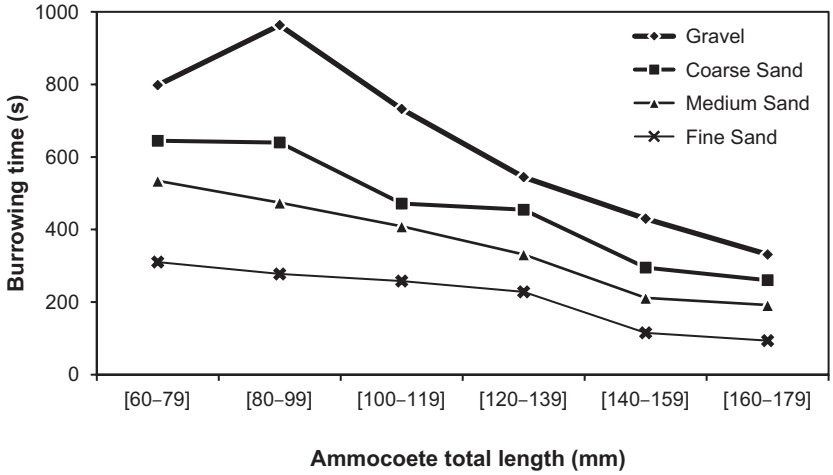

Fig. 3.2 Burrowing performances of larval sea lamprey according to their length class and substrate grain size (Gravel: 2.0–0.39 mm; Coarse Sand: 1.0–1.99 mm; Medium Sand: 0.5–0.99 mm; Fine Sand: 0.25–0.49 mm). (Redrawn from data of Quintella et al. (2007))

ammocoetes (20–60 mm total length) preferred silty-sand substrates (i.e., sediments with a comparatively higher percentage of sand), but also with a relatively large portion of silt (Almeida and Quintella 2002; Fig. 3.1). Medium-size ammocoetes (60–140 mm) were mainly found in gravel-silty-sand substrate, where gravel and silt seem to have an identical contribution to the composition of this more heterogeneous sediment. Larger ammocoetes (140–200 mm) preferred coarse-grained sediments (gravelly-sand and sand; Almeida and Quintella 2002). In the landlocked sea lamprey, Sullivan (2003) likewise showed that as larvae grow, their preference shifts toward larger particle sizes, although even large larvae (>120 mm) are rarely found in coarse substrates such as gravel, cobble, or rubble (Jones 2007). Lampreys going through the process of metamorphosis have also been observed in coarser substrates (see below).

Differences in habitat preference with body size may be related to burrowing abilities. In a laboratory experiment, smaller sea lamprey ammocoetes had lower burrowing performance than larger individuals across all substrate types tested (i.e., gravel, coarse sand, medium sand, and fine sand), but the differences were greater in coarser substrates (Quintella et al. 2007; Fig. 3.2). Additionally, coarser sediment particles increased the time spent on burrowing regardless of larval size, which is likely related to fatigue (Quintella et al. 2007). These authors suggested that smaller ammocoetes are usually associated with fine-grained sediments, because these softer sediments allow younger larvae, with a reduced swimming capacity, to propel the head and branchial region below the surface (Quintella et al. 2007). Larger ammocoetes, on the contrary, may colonize a wider range of sediment types because their burrowing capacities are considerably higher (Quintella et al. 2007). Since the selection of the burrowing sediment is a size-dependent char-

acteristic, the differences observed in the preferences for distinct sediment types within the same age group probably resulted from a reorganization of the ammocoete distribution pattern at the end of each annual growing season. This behavior could be a strategy developed by this species to avoid high densities in the areas colonized by younger individuals, and therefore reduce intraspecific competition for space and food (Almeida and Quintella 2002).

A similar preference by smaller larvae for smaller substrate particle sizes (and a narrower range of preferred particle size) has been identified in other lamprey species. Small (≤50 mm total length) Far Eastern brook lamprey larvae preferentially selected substrate in the 0.125–1 mm diameter range, whereas larger larvae (>50 mm) selected all three substrate size classes (<0.125 mm, 0.125–1 mm, and 1–2 mm diameter) equally (Sugiyama and Goto 2002). In the least brook lamprey, small ammocoetes (<50 mm) had a stronger preference (54.7%) for fine sand compared to large ammocoetes (100–150 mm; 49.7%), and a moderate number of large ammocoetes (17.7%) also selected coarse sand habitat (Smith et al. 2011; see Sect. 3.2.1.1). However, not all studies are in consensus regarding different habitat preferences of ammocoetes of different length classes. Optimal particle size and abundance for American brook lamprey did not change with larval length (Beamish and Lowartz 1996), and Goodwin et al. (2008) similarly found no relationship between particle size and ammocoete length in the Ballinderry River in Northern Ireland.

In the study by Sugiyama and Goto (2002), large Far Eastern brook lamprey larvae also showed a greater preference for deeper substrate and occupied a wider range of water depths (0–38 cm) than the small larvae (0–24 cm). In an extensively sampled river in northern Wisconsin, no difference was detected in density of larger sea lamprey larvae (>51 mm TL) between deep (>0.8 m) and shallow (<0.8 m) water; however, small larval sea lamprey (<51 mm TL) showed a greater preference for shallower water (Treble unpublished data). Earlier studies also suggested that larger sea lamprey ammocoetes are found more commonly in deep water (Wagner and Stauffer 1962; Manion and McLain 1971; Manion and Smith 1978). These results suggest that larvae change habitats as they grow, and size segregation among different habitats has also been found in pouched lamprey ammocoetes in New Zealand streams (Kelso and Todd 1993), and with sea lamprey in Portuguese rivers (Almeida and Quintella 2002).

Spatial segregation may be even more pronounced between larvae and transformers. It has been observed in several different species that as larvae approach metamorphosis, there is a tendency for some to move to coarser substrates (Potter 1970). Richards and Beamish (1981), for example, reported that metamorphosing Pacific lamprey typically occurred in coarser substrate with better oxygenated water than did ammocoetes, and Kelso and Todd (1993) similarly found metamorphosing pouched lamprey in downstream reaches where substrate was coarser and flows were higher. Potter and Brown (1975) suggested that European river lamprey move into faster-flowing areas with more oxygenated sediments sometime during metamorphosis, whereas the non-migratory non-parasitic European brook lamprey may remain in silted areas typical of the larvae until just prior

to spawning. Differences in the ecology of these two species are likely related to differences in their oxygen requirements during and after metamorphosis (see Sect. 3.2.2.6; see Chap. 4). Other studies likewise report transformers in nursery habitat alongside ammocoetes of varying year classes (Potter et al. 1980; Quintella et al. 2003). Not all brook lampreys, however, remain in the ammocoete beds during metamorphosis. Although Beamish and Medland (1988a) reported that "it was not unusual" to find adult and ammocoete mountain brook lamprey in the same areas, as metamorphosis progressed, this species tended to shift to coarser substrates and higher water flows.

Differences in whether or not ammocoetes and transformers are spatially segregated may be related to other features of the habitat. Streams with a high gradient, for example, may show more downstream movement of larvae, especially during periods of flooding, so that age and size of larvae increases with distance from the spawning grounds. Streams with a lower profile may show less movement and less sorting by age and size (Hardisty 1986). Kelso and Todd (1993) suggested that downstream movement of pouched lamprey with age is significant in New Zealand streams since stream gradients are high and flooding is frequent.

During the initial stages of metamorphosis, transformers are more sedentary in comparison to ammocoetes (Quintella et al. 2005). Later on, juveniles burrow less frequently and are found hiding between pebbles, under aquatic vegetation, rocks and other structures (Almeida unpublished data).

3.2.4 Macroenvironmental Statolith Signature

The elemental composition of the habitat in which lampreys are found is reflected in their statoliths. Statoliths are structures in lampreys that are analogous to otoliths in teleost fishes, and the analysis of statolith microchemistry could possibly be used as a means of providing gross population structure of sea lamprey in the Great Lakes (Brothers and Thresher 2004). Previous Great Lakes otolith microchemistry studies have successfully discriminated among local spawning locations in yellow perch *Perca flavescens* (Brazner et al. 2004; Ludsin et al. 2006). The elemental composition of larval sea lamprey statoliths was found to reflect the ambient elemental concentrations of the river systems from which the lampreys originated (Brothers and Thresher 2004). These authors indicated that strontium (Sr) and rubidium (Rb) differences alone were sufficient to correctly assign most larval sea lamprey to their natal rivers, and almost perfectly distinguished between specimens from the St. Marys River (connecting lakes Superior and Huron) and those from the drainages of Michigan's Lower Peninsula, with Sr and Rb differences likely due to the regional differences in the geochemistry of the Canadian Shield and the Michigan basin. Hand et al. (2008) quantified elemental concentration in larval sea lamprey statoliths, and were able to discriminate among larvae from 13 streams located in lakes Michigan, Huron, and Superior with 82 % classification accuracy. However, several streams carried a common signature, and the

ability to discriminate among lakes (when all streams within a lake were grouped into a single category) was only about 60 %. This demonstrates that stream-specific signatures differ within each of these systems, and that local geology, watershed runoff, and pollutant sources may overwhelm regional, basin-wide geology (Hand et al. 2008).

Statolith elemental signatures within river systems are relatively stable over time and are affected only slightly by year-class variability (Brothers and Thresher 2004). However, it appears that statolith elemental signatures may not be stable during the course of the lamprey life cycle. Recent research has found that in newly metamorphosed sea lamprey, the portion of the statolith deposited during the larval stage was enriched in rubidium (Rb), which suggests a chemical reworking of statoliths during metamorphosis (Lochet et al. 2013). Since discriminating among sea lamprey from different streams mostly relies on premetamorphic levels of Rb, strategies for the use of statoliths to identify the natal origin of juvenile and adult sea lamprey should take into account the chemical changes associated with metamorphosis (Lochet et al. 2013).

3.3 Feeding

Ammocoetes feed by trapping small, water-borne particles in mucus within the pharynx (Mallatt 1981), and larval habitats provide a regular supply of the suspended organic matter upon which larval lampreys feed (Sutton and Bowen 1994; Yap and Bowen 2003). Allowing for the effect of other environmental variables, an increase in the amount of organic material often—but not always—corresponds to an increase in larval density (see Sect. 3.2.1.3). Habitat type selected by larval northern brook lamprey had relatively minor consequences for organic matter and amino acids in the diet, but had major consequences for assimilation efficiency for both nutrients, with highest efficiencies occurring in depositional areas where larvae aggregated at low densities (Yap and Bowen 2003). Whereas most suspension feeders meet their food requirement by moving dilute suspensions rapidly across their feeding structures, ammocoetes meet nutrient needs by slowly processing concentrated suspensions (Mallatt 1983). A slow rate of water flow through the pharynx, necessitated by the high resistance of the substrate inhabited and the design of the pharyngeal pump, confines ammocoetes to environments where food suspensions are concentrated (Mallatt 1983). Since the burrowing habit that limits flow rate is necessary for protecting lampreys from predation during the larval stage (Morman et al. 1980), the requirement for concentrated suspensions seems basic to the animal's biology (Mallatt 1983).

The larval lamprey diet consists of a mixture of algae (primarily diatoms), organic detritus, and bacteria (Sutton and Bowen 1994), with the detrital fraction serving as the primary food source (Moore and Beamish 1973; Moore and Potter 1976a, b; Sutton and Bowen 1994; Mundahl et al. 2005). Sutton and Bowen (2009) found that diets of larval northern brook lamprey were dominated by detritus, which ranged from 94 to 99 % of the organic fraction of the gut contents. The remainder

of the contents in the digestive tract was composed of algae and bacteria, which in sum contributed less than 6% of the total diet ash-free dry mass (Sutton and Bowen 2009). Similar observations of organic detritus-dominated diets have been reported for sea lamprey and American brook lamprey during the summer months (Sutton and Bowen 1994; Mundahl et al. 2005). Specific physiological attributes, such as efficient digestion and assimilation and a low metabolic rate, have been identified as adaptations that allow larval lampreys to effectively use this food source (Moore and Beamish 1973; Moore and Mallatt 1980; Sutton and Bowen 1994; Yap and Bowen 2003; Mundahl et al. 2005). Shirakawa et al. (2009) experimentally found positive growth of sub-yearling Arctic lamprey ammocoetes given a diet of fallen leaves and negative growth of those given algae, although comparison to wild ammocoetes suggested a varied diet that may contain leaves and algae. In contrast, another experimental study indicated that larval Pacific lamprey grew more when given diets of algae or salmon carcass analogs and had negative growth when given diets of leaves (Jolley unpublished data). In this latter study, the presence of the unique stable isotope signatures in each of the food items confirmed consumption. Bacteria and organic substances dissolved in water are also likely significant as a food source for larval lampreys (Moore and Potter 1976b). Analysis of sea lamprey larvae gut contents found that microalgae belonging to the class Bacillariophyceae were the major constituents of the algal portion of the diet of ammocoetes (Quintella 2000). Among the diatoms found, the genera *Melosira* and *Navicula* were the two most important algal food items, occurring in more than 95% of the observed gut contents, and corresponding to 86% of the total identified microalgae. The genera *Cyclotella*, *Cymbella*, *Nytzschia*, *Cocconeis*, *Bacillaria*, *Synedra* and *Rhizosolenia* were also classified as preferred algal food items (Fig. 3.3). During the spring and summer periods, as expected, the diversity of microalgae present in the analyzed gut contents was much higher than during autumn and winter (Fig. 3.3). The diversity of algal food items was low throughout the year mainly due to the almost absolute dominance of the genera *Melosira* and *Navicula.*

In British populations of European brook lamprey, Moore and Potter (1976b) found that feeding rate was most intense in early spring (as water temperatures rose from 5 to 12 °C), which was about two months before the spring algal bloom. Rates remained relatively constant throughout the summer, and declined in October, although temperatures (10 °C) were still similar to those observed in spring. Maximal rates of feeding in the spring and summer are, not surprisingly, consistent with high summer growth rates (see Sect. 3.7.2).

No consistent pattern of change in the size of ingested material with length of larvae has been observed. Composition of gut contents among larval individuals of landlocked sea lamprey, anadromous sea lamprey, and American brook lamprey for a given season and river did not change greatly, irrespective of body length (Moore and Beamish 1973). Further, the authors found no consistent pattern of change in size of ingested algae with length of larvae. However, by the end of the larval stage, lampreys must have accumulated sufficiently large lipid reserves to act as an energy source during the subsequent long non-trophic period of metamorphosis (Moore and Potter 1976b), as no lamprey species has been found to feed during the metamorphic period (Hardisty and Potter 1971a; see Chap. 4).

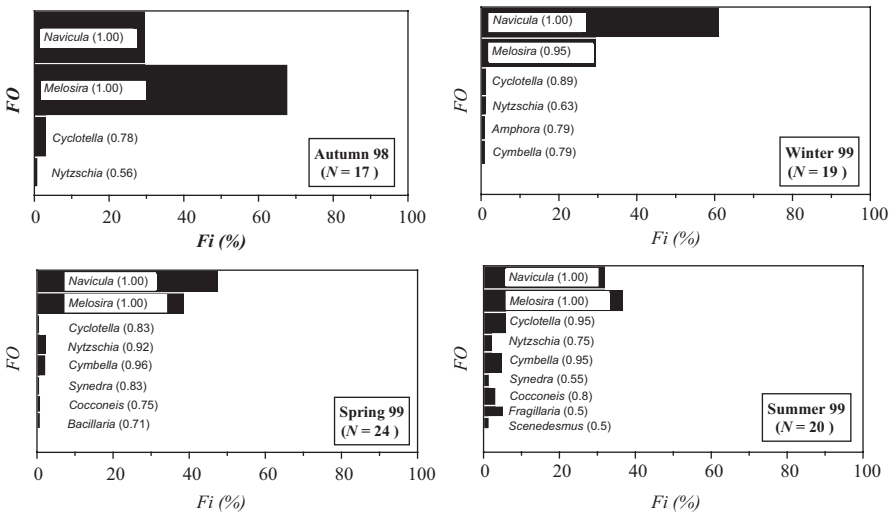

Fig. 3.3 Seasonal evaluation of the microalgal food items found in the diet of sea lamprey larvae in the River Mondego, Portugal, showing numerical frequency (F_i) and frequency of occurrence (*FO*) of the preferential food organisms. In each of the graphics, the width of the bars is proportional to the *FO* indicated between brackets. (Figure adapted from Quintella 2000)

3.4 Lamprey Demographics

3.4.1 Density

As with the effect of various environmental factors on larval distribution and abundance, larval density will depend on the spatial scale at which it is measured. Malmqvist (1983) reviewed several previous studies, and reported that maximum densities in optimal habitats (i.e., on a fine scale) can number hundreds to thousands of individuals per m^2 (e.g., up to 126, 113, and 2,000 larvae/m^2 in northern brook, European brook, and European river lampreys, respectively; Churchill 1945; Malmqvist 1980; Tuunainen et al. 1980). Nursall and Buchwald (1972) reported as many as 284 Arctic lamprey burrows per m^2, and more recent studies in other species also show high maximum densities (e.g., as many as 104 pouched lamprey larvae and more than 100 Pacific lamprey larvae/m^2; Kelso and Todd 1993; Torgersen and Close 2004). Presumably, there is a negative relationship between larval size and density; the 40–2,000 European river lamprey reported per square meter were for young-of-the-year larvae (8–36 mm) in spawning areas (Tuunainen et al. 1980).

Mean densities over larger areas of suitable habitat will be much lower. The earlier studies reviewed by Malmqvist (1983) show average densities to range from < 1 to about 20 larvae/m^2 (e.g., Hansen and Hayne 1962; Kainua and Valtonen 1980; Malmqvist 1980), and more recent studies show similar ranges over this

scale. Beamish and Youson (1987) estimated larval North American river lamprey densities in the Fraser River, British Columbia, to range from 2.8 to 64.3/m^2, with an average of 26–28 larvae/m^2. Mundahl et al. (2006) examined densities and age structures of American brook lamprey larvae in several streams in southeastern Minnesota, where they found that mean densities of lamprey larvae varied from 0.33 to 5.78 larvae/m^2 in the best habitats available. During a survey to characterize microhabitat preferences of European brook and river lampreys in two Portuguese watersheds, Almeida et al. (2011) found a mean (± standard deviation, SD) density of 3.97 ± 4.41 larvae/m^2 ranging from 0.44 to 24.50 larvae/m^2. In a study of nine river basins in the Galicia region of northwest Spain during the summer period between 2007 and 2009, the density of sea lamprey ammocoetes captured with electrofishing was on average 4.9 larvae/m^2, with a mean value per river basin ranging from 0.7 to 13.4 larvae/m^2 (Gradín 2010). In Northern Ireland, Goodwin et al. (2008) found the density of European river and brook lamprey ammocoetes to range from 0.1 to 2.4/m^2 (average 0.79/m^2). In the Gironde-Dordogne River basin in France, Taverny et al. (2012) recovered as many as 9 and 19 sea and *Lampetra* larvae per square meter (averages 3.4 and 7 larvae/m^2, respectively).

In the Laurentian Great Lakes, the Great Lakes Fishery Commission and its agents have successfully reduced sea lamprey populations by approximately 90 % from peak levels (Marsden and Siefkes in press). A moderate to high density of larval sea lamprey in the Great Lakes is now considered to be > 5/m^2 (Steeves et al. 2003). Slade et al. (2003) estimated mean larval catch in 15-m^2 plots of Type I habitat from 214 infested stream reaches in 1996–1998 and calculated mean density to be 1.16 larvae/m^2. In 51 stream reaches in the Lake Superior and Michigan basins, mean density in Type I habitat ranged from 0.01 to 8.45 larvae/m^2 (Lake Superior) and 0.04–10.4 larvae/m^2 (Lake Michigan). Using data from 26 Great Lakes tributaries surveyed between 1991 and 1995, Slade et al. (2003) estimated that sea lamprey density in Type II habitat was approximately 10 % of that in Type I habitats. However, these authors suggested that more recent data estimated density in Type II habitat to be 27.5 % that of Type I habitat; this latter value is similar to the relative densities observed by Zerrenner and Marsden (2005) in Type I and II habitats. In Lewis Creek, Vermont, in the Lake Champlain basin (which has been subject to lampricide treatments since 1990), Zerrenner and Marsden (2005) estimated mean sea lamprey densities in 1999 and 2000 to be 5.02–6.96/m^2 and 1.93–3.30/m^2 in Type I and Type II habitats, respectively. These numbers included larvae and transformers, with transformers making up 2.4–5.8 % of the total number.

Based on the above studies, it is becoming clear that natural densities of lampreys are often much lower than those used in laboratory and in-stream studies, which have shown negative effects of larval density on growth rates and survival (see Sects. 3.6.1 and 3.7.2). Many of these studies use experimental densities that are more in line with maximum observed densities and even the “low-density” treatments are often high relative to naturally observed densities.

3.4.2 Abundance

Over moderate to large scales, the abundance of spawning-run lampreys is more readily measured than that of the stream-resident stages, but counts of downstream-migrating juveniles are now being made as well (e.g., as they pass through dams, salmon smolt traps, or other counting structures; Fish Passage Center 2013) and abundance estimates for even the cryptic larval and metamorphosing stages are also emerging.

The earliest known attempt to estimate total larval abundance within an entire stream system was made by Hansen and Hayne (1962). In the Ogontz River, a small tributary to Lake Michigan, they estimated the total larval lamprey population in a 15-km reach at 136,800. In Ogontz Bay, an estimated 30,100 sea lamprey and 2,900 American brook lamprey larvae were present. These estimates were made in 1959 and 1958, respectively, and Hansen and Hayne (1962) felt that these numbers were not substantially affected by an electromechanical barrier first operated in 1958. For more recent estimates, we used the data provided in Table 3 of Slade et al. (2003) to calculate the total abundance of sea lamprey larvae (age 1+) in 24 and 27 stream reaches in the Lake Superior and Lake Michigan basins, respectively (but note that infested length in this table should be in m, not km; Jeffrey W. Slade, U.S. Fish and Wildlife Service, Ludington, MI, personal communication, 2014). In the Lake Superior basin, total estimated abundance ranged from 136 in a 7.6-km infested reach of the Black Sturgeon River to 311,032 in a 66-km reach of the Goulais River. In the Lake Michigan basin, estimated total abundance ranged from 18 in Arthur Bay (where the infested reach was only 0.16 km long) to almost 1.4 million in a 14.6-km infested reach in the Platte River. Total abundance from these 24 Lake Superior and 27 Lake Michigan stream reaches totaled almost 800,000 and over 2.2 million larvae, respectively. Total larval abundance in a 9.4-km infested reach of Lewis Creek, Vermont, was estimated at 30,089–116,762 between 1990 and 2000 (Zerrenner and Marsden 2005). The streams in these recent estimates, of course, have been subject to periodic lampricide treatments. In a portion of the Black Sturgeon River currently not exposed to lampricide application (i.e., above a barrier), Sea Lamprey Control Centre personnel in 2006 estimated that the total abundance of *Ichthyomyzon* ammocoetes (presumably northern brook lamprey since newly metamorphosed individuals of this species were also captured during sampling) to be more than 14.7 million (Mike Steeves, Fisheries and Oceans Canada, Sea Lamprey Control Centre, Sault Ste. Marie, ON, personal communication, 2014).

Abundance estimates have also been made for metamorphosing lampreys. In the case of the Great Lakes sea lamprey, sea lamprey control efforts aim to kill the larvae before they metamorphose; control agents, therefore, attempt to predict in advance (rather than measure after the fact) the production of transformers in Great Lakes tributaries (Marsden and Siefkes in press). Slade et al. (2003) estimated the potential production of metamorphosed sea lamprey from 57 Lake Superior and 58 Lake Michigan tributaries at 0–82,497 (average 4,318) and 0–103,027 (average

3,566), respectively. In the Black Sturgeon River study mentioned above, it was predicted that 115,066 northern brook lamprey would metamorphose that year (Treble unpublished data).

Counts of downstream-migrating juveniles are available for some species. Beamish and Youson (1987), for example, calculated that as many as 6.5 million juvenile North American river lamprey left the Fraser River in British Columbia in 1979. Beamish and Levings (1991) estimated that, per year, 19,000–176,000 Pacific lamprey juveniles migrated out of one Fraser River tributary, the Nicola River, in 1984–1985 and 1987–1988. For more recent time periods, counts of juvenile lamprey (Pacific lamprey and/or western brook lamprey) as they pass through the mainstem hydrosystem in the Columbia River basin are recorded and are available at the Fish Passage Center (Columbia Basin Fishery Agencies and Tribes 2014).

The above studies are a valuable start to permitting a more thorough appreciation for total lamprey numbers in river systems but, particularly in species other than sea lamprey in the Great Lakes, there is still a need for standardized measurements to be made over time. Detection probability models are currently being used to determine occurrence of Pacific lamprey in the Columbia River basin, which may provide a baseline for comparison with future studies (Jolley et al. 2012; Dunham et al. 2013). In many species of conservation concern, there is evidence of population declines (mostly from the number of upstream migrants or harvest rates; see Chap. 8), but temporal trends in larval and metamorphosing stages are, in general, still poorly documented.

3.4.3 Sex Ratios

A small but variable excess of males has long been observed in spawning-phase lampreys (e.g., Dean and Sumner 1898; Young and Cole 1900; Wigley 1959; Zanandrea 1961). Skewed larval sex ratios have also been reported, and there is some evidence to suggest that sex ratios may be related to larval density. As sea lamprey numbers in the upper Great Lakes were drastically reduced following the initiation of sea lamprey control, the proportion of males correspondingly declined and a preponderance of female larvae (and adults) was soon observed (Smith 1971; Purvis 1979; Torblaa and Westman 1980). Sex ratio variations were also observed among least brook lamprey populations in Maryland, Delaware, Kentucky, Tennessee, and Alabama (which have not been subjected to lampricide treatment), and differences were related to larval density; the proportion of males increased significantly with relative density (Docker and Beamish 1994). In this study, larval sex ratio was not significantly related to water hardness, pH, annual thermal units, or latitude. It has thus been suggested that sex determination in larval lampreys is density dependent (Purvis 1979; Docker and Beamish 1994), occurring during a prolonged period (1–3 years) of sexual indeterminacy (see Docker et al. in press). Beamish (1993) also presented evidence for the existence

of environmental sex determination in the southern brook lamprey. He noted that when conditions for larval growth were favorable, increased occurrence of males was positively correlated with larval density. He found that under poor growth conditions, higher densities were associated with fewer males. Torblaa and Westman (1980) found highly variable sex ratios of larval sea lamprey present in Great Lakes streams with divergent physical and chemical characteristics, which lends credibility to the view that environmental factors may play an important role in sexual differentiation of the ammocoete. However, approximately equal proportions of male and female larvae in other species (i.e., European river and brook lampreys) led Hardisty (1960) to rule out an environmental effect on sex differentiation. In a more recent study examining sea lamprey sex ratios in the Great Lakes, Wicks et al. (1998) were likewise unable to detect an effect of larval density on sex ratio, although this may be because larval densities in Great Lakes streams now remain low due to the control program (Jones et al. 2003). In an experimental study, Docker (1992) also found no significant relationship between rearing density and sea lamprey sex ratio but, in this case, it appears that all experimental densities were high (between 57 and 470 larvae/m^2) compared to natural densities (see Sect. 3.4.1).

The possible adaptive significance of environmental sex determination in lamprey could be compensation for changes in density to favor an equilibrium population. However, only experimental studies at more natural densities can verify a causal relationship between density and sex ratio, and further experimental and field studies may shed some light on density-related differences between the sexes (Docker and Beamish 1994).

Other possible explanations for unequal sex ratios in larval lampreys (i.e., other than environmental sex determination) include sex-specific differences in mortality during the larval stage or differential rates of metamorphosis. In the least brook lamprey, however, sex ratio variations were not likely the result of differential mortality between the sexes since sex ratios varied among streams from the time of gonadal differentiation, and remained relatively constant thereafter (Docker and Beamish 1994). Furthermore, among sea lamprey larvae maintained at various densities for over 3 years, there was no evidence of sex-specific mortality (Docker 1992). There is also no evidence that lampricides applied to Great Lakes streams are selectively toxic to male lamprey (Purvis 1979). There is evidence of sex-specific differences in age at metamorphosis (i.e., that females metamorphose at an older age than males, particularly in non-parasitic species; Purvis 1970; Docker and Beamish 1994; Docker 2009; see Chap. 4) but, since the sex ratio variations observed in the least brook lamprey were established at the time of gonadal differentiation, Docker and Beamish (1994) asserted that differential recruitment to the adult stock was not responsible for the differences in larval sex ratios.

The demographic effects of skewed larval sex ratios (e.g., on overall rates of recruitment and mortality) are unknown, and will probably vary greatly between resident and anadromous species (see Sect. 3.10).

3.5 Movement

Data show that the ammocoete life stage is not entirely at the mercy of the environment. Although dispersal is largely determined by changes in current velocity or water levels, with floods and spring thaw events regarded as a major factor in their redistribution (Hardisty and Potter 1971a), the possibility that ammocoetes may actively seek out more favorable areas for colonization is supported by numerous studies. For example, multiple studies suggest high drift or movement rates soon after sea lamprey larval emergence. The initial migration from the spawning site appears to be a result of a mass emergence from the nest substrate caused by sudden changes in the larvae at the critical stage of yolk absorption (Applegate 1950). Manion and McLain (1971) suggested that age-0 sea lamprey larvae initially remained close to spawning areas and by age 1 had only moved a few hundred meters; they reported a more widespread distribution of larvae by age 2. A more recent study of this species by Derosier et al. (2007), based both on field observations and genetic data (i.e., which allowed the researchers to use distance separating full sibling larvae as a proxy of dispersal distance), does not support this previous observation. According to these authors, age-0 larvae did not cluster near the spawning nests, but dispersed widely soon after emergence, and were equally likely to be found in habitats greater than 150 m from the nest as within 50 m. Genetic data showed that age-0 siblings were found up to 0.9 km from each other within 3 months of emergence (Derosier et al. 2007). Age-1 larvae showed even greater dispersal after a single year; larvae were frequently found greater than 1 km downstream (Derosier et al. 2007). Kelso and Todd (1993), based on the downstream distribution of age-0 pouched lamprey larvae, likewise suggested a more rapid downstream colonization in this species. In addition, age-0 Pacific and western brook lampreys (< 30 mm TL) were locally abundant in tributary mouth depositional areas of the Columbia River and likely originated some distance upstream (Jolley unpublished data).

Various biotic and abiotic factors have been found to affect the movement of larval sea lamprey. Larval movement was observed to occur significantly more often at higher densities and significantly less often at temperatures below 18.5 °C in the laboratory (Derosier et al. 2007). Relatively high movement rates were observed during warmer temperatures, with 20–30 % of larvae emerging from the sediment and drifting during a single night (Derosier et al. 2007). Derosier et al. (2007) hypothesized that the risk of movement for sea lamprey larvae into less suitable habitats in the fall (when temperatures below 18.5 °C are more likely to occur) outweighs the benefits of seeking habitats with lower densities.

Although earlier authors have suggested that larval movement is largely passive (e.g., Applegate 1950), Potter (1980a) countered this argument, citing the tagging studies of Smith and McLain (1962) and Manion (1969) that showed that sea lamprey larvae may occasionally move a short distance upstream. More recently, Quintella et al. (2005), monitoring sea lamprey ammocoetes marked with passive integrated transponders (PIT) tags, similarly observed a considerable proportion of

short movements in an upstream direction. As expected, however, observations during laboratory experiments revealed that ammocoetes spent most of the time motionless and, when active, downstream movements were more frequent and longer compared to upstream movements (Quintella et al. 2005).

Furthermore, although ammocoetes spend most of their time burrowed, they are adept at performing and recovering from vigorous anaerobic exercise. Such attributes could be important when these animals are vigorously swimming or burrowing as they evade predators or forage (Wilkie et al. 2001). Laboratory experiments conducted by Sutphin and Hueth (2010) to measure the swimming performance of Pacific lamprey ammocoetes suggest relatively high swimming capacities of larval lampreys. According to these authors, ammocoetes are able to swim considerable periods of time at a prolonged-sustained level, ranging from 43.0 min when exposed to a low velocity of 0.1 m/s, to 0.4 min when exposed to 0.5 m/s water velocity. The burst swimming speeds of larval Pacific lamprey tended to increase with size of the individuals tested, ranging from 0.3 to 0.8 m/s (Sutphin and Hueth 2010).

As reviewed by Potter (1980a), the downstream movement of larval lampreys occurs mainly at night; this is evident in many species. Abundant nighttime catches of larval Pacific lamprey in drift nets were noted in the Deschutes River in the Columbia River basin (Gadomski and Barfoot 1998), and White and Harvey (2003) found larval drift in this species to occur almost exclusively at night. For European river lamprey larvae in the main channel of the Derwent River in England, nighttime catches were eight times higher than daytime catches; for transformers, nighttime catches were 24 times higher (Bracken and Lucas 2013). Ammocoetes are, in general, more active at night (Almeida et al. 2005), and nocturnal downstream movement likely makes them less vulnerable to predation (Naesje et al. 1986; Harvey 1991). More recent studies also support previous observations that downstream movement is highly seasonal. For example, peak catches of European river lamprey larvae and transformers in the Derwent River occurred in mid-winter and December–April, respectively, and may be coupled with higher winter flows (Bracken and Lucas 2013; see Sect. 3.9.1). White and Harvey (2003) found larger Pacific lamprey ammocoetes to drift almost exclusively during higher flows in spring, and smaller (presumably newly hatched) larvae drifted during the summer.

In several different species, studies have revealed that as larvae approach metamorphosis, they have a tendency to move further downstream into coarser substrates where the water velocity and dissolved oxygen content is higher (see Sect. 3.2.3). In Arctic lamprey, larger (presumably older) individuals have been found in the downstream reaches, closer to river mouths (Heard 1966; Nursall and Buchwald 1972; Bradford et al. 2008). Likewise, as sea lamprey larvae increase in size, movement towards the mouth of streams and lentic areas has been noted (Quintella et al. 2003; Jones 2007), although the rate of movement and the habitats occupied during this migration are unknown (Jones 2007). Kelso and Todd (1993) found that the size of pouched lamprey larvae in two New Zealand streams was greatest at downstream sites, the range in size was greatest downstream, and transformers were typically found in the downstream reaches. Although very small (presumably age-0) Pacific lamprey ammocoetes have also been found close to or in the mouths of rivers that

meet the Columbia River mainstem, the results suggests that these habitats are primarily inhabitated by larger larvae.

3.6 Mortality

Mortality during the larval life stage is an important demographic parameter about which we presently have very limited information (Jones et al. 2009). Sea lamprey mortality is thought to be high in the egg phase and immediately following hatching when ammocoetes disperse from nest sites to suitable larval habitats (Potter 1980a). Available evidence suggests that ammocoetes older than age-0 experience relatively low and uniform mortality throughout the remainder of the larval stage; their propensity to burrow in sediments presumably allows them to avoid predators (Potter 1980a). Metamorphosis, however, may represent a second critical stage in lamprey development (Hardisty and Potter 1971a).

3.6.1 Mortality Factors in Different Life Stages

Available estimates of egg viability suggest reasonably high (but variable) survival in the absence of predation. When sampled shortly before hatch (at approximately 8–13 days after fertilization), 0–90% (average 43.4%) and 57.8–100% (average 84.4%) of the eggs in sea and Pacific lamprey nests, respectively, were found to be viable (Bergstedt et al. 2003; Ward et al. 2012). Under optimal laboratory conditions (i.e., at 18.4 °C), Piavis (1961) found 78% survival to the burrowing stage (17–33 days after fertilization). However, lamprey eggs appear to be preyed upon by a number of fish species, including speckled dace *Rhinichthys osculus* (Brumo 2006) and hornyhead chub *Nocomis biguttatus* (Cochran 2009). Eggs dislodged from the nest seem to be particularly vulnerable. Using an experimental hatching system, Smith and Marsden (2009) found that a high proportion (85%) of sea lamprey eggs was washed out of the nest, and predation rates on eggs outside the nest were high. Brumo (2006), however, found that egg predation did not have a significant effect on the relative survival of Pacific lamprey ammocoete cohorts. Large parasitic lampreys have high fecundity (averaging approximately 45,000–79,000 and 98,000–238,000 eggs per female for landlocked sea and Pacific lampreys, respectively; see Docker et al. in press) and highly variable recruitment (Jones et al. 2003), leading to the potential for offspring to exceed the capacity of early rearing environments to support them (Derosier et al. 2007). Fecundity in small, non-parasitic lamprey species, however, rarely exceeds 2,000 eggs per female (Docker et al. in press); the effect of egg predation on these species is unknown.

As mentioned above, mortality throughout the remainder of the larval stage is thought to be relatively low and uniform. Actual larval survival rates are highly uncertain, but some estimates are available. In the pouched lamprey, Kelso and Todd (1993) calculated annual larval survival rates to be approximately 47% for age 0

and 1 larvae and 77 % for age 1 and 2 larvae. Weise and Pajos (1998) estimated annual survival of one age class of sea lamprey larvae to be 61 % (see below). A recent study, in which sea lamprey larvae >60 mm total length were tagged, released into six Great Lakes tributaries following lampricide treatment, and later recaptured, provided survival rate estimates of 56.8–57.6 % (Johnson et al. 2014). These estimates are somewhat higher than the values (39.5–51.8 %) used in sea lamprey population model simulations (Jones et al. 2009; Irwin et al. 2012), but are generally lower than those (52–96 %) reported by Morman (1987) from cage studies in which predators would have been excluded.

Larval lampreys are eaten by a variety of fishes, amphibians, reptiles, birds, and mammals, and predation on lampreys is known in both marine and freshwater habitats (Cochran 2009). However, predation is thought to be a more important source of mortality in migrating (including downstream-migrating juveniles, see below) and spawning lampreys than in larval lampreys (Cochran 2009). Teleost predators of larval lampreys include species of minnows (Cyprinidae), sticklebacks (Gasterosteidae), eels (Anguillidae), sculpins (Cottidae), walleye and perch (Percidae), salmon and trout (Salmonidae), burbot (Lotidae), pike (Esocidae), and bullheads or catfishes (Ictaluridae) (Hardisty 1961a, b; Heard 1966; Manion 1968; Tuunainen et al. 1980; Maitland 2003; Nakamoto and Harvey 2003; Cochran 2009). Birds known to eat larval lampreys include herons (Ardeidae), gulls (*Larus* spp.), mergansers (*Mergus* spp.), cormorants (Phalacrocoracidae), and terns (e.g., Forster's tern *Sterna forsteri*) (Poe et al. 1991; Maitland 2003; Antolos et al. 2005; Cochran 2009).

The burrowing habits of larval lampreys appear to protect them from predation. According to a laboratory experiment performed by Smith et al. (2012), the availability of fine sand habitat may influence the predation risk of ammocoetes, since survival from predation was found to be highest in fine sand and lower in other substrates (see Sect. 3.2.1.1).

The substrate largely protects larval lampreys from predators, but their burrowing habit does make them vulnerable to large-scale anthropogenic disturbances of the sediment, particularly dredging (e.g., for mining or channel maintenance for flood control and navigation; see Chap. 8). Not only does dredging remove desirable substrate (see Sect. 3.2.1.1), it likely also results in the removal of the ammocoetes themselves. Unlike more mobile fishes that might quickly move from the site of a disturbance, ammocoetes often emerge from the sediment long after operations cease and they are not salvaged. Although poorly studied, these losses may be substantial (e.g., O'Connor 2004, 2006). Channelization also reduces habitat heterogeneity, eliminating or reducing the flow refugia important to larval lampreys (see Sect. 3.2.1).

Larval mortality due to disease is not well-studied (see Chap. 8). Research has largely focused on the impact of potential lamprey pathogens on human health or their potential transmission to commercially-important fishes. One recent study (Kurath et al. 2013), however, showed that Pacific lamprey ammocoetes exposed to common fish rhabdovirus pathogens—infectious hematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV)—showed no evidence of viral infection, replication, or persistence.

Larval density in the stream bed appears to be an important factor determining survival to metamorphosis (Manion and Smith 1978; Malmqvist 1983). Morman (1987) placed sea lamprey in cages in five Michigan streams at two densities (25 and 75 larvae/m^2), and monitored growth for 4½ years. Total mortality of larvae from ages 1 to 5 in the low and high density cages ranged from 4 to 8 % and 32 to 48 %, respectively. Weise and Pajos (1998) measured growth and density using length-frequency data between year classes of sea lamprey during recolonization of a Great Lakes tributary following a lampricide treatment in August 1989. They identified intraspecific competition, found no significant change in density of the 1990 year class during the study, and measured annual mortality of the 1991 year-class (interpreted as intraspecific mortality) as 39 %.

Survival of ammocoetes is also related to temperature, which is reflected in their geographical distribution (see Sect. 3.2.2.5). Van de Wetering and Ewing (1999) found lethal temperatures for larval Pacific lamprey beginning at 28 °C, which is similar to that found in earlier studies for other species: Potter and Beamish (1975) recorded incipient lethal temperatures ranging from 28 to 30.5 °C for northern brook, sea, European brook, and American brook lampreys; and Macey and Potter (1978) determined the incipient lethal temperature of pouched lamprey to be 28.3 °C. Meeuwig et al. (2005), however, reported decreasing survival of Pacific lamprey and western brook lamprey larvae as temperatures reached as little as 22 °C. Acclimation (i.e., to higher temperatures) appears to have little effect on incipient lethal temperature in lampreys. In teleosts, a rise of 3 °C in acclimation temperature generally results in a 1 °C increase in incipient lethal temperature; however, this trend was not observed in larval lampreys (Macey and Potter 1978). In fact, incipient lethal temperature in pouched lamprey acclimated at 5 and 25 °C was 27.3 and 28.2 °C, respectively (Macey and Potter 1978). This small (0.9 °C) rise over a 20 °C range in acclimation temperature is typical of other larval lampreys (Potter and Beamish 1975), and may be related to the low metabolic rate of ammocoetes (Hill and Potter 1970; Potter and Rogers 1972).

Mortality rates are thought to increase again at metamorphosis. Extensive morphological and physiological changes are occurring during this non-trophic period, including dramatic changes to the respiratory system (see Chap. 4) that are assumed to produce a “partial asphyxiation” (Hardisty and Potter 1971a). In addition, dams and other engineering works may represent significant sources of mortality in those species that undergo downstream migration following metamorphosis (see Chap. 8). Lampreys appear to swim lower in the water column than fishes with swim bladders and may thus pass beneath turbine bypass screens designed for juvenile salmonids (Long 1968; Moursund et al. 2003). In addition, those lampreys that encounter salmonid bypass screens may become impinged, leading to elevated mortality rates (Hammond 1979; Moursund et al. 2003; Dauble et al. 2006; Columbia River Inter-Tribal Fish Commission 2008). However, juvenile lampreys that pass through dam turbines may survive relatively better than other fishes. A recent study found no mortality or injury to juvenile Pacific lamprey exposed to a rapid decrease in pressure similar to what would occur during turbine passage; mortality or injury would be expected in 97.5 % of juvenile Chinook salmon *Oncorhynchus*

tshawytscha similarly exposed (Colotelo et al. 2012). Nevertheless, lampreys that survive passage through dams may become disorientated and suffer increased predation rates. Predatory fishes have been observed to congregate downstream of turbine outflows (Lucas and Baras 2001).

3.6.2 Mortality Ascribed to Pollution and Water Quality

Pollution can affect survival of lampreys. Occassional mortalities have been ascribed to pollution, and significant levels of pollution can eliminate whole populations of lampreys from rivers. Sea lamprey and European river lamprey disappeared from the polluted Thames and Clyde rivers in the United Kingdom (see Chap. 8) and, in Portugal, industrial pollution is thought to be mainly responsible for the extirpation of sea lamprey in the Ave River basin (Quintella 2006). According to Almeida et al. (2008), industrial pollution was also the primary factor responsible for the extremely low density of sea lamprey larvae found in the lower reaches of River Cávado in Portugal. Embryos and larvae using toxic sediments are likely more at risk than are juvenile and adult lampreys migrating through polluted waters (see Chap. 8); given that the larvae remain burrowed in river sediments for years, they may be particularly at risk. Pacific lamprey ammocoetes in the Trinity River, California, contained mercury concentrations (legacy mining contaminants) 12–25 times higher than freshwater mussels from the same site, and were well above concentrations considered to be detrimental in other fishes (Bettaso and Goodman 2010). Pacific lamprey larvae were found to be particularly sensitive to pentachlorophenol, one of the pollutants found in the Portland Superfund area of the Willamette River (Andersen et al. 2010). Morman et al. (1980) observed that streams affected by domestic, industrial, or agricultural pollution usually have no larvae or only small, discrete populations. However, Young et al. (1996) found that peaks in abundance of larval sea lamprey in the St. Marys River in 1971 and 1983 were both before and after reported declines in sediment contamination which could have influenced larval populations.

Few studies are available that concern the water quality requirements of lampreys (Maitland 2003). In comparison with the information on teleost fishes, for example, very little is known about the effects on lampreys of acid water and its associated environmental changes. Those studies available, however, suggest there is cause for concern. According to Myllynen et al. (1997), hatching success of European river lamprey and the survival of newly hatched larvae are clearly reduced by the prevailing water quality of the river. It appears that high iron concentration especially, together with acidic pH, reduces the hatchability of the eggs and increases the mortality of newly hatched larvae (Myllynen et al. 1997). These authors also observed that changes in water quality affected European river lamprey populations in Finnish rivers, as larval populations diminished in areas that otherwise should have been suitable for larval growth.

3.7 Duration of Larval Life and Growth Rates

3.7.1 Duration of Larval Life

The duration of the larval period in the life cycle of lampreys has been found to vary among species and within species (Potter 1980a; Youson 2003; Table 3.2). In general, non-parasitic species appear to persist longer in the larval phase and attain greater lengths at metamorphosis than parasitic species, at least when comparing "paired" (i.e., closely-related) parasitic and non-parasitic lampreys (Potter 1980b; Docker 2009). For example, average duration of the larval period in the European river lamprey in the River Teme has been estimated to be 4¼ years, whereas average length of larval life in the European brook lamprey is approximately 6¼ years (Potter 1980a). However, some earlier studies (Knowles 1941; Hardisty 1944) suggest a much shorter larval phase in the latter species (see Table 3.2). Furthermore, this trend is less apparent when comparing across taxa, and considerable variation in age at metamorphosis has been observed among parasitic species. Some species (e.g., pouched lamprey, European river lamprey, and short-headed lamprey *Mordacia mordax*) initiate metamorphosis at relatively young ages (3¼–4¼ years; Potter 1980a) whereas duration of the larval period for the anadromous sea lamprey was estimated at 6–8 years in Canadian rivers (Beamish and Potter 1975) and at approximately 5 years for British populations (Hardisty 1979). The age at metamorphosis of Pacific lamprey has been estimated at 4–8 years, with the majority of transformers being ages 5–7 (Russell 1986; Beamish and Northcote 1989).

For sea lamprey in the Great Lakes watershed, larvae generally range between 2 and 7 years of age at the time they enter metamorphosis (Potter 1980a; Morkert et al. 1998), although metamorphosis in as few as 2 years is unusual. The Chippewa River in Michigan, where many sea lamprey were found to undergo metamorphosis as early as age 2, is a highly productive stream (Morkert et al. 1998). In the Big Garlic River in Michigan, which is a relatively cold, unproductive stream, a known-age population of ammocoetes were not yet undergoing metamorphosis by age 6 (Dawson unpublished data). Higher productivity associated with higher water temperatures may enhance feeding efficiency and growth, resulting in an earlier age-at-metamorphosis (Morman 1987). Relative to more northerly situated river basins, anadromous sea lamprey ammocoetes from the Portuguese River Mondego presented a shorter larval stage duration of 4 years (Quintella et al. 2003; Fig. 3.4). The relationship between age-at-metamorphosis and ammocoete growth rate is discussed further below (Sect. 3.7.2); size at metamorphosis is discussed in Sect. 3.8.1.

3.7.2 Growth Rate of Ammocoetes

The growth rate of ammocoetes is correlated with factors important to the duration of larval life (such as water temperatures) and can vary considerably between geographic regions with different climatic regimes (Potter 1980a). Landlocked sea

Table 3.2 Summary of past research on the determination of lamprey larval stage duration. The methods listed are those used to determine age/growth

Species	Larval stage (years)	Method	Author
Geotria australis, pouched lamprey	4.25	Length–frequency	Potter and Hilliard (1986)
Geotria australis	3.5	Length–frequency	Todd and Kelso (1993)
Ichthyomyzon gagei, southern brook lamprey	3.25–4.25	Length–frequency	F. W. H. Beamish (1982)
Ichthyomyzon greeleyi, mountain brook lamprey	5.2–6.2	Length–frequency	Beamish and Austin (1985)
Ichthyomyzon greeleyi	5.25–6.25	Length–frequency	Potter and Bailey (1972)
Lampetra aepyptera, least brook lamprey	4–5	Length–frequency/ statolith	Docker and Beamish (1994)
Lampetra fluviatilis, European river lamprey	4.5	Length–frequency	Hardisty and Huggins (1970)
Lampetra planeri, European brook lamprey	2–3	Length–frequency	Knowles (1941)
Lampetra planeri	3.5–4	Length–frequency	Hardisty (1944)
Lampetra planeri	6.25	Length–frequency	Hardisty (1961b)
Lethenteron camtschaticum, Arctic lamprey	4	Length–frequency/ statolith	Kucheryavyi et al. (2007)
Mordacia mordax, short-headed lamprey	3.5	Length–frequency	Potter (1970)
Petromyzon marinus, sea lamprey	3.4–3.9	Length–frequency	Applegate (1950)
Petromyzon marinus	5	Length–frequency	Hardisty (1969)
Petromyzon marinus	6	Length–frequency	Lowe et al. (1973)
Petromyzon marinus	6–8	Length–frequency	Beamish and Potter (1975)
Petromyzon marinus	5	Length–frequency	Hardisty (1979)
Petromyzon marinus	5	Cage	Morman (1987)
Petromyzon marinus	2	Length–frequency/ statolith	Morkert et al. (1998)
Petromyzon marinus	3–4	Statolith	Griffiths et al. (2001)
Petromyzon marinus	4	Length–frequency/ statolith	Quintella et al. (2003)

lamprey in the lower Great Lakes (lakes Erie and Ontario) are known to achieve larger sizes more quickly than sea lamprey in the upper lakes (lakes Superior, Michigan, and Huron; Potter 1980a; Hansen et al. 2003; Slade et al. 2003; Hansen and Jones 2009). More favorable climatic conditions are likely to induce higher growth rates. The majority (96 %) of known-age sea lamprey larvae in a warm, high alkalinity Lake Huron tributary (Ogemaw Creek) achieved lengths of 120 mm or more by late summer at age 5, and some ammocoetes were observed undergoing metamorphosis. However, none of the known-age larvae in a cold, low alkalinity Lake Superior tributary (Big Garlic River) achieved a length of 120 mm by late summer at age 6 (Fig. 3.5). Quintella et al. (2003) used length-frequency analyses and statolith readings to measure growth and larval stage duration of the sea lam-

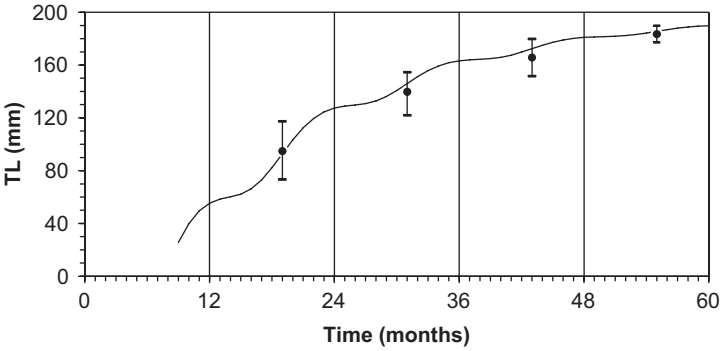

Fig. 3.4 Graphic expression of the seasonal von Bertalanffy growth formula estimated for sea lamprey ammocoetes in the River Mondego, Portugal. Also represented is the mean total length (mm ± SD) of lamprey (●) for each age group assigned with statoliths readings. (This figure was originally published in Quintella et al. (2003) and reproduced with permission of John Wiley & Sons, Inc.)

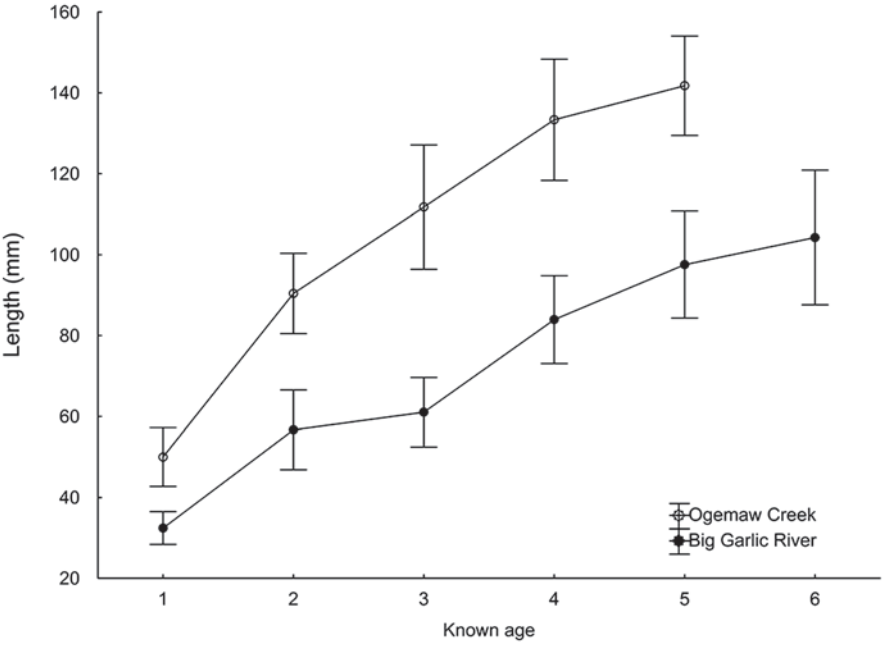

Fig. 3.5 Length at known age for natural populations of sea lamprey in two Michigan streams. Ogemaw Creek is a warm, high alkalinity tributary of Lake Huron, while the Big Garlic River is a cold, low alkalinity tributary of Lake Superior (Dawson unpublished data)

prey in the River Mondego in Portugal. The theoretical growth model based on length-frequency distribution of sea lamprey larvae from this system, confirmed by the number of annuli identified on statoliths, suggested approximately 4 years

of larval life (Fig. 3.4). The seasonal von Bertalanffy growth formula, calculated using the length-frequency distribution of the sea lamprey ammocoetes sampled in River Mondego on a monthly basis and during a 3-year period, displayed a marked seasonal pattern of growth throughout the year with a relatively short period of slow growth (±1.2 months), apparently taking place between January and February, the cooler period of the year (Fig. 3.4). On average, ammocoetes attained 36.7% of the theoretical maximum length (TL_{∞}) in the first year (i.e., 72 mm), 68.6% at the end of the second year (i.e., 136 mm), 84.4% at the end of the third year (i.e., 168 mm), and 92.3% of the TL_{∞} at the end of the fourth year (i.e., 183 mm; Fig. 3.4). In the Quintella et al. (2003) study, the ages assigned from the number of annuli were consistent with ages derived from the theoretical growth model (Fig. 3.4).

Hansen et al. (2003) found that sea lamprey growth varied significantly among Great Lakes streams and years. This observation is consistent with other studies that indicate variability in larval growth likely derives from watershed characteristics, which define the productivity of each stream and contribute to the variation in growth among streams (Manion and Smith 1978; Purvis 1979; Potter 1980a; Holmes 1990; Young et al. 1990a; Morkert et al. 1998; Rodríguez-Muñoz et al. 2001). Within streams, annual environmental characteristics, such as temperature and precipitation, contribute to variability in growth (Manion and McLain 1971; Young et al. 1990b). Lamprey feed slowly but efficiently on organic detritus in streams, accumulating most of their energy during summer when temperatures are warm and food quality is high (Sutton and Bowen 1994; Yap and Bowen 2003; see Sect. 3.3). This is consistent with previous observations by Lowe et al. (1973) that increases in length of larval sea lamprey in Shelter Valley Creek, a small tributary to Lake Ontario, were almost entirely restricted to the warmest months.

Lowe et al. (1973) also observed that further increases in length did not take place during the final year of larval life. This so-called "arrested growth phase" or "rest period" prior to metamorphosis is not due to environmental conditions, but rather suggests that metabolism prior to metamorphosis is focused more on lipidogenesis than somatic growth (O'Boyle and Beamish 1977; Potter 1980a; Bird and Potter 1981; Treble 2006; see Chap. 4). It is not known, however, whether all lamprey larvae undergo a pre-metamorphic arrested growth phase. Such information is critical for predicting rates of metamorphosis and potential recruitment of sea lamprey to the Great Lakes based on the size structure of larval populations (Slade et al. 2003).

Laboratory studies have shown negative effects of larval density on growth rates in lampreys, both in single species (Mallatt 1983; Malmqvist 1983; Murdoch et al. 1992) and multispecies (Murdoch et al. 1991) experiments. Growth of sea lamprey ammocoetes was observed to decrease significantly with increasing density (Murdoch et al. 1992). Over an 8-month period, the authors recorded changes in length (mean ± standard error) of 17.3 ± 3.0, 4.3 ± 4.5, and −1.4 ± 1.0 mm at larval densities of approximately 34, 172, and 345 per m^2, respectively. Rodríguez-Muñoz et al. (2003) assessed the role of population density and waterborne-mediated interference on the growth rate of sea lamprey larvae in two laboratory experiments. The effects of these factors were evaluated by comparing growth of larvae reared

at three different densities (27, 75, and 128 individuals/m^2) or in preconditioned water (i.e., water taken from aquaria containing sea lamprey larvae at the same three densities). The authors found that water conditioning had a negative but weaker effect on growth than larval density, with larvae reared in water preconditioned at the two higher densities having shown a lower mass increase than those growing in water preconditioned at the lowest density. These results suggest that chemical or biological agents released into the surrounding water by conspecifics may influence growth in larval sea lamprey (Rodríguez-Muñoz et al. 2003).

The effect of density on larval growth rate has also been measured in situ through the use of cages (Malmqvist 1983; Morman 1987; Zerrenner 2004). Morman (1987) observed in his long-term cage experiments that sea lamprey ammocoete lengths were significantly greater in the low-density cages (25 larvae/m^2) by the end of the study than in the high density cages (75 larvae/m^2). He also found that some larvae had entered metamorphosis in the low-density cages (presumably as the result of attaining a larger size), while no metamorphosing animals were observed in the high density cages. Zerrenner (2004), however, found growth of sea lamprey larvae held in circular cages (0.16 m^2) in streams for a period of 1 year was not significantly affected by density (25, 50, 100, 150, and 200/m^2).

The experimental densities used in the above studies are generally much higher than average larval densities observed in nature (see Sect. 3.4.1). Nevertheless, comparisons within and among streams have also suggested that larval density influences growth rate at natural densities (Manion and Smith 1978; Morman 1987; Jones et al. 2003). Overall, most evidence supports a general reduction in growth rate of larvae with increasing density.

Negative growth must be experienced by lampreys during the non-trophic metamorphic period, although reductions in weight are often relatively modest (especially compared to reductions observed during the non-trophic upstream spawning migration of some species; see Chap. 5) and changes in length are often complicated by morphological changes associated with metamorphosis (e.g., elongation of the snout and development of the oral disc in parasitic species; Hardisty and Potter 1971b). In metamorphosing landlocked sea lamprey observed between October and early March, Wigley (1959) observed that weight decreased by about 11 % but that length increased by 5 %. Over a similar time period in metamorphosing European river lamprey, Hardisty (1970) observed that weight decreased by about 8 % but length stayed the same. In non-parasitic species, completion of metamorphosis overlaps with gonadal maturation, further complicating comparisons (Hardisty et al. 1970; Hardisty and Potter 1971b). Earlier studies (e.g., in northern brook lamprey; Leach 1940) showed overall reductions in length and weight between transformation and sexual maturity, but Beamish and Medland (1988a) showed that neither length nor weight of mountain brook lamprey males or females changed significantly between metamorphic stages 1 and 7. In the latter study, condition factor increased significantly between stages 1 and 2, and did not change significantly after stage 2. Only at sexual maturity (stage 8) in females did weight change significantly, but it also increased. Beamish and Medland (1988a) concluded that lipid reserves used during the 3.3–4.7 months required to complete metamorphosis

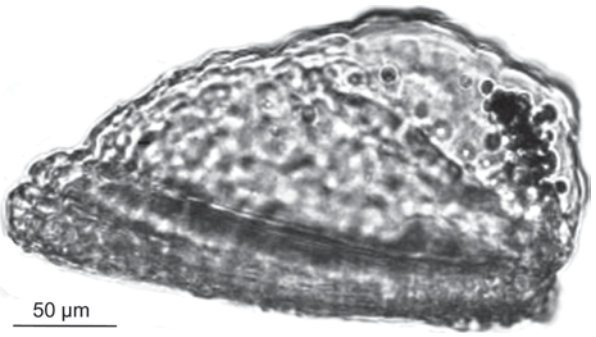

Fig. 3.6 Photograph of a statolith from a larval sea lamprey. (Photo: Nuno Andrade)

were replaced by water so that total length and body weight did not change. More research is required to determine the energetic costs of metamorphosis.

3.7.3 Reliable Aging Methods Required to Estimate Duration of Larval Life and Growth

Analyses that require age-composition data, such as growth and duration of the larval life stage, have been generally avoided due to the unreliability of age-assessment methods for larval lampreys. For a review of past research on larval stage duration, see Sect. 3.7.1 and Table 3.2. Historically, sea lamprey ammocoetes have been assigned ages using visual assessment of length-frequency distributions (Hardisty and Potter 1971a; Beamish and Medland 1988b). Determining age based on length-frequency distributions is subjective because of heterogeneity in larval growth rates within and across streams, and the resulting overlap in lengths between age classes introduces uncertainty into the estimates of population age composition (Potter 1980a). The great individual variability in growth rates of ammocoetes and the consequent wide scatter of lengths within the same age class have frequently made it difficult to identify with certainty the individuals belonging to the older age groups in the length-frequency distribution (Hardisty and Potter 1971a; Hardisty 1979). Even the age at transformation of a re-established population of ammocoetes (e.g., after lampricide treatment) is difficult to determine if residual larvae are also present in the stream (Purvis 1979). Only a small percentage of a year class may transform during the year metamorphosis begins, and it is often impossible to separate these animals from the small residual population when the length frequencies of the two groups overlap (Purvis 1979).

Statoliths, the only calcareous structure in lampreys, have been used as an alternative technique to age ammocoetes (e.g., Volk 1986; Beamish and Medland 1988b; Hollett 1998). Statoliths display alternating narrow opaque bands, which represent prolonged slow growth during winter, with the translucent band deposited during rapid growth periods associated with increased temperatures and feeding (Beamish and Medland 1988b; Barker et al. 1997; Fig. 3.6). When determining age using statoliths, each opaque band or annulus is counted as representing 1 year

(Volk 1986). Most studies provided similar age estimates when comparing ages determined from statoliths and length-frequency distribution (Volk 1986; Beamish and Medland 1988b; Docker and Beamish 1994; Barker et al. 1997; Morkert et al. 1998; Griffiths et al. 2001; Quintella et al. 2003). Meeuwig and Bayer (2005) did report some modal separation for western brook lamprey from Washington, but more year classes were observed by aging statoliths than were apparent from the length-frequency histogram. However, the reliability of the use of statoliths to accurately assess the age of ammocoetes is not consensual among lamprey researchers.

To use statoliths as a method of assessing age of larval sea lamprey, the structures must be validated so that the banding pattern can be repeatedly visualized by a reader and represents the true age of the ammocoete over multiple years in contrasting streams (Jones 2007). Most studies using statoliths to estimate larval lamprey population age composition measure the precision (the deviation of each age assignment from the mean age assigned by readers) as an assessment of error and not the accuracy (deviation of age assignment from the true age; Dawson et al. 2009). Recently, Dawson et al. (2009) established known-age populations of sea lamprey in two contrasting Great Lakes streams and assessed the precision and accuracy of statolith aging over multiple years. The authors found that the precision average percent error (APE) ranged from 12.6 to 19.7%, and was smaller than the accuracy APE (bias) that ranged from 24.3 to 36.2 %. These results indicate a lack of accuracy in age assessments using statoliths (Fig. 3.7). Accurate estimates of age composition are better obtained by combining length-frequency information with a small sample of bias-corrected statolith annuli counts in a statistical model of growth of lamprey ammocoetes (Dawson et al. 2009). However, the bias in annuli counts by readers is variable by stream and by age. Additional research combining length-frequency information with statolith size data from the population did not improve estimates of age composition (Dawson unpublished data). Thus, the use of statoliths as a tool to reliably age lampreys is in question.

3.8 Onset of Metamorphosis

The microphagous period of the larval life stage and the parasitic juvenile stage (or, in the case of non-parasitic species, the non-trophic adult period) are separated in all lampreys by a non-feeding period (the duration of which varies within and among species) and a radical transformation (Potter et al. 1980). The transformation of larval lampreys represents one of the few “true” vertebrate metamorphoses, as most organ systems undergo some sort of reorganization to facilitate the impending change in lifestyle (see Chap. 4). In some ways, however, lamprey metamorphosis in anadromous species is similar to the smolting period of salmonids, in which juvenile animals prepare for life in a marine environment after hatching and rearing in fresh water, by developing silvery coloration, large eyes, and the ability to osmoregulate in a marine environment (Potter and Huggins 1973).

The precise onset of metamorphosis is difficult to detect in larval lampreys because many internal changes may be occurring before it is externally apparent. As a

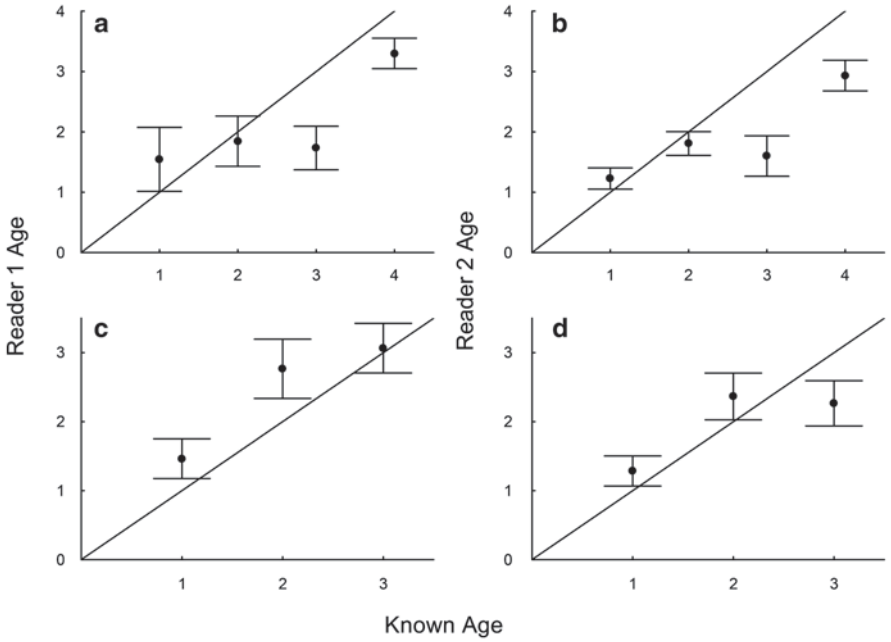

Fig. 3.7 Age bias plots constructed from sea lamprey statoliths evaluated from two "known-age" populations that were aged by two readers. Panels (**a**) and (**b**) compare the average age coded by each reader to the true age when evaluating the slow-growing Big Garlic River population. Panels (**c**) and (**d**) compare the average age coded by each reader to the true age when evaluating the fast-growing Ogemaw Creek population. *Error bars* indicate 95% confidence intervals surrounding the average age assigned by each reader. This figure was originally published in Dawson et al. (2009) and reproduced with permission of Taylor & Francis Ltd. (http://www.tandf.co.uk/journals)

result, older studies of lamprey metamorphosis may not have recognized the earliest stages and thus there is a great deal of variability in the estimated duration of the metamorphic period for some species (Potter 1980b). However, two components are required before lampreys can enter into metamorphosis: a suitable water temperature regime prior to the onset of metamorphosis and sufficient size/lipid reserves to provide enough energy to support all of the developmental changes that occur while the animal ceases to feed (Youson et al. 1993). The process of metamorphosis is described in detail in Manzon et al. (see Chap. 4), but aspects relevant to the ecology of the stream-resident stage of the lamprey life cycle are discussed below.

3.8.1 Size of Metamorphosing Lampreys

Metamorphosis generally occurs at lengths ranging from approximately 90 to 170 mm (Potter 1980a; Docker 2009; see Chap. 4). However, there is considerable variability in ammocoete length and weight at the time of metamorphosis between

different lamprey species, as well as within a single species. Temperature and stream productivity influence growth rates of larval populations (see Sect. 3.7.2), adding inter- and intra-annual variation in lamprey size at metamorphosis (Holmes 1990; Young et al. 1990a). Population density (both conspecifics and heterospecifics) influences the size at which larval lampreys initiate metamorphosis as competition for available food resources may negatively influence larval growth rate (Murdoch et al. 1992; Rodríguez-Muńoz et al. 2003). This is evident in the Great Lakes basin, where transforming lamprey that are residuals from a lampricide treatment (and thus are at lower density) are routinely observed to be larger than those observed during the lampricide treatment a year or two prior (Purvis 1980). Further, the landlocked variety of sea lamprey found in the Great Lakes enters metamorphosis at a larger size than the anadromous form from which it is derived (Potter et al. 1978).

As mentioned above (Sect. 3.7.2), an arrested growth phase, whereby there is an increase in weight and lipid content—but not length—has been observed in some lamprey species prior to metamorphosis. Consequently, lampreys preparing for metamorphosis are presumably "fatter" for a given length than are other larvae. A condition factor criterion (combined with length and weight criteria) has thus been used to identify presumptive metamorphosing sea lamprey larvae (Holmes and Youson 1994, 1997, 1998; see Chap. 4). However, condition factor has performed well in predicting metamorphosis in close proximity to the event (Youson 2003), but not many months in advance of its onset (Treble et al. 2008). Using a mark-recapture technique, Treble et al. (2008) determined that the factors most likely to predict onset of metamorphosis of larval Great Lakes sea lamprey in eight streams were weight, age, larval density, stream temperature, and geographic location.

A general trend across the lamprey genera suggests that parasitic species metamorphose at a smaller length and/or weight than closely-related non-parasitic species (Vladykov and Kott 1979; Docker 2009; see Chap. 4). This may be related to the fact that while parasitic species feed for a few months to several years after metamorphosis before their gonads ripen, non-parasitic lampreys become sexually mature shortly after metamorphosis, without any further intake of food. Although not as fecund as parasitic species, this would still represent a significant increase in energy requirements (see Sect. 3.7.1). However, the ability to distinguish between species of ammocoetes from the same genus is often difficult and thus usually limits comparisons to allopatric populations (Docker 2009), where paired parasitic and non-parasitic species may be subject to different environmental conditions. Furthermore, the trend observed between paired species should not necessarily be taken to mean that parasitic species in general metamorphose at a smaller size than non-parasitic species. As with age at metamorphosis (see Sect. 3.7.1), considerable variation in size at metamorphosis has been observed among parasitic species. Pouched lamprey, for example, metamorphose at a relatively small size (c. 90 mm; Potter 1980b), whereas anadromous and landlocked sea lamprey metamorphose at much larger sizes (c. 130 and 140 mm, respectively; see Chap. 4). Likewise, even within a population, there can be differences among individuals in size at metamorphosis; in the non-parasitic species at least, there is evidence that females metamorphose at a larger size than males (see Docker 2009).

3.8.2 Seasonal Incidence and Duration of Metamorphosis

A generally accepted fact is that the onset of metamorphosis is highly synchronous in a given species within a particular river system (within 3–4 weeks), despite the variability observed inter-annually (Potter 1980a). This is related to latitude, as well as local environmental conditions (Potter et al. 1978; Youson et al. 1993; see Chap. 4). In both Northern and Southern hemispheres, transformation begins during the spring or summer months, when water temperatures are the most favorable, and is generally completed by winter or early the following spring (e.g., Richards and Beamish 1981; Potter et al. 1982; Potter and Hilliard 1986; Holmes and Lin 1994; Quintella et al. 2003; McGree et al. 2008). Beamish and Medland (1988a) compared timing of metamorphosis in six Northern Hemisphere lamprey species, and observed that those occurring at lower latitudes tend to enter metamorphosis later than those at higher latitudes (Beamish and Medland 1988a). Southern brook lamprey, for example, were found to enter metamorphosis as late as early September (Beamish and Thomas 1984), whereas more northerly species such as Arctic lamprey, Pacific lamprey, and European river lamprey generally initiate metamorphosis in July (Beamish and Medland 1988a; Youson et al. 1993; Kucheryavyi et al. 2007; McGree et al. 2008). There is also variability in the timing of metamorphosis between conspecifics at the latitudinal extremes of their ranges. Tennessee populations of American brook lamprey start metamorphosis around mid-August (Seagel and Nagel 1982), whereas populations of the same species in the more northerly Great Lakes basin are found to start metamorphosis as early as late June (Holmes et al. 1999). For sea lamprey, initiation of metamorphosis typically occurs in mid-summer (August/September) in Portugal (Quintella et al. 2003), and early to mid-July at more northerly latitudes (Potter 1980b; Youson et al. 1993). However, there do appear to be exceptions to this pattern. For example, the Mexican lamprey *Tetrapleurodon spadiceus* and Mexican brook lamprey *T. geminis* enter metamorphosis in April (Cochran et al. 1996), and the precocious or Australian brook lamprey *Mordacia praecox* initiates metamorphosis in October or November, the equivalent of spring in the Northern Hemisphere (Potter 1970); all three species occur at low latitudes (20° N, 20° N, and 35–44° S, respectively).

Lamprey metamorphosis generally lasts 3–4 months, but can be variable among species. Comparisons among studies, however, may be complicated by differences in defining the exact point of completion of metamorphosis. Although metamorphosis was initially divided into five (Manion and Stauffer 1970) and later seven (Bird and Potter 1979; Youson and Potter 1979) stages based on the sequential changes of five key morphological features (see Chap. 4), some studies report instead when initiation of downstream migration and commencement of parasitic feeding occurs; these ecological transitions may occur sometime after the morphological completion of metamorphosis (see Sect. 3.9). Furthermore, they are not applicable to non-parasitic species. The duration of the metamorphic phase does not appear to be related to latitude, temperature, or whether the animal follows a parasitic or non-parasitic life history. Duration of metamorphosis may be as short as 3–3.5 months in

Siberian brook lamprey *Lethenteron kessleri* (Poltorykhina 1971), European river lamprey (Bird and Potter 1979), and anadromous sea lamprey (Reis-Santos et al. 2008), or as long as 7–8.2 months in European brook lamprey (Bird and Potter 1979) and 9 months in North American river lamprey (Beamish and Youson 1987).

3.9 Downstream Migration

After metamorphosis is complete, some species (e.g., European brook lamprey) remain burrowed in the substrate until ready to spawn, whereas others (e.g., sea lamprey, European river lamprey, short-headed lamprey) burrow less often, instead preferring to hide under logs and rocks during the day (generally in areas with appreciable water flow) and moving at night (Potter 1970; Potter and Huggins 1973). In Pacific lamprey in the Fraser River, British Columbia, 99% of all downstream migrants were captured at night (Beamish and Levings 1991). The downstream migration of newly metamorphosed lampreys applies almost exclusively to parasitic species migrating to feeding grounds, although presumably a small number of brook lampreys migrate downstream to colonize new tributaries in landlocked populations. Anadromous parasitic lampreys migrate to the marine environment, although several species that are typically anadromous also appear to exist as freshwater-resident populations or individuals. The best known example, of course, is the Great Lakes sea lamprey, but other examples include populations of Arctic and European river lampreys that feed in other large lakes (e.g., Heard 1966; Nursall and Buchwald 1972; Adams et al. 2008; see Docker and Potter in press). Although poorly studied, these and other freshwater-resident parasitic lampreys undergo more limited downstream migrations than most anadromous lampreys. This, presumably, is particularly true of those species that feed predominantly in river rather than lake systems (e.g., chestnut lamprey *Ichthyomyzon castaneus*, Ohio lamprey *Ichthyomyzon bdellium*, Carpathian lamprey *Eudontomyzon danfordi*; Renaud 2011). The following sections, therefore, deal only with anadromous species and the well-studied Great Lakes sea lamprey.

3.9.1 Environmental Triggers and Timing of Downstream Migration

Unlike the onset of metamorphosis, which is highly synchronized within a population, the timing of downstream migration can be quite variable (Potter et al. 1982), and evidence suggests that it is triggered by environmental cues. The most significant environmental trigger appears to be increases in river flow resulting from freshet events (Bird et al. 1994; Quintella et al. 2005; Columbia River Inter-Tribal Fish Commission 2011). Downstream migration of European river lamprey juveniles in Germany shows a peak in the spring (Thiel and Salewski 2003). In a

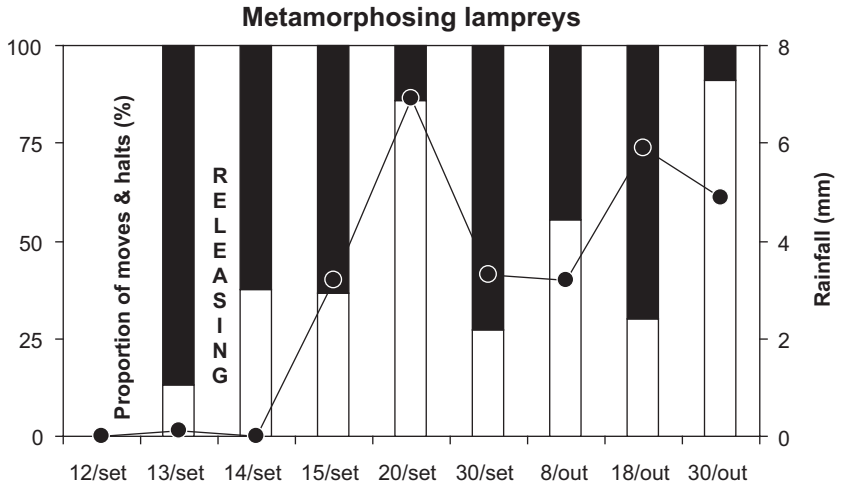

Fig. 3.8 Relationship between the rainfall (-●-) observed in a tributary of River Mondego, Portugal, and the proportion of moves (□) and halts (■) for metamorphosing sea lamprey. Dates (day/month) are given on the horizontal axis (*set* = Sept; *out* = Oct). (This figure was originally published in Quintella et al. (2005) and reproduced with permission of John Wiley & Sons, Inc.)

tributary of the River Mondego, Portugal, a significant increase in the proportion of downstream movements of transformers followed rainfall episodes (Quintella et al. 2005; Fig. 3.8). Downstream movement of juvenile North American river (Beamish and Youson 1987) and Pacific (Beamish and Levings 1991) lampreys likewise appears to coincide with increased discharge. Beamish and Youson (1987), in fact, suggested that the prolonged period of metamorphosis observed in the North American river lamprey (see Sect. 3.8.2) may have evolved in response to the pattern of discharge in the Fraser River (i.e., that this species delays completion of metamorphosis until flow increases in the spring). In Pacific lamprey in the Fraser River, downstream migration started in late September, with the largest number of migrants captured in mid-March to mid-May (Beamish and Levings 1991). Counts of juvenile lamprey (presumably mostly Pacific lamprey) as they pass through the mainstem dams and upriver salmon smolt traps in the Columbia River basin likewise show spring peaks of migration that coincide with periods of increased flow (Columbia Basin Fishery Agencies and Tribes 2014). There may be other peaks in winter as large pulses of juvenile lamprey have been observed in salmon smolt traps on the Umatilla River, a Columbia River tributary (Mary L. Moser, Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, Seattle WA, personal communication, 2014).

Although increases in stream discharge appear to be the primary trigger for the initiation of downstream migration, water temperature is also an important cue (Potter and Huggins 1973). The downstream migration of newly-transformed juvenile sea lamprey is monitored annually on the St. Marys River, which connects Lake Superior and Lake Huron. As water levels on this river are controlled, tem-

perature is the primary cue for downstream migrants, with the timing of the peak of the run varying from year to year, but generally occurring after water temperatures fall below 8 °C in any given year (Treble unpublished data). Applegate (1950) observed that downstream migration of metamorphosed Great Lakes sea lamprey peaked in the fall (around November) and again in spring (around April). Those that enter the lakes in the fall can start the parasitic feeding phase 3–7 months earlier than the spring outmigrants, which overwinter in the stream substrate without feeding (Swink 1995). A recent study by Swink and Johnson (2014), however, found no significant differences in survival or growth over the entire feeding phase (i.e., between downstream migration and spawning) in fall versus spring migrants (see Chap. 4).

3.9.2 Salinity Tolerance

Because all lamprey species spend the majority of their life as ammocoetes burrowed in the sediments of freshwater streams, the ability to tolerate brackish and salt water is a critical developmental phase in the life cycle of anadromous species. The process of marine osmoregulation consists of swallowing sea water, uptake of water and ions across the gut wall, and excretion of excess ions through the gills and opisthonephros (kidney) (Bartels and Potter 2004). Some of the developmental characteristics that allow this include the opening of the foregut and development of numerous lateral folds, as well as the presence of numerous mitochondrial-rich cells (also known as chloride cells) containing Na^+/K^+-ATPase transporting enzyme in the gill filaments (Potter and Huggins 1973; Richards and Beamish 1981; Reis-Santos et al. 2008).

Osmoregulatory efficiency correlates well with the expression of branchial Na^+/K^+-ATPase and chloride cell proliferation, which all increase during metamorphosis (Bartels and Potter 2004; Reis-Santos et al. 2008). Several studies have demonstrated that larval lampreys are generally unable to osmoregulate in water with salinities higher than 10 practical salinity units (psu) or ‰ (e.g., Hardisty 1956; Beamish et al. 1978; Morris 1980; Reis-Santos et al. 2008), which corresponds to c. 28 % full strength sea water. Pacific lamprey larvae have been found to tolerate salinities up to 10 psu for 14 days, although Pacific and western brook lamprey larvae have been observed in tidally influenced areas experiencing up to 15 psu (Gregory S. Silver, U.S. Fish and Wildlife Service, Vancouver, WA, personal communication, 2014). Salinity tolerance (in anadromous species at least, see below) develops during metamorphosis. In anadromous sea lamprey, early transformers (stages 3–5) were already capable of tolerating transfer to water salinity of 25 psu or 70 % full strength sea water, although these early transformers did not survive at full strength sea water and, even at 25 psu, they were inactive during the first two days (Reis-Santos et al. 2008). All later staged transformers (stage 6) in this study survived in full strength sea water. In Pacific lamprey, Richards and Beamish (1981) reported that individuals in stage 5 of metamorphosis were able to survive salinities > 13.4 psu

(>38% full strength sea water), and those in stage 6 survived a direct transfer to 30 psu (85% sea water). Thus, it appears that the ability to acclimate to full strength sea water is developed around metamorphic stage 6 (see Chap. 4), which coincides with the formation of the foregut in these two species (Richards and Beamish 1981; Reis-Santos et al. 2008). Once metamorphosis is complete (i.e., at stage 7), survival rates are high with either acclimated (Potter et al. 1980; Beamish et al.1978; Clarke and Beamish 1988; Reis-Santos et al. 2008) or direct (Potter and Beamish 1977) transfer to full strength sea water. Similar results were observed in young adults of European river and pouched lampreys (Potter and Huggins 1973; Potter et al. 1980).

Freshwater-resident populations of normally anadromous species (see Docker and Potter in press) likely retain some ability to osmoregulate in salt water. Youson and Freeman (1979) demonstrated that feeding adults of the landlocked sea lamprey still contained well developed chloride cells in their gills. Tolerance to salt water, however, appears to be related to body size; only when landlocked sea lamprey juveniles reached almost 280 mm in length did they survive direct transfer to full seawater for periods of approximately 2 weeks (Mathers and Beamish 1974). Non-parasitic lampreys, which remain in fresh water throughout their lives, appear to be less tolerant of salinity than parasitic (and certainly anadromous) species (e.g., European brook versus European river lamprey; Hardisty 1956). However, some non-parasitic lampreys (those that are, presumably, recently derived from anadromous parasitic ancestors) still retain well developed chloride cells in their gills (e.g., American brook lamprey; Bartels et al. 2011) and may be capable of limited osmoregulation in sea water. This might enable transformers of these species, if swept downstream into estuaries, to survive and swim back into rivers. As discussed above (Sects. 3.2.3 and 3.5), metamorphosed individuals typically occur in the downstream reaches of rivers; in streams where high flow events are frequent, they may be vulnerable to being swept out of the stream altogether.

3.10 Potential Compensatory Effects of Sea Lamprey Control

Compensatory mechanisms refer to processes that increase birth rates or decrease death rates when population density decreases. Thus, there is concern that the successes achieved in sea lamprey control in the Great Lakes may, in part, be counteracted by compensatory mechanisms (Jones et al. 2003). For example, increased growth as the result of lampricide-induced decreases in density could, in turn, result in higher survival or earlier age at metamorphosis. The consequences to the sea lamprey control program of the former are clear, and shortening the duration of the larval period would necessitate more frequent lampricide applications per stream. However, there is little evidence of a strong, repeatable influence of density-dependent compensatory mechanisms in Great Lakes sea lamprey populations. Some studies have suggested accelerated growth and time to metamorphosis as a result

of lower densities following lampricide application (e.g., Purvis 1979; Weise and Pajos 1998; Morkert et al. 1998). Purvis (1979), for example, noted that metamorphosing lamprey were consistently larger (longer) in post-treatment populations than in pre-treatment populations in the same stream, suggesting that growth rates were accelerated following treatment. However, since there is no age-composition data on these larvae, it is impossible to determine whether the increased size is indicative of size- or age-dependent differences in treatment mortality or potential changes in the duration of the larval stage (Jones et al. 2003). In the Lake Champlain basin, Zerrenner and Marsden (2006) reported that transformation occurred at a smaller size (131 mm vs. 143 mm) and at a younger age (100 % age 4 vs. 100 % age 5; larvae were aged using statoliths) in a tributary following lampricide treatment (albeit where densities were still high) relative to a tributary that had never been chemically treated. These authors suggested that this may be due to selection for early transformation in larvae due to exposure to lampricide treatments.

Other studies, however, do not suggest compensatory effects. Jones et al. (2003) compared the average sizes reached by successive sea lamprey cohorts following treatment of a stream with lampricide to evaluate whether lower density would lead to mean lengths of ammocoetes in the first cohort being greater than in later cohorts. These authors found that lengths of age-0 and age-1 ammocoetes were not consistently greater in the first year following treatment. Griffiths et al. (2001) similarly found that the daily growth of larvae in lampricide-treated streams was similar to that in populations that were never exposed to lampricides. Johnson et al. (2014), who monitored tagged sea lamprey larvae released into six Great Lakes tributaries following lampricide treatment, found that survival and size at metamorphosis of these residual larvae were very similar to those of untreated populations. These results suggest that other factors that vary from year to year in the natural environment, such as weather and timing of hatching, may be at least as important as ammocoete density in controlling cohort mean lengths (Jones et al. 2003).

A large shift in sex ratios of both adult and larval Great Lakes sea lamprey was observed in all three of the upper Great Lakes and a Lake Champlain stream (Zerrenner and Marsden 2005) after the implementation of sea lamprey control (Purvis 1979; Heinrich et al. 1980). This shift is presumably a demographic response of the lamprey populations to reductions in their overall abundance (Jones et al. 2003; see Sect. 3.4.3). Prior to control, adult lamprey populations in all three lakes were predominantly (54–70 %) male (Smith 1971). After the control program had successfully reduced the lamprey populations in these lakes to far below their pre-treatment levels (e.g., estimated reductions of 76–92 % in Lake Superior; Smith and Tibbles 1980), sex ratios shifted sharply to a predominance of females (with males comprising only 21–44 %; Purvis 1979). The most pronounced shifts in sex ratios of sea lamprey larvae and transformers have been toward an increased proportion of females occurring predominantly in high-density streams supporting disproportionate numbers of males during original treatments (Torblaa and Westman 1980). Sex compositions in streams where relatively low densities of larvae existed during original treatments remained remarkably stable since the advent of chemical control (Torblaa and Westman 1980). If the supply of eggs (number of females)

is correlated with the production of larvae and the supply of males does not limit recruitment, then a shift from a preponderance of males to a preponderance of females should, all else being equal, tend to compensate for a reduction in overall numbers of adults (Jones et al. 2003). Whether this is indeed the case, however, will depend on the mating system of sea lamprey (see Chap. 6).

3.11 Conclusions

Remarkable similarities exist across lamprey species with respect to the larval stage of their life cycle and the process of metamorphosis; thus, ecological requirements of this group are also similar. Habitat variables corresponding to larval lamprey abundance are different depending on the spatial scale examined and the size of the ammocoete. On the river-basin scale, the existence of suitable conditions for ammocoete colonization is dependent on stream gradients, which will, in turn, determine the overall velocity of the current and, consequently, the type of substrate particles that are deposited (Hardisty and Potter 1971a). Recent studies have also indicated that the spatial context of biological factors, such as the spawning distribution of adults, also plays an important role in larval distribution. Evidence exists that freshet events, larval density, and temperature can affect movement of larval lampreys, with movements occurring primarily in a downstream direction, as evidenced by tagging studies and the accumulation of larger larvae in lentic areas near the mouths of streams (Jones 2007). Nevertheless, it is misleading to regard ammocoetes as entirely at the mercy of their environment in terms of dispersal or foraging, as they are capable of moving short distances in the upstream direction against slow currents (Quintella et al. 2005). Environmental characteristics such as temperature, precipitation, and water chemistry contribute to variability of larval growth within streams (Manion and McLain 1971; Young et al. 1990b), as do other factors such as larval density (Mallatt 1983; Malmqvist 1983; Murdoch et al. 1992). Estimation of the growth rate and duration of the larval stage of lampreys is complicated by the unreliability of age-assessment methods for larval lampreys.

All species of lampreys go through a true vertebrate metamorphosis to change from the larval to the adult form (Youson 2003; see Chap. 4). The start of metamorphosis is highly synchronous (within 3 to 4 weeks) within a population and is related, in large part, to the latitude of the population in question, as well as by local environmental conditions (Potter et al. 1978; Youson et al. 1993). There is evidence that the synchronous nature of the downstream migration of anadromous species is triggered by environmental cues, namely increases in river flow resulting from heavy rain and/or spring thaw events (Bird et al. 1994; Quintella et al. 2005). Unlike the timing of metamorphosis, the duration of the metamorphic phase does not appear to be related to latitude, temperature, or whether the animal follows a parasitic or non-parasitic life history, with the duration of metamorphosis recorded as occurring in as little as 3 months and as long as 9 months in different species. Considerable variability also exists in ammocoete length, weight, and age at the

onset of metamorphosis, both between and within species. Large lipid reserves are required to undergo the process of metamorphosis, although the temporal pattern of lipid deposition varies between ammocoetes of different species (Bird and Potter 1981). It is following metamorphosis that the ecology (and hence physiology) of species with different adult life history types diverge; in anadromous species, it appears that the ability to acclimate to full strength sea water develops around metamorphic stage 6 (see Chap. 4). Evidence exists of environmental sex determination in lampreys (Docker and Beamish 1994). A large shift in sex ratios of both adult and larval Great Lakes sea lamprey was observed in all three of the upper Great Lakes after the implementation of the sea lamprey control program during the 1960s (Purvis 1979; Heinrich et al. 1980), which likely resulted from a demographic response of the lamprey populations to reductions in their overall abundance (Jones et al. 2003). Understanding the ecology of larval and metamorphosing lampreys has been critical for control of the invasive sea lamprey in the Great Lakes, and is becoming increasingly more important for the management of many threatened and endangered lamprey species worldwide.

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References

Adams CE, Bissett N, Newton J, Maitland PS (2008) Alternative migration and host parasitism strategies and their long-term stability in river lampreys from the River Endrick, Scotland. J Fish Biol 72:2456–2466

Allen JRL (1984) Sedimentary structures-their character and physical basis, vol 1. Elsevier, Amsterdam

Almeida PR, Quintella BR (2002) Larval habitat of the sea lamprey (*Petromyzon marinus* L.) in the River Mondego (Portugal). In: Collares-Pereira MJ, Coelho MM, Cowx IG (eds) Freshwater fish conservation: options for the future. Fishing News Books, Blackwell, Oxford

Almeida PR, Silva HT, Quintella BR (2000) The migratory behavior of the sea lamprey *Petromyzon marinus* L., observed by acoustic telemetry in River Mondego (Portugal). In: Moore A, Russel I (eds) Advances in fish telemetry. CEFAS, Suffolk

Almeida PR, Quintella BR, Dias NM, Andrade N (2002a) The anadromous sea lamprey in Portugal: biology and conservation perspectives. Proceedings of the international congress on the biology of fish: the biology of lampreys, Vancouver, pp 49–58

Almeida PR, Silva HT, Quintella BR (2002b) The spawning migration of the sea lamprey (*Petromyzon marinus* L.), in the River Mondego (Portugal). In: Pardal MA, Marques JC, Graça MA (eds) Aquatic ecology of the Mondego river basin. Global importance of local experience. Imprensa da Universidade de Coimbra, Coimbra, pp 381–386

Almeida PR, Paulo-Martins C, Andrade NO, Quintella BR (2005) Influence of the light-dark cycle in the diel activity rhythms of sea lamprey's ammocoetes. In: Spedicato MT, Lembo G, Marmulla G (eds) Aquatic telemetry: advances and applications. FAO/COISPA, Rome, pp 225–230

Almeida PR, Tomaz G, Andrade NO, Quintella BR (2008) Morphological analysis of geographic variation of sea lamprey ammocoetes in Portuguese river basins. Hydrobiologia 602:47–59

Almeida PR, Maia C, Quintella BR et al (2011) National plan to conserve European River and Brook lampreys in Portugal. Final report. EDP-Energias de Portugal, S.A., Lisbon, Portugal
Andersen HB, Caldwell RS, Toll J, Do T, Saban L (2010) Sensitivity of lamprey ammocoetes to six chemicals. Arch Environ Contam Toxicol 59:622–631
Antolos M, Roby DD, Lyons DE et al (2005) Caspian tern predation on juvenile salmonids in the Mid-Columbia River. Trans Am Fish Soc 134:466–480
Applegate VC (1950) Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. US Fish and Wildlife Service Special Scientific Report 55, Washington, DC
Barker LA, Morrison BJ, Wicks BJ, Beamish FWH (1997) Age discrimination and statolith diversity in sea lamprey from streams with varying alkalinity. Trans Am Fish Soc 126:1021–1026
Bartels H, Potter IC (2004) Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys. Implications for osmoregulation in fresh and seawater. J Exp Biol 207:3447–3462
Bartels H, Fazekas U, Youson JH, Potter IC (2011) Changes in the cellular composition of the gill epithelium during the life cycle of a nonparasitic lamprey: functional and evolutionary implications. Can J Zool 89:538–545
Baxter EW (1954) Studies on the biology of lampreys. PhD thesis, University of London, London
Baxter EW (1957) Lamprey distribution in streams and rivers. Nature 180:1145
Beamish FWH (1982) Biology of the southern brook lamprey, *Ichthyomyzon gagei*. Environ Biol Fish 7:302–320
Beamish FWH (1993) Environmental sex determination in southern brook lamprey, *Ichthyomyzon gagei*. Can J Fish Aquat Sci 50:1299–1307
Beamish FWH, Austin LS (1985) Growth of the mountain brook lamprey, *Ichthyomyzon greeleyi*. Hubbs and Trautman. Copeia 1985:881–890
Beamish FWH, Jebbink JA (1994) Abundance of lamprey larvae and physical habitat. Environ Biol Fish 39:209–214
Beamish FWH, Lowartz S (1996) Larval habitat of American brook lamprey. Can J Fish Aquat Sci 53:693–700
Beamish FWH, Medland TE (1988a) Metamorphosis of the mountain brook lamprey *Ichthyomyzon greeleyi*. Environ Biol Fish 23:45–54
Beamish FWH, Medland TE (1988b) Age determination for lampreys. Trans Am Fish Soc 117:63–71
Beamish FWH, Potter IC (1975) The biology of the anadromous sea lamprey (*Petromyzon marinus*) in New Brunswick. J Zool 177:57–72
Beamish FWH, Thomas EJ (1984) Metamorphosis of the southern brook lamprey, *Ichthyomyzon gagei*. Copeia 1984:502–515
Beamish FWH, Strachan PD, Thomas E (1978) Osmotic and ionic performance of the anadromous sea lamprey, *Petromyzon marinus*. Comp Biochem Physiol 60A:435–443
Beamish RJ (1982) Lampetra macrostoma, a new species of freshwater parasitic lamprey from the west coast of Canada. Can J Fish Aquat Sci 39:736–747
Beamish RJ, Levings CD (1991) Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata,* in a tributary of the Fraser River, British Columbia. Can J Fish Aquat Sci 48:1250–1263
Beamish RJ, Northcote TJ (1989) Extinction of a population of anadromous parasitic lamprey, *Lampetra tridentata*, upstream of an impassable dam. Can J Fish Aquat Sci 46:420–425
Beamish RJ, Wade J (2008) Critical habitat and the conservation ecology of the freshwater parasitic lamprey, *Lampetra macrostoma*. Can Field Nat 122:327–337
Beamish RJ, Youson JH (1987) Life history and abundance of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the Strait of Georgia. Can J Fish Aquat Sci 44:525–537
Bergstedt RA, Genovese JH (1994) New techniques for sampling sea lamprey larvae in deepwater habitats. N Am J Fish Manag 14:449–452

Bergstedt RA, McDonald RB, Twohey MB, Mullett KM, Young RJ, Heinrich JW (2003) Reduction in sea lamprey hatching success due to release of sterilized males. J Great Lakes Res 29(Suppl 1):435–444

Bettaso JB, Goodman DH (2010) A comparison of mercury contamination in mussel and ammocoete filter feeders. J Fish Wildl Manag 1:142–145

Bird DJ, Potter IC (1979) Metamorphosis in paired species of lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). 1. A description of the timing and stages. J Linn Soc Lond Zool 65:127–143

Bird DJ, Potter IC (1981) Proximate body composition of the larval, metamorphosing and downstream migrant stages in the life cycle of the Southern Hemisphere lamprey, *Geotria australis*. Environ Biol Fish 6:285–297

Bird DJ, Potter IC, Hardisty MW, Baker BI (1994) Morphology, body size and behavior of recently-metamorphosed sea lampreys, *Petromyzon marinus*, from the lower River Severn, and their relevance to the onset of parasitic feeding. J Fish Biol 44:67–74

Bracken F, Lucas M (2013) Potential impacts of small-scale hydroelectric power generation on downstream moving lampreys. River Res Appl 29:1073–1081

Bradford MJ, Duncan J, Jang JW (2008) Downstream migrations of juvenile salmon and other fishes in the Upper Yukon River. Arctic 61:255–264

Brazner JC, Campana SE, Tanner DK (2004) Habitat fingerprints for Lake Superior coastal wetlands derived from elemental analysis of yellow perch otoliths. Trans Am Fish Soc 133:692–704

Brothers E, Thresher R (2004) Statolith chemical analysis as a means of identifying stream origins of lampreys in Lake Huron. Trans Am Fish Soc 133:1107–1116

Brown LR, Moyle PB (1993) Distribution, ecology, and status of the fishes of the San Joaquin River drainage, California. Calif Fish Game 79:96–114

Brumo AF (2006) Spawning, larval recruitment, and early life survival of Pacific lamprey in the South Fork Coquille River, Oregon. MS thesis, Oregon State University, Corvallis, OR

Christie GC, Adams JV, Steeves TB et al (2003) Selecting Great Lakes streams for lampricide treatment based on larval sea lamprey surveys. J Great Lakes Res 29(Suppl 1):152–160

Churchill WS (1945) The brook lamprey in the Brule River. Trans Wisc Acad Sci Arts Lett 37:337–346

Claire CW, Cochnauer TG, LaBar GW (2007) Pacific lamprey ammocoete habitat utilization in Red River, Idaho. In: Brouder MJ, Scheurer JA (eds) Status, distribution, and conservation of freshwater fishes of western North America: a symposium proceedings. American Fisheries Society, Symposium 53, Bethesda, pp 151–161

Clarke WC, Beamish RJ (1988) Response of recently metamorphosed anadromous parasitic lamprey (*Lampetra tridentata*) to confinement in fresh water. Can J Fish Aquat Sci 45:42–47

Cochran PA (2009) Predation on lampreys. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 139–151

Cochran PA, Lyons J (2004) Field and laboratory observations on the ecology and behavior of the silver lamprey (*Ichthyomyzon unicuspis*) in Wisconsin. J Freshw Ecol 19:245–253

Cochran PA, Lyons J, Merino-Nambo E (1996) Notes on the biology of the Mexican lampreys *Lampetra spadicea* and *L. geminis* (Agnatha: Petromyzontidae). Ichthyol Explor Freshw 7:173–180

Colotelo AH, Pflugrath BD, Brown RS et al (2012) The effect of rapid and sustained decompression on barotrauma in juvenile brook lamprey and Pacific lamprey: implications for passage at hydroelectric facilities. Fish Res 129:17–20

Columbia Basin Fishery Agencies and Tribes (2014) Fish Passage Center. http://www.fpc.org/lamprey/lamprey_home.html. Accessed 27 Feb 2014

Columbia River Inter-Tribal Fish Commission (2008) Tribal Pacific Lamprey Restoration Plan for the Columbia River Basin. http://www.critfc.org/text/lamprey/restor_plan.pdf. Accessed 1 Feb 2010

Columbia River Inter-Tribal Fish Commission (2011) Tribal Pacific Lamprey Restoration Plan for the Columbia River Basin. http://www.critfc.org/wp-content/uploads/2012/12/lamprey_plan.pdf. Accessed 24 April 2014

Dauble DD, Moursund RA, Bleich MD (2006) Swimming behavior of juvenile Pacific lamprey, *Lampetra tridentata*. Environ Biol Fish 75:167–171

Dawson HA, Jones ML (2009) Factors affecting recruitment dynamics of Great Lakes sea lamprey (*Petromyzon marinus*) populations. J Great Lakes Res 35:353–360

Dawson HA, Jones ML, Scribner KT, Gilmore SA (2009) An assessment of age determination methods for Great Lakes larval sea lampreys. N Am J Fish Manag 29:914–927

Dean B, Sumner FB (1898) Notes on the spawning habits of the brook lamprey (*Petromyzon wilderi*). Trans N Y Acad Sci 16:321–324

Dendy JS, Scott DC (1953) Distribution, life history, and morphological variations of the southern brook lamprey, *Ichthyomyzon gagei*. Copeia 1953:152–162

Derosier AL, Jones ML, Scribner, KT (2007) Dispersal of sea lamprey larvae during early life: relevance for recruitment dynamics. Environ Biol Fish 78:271–284

Docker MF (1992) Labile sex determination in lampreys: the effect of larval density and sex steroids on gonadal differentiation. PhD thesis, University of Guelph, Guelph, ON

Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 71–114

Docker MF, Beamish FWH (1994) Age, growth, and sex ratio among populations of least brook lamprey, *Lampetra aepyptera*, larvae: an argument for environmental sex determination. Environ Biol Fish 41:191–205

Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Docker MF, Beamish FWH, Bryan MB, Spice EK (in press) The lamprey gonad. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Dunham JB, Chelgren ND, Heck MP, Clark SM (2013) Comparison of electrofishing techniques to detect larval lampreys in wadeable streams in the Pacific Northwest. N Am J Fish Manag 33:1149–1155

Farlinger SP, Beamish RJ (1984) Recent colonization of a major salmon-producing lake in British Columbia by the Pacific lamprey (*Lampetra tridentata*). Can J Fish Aquat Sci 41:278–285

Ferreira AF, Quintella BR, Maia C et al (2013) Influence of macrohabitat preferences on the distribution of European brook and river lampreys: implications for conservation and management. Biol Conserv 159:175–186

Fish Passage Center (2013) Fish Passage Center annual report, 2012. http://www.fpc.org/documents/FPC_Annual_Reports.html. Accessed 24 April 2014

Fodale MF, Bronte CR, Bergstedt RA, Cuddy DW, Adams JV (2003) Classification of lentic habitat for sea lamprey (*Petromyzon marinus*) larvae using a remote seabed classification device. J Great Lakes Res 29:190–203

Fortin C, Ouellet M, Cartier I, Banville D, Renaud C (2007) Biologie et situation de la lamproie du nord, *Ichthyomyzon fossor*, au Québec. Can Field Nat 121:402–411

Gadomski DM, Barfoot CA (1998) Diel and distributional abundance patterns of fish embryos and larvae in the lower Columbia and Deschutes rivers. Environ Biol Fish 51:353–368

Goodwin CE, Dick JTA, Rogowski DL, Elwood RW (2008) Lamprey (*Lampetra fluviatilis* and *Lampetra planeri*) ammocoete habitat associations at regional, catchment and microhabitat scales in Northern Ireland. Ecol Freshw Fish 17:542–553

Gradín FC (2010) Estado de conservación das poboacións de lamprea mariña en ríos de Galicia. Xunta de Galicia. Consellería do Medio Rural. Dirección Xeral de Conservación da Natureza, Galicia

Griffiths RW, Beamish FWH, Morrison BJ, Barker LA (2001) Factors affecting larval sea lamprey growth and length at metamorphosis in lampricide-treated streams. Trans Am Fish Soc 130:289–306

Gunckel SL, Jones KK, Jacobs SE (2009) Spawning distribution and habitat use of adult Pacific and western brook lampreys in Smith River, Oregon. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 173–189

Hammond RJ (1979) Larval biology of the Pacific lamprey, *Entosphenus tridentatus* (Gairdner) of the Potlatch River, Idaho. MS thesis, University of Idaho, Moscow

Hand CP, Ludsin SA, Fryer BJ, Marsden JE (2008) Statolith microchemistry as a technique for discriminating among Great Lakes sea lamprey (*Petromyzon marinus*) spawning tributaries. Can J Fish Aquat Sci. 65:1153–1164

Hansen MJ, Hayne DW (1962) Sea lamprey larvae in Ogontz Bay and Ogontz River, Michigan. J Wildl Manag 26:237–247

Hansen, GJA, Jones ML (2009) Variation in larval sea lamprey demographics among Great Lakes tributaries: a mixed-effects model analysis of historical survey data. J Great Lakes Res 35:591–602

Hansen MJ, Adams JV, Cuddy DW et al (2003) Optimizing larval assessment to support sea lamprey control in the Great Lakes. J Great Lakes Res 29(Suppl 1):113–129

Hardisty MW (1944) The life history and growth of the brook lamprey. (*Lampetra planeri*). J Anim Ecol 13:110–122

Hardisty MW (1956) Some aspects of osmotic regulation in lampreys. J Exp Biol 33:431–447

Hardisty MW (1960) Sex ratios of ammocoetes. Nature 186:988–989

Hardisty MW (1961a) Studies on an isolated spawning population of brook lampreys (*Lampetra planeri*). J Anim Ecol 30:339–355

Hardisty MW (1961b) The growth of larval lampreys. J Anim Ecol 30:357–371

Hardisty MW (1969) Information on the growth of the ammocoete larva of the anadromous sea lamprey, *Petromyzon marinus* in British rivers. J Zool 159:139–144

Hardisty MW (1970) The relationship of gonadal development to the life cycles of the paired species of lamprey, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). J Fish Biol 2:173–181

Hardisty MW (1979) Biology of the cyclostomes. Chapman and Hall, London

Hardisty MW (1986) General introduction. In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part I, (*Petromyzontiformes*). AULA, Wiesbaden, pp 19–83

Hardisty MW, Huggins RJ (1970) Larval growth in the river lamprey, *Lampetra fluviatilis*. J Zool 161:549–559

Hardisty MW, Potter IC (1971a) The behavior, ecology and growth of larval lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 85–125

Hardisty MW, Potter IC (1971b) The general biology of adult lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 127–206

Hardisty MW, Potter IC, Sturge R (1970) A comparison of the metamorphosing and macrophthalmia stages of the lampreys, *Lampetra fluviatilis* and *L. planeri*. J Zool 162:383–400

Harvey BC (1991) Interaction of abiotic and biotic factors influences larval fish survival in an Oklahoma stream. Can J Fish Aquat Sci 48:1476–1480

Harvey J, Cowx I (2003) Monitoring the river, brook, and sea lamprey, *Lampetra fluviatilis*, *L. planeri*, and *Petromyzon marinus*. Conserving Natura 2000 Rivers. Conservation Techniques Series No 5 English Nature, Peterborough. http://www2.hull.ac.uk/discover/pdf/lamprey_monitoring.pdf. Accessed 5 Sept 2011

Heard WR (1966) Observations on lampreys in the Naknek River system of Southwest Alaska. Copeia 1966:332–339

Heinrich JW, Weise JG, Smith BR (1980) Changes in the biological characteristics of the sea lamprey (*Petromyzon marinus*) as related to lamprey abundance, prey abundance, and sea lamprey control. Can J Fish Aquat Sci 37:1861–1871

Heinrich JW, Mullett KM, Hansen MJ et al (2003) Sea lamprey abundance and management in Lake Superior, 1957 to 1999. J Great Lakes Res 29(Suppl 1):566–583

Hill BJ, Potter IC (1970) Oxygen consumption in ammocoetes of the lamprey *Ichthyomyzon hubbsi* Raney. J Exp Biol 53:47–57
Hollett AK (1998) Condition factor and statolith aging in assessment of metamorphosis in sea lampreys (*Petromyzon marinus*), in the Great Lakes. MSc thesis, University of Toronto, Toronto
Holmes JA (1990) Sea lamprey as an early responder to climate change in the Great Lakes basin. Trans Am Fish Soc 119:292–300
Holmes JA, Lin P (1994) Thermal niche of larval sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 53:253–262
Holmes JA, Youson JH (1994) Fall condition factor and temperature influence the incidence of metamorphosis in sea lampreys, *Petromyzon marinus*. Can J Zool 72:1134–1140
Holmes JA, Youson JH (1997) Laboratory study of the effects of spring warming and larval density on the metamorphosis of sea lampreys. Trans Am Fish Soc 126:647–657
Holmes JA, Youson JH (1998) Extreme and optimal temperatures for metamorphosis in sea lampreys. Trans Am Fish Soc 127:206–211
Holmes JA, Chu H, Khanam S, Manzon R, Youson JH (1999) Spontaneous and induced metamorphosis in the American brook lamprey, *Lampetra appendix*. Can J Zool 77:959–971
Irwin BJ, Liu W, Bence JR, Jones ML (2012) Defining economic injury levels for sea lamprey control in the Great Lakes basin. N Am J Fish Manag 32:760–771
Jellyman DH, Glova GJ (2002) Habitat use by juvenile lampreys (*Geotria australis*) in a large New Zealand river. N Z J Mar Fresh Res 36:503–510
Johnson NS, Swink WD, Brenden TO, Slade JW, Steeves TB, Fodale MF, Jones ML (2014) Survival and metamorphosis of low-density populations of larval sea lampreys (*Petromyzon marinus*) in streams following lampricide treatment. J Great Lakes Res 40:155–163
Jolley JC, Silver GS, Whitesel TA (2012) Occupancy and detection of larval Pacific lampreys and *Lampetra* spp. in a large river: the Lower Willamette River. Trans Am Fish Soc 141:305–312
Jolley JC, Silver GS, Whitesel TA (2013) Occurrence, detection, and habitat use of larval lamprey in the Lower White Salmon River and mouth: post-Condit Dam removal. US Fish Wildl Serv, Columbia River Fisheries Program Office, 2012 Annual Report, Vancouver, WA, 22 pp
Jones ML (2007) Toward improved assessment of sea lamprey population dynamics in support of cost-effective sea lamprey management. J Great Lakes Res 33(Spec Iss 2):35–47
Jones ML, Bergstedt RA, Twohey MB et al (2003) Compensatory mechanisms in Great Lakes sea lamprey populations: implications for alternative control strategies. J Great Lakes Res 29(Suppl 1):113–129
Jones ML, Irwin BJ, Hansen GJA et al (2009) An operating model for the integrated pest management of Great Lakes sea lampreys. Open Fish Sci J 2:59–73
Kainua K, Valtonen T (1980) Distribution and abundance of European river lamprey (*Lampetra fluviatilis*) larvae in three rivers running into Bothnian Bay, Finland. Can J Fish Aquat Sci 37:1960–1966
Kan TT (1975) Systematics, variation, distribution, and biology of lampreys of the genus *Lampetra* in Oregon. PhD thesis, Oregon State University, Corvallis, OR
Kelso JRM, Todd PR (1993) Instream size segregation and density of *Geotria australis* ammocoetes in two New Zealand streams. Ecol Freshw Fish 2:108–115
Kirchhofer A (1995) Concept of conservation for the European brook lamprey (*Lampetra planeri*) in Switzerland. Fischokologie 8:93–108
Knowles FGW (1941) The duration of larval life in ammocoetes and an attempt to accelerate metamorphosis by injections of an anterior-pituitary extract. Proc Zool Soc Lond A 111:101–109
Kucheryavyi AV, Savvaitova KA, Pavlov DS et al (2007) Variations of life history strategy of the Arctic lamprey *Lethenteron camtschaticum* from the Utkholok River (Western Kamchatka). J Ichthyol 47:37–52
Kurath G, Jolley JC, Thompson TM et al (2013) Ammocoetes of Pacific lamprey are not susceptible to common fish rhabdoviruses of the U.S. Pacific Northwest. J Aquat Anim Health 25:274–280
Larson GL, Christie GC, Johnson DA et al (2003) The history of sea lamprey control in Lake Ontario and updated estimates of suppression targets. J Great Lakes Res 29(Suppl 1):637–654

Lavis DS, Hallett A, Koon EM, McAuley TC (2003) History of and advances in barriers as an alternative method to suppress sea lampreys in the Great Lakes. J Great Lakes Res 29(Suppl 1):362–372

Leach WJ (1940) Occurrence and life history of the northern brook lamprey, *Ichthyomyzon fossor*, in Indiana. Copeia 1940:21–34

Lee DS (1989) Proximate determinants of larval lamprey habitat selection. PhD thesis, Michigan State University, East Lansing

Lee DS, Weise JG (1989) Habitat selection of lentic larval lampreys: preliminary analysis based on research with a manned submersible. J Great Lakes Res 15:156–163

Lochet A, Marsden JE, Fryer BJ, Ludsin SA (2013) Instability of statolith elemental signatures revealed in newly metamorphosed sea lamprey (*Petromyzon marinus*). Can J Fish Aquat Sci 70:565–573

Long CW (1968) Diel movement and vertical distribution of juvenile anadromous fish in turbine intakes. Fish Bull 66:599–609

Lorion CM, Markle DF, Reid SB, Docker MF (2000) Redescription of the presumed-extinct Miller Lake lamprey, *Lampetra minima*. Copeia 2000:1019–1028

Lowe DR, Beamish FWH, Potter IC (1973) Changes in the proximate body composition of the landlocked sea lamprey *Petromyzon marinus* (L.) during larval life and metamorphosis. J Fish Biol 5:673–682

Lucas MC, Baras E (2001) Migration of freshwater fishes. Blackwell, Oxford

Ludsin SA, Fryer BJ, Gagnon JE (2006) Comparison of solution-based versus laser ablation inductively coupled plasma mass spectrometry for analysis of larval fish otolith microelemental composition. Trans Am Fish Soc 135:218–231

Luzier CL, Schaller HA, Bostrom JK et al (2011) Pacific lamprey (*Entosphenus tridentatus*) assessment and template for conservation measures. US Fish and Wildlife Service, Portland

Macey DJ, Potter IC (1978) Lethal temperatures of ammocoetes of the Southern Hemisphere lamprey, *Geotria australis* Gray. Environ Biol Fish 3:241–243

Maitland PS (2003) Ecology of the river, brook and sea lamprey. Conserving Natura 2000, Rivers Ecology Series No 5. English Nature, Peterborough

Mallatt J (1981) The suspension feeding mechanism of the larval lamprey *Petromyzon marinus*. J Zool 194:103–142

Mallatt J (1983) Laboratory growth of larval lampreys *(Lampetra (Entosphenus) tridentata* Richardson) at different food concentrations and animal densities. J Fish Biol 22:293–301

Malmqvist B (1980) Habitat selection of larval brook lampreys (*Lampetra planeri*, Bloch) in a south Swedish stream. Oecologia 45:35–38

Malmqvist B (1983) Growth, dynamics, and distribution of a population of the brook lamprey *Lampetra planeri* in a south Swedish stream. Holarct Ecol 6:404–412

Manion PJ (1968) Production of sea lamprey larvae from nests in two Lake Superior streams. Trans Am Fish Soc 97:484–486

Manion PJ (1969) Evaluation of lamprey larvicides in the Big Garlic River and Saux Head Lake. J Fish Res Board Can 26:3077–3082

Manion PJ, McLain AL (1971) Biology of larval sea lampreys (*Petromyzon marinus*) of the 1960 year class, isolated in the Big Garlic River, Michigan 1960–65. Great Lakes Fishery Commission, Technical Report 16, Ann Arbor

Manion PJ, Smith BR (1978) Biology of larval and metamorphosing sea lampreys, *Petromyzon marinus*, of the 1960 year class, isolated in the Big Garlic River, Michigan Part II, 1966–72. Great Lakes Fishery Commission, Technical Report 30, Ann Arbor

Manion PJ, Stauffer TM (1970) Metamorphosis of the landlocked sea lamprey, *Petromyzon marinus*. J Fish Res Board Can 27:1735–1746

Marsden JE, Siefkes MJ (in press) Sea lamprey control in the Great Lakes, Finger Lakes, and Lake Champlain. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Mateus CS, Rodríguez-Muñoz R, Quintella BR, Alves MJ, Almeida PR (2012) Lampreys of the Iberian Peninsula: distribution, population status and conservation. Endang Spec Res 16:183–198

Mathers JS, Beamish FWH (1974) Changes in serum osmotic and ionic concentration in landlocked *Petromyzon marinus*. Comp Biochem Physiol 49A:677–688

McGree M, Whitesel TA, Stone J (2008) Larval metamorphosis of individual Pacific lampreys reared in captivity. Trans Am Fish Soc 137:1866–1878

Meckley TD, Wagner CM, Luehring MA (2012) Field evaluation of larval odor and mixtures of synthetic pheromone components for attracting migrating sea lampreys in rivers. J Chem Ecol 38:1062–1069

Meeuwig MH, Bayer JM (2005) Morphology and aging precision of statoliths from larvae of Columbia River Basin lampreys. N Am J Fish Manag 25:38–48

Meeuwig MH, Bayer JM, Seelye JG (2005) Effects of temperature on survival and development of early life stage Pacific and western brook lampreys. Trans Am Fish Soc 134:19–27

Milliman JD, Qin YS, Ren ME, Saito Y (1987) Man's influence on the erosion and transport of sediment by Asian Rivers: the Yellow River (Huanghe) example. J Geol 95:751–762

Moore JW, Beamish FWH (1973) Food of larval sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). J Fish Res Board Can 30:7–15

Moore JW, Mallatt J (1980) Feeding of larval lamprey. Can J Fish Aquat Sci 37:1658–1664

Moore JW, Potter IC (1976a) A laboratory study on the feeding of the brook lamprey *Lampetra planeri* (Bloch). J Anim Ecol 45:81–90

Moore JW, Potter IC (1976b) Aspects of feeding and lipid deposition and utilization in the lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). J Anim Ecol 45:699–712

Moore HH, Schleen LP (1980) Changes in spawning runs of sea lamprey (*Petromyzon marinus*) in selected streams of Lake Superior after chemical control. Can J Fish Aquat Sci 37:1851–1860

Morkert SB, Swink WD, Seelye JG (1998) Evidence for early metamorphosis of sea lampreys in the Chippewa River, Michigan. N Am J Fish Manag 18:966–971

Morman RH (1987) Relationship of density to growth and metamorphosis of caged larval sea lampreys, *Petromyzon marinus* Linnaeus, in Michigan streams. J Fish Biol 30:173–181

Morman RH, Cuddy DW, Rugen PC (1980) Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:1811–1826

Morris R (1980) Blood composition and osmoregulation in ammocoete larvae. Can J Fish Aquat Sci 37:1665–1679

Morse TJ, Ebener MP, Koon EM et al (2003) A case history of sea lamprey control in Lake Huron: 1979 to 1999. J Great Lakes Res 29(Suppl 1):599–614

Moser ML, Close DA (2003) Assessing Pacific lamprey status in the Columbia River basin. Northwest Sci 77:116–125

Moursund RA, Dauble DD, Langeslay M (2003) Turbine intake diversion screens: investigating effects on Pacific lamprey. Hydro Rev 22:40–46

Mueller R, Arntzen E, Nabalek M et al (2012) Laboratory testing of a modified electrofishing system designed for deepwater juvenile lamprey sampling. Trans Am Fish Soc 141:841–845

Mullett KM (1997) Agreement of observers classifying larval sea lamprey (*Petromyzon marinus*) habitat. MS thesis, Northern Michigan University, Marquette

Mundahl ND, Erickson C, Johnston MR, Sayeed GA, Taubel S (2005) Diet, feeding rate, and assimilation efficiency of American brook lamprey larvae. Environ Biol Fish 72:67–72

Mundahl ND, Sayeed G, Taubel S et al (2006) Densities and habitat of American brook lamprey (*Lampetra appendix*) larvae in Minnesota. Am Midl Nat 156:11–22

Murdoch SP, Beamish FWH, Docker MF (1991) Laboratory study of growth and interspecific competition in larval lampreys. Trans Am Fish Soc 120:653–656

Murdoch SP, Docker MF, Beamish FWH (1992) Effect of density and individual variation on growth of sea lamprey *(Petromyzon marinus)* larvae in the laboratory. Can J Zool 70:184–188

Myllynen K, Ojutkangas E, Nikinmaa M (1997) River water with high iron concentration and low pH causes mortality of lamprey roe and newly hatched larvae. Ecotoxicol Environ Saf 36:43–48

Naesje TF, Johnson B, Sandlund OT (1986) Drift of cisco and whitefish larvae in a Norwegian river. Trans Am Fish Soc 115:89–93
Nakamoto RJ, Harvey BC (2003) Spatial, seasonal, and size-dependent variation in the diet of Sacramento pikeminnow in the Eel River, Northwestern California. Calif Fish Game 89:30–45
Neeson TM, Koonce JF, Whiting PJ (2007) Predicting sea lamprey (*Petromyzon marinus*) ammocoete habitat using Geographic Information Systems. J Great Lakes Res 33:546–553
Neeson TM, Wiley MJ, Adlerstein SA, Riolo RL (2011) River network structure shapes interannual feedbacks between adult sea lamprey migration and larval habitation. Ecol Model 222:3181–3192
Neeson TM, Adlerstein SA, Wiley MJ (2012a) Towards a process domain-sensitive substrate habitat model for sea lampreys in Michigan rivers. Trans Am Fish Soc 141:313–326
Neeson TM, Wiley MJ, Adlerstein SA, Riolo RL (2012b) How river network structure and habitat availability shape the spatial dynamics of larval sea lampreys. Ecol Model 226:62–70
Nursall JR, Buchwald D (1972) Life history and distribution of the arctic lamprey (*Lethenteron japonicum* (Martens)) of Great Slave Lake, N.W.T. J Fish Res Board Can, Tech Rep 304
O'Boyle RN, Beamish FWH (1977) Growth and intermediary metabolism of larval and metamorphosing stages of the landlocked sea lamprey *Petromyzon marinus* (L.). Environ Biol Fish 2:103–120
O'Connor W (2004) A survey of juvenile lamprey populations in the Moy Catchment. Ir Wildl Man 15:1–36
O'Connor W (2006) A survey of juvenile lamprey populations in the Boyne catchment. Ir Wildl Man 24:1–45
Ojutkangas E, Aronen K, Laukkanen E (1995) Distribution and abundance of river lamprey (*Lampetra fluviatilis*) ammocoetes in the regulated river Perhonjoki. Regul River 10:239–245
Piavis GW (1961) Embryological stages in the sea lamprey and effects of temperature on development. US Fish Wildl Serv Fish Bull 61:111–143
Poe TP, Hansel HC, Vigg S, Palmer DE, Prendergast LA (1991) Feeding of predaceous fishes on out-migrating juvenile salmonids in John Day Reservoir, Columbia River. Trans Am Fish Soc 120:405–420
Poltorykhina AN (1971) Metamorphosis of the Arctic brook lamprey (*Lampetra japonica kessleri* [Anikin]) in upper Irtysh. J Ichthyol 11:281–285
Potter IC (1970) The life cycles and ecology of Australian lampreys of the genus *Mordacia*. J Zool 161:487–511
Potter IC (1980a) Ecology of larval and metamorphosing lampreys. Can J Fish Aquat Sci 37:1641–1657
Potter IC (1980b) The Petromyzoniformes with particular reference to paired species. Can J Fish Aquat Sci 37:1595–1615
Potter IC, Bailey JR (1972) The life cycle of the Tennessee brook lamprey, *Ichthyomyzon hubbsi* Copeia 1972:470–476
Potter IC, Beamish FWH (1975) Lethal temperatures in ammocoetes of four species of lamprey. Acta Zool 56:85–91
Potter IC, Beamish FWH (1977) The freshwater biology of adult anadromous sea lampreys *Petromyzon marinus*. J Zool 181:113–130
Potter IC, Brown ID (1975) Changes in haemoglobin electropherograms during the life cycle of two closely related lampreys. Comp Biochem Physiol 51B:517–519
Potter IC, Hilliard RW (1986) Growth and the average duration of larval life in the southern hemisphere lamprey, *Geotria australis* Gray. Experientia 42:1170–1173
Potter IC, Huggins RJ (1973) Observations on the morphology, behavior and salinity tolerance of downstream migrating river lamprey (*Lampetra fluviatilis*). J Zool 169:365–379
Potter IC, Rogers MJ (1972) Oxygen consumption in burrowed and unburrowed ammocoetes of *Lampetra planeri*. Comp Biochem Physiol 41:427–432
Potter IC, Hill BJ, Gentleman S (1970) Survival and behavior of ammocoetes at low oxygen tensions. J Exp Biol 53:59–73

Potter IC, Wright GM, Youson JH (1978) Metamorphosis in the anadromous sea lamprey, *Petromyzon marinus* L. Can J Zool 56:561–570
Potter IC, Hilliard RW, Bird DJ (1980) Metamorphosis in the Southern Hemisphere lamprey, *Geotria australis* Gray. J Zool 190:405–430
Potter IC, Hilliard RW, Bird DJ (1982) Stages in metamorphosis. In: Hardisty MW, Potter IC (eds) The biology of lampreys. Academic Press, London, pp 137–164
Potter IC, Hilliard RW, Bradley JS, McKay RJ (1986) The influence of environmental variables on the density of larval lampreys in different seasons. Oecologia 70:433–440
Purvis HA (1970) Growth, age at metamorphosis and sex ratio of northern brook lampreys in a tributary of southern Lake Superior. Copeia 1970:326–332
Purvis HA (1979) Variation in growth, age at transformation, and sex ratio of sea lampreys re-established in chemically treated tributaries of the Upper Great Lakes. Great Lakes Fishery Commission, Technical Report 35, Ann Arbor
Purvis HA (1980) Effects of temperature on metamorphosis and the age and length at metamorphosis in sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:1827–1834
Quintella BR (2000) Ecology of the sea lamprey (*Petromyzon marinus* L.) larval phase in the River Mondego. BSc thesis, University of Lisbon, Portugal
Quintella BR (2006) Biology and conservation of sea lamprey (*Petromyzon marinus* L.) in Portugal. PhD thesis, University of Lisbon, Portugal
Quintella BR, Andrade NO, Almeida PR (2003) Distribution, larval stage duration and growth of the sea lamprey ammocoetes, *Petromyzon marinus* L., in a highly modified river basin. Ecol Freshw Fish 12:1–8
Quintella BR, Andrade NO, Espanhol R, Almeida PR (2005) The use of PIT telemetry to study movements of ammocoetes and metamorphosing sea lampreys in river beds. J Fish Biol 66:97–106
Quintella BR, Andrade NO, Dias NM, Almeida PR (2007) Laboratory assessment of sea lamprey larvae burrowing performance. Ecol Freshw Fish 16:177–182
Reighard J, Cummins H (1916) Description of a new species of lamprey of the genus *Ichthyomyzon*. Occas Pap Mus Zool Univ Mich 31:1–12
Reis-Santos P, McCormick SD, Wilson JM (2008) Ionoregulatory changes during metamorphosis and salinity exposure of juvenile sea lamprey. J Exp Biol 211:978–988
Renaud CB (2011) Lampreys of the world. An annotated and illustrated catalogue of lamprey species known to date. FAO Species Catalogue for Fishery Purposes 5, FAO, Rome
Richards JE, Beamish FWH (1981) Initiation of feeding and salinity tolerance in the Pacific lamprey *Lampetra tridentata*. Mar Biol 63:73–77
Rodríguez-Muñoz R, Nicieza AG, Braña F (2001) Effects of temperature on developmental performance, survival, and growth of sea lamprey embryos. J Fish Biol 58:475–486
Rodríguez-Muñoz R, Nicieza AG, Braña F (2003) Density-dependent growth of sea lamprey larvae: evidence for chemical interference. Funct Ecol 17:403–408
Roni P (2002) Habitat use by fishes and Pacific giant salamanders in small western Oregon and Washington streams. Trans Am Fish Soc 131:743–761
Russell JE (1986) Ecology of the Pacific lamprey (*Lampetra tridentata*) colonizing Babine Lake, British Columbia, Canada. MSc thesis, University of Guelph, ON
Russell JE, Beamish FWH, Beamish RJ (1987) Lentic spawning by the Pacific lamprey, *Lampetra tridentata*. Can J Fish Aquat Sci 44:476–478
Seagel HH, Nagel JW (1982) Life cycle and fecundity of the American brook lamprey, *Lampetra appendix*, in Tennessee. Copeia 1982:362–366
Seversmith HF (1953) Distribution, morphology, and life history of *Lampetra aepyptera*, a brook lamprey, in Maryland. Copeia 1953:225–232
Shirakawa H, Yanai S, Kouchi K (2009) Habitat selection of fluvial lamprey larvae *Lethenteron japonicum* change with growth stage. Ecol Civil Eng 12:87–98
Shirakawa H, Yanai S, Goto A (2013) Lamprey larvae as ecosystem engineers: physical and geochemical impact on the streambed by their burrowing behavior. Hydrobiologia 701:313–322

Silver GS, Luzier CW, Whitesel TA (2008) Investigation of larval Pacific lamprey habitat use in the mainstem Columbia River and Willamette River. US Fish and Wildlife Service, Columbia River Fisheries Program Office, 2007 Annual Report, Vancouver

Slade JW, Adams JV, Christie GC et al (2003) Techniques and methods for estimating abundance of larval and metamorphosed sea Lampreys in Great Lakes tributaries, 1995 to 2001. J Great Lakes Res 29(Suppl 1):137–151

Smith BR (1971) Sea lampreys in the Great Lakes of North America. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, New York, pp 207–247

Smith DM (2009) Habitat selection and predation risk in larval lampreys. MS thesis, West Virginia University, Morgantown

Smith SJ, Marsden JE (2009) Factors affecting sea lamprey (*Petromyzon marinus*) egg survival. N Am J Fish Manag 29:859–868

Smith BR, McLain AL (1962) Estimation of the brook and sea lamprey ammocete populations of three streams. Great Lakes Fishery Commission, Technical Report 4, Ann Arbor

Smith BR, Tibbles JJ (1980) Se lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936–1978. Can J Fish Aquat Sci 37:1780–1801

Smith DM, Welsh SA, Turk PJ (2011) Selection and preference of benthic habitat by small and large ammocoetes of the least brook lamprey (*Lampetra aepyptera*). Environ Biol Fish 91:421–428

Smith DM, Welsh SA, Turk PJ (2012) Available benthic habitat type may influence predation risk in larval lampreys. Ecol Freshw Fish 21:160–163

Steeves TB, Slade JW, Fodale MF, Cuddy DW, Jones ML (2003) Effectiveness of using backpack electroshocking gear for collecting sea lamprey (*Petromyzon marinus*) larvae in Great Lakes tributaries. J Great Lakes Res 29(Suppl 1):161–173

Stone J, Barndt S (2005) Spatial distribution and habitat use of Pacific lamprey (*Lampetra tridentata*) ammocoetes in a western Washington stream. J Freshwat Ecol 20:171–185

Sugiyama H, Goto A (2002) Habitat selection by larvae of a fluvial lamprey, *Lethenteron reissneri*, in a small stream and an experimental aquarium. Ichthyol Res 49:62–68

Sullivan WP (2003) Substrate as a correlate of density and distribution of larval sea lampreys in streams. MSc thesis, University of Guelph, Guelph, ON

Sutphin ZA, Hueth CD (2010) Swimming performance of larval Pacific lamprey (*Lampetra tridentata*). Northwest Sci 84:196–200

Sutton TM, Bowen SH (1994) Significance of organic detritus in the diet of larval lampreys in the Great Lakes basin. Can J Fish Aquat Sci 51:2380–2387

Sutton TM, Bowen SH (2009) Diel feeding by larval northern brook lampreys in two northern Michigan streams. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fishery Society, Symposium 72, Bethesda, pp 153–164

Swink WD (1995) Growth and survival of newly parasitic sea lampreys at representative winter temperatures. Trans Am Fish Soc 124:380–386

Swink WD, Johnson NS (2014) Growth and survival of Sea Lampreys from metamorphosis to spawning in Lake Huron. Trans Am Fish Soc 143:380–386

Taverny C, Lassalle G, Ortusi I et al (2012) From shallow to deep waters: habitats used by larval lampreys (genus *Petromyzon* and *Lampetra*) over a western European basin. Ecol Freshw Fish 21:87–99

Thiel R, Salewski V (2003) Distribution and migration of lampreys in the Elbe estuary (Germany). Limnologica 33:214–226

Thomas MLH (1962) Observations on the ecology of ammocoetes of *Petromyzon marinus* L. and *Entosphenus* (*Lampetra*) *lamottei* (LeSueur) in the Great Lakes watershed. MSc thesis, University of Toronto, ON

Todd PR, Kelso JRM (1993) Distribution, growth and transformation timing of larval *Geotria australis* in New Zealand. Ecol Freshw Fish 2:99–107

Torblaa RL, Westman RW (1980) Ecological impacts of lampricide treatments on sea lamprey (*Petromyzon marinus*) ammocoetes and metamorphosed individuals. Can J Fish Aquat Sci 37:1835–1850

Torgersen CE, Close DA (2004) Influence of habitat heterogeneity on the distribution of larval Pacific lamprey (*Lampetra tridentata*) at two spatial scales. Freshw Biol 49:614–630
Treble AJ (2006) Choosing streams for sea lamprey control: using alternative models of metamorphosis to optimize the stream selection process. MS thesis, Michigan State University, East Lansing
Treble AJ, Jones ML, Steeves TB (2008) Development and evaluation of a new predictive model for metamorphosis of Great Lakes larval sea lamprey (*Petromyzon marinus*) populations. J Great Lakes Res 34:404–417
Tuunainen P, Ikonen E, Auvinen H (1980) Lampreys and lamprey fisheries in Finland. Can J Fish Aquat Sci 37:1953–1959
Van de Wetering SJ, Ewing R (1999) Lethal temperatures for larval Pacific lamprey, *Lampetra tridentata*. Confederated Tribes of the Siletz Indians, Siletz
Vladykov VD, Kott E (1979) Satellite species among the holarctic lampreys (Petromyzonidae). Can J Zool 57:860–867
Volk EC (1986) Use of calcareous otic elements (statoliths) to determine age of sea lamprey ammocoetes (*Petromyzon marinus*). Can J Fish Aquat Sci 43:718–722
Wagner WC, Stauffer TM (1962) Sea lamprey larvae in lentic environments. Trans Am Fish Soc 91:384–387
Ward DL, Clemens BJ, Clugston D et al (2012) Translocating adult Pacific lamprey within the Columbia River Basin: state of the science. Fisheries 37:351–361
Weise JG, Pajos TA (1998) Intraspecific competition between larval sea lamprey year-classes as Salem Creek was recolonized, 1990–1994, after a lampricide application. N Am J Fish Manag 18:561–568
White JL, Harvey BC (2003) Basin-scale patterns in the drift of embryonic and larval fishes and lamprey ammocoetes in two coastal rivers. Environ Biol Fish 67:369–378
Wicks BJ, Barker LA, Morrison BJ, Beamish FWH (1998) Gonadal variation in Great Lakes sea lamprey, *Petromyzon marinus*, larvae. J Great Lakes Res 24:962–968
Wigley RL (1959) Life history of the sea lamprey of Cayuga Lake, New York. US Fish Wildl Serv Fish Bull 59:559–617
Wilkie MP, Bradshaw PG, Joanis V, Claude JF, Swindell SL (2001) Rapid metabolic recovery following vigorous exercise in burrow-dwelling larval sea lampreys (*Petromyzon marinus*). Physiol Biochem Zool 74:261–272
Winberg GG (1956) Rate of metabolism and food requirements of fishes. J Fish Res Board Can Transl Ser 194:1–102
Yamazaki Y (2007) Microhabitat use by the larvae of two cryptic lamprey species in *Lethenteron reissneri* in a sympatric area. Ichthyol Res 54:24–31
Yap MR, Bowen SH (2003) Feeding by northern brook lamprey (*Ichthyomyzon fossor*) on sestonic biofilm fragments: habitat selection results in ingestion of a higher quality diet. J Great Lakes Res 29(Suppl 1):15–25
Young RT, Cole LJ (1900) On the nesting habits of the brook lamprey (*Lampetra wilderi*). Am Nat 34:617–620
Young RJ, Kelso JRM, Weise JG (1990a) Occurrence, relative abundance, and size of landlocked sea lamprey (*Petromyzon marinus*) ammocoetes in relation to stream characteristics in the Great Lakes. Can J Fish Aquat Sci 47:1773–1778
Young RJ, Houston KA, Weise JG, Kelso JRM (1990b) The effect of environmental variables on the population dynamics of sea lamprey, *Petromyzon marinus*. Can Tech Rep Fish Aquat Sci 61:1736
Young RJ, Christie GC, McDonald RB et al (1996) Effects of habitat change in the St. Marys River and northern Lake Huron on sea lamprey (*Petromyzon marinus*) populations. Can J Fish Aquat Sci 53:99–104
Youson JH (2003) The biology of metamorphosis in sea lampreys: endocrine, environmental and physiological cues and events: application to lamprey control. J Great Lakes Res 29(Suppl 1):26–49

Youson JH, Freeman PA (1979) Morphology of gills of larval and parasitic adult sea lamprey, *Petromyzon marinus* L. J Morph 149:73–103
Youson JH, Potter IC (1979) A description of the stages in the metamorphosis of the anadromous sea lamprey, *Petromyzon marinus* L. Can J Zool 57:1808–1817
Youson JH, Holmes JA, Guchardi JA et al (1993) Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 50:2448–2456
Yun SS, Wildbill AJ, Siefkes MJ et al (2011) Identification of putative migratory pheromones from Pacific lamprey (*Lampetra tridentata*). Can J Fish Aquat Sci 68:2194–2203
Zanandrea G (1961) Studies on European lampreys. Evolution 15:523–534
Zerrenner A (2004) Effect of density and age on larval sea lamprey growth and survival in three Lake Champlain streams. J Freshw Ecol 19:515–519
Zerrenner A, Marsden JE (2005) Influence of larval density on sea lamprey transformer life history characteristics. Trans Am Fish Soc 134:687–696
Zerrenner A, Marsden JE (2006) Comparison of larval sea lamprey life history characteristics in a lampricide-treated tributary and untreated tributary system of Lake Champlain. Trans Am Fish Soc 135:1301–1311

Chapter 4
Lamprey Metamorphosis

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Abstract Among vertebrates, true metamorphosis is restricted to amphibians, two groups of bony fishes, and lampreys. This chapter provides a comprehensive review of the ecology, morphology, physiology, and molecular biology of lamprey metamorphosis. The lamprey life cycle includes an embryonic period, a larval period ending with metamorphosis, a parasitic or non-parasitic juvenile period, and an adult reproductive period. Lamprey metamorphosis is influenced by endogenous and exogenous factors, most significantly a rise in spring water temperature, the accumulation of sufficient lipid reserves for the non-trophic metamorphic phase, and thyroid hormones. In lampreys, thyroid hormones appear to have a dual role, whereby high levels promote larval growth and a subsequent sharp decline is important for development and metamorphosis. As with other true metamorphoses, dramatic biochemical, cellular, and morphological changes occur during lamprey metamorphosis. The external changes are striking and include the development of an oral (suctorial) disc and eyes, restructuring of the branchial region, and changes in the fins and body coloration. Internal changes include major modifications to the digestive system (new esophagus, remodeled intestine, and loss of hepatic biliary tree and gall bladder). The larval kidneys regress while the definitive juvenile kidney develops de novo. Numerous changes are also observed in the respiratory and skeletal systems in preparation for the juvenile and spawning periods.

Keywords Condition factor · Hypothalamic-pituitary · Life history · Lipid · Metamorphosis · Temperature · Thyroid hormones

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4.1 Introduction

The jawless fishes (the so-called agnathans) are represented by two extant orders: the Myxiniformes (class Myxini, the hagfishes) and the Petromyzontiformes (class Petromyzontida, the lampreys; Nelson 2006). As the oldest living members of the subphylum Craniata or Vertebrata, hagfishes and lampreys represent great models for understanding vertebrate evolution (see Chap. 1; Lee and McCauley in press) and the phylogenetic relationships between these agnathans and other vertebrates has been, and continues to be, of great interest (e.g., Forey and Janvier 1993, 1994; Delarbre et al. 2002). Most recent molecular data suggest that hagfishes and lampreys form a monophyletic group (Heimberg et al. 2010; Janvier 2010); however, they differ markedly in their life history strategies. Hagfishes are direct developers that reside exclusively in a marine environment. In contrast, there are both freshwater and anadromous lamprey species (see Chap. 2; Docker and Potter in press), all of which are indirect developers that undergo a true metamorphosis prior to sexual maturation. Dawson et al. (see Chap. 3) discussed the ecology of metamorphosing lampreys; the present chapter provides a review of the process of metamorphosis, specifically the morphology, physiology, and molecular and cellular biology of lamprey metamorphosis, with a focus on research that has occurred following the first comprehensive reviews of the topic (Potter 1980; Youson 1980). There are several recent reviews on various aspects of lamprey metamorphosis (e.g., Youson 2003; Youson and Manzon 2012) and this chapter consolidates and supplements this information. Given the breadth of the topic and volume of information, this chapter will provide summaries of topics covered in depth elsewhere and the reader is referred to other reviews on specific aspects of metamorphosis.

4.2 The Significance of Metamorphosis

Indirect development (i.e., with a “true” or first metamorphosis between the dramatically different larval and adult phases; Youson 1988) is relatively rare among vertebrates (but see Laudet 2011). Although it is a developmental strategy seen in a wide variety of invertebrates and one of the two non-vertebrate chordate lineages (i.e., tunicates), in vertebrates, only the life cycles of some amphibians and fishes include a metamorphosis. At least 70% of the more than 6,000 extant amphibian species show indirect development (Pough et al. 2013), but relatively few of the approximately 28,000 described species of fish (Nelson 2006) undergo metamorphosis (Youson 1988, 2004). Apart from lampreys, only two other groups of fishes include a true metamorphosis as part of their life history: the approximately 860 species in the actinopterygian subdivision Elopomorpha (tarpons, ten pounders, true eels) and all 680 species (approximately) in the order Pleuronectiformes (flatfishes) of the subdivision Euteleostei (see Youson 1988; Manzon 2011). However, despite its relative paucity, indirect development is an effective developmental strategy for those vertebrates that employ it as part of their life history. This effectiveness is

demonstrated by the lampreys which are thought to have undergone little change over the past 350 million years (Forey and Janvier 1994; Gess et al. 2006).

4.2.1 Life Cycle and Life History Types

The lamprey life cycle consists of an embryonic period, a larval period which ends with a true metamorphosis, a juvenile period, and an adult (i.e., sexually mature) period. Adult lampreys spawn in freshwater streams where eggs are laid and externally fertilized in nests that the adults make by moving rocks with their oral discs (see Chap. 6). Embryonic development is rapid, with prolarvae hatching and burrowing in the soft sand and silt of the stream bed at 15–17 days post-fertilization (PF). By approximately day 33 PF, exogenous feeding on algae, detritus, diatoms, and desmids begins as the yolk sac is depleted (Piavis 1961; Richardson et al. 2010). Larvae are relatively sedentary within their burrows, but do undergo periodic movements in their natal stream (see Chap. 3). As larvae cannot survive even dilute salt water, they remain outside any tidal influences or brackish water (Beamish et al. 1978; Beamish 1980). However, larvae which reside in streams that empty into freshwater lakes are often found at the mouth and, on occasion, even burrowed in the lake sediments. The length of the larval period varies both between and within species, generally ranging from 2 to 7 years, but can last longer than 12 years (Potter 1980; see Chap. 3). When the appropriate conditions have been met (see Sect. 4.4.2.1), the metamorphic phase of the larval period begins in early to mid-summer (i.e., July–August in Northern Hemisphere species and January–February in Southern Hemisphere species; Potter et al. 1978; Youson 2003). However, timing tends to vary to some extent with temperature and latitude (see Sect. 4.4.1); lampreys at higher latitudes generally begin metamorphosis earlier than those in more southern regions (Beamish and Medland 1988). Metamorphosis is highly synchronized within a population and lasts approximately 4 months (Hardisty 2006; see Chap. 3).

The completion of metamorphosis marks the onset of the juvenile period. Juvenile lampreys use one of two general life history strategies, parasitic or non-parasitic. Of the 41–44 recognized species of lampreys, 23–26 are non-parasitic and 18 are parasitic (see Chaps. 2 and 8). In many instances, parasitic and non-parasitic species are so morphologically and genetically similar that they are grouped as paired species (e.g., European brook and river lampreys, *Lampetra planeri* and *L. fluviatilis*, respectively; see Chap. 2; Docker and Potter in press). In these cases, an ancestral parasitic species is thought to have given rise to the morphotype with the non-parasitic life history (see Youson 2004; Docker 2009).

Non-parasitic lampreys do not feed as juveniles or adults and begin sexual maturation almost immediately after metamorphosis (Docker 2009). In the spring following metamorphosis, non-parasitic lampreys undergo a short migration to the upstream spawning grounds where they spawn and then die (see Chap. 5). Parasitic lampreys, in contrast, undergo a period of feeding on blood, body fluids and/or tissue of a host fish prior to sexual maturation and spawning (see Renaud and Cochran

in press). Parasitic feeding can take place in the natal stream or a large body of water and generally lasts 1–2 years. In the case of anadromous species (e.g., sea lamprey *Petromyzon marinus* and pouched lamprey *Geotria australis*), parasitic juveniles migrate downstream to the ocean to feed where they can travel great distances prior to returning to fresh water for upstream migration, sexual maturation, and spawning. As is the case with non-parasitic lampreys, parasitic lampreys die shortly after spawning. The duration and distance of the spawning migration is species-dependent and varies with life history. The spawning migration can be either very short (1–2 km; e.g., non-parasitic European brook lamprey or American brook lamprey *Lethenteron appendix*) or quite long (1,000–2,000 km; e.g., pouched lamprey; Arctic lamprey *Lethenteron camtschaticum*, and Caspian lamprey *Caspiomyzon wagneri*; see Chap. 5).

4.2.2 Significance of Metamorphosis in Relation to Reproduction and Evolution

When considering animals that undergo an indirect development, one often questions what might have been the selective advantage that led to the evolution of this life history strategy. This question is particularly relevant when considering the fact that organisms are highly vulnerable during the metamorphic phase of their life cycle (see Chap. 3). In most instances, indirect development likely offers a range of selective advantages rather than a single easily defined advantage. Indirect development offers individuals the opportunity to exploit different environments (e.g., aquatic versus terrestrial, pelagic versus benthic) in different ways (e.g., filter versus parasitic feeding). Plasticity in developmental timing also appears to be a significant advantage of indirect development, enabling individuals to continue to exploit the larval ecological niche when conditions are favorable or metamorphose early when conditions are poor (Youson 2004). Extreme examples of such a shift in the relative duration of larval and adult phases are seen in some amphibians, either neoteny or paedomorphosis where individuals reach sexual maturity without undergoing metamorphosis (see Denver et al. 2002; Johnson and Voss 2013) or direct development where the free-living larval stage has been omitted (see Callery et al. 2001).

No lamprey species show direct development. However, the observed neoteny in amphibians raises the question of whether metamorphosis is required for sexual maturation in lampreys, especially in the non-parasitic species (i.e., those that do not feed post-metamorphosis). In short, the answer is yes. Paedomorphosis has been reported in two different species of lampreys, the Po brook lamprey *Lampetra zanandreai* and least brook lamprey *Lampetra aepyptera* (Zanandrea 1957; Walsh and Burr 1981). However, these claims have been thoroughly reviewed and it was concluded that these authors were either examining a disrupted asynchronous metamorphosis or post-metamorphic juveniles (Vladykov 1985; Youson 2003), and that paedomorphosis is not a life history strategy used by lampreys. Moreover, despite efforts with an array of hormone treatments, scientists have not been able to initiate

precocious sexual maturation in larval lampreys (Hardisty 2006). Finally, if one considers the biology, behavior, and ecology of larval versus juvenile and adult lampreys, it is clear that larvae are not physically suited for spawning (Hardisty 2006). For instance, the sedentary larvae with a modest metabolism, limited swimming abilities, and small heart would not be capable of withstanding migration through the fast-moving headwaters to the spawning grounds (see Chap. 5). This migration would be particularly difficult without a suctorial oral disc to anchor itself in the fast-moving waters. Likewise, adults use the oral disc in nest building and mate pairing during fertilization (see Chap. 6). It is perhaps for the aforementioned reasons that non-parasitic lampreys, which are considered to have evolved from an ancestral parasitic species (Docker 2009), have never dispensed with metamorphosis or the characteristic oral disc.

An extensive discussion of the evolution of non-parasitic lampreys is beyond the scope of this chapter, and thus readers are referred to Docker and Potter (in press), as well as other discussions of the topic (Hardisty and Potter 1971a; Hubbs and Potter 1971; Beamish 1985; Youson 2004; Docker 2009). However, the ideas of developmental plasticity and heterochrony are worth discussing briefly as they may be important factors in the evolution of lamprey metamorphosis and of the non-parasitic life history. There are various uses of the term heterochrony; for the purposes of this discussion, it is defined as a change in relative developmental timing (whether due to an environmental perturbation, experimental induction, or a genetic mutation) that results in the potential for new traits to evolve. The appearance of a non-parasitic life history may be the result of a prolonged larval period and delayed metamorphosis (i.e., plasticity in developmental timing or heterochrony) that produces larger larvae which become sexually mature immediately following metamorphosis (Youson 2004; Hardisty 2006; Docker 2009). This viewpoint is consistent with the idea that over the course of lamprey evolution, the larval period has been extended (Hardisty 1979) and observations that the total life span of the parasitic and non-parasitic individuals in paired species is similar in duration. Thus, whereas parasitic species spend 1–2 years feeding post-metamorphosis, non-parasitic species may spend an additional 1–2 years filter feeding as larvae prior to metamorphosis (see Docker 2009).

A particularly interesting case where heterochrony may have led to polymorphism in life history type is that of *Lampetra richardsoni* var. *marifuga* of Morrison Creek, British Columbia, Canada (hereafter called "marifuga" since varieties published after 1960 are not recognized in zoological nomenclature; see Renaud et al. 2009). So named due to its inability to survive in sea water, "marifuga" is a potentially parasitic morph of the non-parasitic western brook lamprey *L. richardsoni* which is distributed throughout western North America (Beamish 1985, 1987). The anadromous North American or western river lamprey *L. ayresii* is the widely distributed parasitic, paired species of *L. richardsoni* and is morphologically and physiologically distinct from "marifuga" (Beamish 1987). Whether "marifuga" represents a genetic polymorphism or is simply a phenotypic polymorphism remains to be determined. Youson (2004) proposed five different scenarios to describe the possible relationships between these three lampreys within the context of life history evolution, including one in which individuals in this population alter the timing and

rate of metamorphosis and sexual maturation in response to environmental signals, ultimately affecting adult life history type.

Heterochrony likely played a role in the first appearance of metamorphosis in lamprey ancestors. In an earlier essay, Youson (2004) proposed that ancestral lampreys were marine and capable of reproduction in a larval-like form. Invasion of iodine-poor fresh water was facilitated in part by the endostyle's ability to efficiently concentrate the iodide that is required for thyroid hormone (TH) synthesis (see Sect. 4.4.2.2) and was a factor in the evolution of metamorphosis. With the delay of sexual maturation, metamorphosis evolved giving rise to a sedentary benthic adult (Youson 2004). The interaction between the thyroid and reproductive endocrine axes is still present in modern day lampreys (Youson and Sower 2001; Sower et al. 2009; see Chap. 7). Given the interconnectedness of the thyroid and reproductive axes, it is possible that some environmental cue triggered another delay in sexual maturation and the appearance of the free swimming, parasitic juvenile. Although all evidence suggests that, among modern lampreys, the non-parasitic life history is more derived, this recent "re-evolution" of non-parasitism appears to have involved a heterochronic shift in the timing of metamorphosis relative to sexual maturation, either through a prolongation of the larval period (see Docker 2009) or as a result of precocious sexual maturation during metamorphosis and the juvenile period (Youson 2004).

4.3 External Morphology and Staging

Lamprey metamorphosis lasts 3–4 months and is divided into seven stages (1, the earliest and 7, the latest) based on the sequential changes of several key external morphological features (Figs. 4.1 and 4.2). Five stages of metamorphosis were originally described for the landlocked sea lamprey (Manion and Stauffer 1970), but the seven stages subsequently described for the European river and brook lampreys (Bird and Potter 1979) and the sea lamprey (Youson and Potter 1979; Figs. 4.1 and 4.2) have now been universally adopted (Potter et al. 1982). These seven stages have been used to describe the metamorphosis of a variety of species including the southern brook lamprey *Ichthyomyzon gagei* (Beamish and Thomas 1984), the Far Eastern brook lamprey *Lethenteron reissneri* (Tsuneki and Ouji 1984), the mountain brook lamprey *Ichthyomyzon greeleyi* (Beamish and Austin 1985), the American brook lamprey (Holmes et al. 1999), and the Pacific lamprey *Entosphenus tridentatus* (McGree et al. 2008). What follows is a brief overview (summarized from Youson and Potter 1979) of the main external features used to identify the seven stages of metamorphosis in sea lamprey; the criteria in use prior to 1979 have been reviewed elsewhere (Youson 1980; Potter et al. 1982).

Staging of lamprey metamorphosis is based on the sequential changes of five key morphological features: (1) changes in the appearance, shape, and size of the eye; (2) remodeling of the larval buccal funnel (oral hood) and prebranchial region into the adult oral disc and snout; (3) growth and differentiation of the fins; (4) changes

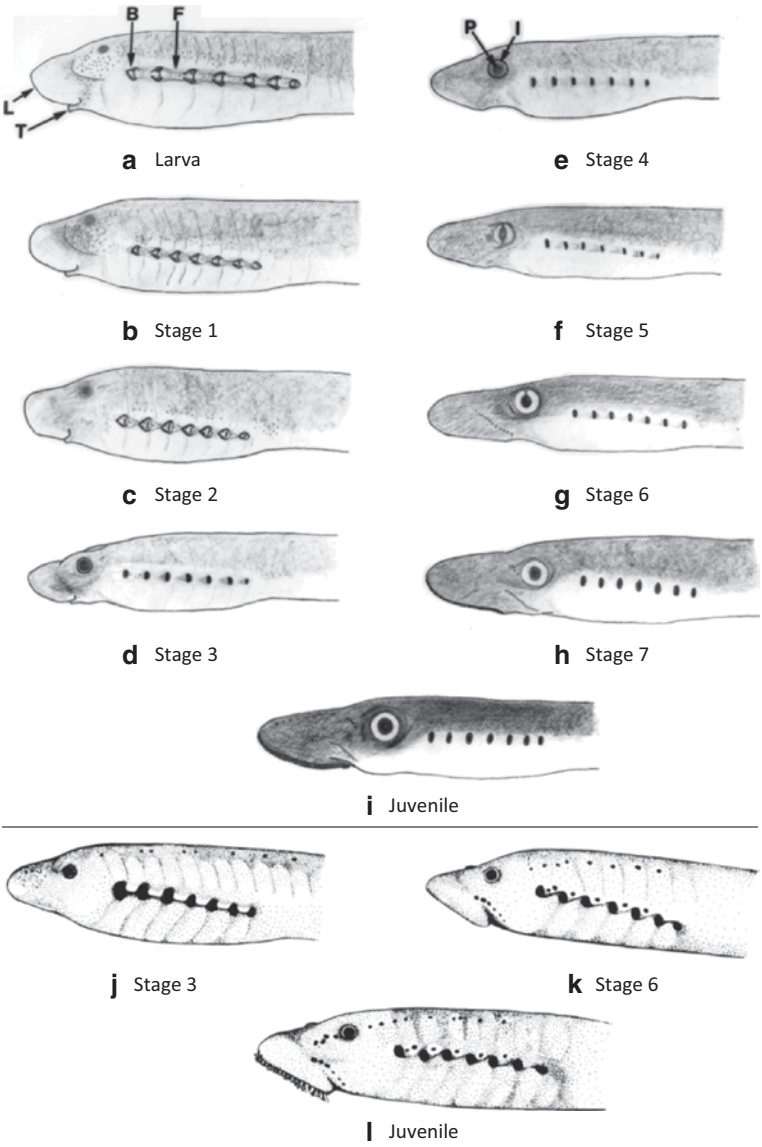

Fig. 4.1 Lateral view of the anterior region of larval, metamorphic (*stages 1* through *7*), and post-metamorphic (*juvenile*) sea lamprey *Petromyzon marinus* (**a–i**). For comparison, lateral views of metamorphic *stage 3* (**j**) and *6* (**k**) and post-metamorphic (*juvenile*) southern brook lamprey *Ichthyomyzon gagei* are also shown. The sea lamprey figure was originally published in Youson and Potter (1979) © 2008 Canadian Science Publishing or its licensors. Reproduced with permission. The southern brook lamprey images were originally published in Beamish and Thomas (1984) and reproduced with permission of Allen Press. *B* branchiopore, *F* furrow, *L* lateral lip of oral hood, *P* pupil, *T* transverse lip of oral hood

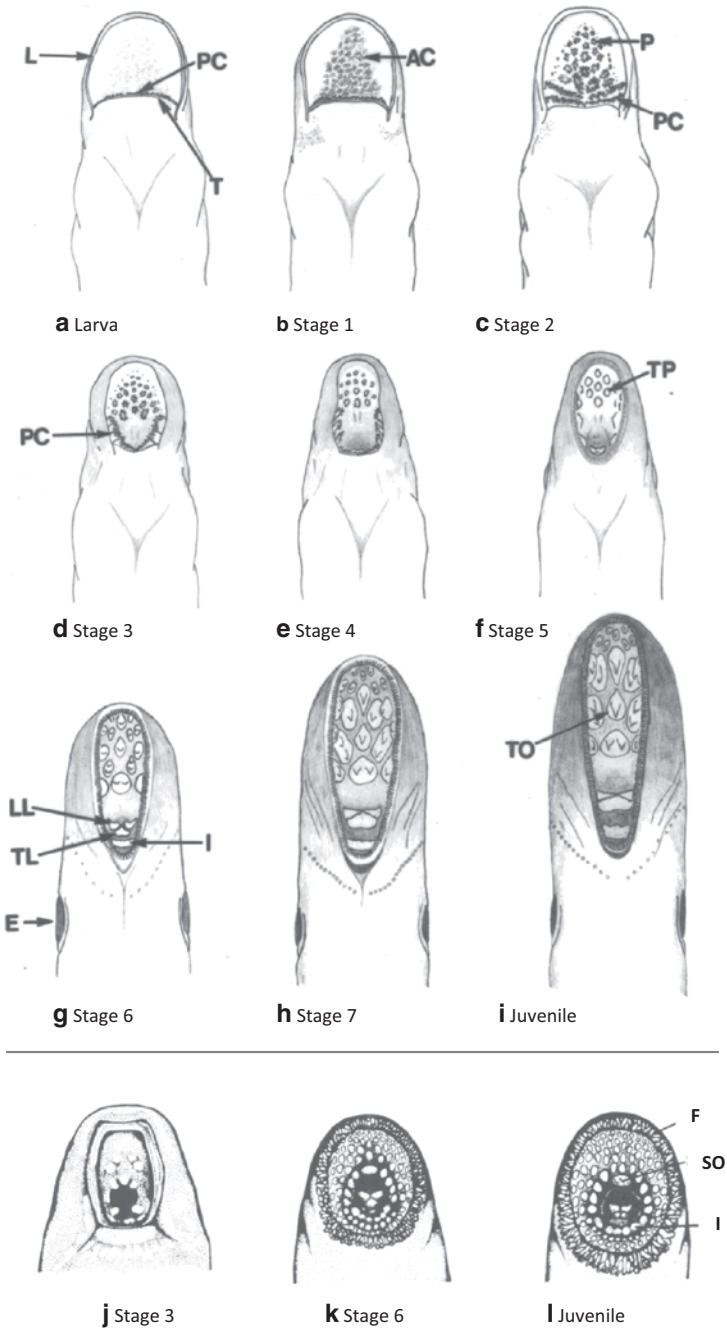

Fig. 4.2 Ventral view of the anterior region of larval, metamorphic (*stages 1* through *7*), and post-metamorphic (*juvenile*) sea lamprey (**a–i**). For comparison, lateral views of metamorphic *stage 3* (**j**) and *6* (**k**) and post-metamorphic (*juvenile*) southern brook lamprey are also shown. The sea lamprey figure was originally published in Youson and Potter (1979) © 2008 Canadian Science

in body coloration; and (5) changes in the shape of the branchiopores and branchial region as a whole (Potter et al. 1982; Figs. 4.1 and 4.2). As noted below, the earliest stages are best defined by the changes in the eye and buccal funnel.

Larval lampreys possess a U-shaped oral hood with a thin, curved lip at the anterior end that is continuous with thin lateral lips that extend beyond and overlap the posterior transverse lip (Fig. 4.2a). The inner surface of the oral hood contains numerous small and uniformly distributed cirrhi-like projections. The rudimentary eyes are visible as small, dark patches and the seven branchiopores are triangular in shape and connected by a prominent longitudinal furrow (Fig. 4.1a). Animals in stage 1 of metamorphosis are superficially very similar in appearance to larvae; however, with experience, they can consistently and readily be distinguished from larvae. The eyes, which now appear in lateral view as small, oval (elliptical) dark grayish patches, are the key identifying feature of stage 1 (Fig. 4.1b). At stage 2 of metamorphosis, the eyes are more conspicuous as they are now larger, darker, and rounder (Fig. 4.1c). The lips of the oral hood have begun to thicken and the inner surface contains papilla-like projections (Fig. 4.2c). The eye at stage 3 of metamorphosis is now very prominent and, for the first time, a dark pupil and light iris are visible (Fig. 4.1d). The lips of the oral hood have thickened such that they form a rectangular opening into the oral cavity and the snout has a characteristic "pug-like" appearance. A tongue-like piston may be present in some species (Fig. 4.2d). Stage 4 of metamorphosis is characterized by the lateral edges of the oral hood fusing with the posterior transverse lip to form a continuous ring of tissue, the oral disc (Fig. 4.2e). The oral papilla-like and cirrhi-like projections are reduced in number and a tongue-like piston is present. Stage 5 is characterized by the large eye with complete light iris (Fig. 4.1f) and the appearance of tooth precursors in the oral disc which are visible as raised points (Fig. 4.2f). Rudimentary fimbriae are present around the edge of the oral disc. In addition, the branchiopores have begun to acquire a more oval appearance, but the longitudinal furrow between successive pores is still prominent (Fig. 4.1f). The oral disc has enlarged significantly in stage 6; teeth and lingual laminae are clearly present as are the small fimbriae around the perimeter of the oral disc (Fig. 4.1g). The eyes are also very prominent at stage 6 and can be seen protruding laterally when the animal is viewed from the ventral surface (Fig. 4.2g). The animal has begun to take on the adult pattern of coloring. Stage 7 marks the completion of metamorphosis; teeth are hardened and end in sharp points, the lingual laminae have fine serrations, fimbriae are well-developed and conspicuous, the branchiopores are oval and longitudinal furrows are no longer present (Figs. 4.1h, 4.2h), and coloration closely approximates that of the juvenile (Fig. 4.1i).

 AC anterior oral cirrhi; *E* eye, *F* oral fimbriae, *I* infraoral lamina, *L* lateral lip of oral hood, *LL* longitudinal lingual lamina, *P* papilla, *PC* posterior oral cirrhi, *SO* supra lingual lamina, *T* transverse lip of oral hood, *TL* transverse lingual lamina, *TO* teeth of oral disc, *TP* tooth precursor

4.4 Regulation of Metamorphosis

Both spontaneous (natural) and induced metamorphoses have been described in lampreys. Spontaneous metamorphosis is a synchronous developmental process of extensive morphological, physiological, and behavioral changes (Youson 1988; Youson 2003) beginning in the summer months and characterized by a sharp decline in serum thyroid hormone (TH) levels in the early stages of metamorphic development (e.g., Wright and Youson 1977; Lintlop and Youson 1983b; Leatherland et al. 1990; Youson et al. 1994; see Sect. 4.4.2.2). Precocious metamorphosis has been induced in some lamprey species, out of season, following goitrogen treatments (Hoheisel and Sterba 1963; Suzuki 1986, 1989; Holmes and Youson 1993; Holmes et al. 1999) which depress serum TH levels (Suzuki 1986, 1989; Youson et al. 1995b; Manzon and Youson 1997; Manzon et al. 1998) as in the early stages of spontaneous metamorphosis. However, induced metamorphosis in lampreys usually results in incompletely developed animals that can neither feed nor reproduce (Hoheisel and Sterba 1963; Holmes and Youson 1993), and the outcomes of induction are not consistent across species or with the different types of goitrogens (e.g., Suzuki 1986; Leatherland et al. 1990; Manzon et al. 2001). In this section, the cues that modulate or control metamorphosis will be reviewed, as this is a highly seasonal event influenced by various environmental and physiological factors and controlled and mediated by the brain and possibly hypothalamic-pituitary axis (see Chap. 7). The focus will be on spontaneous metamorphosis; however, discussion and comparisons with induced metamorphosis will be made as it represents a useful tool to investigate the endocrine regulation of metamorphosis (see Table 4.1).

4.4.1 Environmental Factors in Metamorphosis

Metamorphosis in Northern Hemisphere lampreys generally begins in the summer months, but variations in the timing of metamorphosis in several species have been reported (Heard 1966; Bird and Potter 1979; Youson and Potter 1979; Maitland 1980; Potter et al. 1980; Beamish and Thomas 1984; Hardisty 2006; see Table 4.2). The timing of metamorphosis appears to co-vary with seasonal environmental gradients and, as a result, much research activity on cues of metamorphosis has focused on environmental factors with strong seasonal signals: temperature and photoperiod.

4.4.1.1 Temperature

Water temperature is the principal environmental cue of spontaneous metamorphosis in lampreys. The results of both laboratory and field studies provide ample evidence that the commencement of metamorphosis, rate of development during metamorphic events, and the incidence of metamorphosis within a lamprey population are strongly influenced by temperature. The results of constant temperature

Table 4.1 Modulators of lamprey metamorphosis. Summary of the key exogenous and endogenous factors in lamprey metamorphosis, their role or potential role in metamorphosis, the supporting evidence, and associated references

Modulator	Nature of change/role in metamorphosis	Supporting evidence	Reference
Temperature	Warm water temperature Rise from cold winter to warm summer temperature	Incidence of metamorphosis is highest when lampreys experience a rise in temperature Magnitude of rise has little effect on the incidence of metamorphosis (see Sect. 4.4.1.1)	Potter (1970); Purvis (1980); Youson (1993); Holmes and Youson (1994, 1997, 1998); Holmes et al. (1994)
Thyroid hormones (THs)	Increase throughout the larval period followed by a rapid and dramatic decrease early in metamorphosis	TH levels decrease early in metamorphosis in multiple species Exogenous TH disrupts natural metamorphosis (see Sect. 4.4.2.2) See "Goitrogens"	Wright and Youson (1977); Lintlop and Youson (1983b); Leatherland et al. (1990); Youson (1994); Holmes et al. (1999)
Goitrogens (i.e., antithyroid agents)	Suppress TH synthesis and serum levels Induce precocious metamorphosis	Induction of metamorphosis occurs in larvae that have not attained the necessary condition factor (Sect. 4.4.2.1) at a time of year when metamorphosis does not occur Incidence of metamorphosis is correlated to the magnitude of the decline in TH levels Exogenous TH treatments maintain elevated TH concentrations and block goitrogen-induced metamorphosis (see Sect. 4.4.2.2)	Hoheisel and Sterba (1963); Suzuki (1986); Holmes and Youson (1993); Manzon and Youson (1997, 2002); Manzon et al. (1998, 2001)
TH deiodinases	Larval and metamorphic phases are dominated by outer-ring (ORD, TH activation) and inner-ring (IRD, TH inactivation) activities, respectively	ORD activity is highest in the larval period and decreases early in metamorphosis IRD activity is low in the larval period, increases during metamorphosis, and peaks at stage 7 (see Sect. 4.4.2.2)	Eales et al. (1997, 2000); Stilborn et al. (2009)

Table 4.1 (continued)

Modulator	Nature of change/role in metamorphosis	Supporting evidence	Reference
TH distributor proteins	Likely have no key function in metamorphosis	Serum TH binding capacity does not change significantly during metamorphosis (see Sect. 4.4.2.2)	Gross and Manzon (2011)
TH receptors (TRs)	Increase during tissue morphogenesis	Preliminary data suggest TR (PmTR1) expression is elevated during tissue morphogenesis (see Sect. 4.4.2.2)	Manzon (2006)
Lipids	Increase in lipid content	Lipid content increases from 4 to 14% in premetamorphic individuals	Lowe et al. (1973); Beamish and O'Boyle (1977); Elliot and Youson (1987, 1991); Holmes and Youson (1994); Kao et al. (1997a, b, 1998, 1999b); Hollett (1998); Yaghoubian et al. (2001); Henson et al. (2003); Manzon (2011)
	Increase in CF	CF is a reliable, albeit population-specific, predictor of metamorphosis in lampreys	
	Lipogenic and lipolytic enzymes	The premetamorphic and metamorphic phases are dominated by lipogenic and lipolytic enzymes, respectively Insulin and somatostatin levels increase at stage 4	
	Leptin-like protein	16 kDa protein with leptin-like immunoreactivity appears in metamorphosis (see Sects. 4.4.2.1 and 4.4.2.2)	
GnRH-I and -III	Increase at mid- and late metamorphosis	Correlative immunohistochemical, radioimmunoassay, and gene expression data. Levels begin to increase mid-metamorphosis and peak at the end of metamorphosis in multiple species (see Sect. 4.4.2.2)	Youson and Sower (1991, 2001); Wright et al. (1994); Youson et al. (1995a, 2006); Tobet et al. (1996, 1997); Root et al. (2005)

Table 4.2 Comparison of the timing of the onset of metamorphosis among 16 lamprey species at different latitudes

Species	Location	Latitude	Timing of metamorphosis	Adult feeding type	Migratory type	Reference
Northern Hemisphere						
Sea lamprey *Petromyzon marinus*	Atlantic (North America)	30–53° N	Mid-July	Parasitic	Anadromous	Potter (1980)
	Atlantic (Europe)	40–70° N	July	Parasitic	Anadromous	Maitland (1980)
	Laurentian Great Lakes	42–48° N	Early–mid-July	Parasitic	Freshwater	Youson et al. (1993)
Pacific lamprey *Entosphenus tridentatus*	Columbia River basin	30–52° N	Early–mid-July	Parasitic	Anadromous	Hart (1973); McGree et al. (2008)
Arctic lamprey *Lethenteron camtschaticum*	Barents Sea watershed	65–70° N	March–April, early June	Parasitic	Anadromous	Holčík (1986a); Hardisty (2006)
Caspian lamprey *Caspiomyzon wagneri*	Iranian rivers	35° N	October–early December	Parasitic	Anadromous	Holčík (1986b)
	Kura River	42° N	Late August–mid-September			
	Volga River	47° N	Mid-July			
European river lamprey *Lampetra fluviatilus*	British rivers	52° N	Mid-July	Parasitic	Anadromous & freshwater	Hardisty (1986a)
	Baltic Sea watershed	60° N	August			
European brook lamprey *Lampetra planeri*	British rivers	40–65° N	Late June–July	Non-parasitic	Freshwater	Hardisty (1986b)
Carpathian lamprey *Eudontomyzon danfordi*	Danube River	46–49° N	July–August	Parasitic	Freshwater	Renaud and Holčík (1986)
Ukrainian brook lamprey *Eudontomyzon mariae*	Adriatic, Aegean, Azov, Baltic, Black Sea watersheds	40–53° N	Mid–late July	Non-parasitic	Freshwater	Holčík and Renaud (1986)

Table 4.2 (continued)

Species	Location	Latitude	Timing of metamorphosis	Adult feeding type	Migratory type	Reference
Macedonia brook lamprey *Eudontomyzon hellenicus*	Strimonas River, Greece	41° N	October	Non-parasitic	Freshwater	Renaud (1986)
	Loúros River, Greece	40° N	November			
Po brook lamprey *Lampetra zanandreai*	Po River watershed	45° N	August	Non-parasitic	Freshwater	Bianco (1986)
Mexican lamprey *Tetrapleurodon spadiceus*	Central Mexican plateau	20° N	April	Parasitic	Freshwater	Cochran et al. (1996)
Mexican brook lamprey *Tetrapleurodon geminis*	Central Mexican plateau	20° N	April	Non-parasitic	Freshwater	Cochran et al. (1996)
Southern brook lamprey *Ichthyomyzon gagei*	Southern Mississippi valley	32–33° N	Late August–early September	Non-parasitic	Freshwater	Beamish and Thomas (1984)
Mountain brook lamprey *Ichthyomyzon greeleyi*	Western North Carolina	35° N	Early–mid-August	Non-parasitic	Freshwater	Beamish and Medland (1988)
Southern Hemisphere						
Short-headed lamprey *Mordacia mordax*	Moruya River, Australia	35–44° S	Late Feburary–early March	Parasitic	Anadromous	Potter (1970)
Precocious lamprey *Mordacia praecox*	Moruya River, Australia	35–44° S	Late October–November	Non-parasitic	Freshwater	Potter (1970)

experiments on the sea lamprey (Youson et al. 1993) and the short-headed lamprey *Mordacia mordax* (Potter 1970) support the conclusion that the initiation of metamorphosis in lampreys can be altered by varying environmental temperature. Other studies with seasonally adjusted water temperatures consistently show that the spring rise in temperature from the winter minimum is a critical component of the temperature cycle required for the normal commencement of metamorphosis in larvae that are physiologically prepared (i.e., have sufficient lipid reserves; see Sect. 4.4.2.1) for this event. In addition, warmer water temperatures lead to a faster rate of metamorphosis within the same year so that metamorphosing lampreys may be ready to migrate downstream in the fall rather than the spring and recruit sooner to the juvenile period of the life cycle. These environmental studies (briefly reviewed in this section) and those addressing the role of lipids in metamorphosis (see Sect. 4.4.2.1) highlight both the integration and complexity of the exogenous and endogenous factors involved in this event and the vulnerability of lampreys at this time in their life cycle. Throughout this section the term population will be used to mean a group of larval lampreys within a specific stream.

The number of lampreys metamorphosing (i.e., the incidence of metamorphosis) is sensitive to differences in water temperature 1–2 months prior to the normal commencement of metamorphosis (e.g., Potter 1970; Potter et al. 1978; Purvis 1980; Youson et al. 1993). For example, the incidence of metamorphosis among sea lamprey from the Big Garlic River confined to cages in Lake Superior (7–11 °C) was 5–10 %, which was much lower than 46–76 % of caged animals held in the Big Garlic River itself (14–16 °C) and 75–100 % among animals held in aquaria in the laboratory (20–21 °C; Purvis 1980). Youson et al. (1993) reported that 60 % of sea lamprey larvae kept at a constant 13 °C and 84 % kept at 21 °C from June through August commenced metamorphosis in July, that development in 21 °C water was about 1 month in advance (to stage 3 of the seven stages of metamorphosis described in Sect. 4.3) of those in cold water (stage 1–2), and that these differences in the incidence and rate of development were statistically significant. These lamprey, although held at constant temperatures in the laboratory from June to August, would have been exposed to ambient winter and spring temperatures prior to capture. When evaluating the effect of winter and early spring temperature on the incidence of metamorphosis (i.e., in the 9 months leading up to metamorphosis and not just immediately before metamorphosis), Holmes and Youson (1994) and Holmes et al. (1994) found that sea lamprey of premetamorphic size (≥ 120 mm and 3.0 g, with condition factor ≥ 1.50; see Sect. 4.4.2.1) held at a constant temperature (either 9 or 21 °C) throughout the winter and spring did not initiate metamorphosis the following summer, whereas 53–55 % of the sea lamprey held in temperature regimes simulating seasonal stream temperatures metamorphosed. Collectively these studies support the conclusion that the incidence of metamorphosis in sea lamprey is strongly related to environmental temperature in the winter and spring prior to the event, particularly to a spring increase in temperature.

These earlier studies, however, did not distinguish between the rate of increase in temperature and the magnitude of temperature change (ΔT). In a study designed to address the ΔT of the spring temperature regime, Holmes and Youson (1997) kept

larvae of premetamorphic size at temperature regimes rising from 8 °C to either 13 or 21 °C in spring and found no significant difference in the proportion of sea lamprey larvae initiating metamorphosis (56 %); as with the above studies, only one control larva kept at a constant 8–9 °C commenced metamorphosis. However, by August, the metamorphosing sea lamprey subjected to a large ΔT in spring (ΔT 13 °C, from 8 to 21 °C) were smaller than metamorphosing lamprey subjected to a small ΔT in spring (ΔT 5 °C, from 8 to 13 °C), possibly because the energy requirements for maintenance increase with temperature (Holmes and Lin 1994). The implications of these differences in size (assuming that they persist through to the end of the metamorphic event) on the timing of downstream migration into the Great Lakes, success of first feeding, and the impacts of sea lamprey predation on prey fish populations in the Great Lakes have not been formally investigated (see below).

The range and optimal temperature for metamorphosis in sea lamprey were investigated experimentally by Holmes and Youson (1998) in a 3-month study in which larvae of premetamorphic size were exposed to one of five temperatures (9, 13, 17, 21, 25 °C) from June through August. Consistent with previous studies, no larvae metamorphosed at 9 °C; at 13, 17, 21, and 25 °C, the incidence of metamorphosis averaged 22, 50, 80, and 58 %, respectively (Fig. 4.3). By August, the median stage of metamorphic development for each temperature was stage 2 at 13 °C, stage 4 at 17 °C and 21 °C, and stage 3 at 25 °C (Fig. 4.3). The fact that more larvae on average initiated metamorphosis at 21 °C, coupled with the lower variability in the incidence of metamorphosis among replicate tanks, the higher rate of development, and the absence of mortality at this temperature, led Holmes and Youson (1998) to conclude that 21 °C is near the optimal temperature for metamorphosis of sea lamprey. This inference is consistent with the conclusion of Holmes and Lin (1994) that 21 °C was the optimal temperature for growth and survival of larval sea lamprey in tributary streams of the Great Lakes (although it should be noted that Holmes and Youson (1997) found that sea lamprey metamorphosing at 21 °C were smaller than those at 13 °C; see above). The upper thermal limit for sea lamprey metamorphosis could not be determined from these results, although Holmes and Youson (1998) note that it must be between 25 °C and the reported upper incipient lethal temperature of 31.4 °C (Potter and Beamish 1975).

The above studies investigating the role of temperature in lamprey metamorphosis are mostly short-term studies (June to August), but their results are consistent with the few longer-term studies that have been completed (e.g., Holmes et al. 1994). The evidence from these long-term studies supports the view that a rise in water temperature from a winter low (particularly in the month immediately prior to metamorphosis, i.e., around June in Northern Hemisphere lampreys) is the most important environmental cue of metamorphosis in lampreys. Low temperatures during the winter appear to be necessary to ensure that physiological conditioning occurs, namely, an increase in lipid concentration from approximately 4 to 14 % of the wet body weight (Lowe et al. 1973; O'Boyle and Beamish 1977; see Sect. 4.4.2.1). However, if water temperature does not rise in the spring (e.g., above about 9 °C for sea lamprey), then larvae are unlikely to initiate metamorphosis that summer (Holmes and Youson 1998), despite being physiologically prepared (hormonally

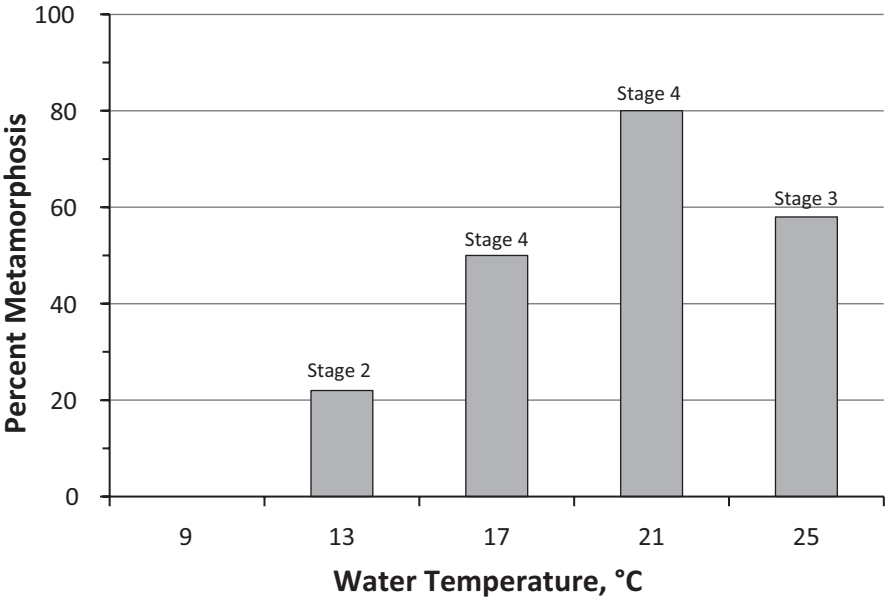

Fig. 4.3 The observed incidence (*percent*) of metamorphosis in sea lamprey maintained in the laboratory at five different temperatures. Lamprey (50 per treatment temperature) were housed at various temperatures from June 6 to August 15, at which time the stage of metamorphosis was determined. The median metamorphic stage for each temperature is indicated above each bar. (Data from Holmes and Youson 1998)

and with the appropriate lipid stores). An unusually cool spring can therefore delay metamorphosis by a year or more, depending on temperatures in succeeding springs. In contrast, an early, warm spring may be sufficient to trigger metamorphosis in larvae that are close to the optimal size and condition and hence result in a greater number of animals entering metamorphosis (Youson 2003), although temperatures that are considerably higher than normal (i.e., 25 °C versus the optimal 21 °C) would be expected to decrease the incidence of metamorphosis (Holmes and Youson 1998). Lower or higher than normal temperatures also appear to elicit a more variable response in a population of lampreys and slower development once metamorphosis has been initiated (see above).

Possible long-term effects of such differences in the rate of development during metamorphosis, as noted above, have not been formally investigated. Once metamorphosis is complete, juvenile lampreys of parasitic species generally migrate downstream to large lakes or the ocean in the late fall or early spring (Potter 1980; see Chap. 3). It is not known, however, whether autumn migrants are largely those lampreys that have completed metamorphosis earlier than their slower developing counterparts, and indeed whether there is any advantage (e.g., in terms of survival, growth, or fecundity) to this "head start" (Beamish and Hanson 1987). In terms of possible benefits, both Applegate (1950) and Parker and Lennon (1956) found that free swimming, newly parasitic sea lamprey grew little in winter, suggesting

that autumn migrants have little growth advantage over spring migrants. Feeding experiments by Swink (1995) suggested that autumn migrants could have an advantage in growth and survival if their food supply is adequate during the winter period; when both groups were provided with longnose sucker *Catostomus catostomus*, newly metamorphosed sea lamprey held at temperatures representative of the deeper waters of Lake Huron during winter stratification (4 °C) showed better survival (60 % versus 30 %) and 2.5–3 times the growth rate by the following June compared to those held at temperatures representative of tributary streams (≤ 1 °C). If prey is limited, however, autumn migrants may have little growth advantage over spring migrants and might, in fact, be at a disadvantage relative to newly metamorphosed sea lamprey that remain burrowed over winter in the stream substrate if the autumn migrants expend more energy swimming in the lake and suffer more predation (Swink 1995). Considered over the entire free swimming lake phase, there appear to be no significant differences in growth or survival between autum and spring migrants. Between autumn 1997 and spring 2000, Swink and Johnson (2014) marked over 2,700 newly metamorphosed sea lamprey during their outmigration from Black Mallard Creek, a tributary to Lake Huron; evaluation of growth and survival in marked individuals recaptured as upstream migrating adults in this and other Lake Huron tributaries showed no significant differences between autumn and spring outmigrants.

4.4.1.2 Density

Since premetamorphic growth and lipid accumulation in larval lampreys are important for proper initiation of metamorphosis (see Sect. 4.4.2.1), then competition for food resources or space, as measured by the number of larvae per unit area of habitat, might be expected to play a role in the commencement of metamorphosis. Crowding would be expected to decrease growth rates and delay metamorphosis, whereas larvae in low density populations would be expected to experience higher growth rates and metamorphose at a younger age. Many laboratory and field studies have indeed reported that increasing population density adversely affects the growth in length or mass of lamprey larvae (e.g., Mallatt 1983; Malmqvist 1983; Morman 1987; Murdoch et al. 1992; Rodríguez-Muñoz et al. 2003; see Chap. 3). Food is generally not considered to be a limiting resource for lampreys (Moore and Beamish 1973; Moore and Mallatt 1980; Malmqvist 1983; Murdoch et al. 1992), but high density may reduce growth rates through chemical inhibition. In addition to finding reduced growth rates at high larval rearing densities, Rodríguez-Muñoz et al. (2003) found that water alone from the two higher densities also had a negative (albeit weaker) effect on larval growth. However, the identity and mode of action of chemical growth inhibitors in lampreys is not known.

Fewer studies have examined the effect of density specifically on rates of metamorphosis, and the results are equivocal. Some empirical studies of lamprey populations re-established in chemically-treated streams in the Great Lakes reported accelerated growth and time to metamorphosis as a result of lower densities following

lampricide application (e.g., Purvis 1970, 1979; Morkert et al. 1998), but others do not (e.g., Griffith et al. 2001; Zerrenner and Marsden 2006). In the most comprehensive experimental study to date, Morman (1987) monitored caged sea lamprey larvae for more than 4 years in Michigan streams and found growth depression at high density and metamorphosis only at low density. However, in contrast to this long-term field study, a 9-month laboratory study by Holmes and Youson (1997) reported that high density during the premetamorphic interval did not significantly affect the incidence of metamorphosis in sea lamprey (regardless of water temperature regime). These results were somewhat surprising, given that the fall and winter period prior to metamorphosis is thought to be important for the lipid accumulation necessary for metamorphosis. However, these authors also found that density did not affect the size of metamorphosing or non-metamorphosing larvae, and suggested that the uniform laboratory environment may have prevented premetamorphic sea lamprey larvae from competing for high quality habitats where lipid deposition occurs.

Clearly, more research into the effect of larval density on the rate of lamprey metamorphosis is needed, given its important management implications. If reduced densities as the result of lampricide treatments result in a decrease in age at metamorphosis in sea lamprey, more frequent lampricide treatments would be required (Zerrenner and Marsden 2006). On the other hand, high densities of native lamprey larvae (either as the result of restocking following treatment or through increased lampricide species-specificity; see Marsden and Siefkes in press) could potentially temper this effect (Murdoch et al. 1992).

4.4.1.3 Photoperiod

The role of light stimuli on metamorphosis in lampreys has received attention because photoperiod exhibits a strong seasonal signal. Eddy (1969) and Cole and Youson (1981) reported that removal of the photosensitive pineal complex prevented metamorphosis in European brook lamprey and anadromous sea lamprey, respectively, and suggested that the pineal complex has a role in the seasonal timing of metamorphosis, possibly through a metabolic mechanism. Potter (1970) and Cole and Youson (1981) found that light had little effect on the incidence of metamorphosis in the short-headed lamprey or anadromous sea lamprey, respectively, but these studies used continuous light and dark regimes, which are atypical of the normal photoperiod experienced by lampreys in their native habitats. Nevertheless, more recent studies comparing the effects of continuous dark to a 15-h light:9-h dark regime (Youson et al. 1993) and the 15-h light:9-h dark regime to the natural ambient photoperiod regime in northern Michigan (Holmes et al. 1994) still found that photoperiod did not have detectable effects on the incidence of metamorphosis in landlocked sea lamprey. Thus, although the pineal complex may be necessary for metamorphosis, the seasonal induction of metamorphosis may not be sensitive to differences in photoperiod (Youson 2003). All evidence to date supports the hypothesis that spring temperature is the primary environmental factor controlling metamorphic processes.

4.4.2 Endogenous Factors in Metamorphosis

4.4.2.1 Size, Condition Factor, and Lipid Accumulation

Larval size has long been known to be an important criterion for predicting the commencement of metamorphosis in lampreys. For example, the smallest lengths at which the initiation of metamorphosis has been observed in anadromous and landlocked sea lamprey populations are 110 and 120 mm, respectively, and there is strong empirical evidence that larvae smaller than these lengths do not begin metamorphosis (Potter 1980; Smith and Tibbles 1980). The average length of metamorphosing sea lamprey in an anadromous population in New Brunswick was 129 mm (Potter et al. 1978), and that for landlocked sea lamprey is approximately 140 mm (Potter 1980; Zerrenner and Marsden 2006), although size at metamorphosis varies among streams and years (e.g., Griffiths et al. 2001; Zerrenner and Marsden 2005). Considerable variation in size at metamorphosis has also been observed among other lamprey species. For example, metamorphosis has been observed in individuals measuring less than 100 mm in European river lamprey (Bird and Potter 1979) and at 200 mm or more in other species (e.g., Ukrainian brook lamprey *Eudontomyzon mariae*; Holčík and Renaud 1986). In general, it appears that parasitic species tend to metamorphose at smaller sizes than non-parasitic species (reviewed in Docker 2009; see Chap. 3). Within the non-parasitic species at least, there is also evidence that females metamorphose at a larger size than males (see Docker 2009). Although a few studies (e.g., Applegate and Thomas 1965) have reported a similar sexual dimorphism in size at metamorphosis in sea lamprey, most studies have found no such difference (e.g., Zerrenner and Marsden 2005).

Some lamprey larvae of metamorphosing length go through an interval of physiological conditioning prior to metamorphosis that has been labeled a "rest period" (e.g., Leach 1940) or an "arrested growth" phase (e.g., Hardisty and Potter 1971b; Potter 1980; Youson 1988). During this period, the length of the larva does not change noticeably but lipid levels increase from about 4 to 14 % of the body weight (Lowe et al. 1973; O'Boyle and Beamish 1977). This increase in lipid results in extensive fat deposits which are the primary source of energy to the animal during the non-trophic interval of metamorphosis (Youson et al. 1979) and is reflected by an increase in weight of premetamorphic larvae in the months prior to the commencement of metamorphosis.

Condition factors ($CF = W/L^3 \times 10^6$, where W is the weight in grams and L is the length in millimeters) are often used to compare the length and weight of individual fish or a sample of fish because differences in CF are believed to be associated with nutritional condition of the fish (Ricker 1975). A condition factor criterion combined with size (length and weight) criteria have been used to successfully identify presumptive metamorphosing sea lamprey larvae in geographically distinct anadromous and Great Lakes populations (e.g., Potter et al. 1978; Youson et al. 1993; Holmes et al. 1994). Potter et al. (1978) were the first to establish that anadromous sea lamprey enter metamorphosis only after reaching at least 110 mm and 2.0 g in size and a $CF \geq 1.46$ based on data from a population in Dennis Stream, a tributary

of the St. Croix River in New Brunswick. Youson et al. (1993) found that these criteria overestimated metamorphosis among larvae from a population in the Chippewa River, a tributary to Lake Huron, and concluded that a presumptive metamorphosing sea lamprey in landlocked populations around the Great Lakes should be at least 120 mm long, weigh 3.0 g, and have a CF ≥ 1.50. These larger criteria were subsequently used to identify presumptive metamorphosing larvae in a series of carefully controlled laboratory studies investigating the role of temperature, density, starvation, and photoperiod on metamorphosis in Great Lakes populations of sea lamprey (Youson et al. 1993; Holmes et al. 1994; Holmes and Youson 1998; see Sect. 4.4.1). The length criterion identifies larvae in the arrested growth phase of the larval period, while the weight and CF criteria identify those larvae of the appropriate mass and with sufficient lipid reserves to enter metamorphosis. These criteria were used in June to predict metamorphosis in July and August; Holmes and Youson (1997) demonstrated that the same length and weight criteria (120 mm and 3.0 g, respectively) combined with a CF ≥ 1.45 could be applied in the fall period to correctly predict metamorphosis the following July. The lower CF criterion in the fall can likely be attributed to ongoing lipid accumulation between the fall and metamorphosis the following summer (Lowe et al. 1973; O'Boyle and Beamish 1977).

The success of these size and CF criteria in predicting which larvae will enter metamorphosis under suitable thermal regimes in the laboratory (and which will not) is high (Table 4.3). Correct classification of metamorphosing and non-metamorphosing larvae is highest for those exposed to ambient temperatures (85–99 %) and, as described in Sect. 4.4.1.1, for those held at 21 °C following a spring rise in temperature (40–88 %). When held at a constant 21 °C from November to August, none of the larvae meeting the size and CF criteria underwent metamorphosis (Holmes et al. 1994), presumably because elevated winter temperatures resulted in metabolic demands of sufficient magnitude to reduce lipids to levels that are inadequate to support metamorphosis the following spring.

Although these criteria appear to work well in predicting metamorphosis under carefully controlled laboratory conditions, they have not been as useful in other circumstances. Two empirical field studies have tested the utility of 120 mm, 3.0 g, and a CF ≥ 1.5 to define a pool of presumptively metamorphosing larvae in "wild" populations of sea lamprey in the Great Lakes (Hollett 1998; Henson et al. 2003). Hollett (1998) collected sea lamprey larvae ≥ 120 mm and 3.0 g in size from six streams in southern Ontario, tagged them with coded wire tags, and released them into their natal streams in the fall of 1995 (three streams) or spring of 1996 (three streams). Recaptures occurred in the fall of 1996 following chemical treatment of these streams for Great Lakes sea lamprey control purposes. The number of sea lamprey larvae tagged varied from a low of 30 in Cannon Creek to 344 in Gordon Creek and the rate of recapture varied from 8.8 % in Wilmot Creek to 30.8 % in Richardson Creek. In all six streams, the observed rate of metamorphosis was significantly different than predicted. Correct predictions of presumptively metamorphic and non-metamorphic larvae ranged from 33 % in Cannon Creek to 73 % in Gordon Creek; correct prediction rates in the fall ranged from 40 to 73 % and from 33 to 42 % in

Table 4.3 Proportion of sea lamprey correctly (C) and incorrectly (I) predicted to undergo metamorphosis or remain larvae at different temperatures. At the beginning of the experiment, all larvae were at least 120 mm and 3.0 g in size and predictions were based on condition factor (CF), but a presumptive metamorphosing animal had a CF ≥ 1.50 at the start of the experiment and an individual predicted to remain a larva had a CF < 1.50

		9 °C		13 °C		17 °C		21 °C		25 °C		Ambient	
Study	Category	C	I	C	I	C	I	C	I	C	I	C	I
Youson et al. (1993) Constant 13 or 21 °C June–August (i.e., with ambient temperature prior to June)	Metamorphosing			90	10			88	12				
	Larva			80	20			61	39				
Holmes et al. (1994) Constant 21 °C or ambient November–August	Metamorphosing							0	100			98	2
	Larva							86	14			99	1
Holmes and Youson (1997) 4–9 °C September–May; increased to 13 °C or ambient (21 °C) June–Aug	Metamorphosing			94	6							87	13
	Larva			93	7							85	15
Holmes and Youson (1998) Constant 9, 13, 17, 21, or 25 °C June–August (i.e., with ambient temperature prior to June)	Metamorphosing	0	100	26	74	61	39	87	13	74	26		
	Larva	24	76	18	82	36	64	40	60	52	48		

the spring. Hollett (1998) noted that the size and CF criteria tended to predict more larvae should commence metamorphosis than were observed to do so, although part of this difference could be observational bias associated with sampling lampreys in streams. Henson et al. (2003) used sequentially numbered coded wire tags (for individual identification) to tag 574 and 1,029 sea lamprey larvae collected in two tributaries to Lake Superior in Michigan, the Amnicon and Marengo rivers, respectively, in May 1994. Recaptures were made with backpack electrofishing and consisted of 50 animals in the Amnicon River and 204 in the Marengo River in October and August 1994, respectively. Of the 50 animals recovered in the Amnicon River, 25 of the 33 (76%) that were predicted to enter metamorphosis based on length, weight, and CF had begun metamorphosis by August 1994. However, the ability to predict which larvae in this river would not enter metamorphosis was considerably poorer; 80% of larvae that did not meet the size and CF criteria nevertheless had entered metamorphosis by August. In the Marengo River, these criteria resulted in misidentification for both categories: 80% of those meeting the size and CF criteria failed to metamorphose as predicted and 69% of the larvae that did not meet the criteria did metamorphose. To help explain these results, Henson et al. (2003) noted that the daily mean water temperatures in July and August were significantly higher in the Amnicon River (a large, stable stream) than in the spring-fed Marengo River; they suggested that these temperature differences influenced larval growth and the incidence of metamorphosis between the May tagging and recovery in August or October. Non-metamorphic and metamorphic larvae in the Marengo River decreased in length and weight between May and mid-August, whereas metamorphic sea lamprey recaptured in the Amnicon River showed slight increases in length but overall decreases in weight between May and October.

The results of these field studies show that the laboratory model of metamorphosis (size and CF criteria) is useful for defining a potential pool of presumptively metamorphic larvae in a wild population of sea lamprey, but further work is needed to define the probability that a presumptively metamorphic larva will commence metamorphosis, particularly since it appears that the criteria for predicting metamorphosis can vary among streams and perhaps among years (Henson et al. 2003). Thus, following up on these field results, Treble et al. (2008) developed a predictive model of sea lamprey metamorphosis based on a combination of individual (length, weight, age) and population (density) traits plus habitat characteristics (stream temperature, pH, conductivity, geographic location). The model was based on mark-recapture data from eight streams throughout the Great Lakes watershed in which larvae marked with coded-wire tags in the fall of year t were recaptured the following year, $t+1$, during lampricide treatments when metamorphic outcomes could be determined. In purely predictive terms, the best model for predicting when individual lamprey are likely to metamorphose using multiple logistic regression included the following independent variables: weight, age, time-integrated optimal temperature between 19 and 23 °C, latitude, longitude, and average larval density in moderate quality habitat. This model was not considered practical because some of the data inputs (age, time-integrated temperatures) are not routinely collected during control operations and the goal of the study was to develop a tool for fore-

casting the incidence of metamorphosis as a guide in the allocation of lampricide treatments among streams in the Great Lake basin. Thus, a second model based on larval length, latitude, longitude, drainage area, average larval density in moderate quality habitat, and lamprey production category (a measure of the regularity with which lampricide treatments are required) was developed and shown to improve predictions of metamorphosis in sea lamprey by 20% (Treble et al. 2008). Further validation of these models is required, but they provide further confirmation of the importance of factors such as weight, temperature, and density on the metamorphic process in lampreys.

Studies trying to improve the ability to predict when a substantial number of individuals in a population will undergo metamorphosis understandably focus on landlocked sea lamprey. Holmes et al. (1999), however, examined size and CF criteria in metamorphosing American brook lamprey. They reported that premetamorphic and spontaneously metamorphosing American brook lamprey larvae in a Lake Ontario tributary were much larger (minimum length 155 mm; minimum weight 5.40 g)—but with a minimum CF of 1.25—than those of the sea lamprey at the same stage of development. These findings are consistent with the trend reported above, where non-parasitic species (especially females) are generally larger at metamorphosis (Docker 2009). Non-parasitic species may need to be larger prior to the onset of metamorphosis since, in these lampreys, the non-trophic period (which begins at metamorphosis) extends through to sexual maturation and spawning; in females, fecundity will therefore be determined by size at metamorphosis. However, there appears to be considerable variation in size at metamorphosis in American brook lamprey. Hoff (1988) observed that American brook lamprey spawning in the Mashpee River in Massachusetts ranged in length from 100 to 109 mm; these individuals would have been somewhat larger at the onset of metamorphosis but would have been considerably smaller than 155 mm. More studies are needed to better understand the underlying difference in size at metamorphosis among and within species.

4.4.2.2 Endocrine Factors

Endocrine regulation of development in general, and metamorphosis in particular, has been of great interest to biologists for the past century and much of the work has centered on the vertebrate thyroid axis. The importance of the thyroid in development was first discovered when Gudernatsch (1912) showed that equine thyroid tissue could initiate metamorphosis in tadpoles. This seminal experiment led to the identification of the thyroid hormones (THs), thyroxine (T_4, 3,5,3′,5′-tetraiodothyronine) and 3,5,3′-triiodothyronine (T_3), as developmental morphogens. Today there is no doubt that T_4, and the more biologically active T_3, are the mandatory developmental morphogens driving virtually all the gene expression cascades, directly or indirectly, necessary for the transformation of the fish-like tadpole into a frog (reviewed in Shi 2000; Tata 2006; Brown and Cai 2007). Moreover, peripheral controls of the thyroid axis have been shown to modulate TH action on a

cell and tissue level and to facilitate the differential timing of tissue morphogenesis (Berry et al. 1998; Brown 2005). Consistent with these findings, studies have shown that environmental factors that alter metamorphic timing in amphibians often do so via the hypothalamic-pituitary regulation of the thyroid axis (Denver 1998; Denver et al. 2002). Finally, several other hormones have been shown to act synergistically with, or modulate the action of, THs on amphibian metamorphosis, including glucocorticoids, prolactin, and growth hormone (reviewed in Buchholz et al. 2011). The amphibian model has represented an excellent foundation from which to study the hormonal regulation of fish metamorphosis and life history transitions. Although an in-depth discussion is beyond the scope of this chapter, the thyroidal regulation of teleost metamorphosis and life history transitions is roughly consistent with that observed in amphibians. For a detailed review of the aforementioned topic, the reader is referred to other reports (Yamano 2005; Blanton and Specker 2007; Power et al. 2008; Manzon 2011). Evidence clearly supports the notion that THs are important for metamorphosis in lampreys; however, the precise nature of the role of THs in lamprey metamorphosis remains to be elucidated.

Thyroid

Evidence supporting the thyroidal regulation of anuran metamorphosis stimulated numerous studies in fishes including the potential role of THs in lamprey metamorphosis. Early investigations focused on the effects of treatment with iodinated compounds, and thyroid, hypothalamic, or pituitary extracts on lamprey metamorphosis (Horton 1934; Young and Bellerby 1935; Knowles 1941; Leach 1946). Despite numerous attempts, various treatment regimes, and the use of immediately premetamorphic larvae (Knowles 1941), in no instance did these treatments trigger a morphogenic change indicative of metamorphosis. The first data suggesting that THs might function in lamprey metamorphosis were provided by Hoheisel and Sterba (1963). They induced a precocious metamorphosis in 1-, 2- and 3-year old larval European brook lamprey, following treatment with the anti-thyroid agent (goitrogen) potassium perchlorate ($KClO_4$), although they did not observe complete metamorphosis. The effects of goitrogens on endostyle (the larval lamprey TH-producing gland) morphology and iodine metabolism clearly indicated that $KClO_4$ and other goitrogens inhibited thyroidal activity (Jones 1947; Klenner and Schipper 1954; Clements-Merlini 1962; Barrington and Sage 1963a, b). Thus, these somewhat paradoxical data suggested a possible link between the inhibition of the thyroid and lamprey metamorphosis and were contradictory to findings in anurans.

That the suppression of the thyroid axis might be associated with the onset of spontaneous metamorphosis was subsequently supported by observations that serum T_4 and T_3 concentrations decrease sharply in the early stages of metamorphosis (see Table 4.1). In the sea lamprey, serum T_4 and T_3 concentrations decrease to c. 25 % and c. 7 %, respectively, of larval levels (Wright and Youson 1977; Lintlop and Youson 1983b; Youson et al. 1994). Leatherland et al. (1990) reported more modest, but significant, declines in the pouched lamprey where T_4 and T_3 concentrations

are 27% and 30% of larval values by stage 2 of metamorphosis. Likewise, in the American brook lamprey, serum T_4 and T_3 concentrations are elevated in premetamorphic larvae and significantly reduced by stage 2 (Holmes et al. 1999). However, unlike other lamprey species studied to date, the peak in serum T_4 concentrations occurred in stage 1 of metamorphosis in this species rather than in the immediately premetamorphic larvae (Holmes et al. 1999). The observation that serum T_4 and T_3 levels gradually increase throughout the protracted larval period (Youson et al. 1994; Holmes et al. 1999) is also significant as it suggests that THs play a yet to be defined, but important, function in larval lamprey growth and development.

To gain a better understanding of the function of THs in lamprey metamorphosis and the role of the precipitous decline, several researchers employed chemical thyroid ablation experiments (goitrogen treatments) akin to those of Hoheisel and Sterba (1963). The first of such experiments were performed by Suzuki (1986, 1987, 1989) who reported complete metamorphosis following treatment of large larval Far Eastern brook lamprey with $KClO_4$ or sodium perchlorate ($NaClO_4$). Holmes and Youson (1993) improved on this work by making use of larger sample sizes, replicate tanks, larvae of different year classes (lengths), and ensuring that their study was conducted in the winter months when metamorphosis does not occur naturally. This later study showed that $KClO_4$ could induce metamorphosis in lamprey which were not of immediately premetamorphic size or condition (i.e., 120 mm, 3 g and CF > 1.5), at a time of year when metamorphosis does not occur. Holmes and Youson (1993), however, found that precociously induced metamorphosis differed in some respects from spontaneous metamorphosis. For example, some metamorphosing individuals, especially those from the two smallest size groups (65–95 mm and 110–119 mm), could not be placed in a definitive metamorphic stage because changes to the eyes and oral disc appeared to be uncoordinated. Furthermore, although one larva in the largest size class (>130 mm) progressed to stage 5 and one to stage 6, metamorphosis in the other individuals did not proceed beyond stage 4 and, in the smallest size groups, did not proceed beyond stage 2. This is not surprising, however, given that metamorphosis was induced in the winter months, when it does not normally occur, and at sizes much smaller than would occur spontaneously; there is presumably interaction between the seasonal, ontogenetic, physiological, and endocrine regulators of metamorphosis. It is also noteworthy that thyroid hormone-induced metamorphosis in amphibians often does not perfectly mimic natural metamorphosis (e.g., Etkin 1935, 1964, 1968).

A subsequent study by these investigators further implicated the decline in serum TH levels in the initiation of metamorphosis by showing that serum T_3 concentrations in all $KClO_4$-treated size groups (65–95 mm, 100–119 mm, >130 mm) were significantly lower (91–95%) than in the controls (Youson et al. 1995b); decreases in T_4 concentrations (27–32%) were only significant for the two smaller size groups. Likewise, $KClO_4$ treatment of larval American brook lamprey resulted in significant declines in serum T_4 and T_3 concentrations and the induction of precocious metamorphosis (Holmes et al. 1999). The phenomenon of goitrogen-induced metamorphosis in lampreys, however, is not universal; exposure of pouched

Treatment	Incidence of Metamorphosis	Serum T4	Serum T3
T4	0		
T3	0		
KClO4	80%		
T4 + KClO4	0		
T3 + KClO4	0		

Fig. 4.4 The observed incidence of metamorphosis, and relative changes in serum thyroxine (T_4) and triiodothyronine (T_3) concentrations in larval sea lamprey during the winter months following treatment with thyroxine (T_4) or triiodothyronine (T_3) in the presence or absence of the goitrogen potassium perchlorate ($KClO_4$) or with $KClO_4$ alone. Treatment concentrations were as follows: T_4 0.56 μM or 1.12 μM; T_3 0.37 μM or 1.48 μM; $KClO_4$ 0.05%. The direction of the *arrow* indicates an increase or decrease in hormone concentration with the size of the *arrow* indicating the relative size of the increase or decrease. (Adapted from Manzon et al. 1997)

lamprey to the goitrogen propylthiouracil for 70 days significantly depressed serum TH and hepatic T_3 concentrations but did not initiate metamorphosis (Leatherland et al. 1990).

Through a series of comprehensive ablation and replacement thyroid experiments, Manzon and co-workers showed that a decline in serum T_4 and T_3 concentrations is essential for goitrogen-induced metamorphosis. By maintaining elevated TH concentrations with exogenous T_4 or T_3, $KClO_4$-induced metamorphosis is completely blocked (Manzon and Youson 1997; Manzon et al. 1998; Fig. 4.4). Likewise, exogenous T_3 can disrupt the normal progression of spontaneous metamorphosis in immediately premetamorphic sea lamprey (Youson et al. 1997). That the induction of lamprey metamorphosis is not unique to the perchlorate anion (ClO_4^-), but rather more generally related to a decline in TH concentrations, was confirmed with observations that several different goitrogens, including methimazole (MMI), potassium thiocyanate and $NaClO_4$, could induce metamorphosis in sea lamprey and that the incidence of metamorphosis was correlated with the magnitude of the decline in serum T_4 and T_3 concentrations (Manzon et al. 2001). However, it is noteworthy that although MMI treatment induced metamorphosis, it also resulted in a high incidence of mortality. Finally, studies showing that $KClO_4$ can directly inhibit iodide uptake and incorporation by endostyles in vitro indicate that the decline in THs is not merely a consequence of some non-specific action but that goitrogens are acting at the level of the endostyle (Manzon and Youson 2002). In summary, these ablation and replacement experiments strongly support the notion that a decline in serum TH concentrations is essential for induced metamorphosis and the normal progression of spontaneous metamorphosis.

Serum TH concentrations represent only one metric to describe thyroid status in fishes. The vertebrate thyroid system consists of numerous regulatory points which act collectively to regulate TH action. Included among these are the

hypothalamic-pituitary (HP) axis which regulates the synthesis and secretion of T_4, and to a lesser extent T_3, from thyroid tissue, serum TH distributor proteins (THDP) which transport TH through the blood and regulate hormone availability, cytosolic deiodinases which regulate TH action via the conversion of T_4 to the more biologically active T_3 and the inactivation of both T_4 and T_3, and finally the TH nuclear receptors (TRs) and their heterodimeric partners, the retinoid-X-receptors (RXRs), which act as ligand-regulated transcription factors to modulate gene expression. For a more in-depth treatment of the thyroid system and its regulation in fishes, the reader is referred to other recent reviews (Leatherland 1994; Eales 1997; Blanton and Specker 2007; Youson 2007; Manzon 2011). Knowledge of the role of these regulatory points in the modulation of TH action is critical to our understanding of the function of THs in lamprey development.

It has been postulated that the decline in serum TH at the onset of metamorphosis may be a consequence of a decrease in the binding and transport capacity of the serum and an increase in tissue uptake. Lintlop and Youson (1983a) showed that the T_3 binding capacity of hepatic nuclei is slightly elevated during metamorphosis. However, they concluded that this could not account for the dramatic decline in serum TH concentrations. Likewise, Gross and Manzon (2011) concluded that despite changes in the type and number of serum THDP throughout the lamprey life cycle, a change in total serum TH binding capacity is not responsible for the decline in serum TH concentrations (Fig. 4.5). TH binding studies show that larval sea lamprey serum contains a single dominant THDP, the lamprey-specific albumin AS (for ammocoete spot; Gross and Manzon 2011). AS is replaced by the albumin SDS-1 (for sodium dodecyl sulfate fraction I) and the glycolipoprotein band CB-III (for Cibroacron Blue) at or immediately following stage 7 of metamorphosis in parasitic juveniles and adults (Gross and Manzon 2011; Fig. 4.5). This shift from AS to SDS-1 and CB-III using TH binding as a metric is consistent with other reports on these proteins using immunometric methodologies (Filosa et al. 1982, 1986; reviewed in Youson 2003). This observed shift in type and number of THDP is consistent with observations in other vertebrates (Richardson et al. 2005; Richardson 2008). However, the lack of an increase in total binding capacity is not consistent with Richardson's augmented serum THDP hypothesis which states that major developmental events coincide with a increase in the type and number of serum THDP and the total TH binding capacity to ensure a sufficient pool of TH (Richardson 2008). Surprisingly, although the complementary DNA (cDNA) for the THDP transthyretin (TTR) was cloned from both sea lamprey and American brook lamprey and was shown to be upregulated in the liver during metamorphosis, TTR was not identified in the sera from either species (Manzon et al. 2007; Gross and Manzon 2011). Serum TTR levels are developmentally regulated in both bullfrog *Lithobates catesbeianus* (formerly known as *Rana catesbeiana*) and sea bream *Sparus aurata*. Bullfrog TTR is absent from larval and adult serum, but transiently appears during metamorphosis (Yamauchi et al. 1993) and adult sea bream serum TTR levels are elevated relative to early developmental stages (Morgado et al. 2007). It is likely that the shift in lamprey serum proteins is more analogous to the shift from an embryonic α-fetoprotein

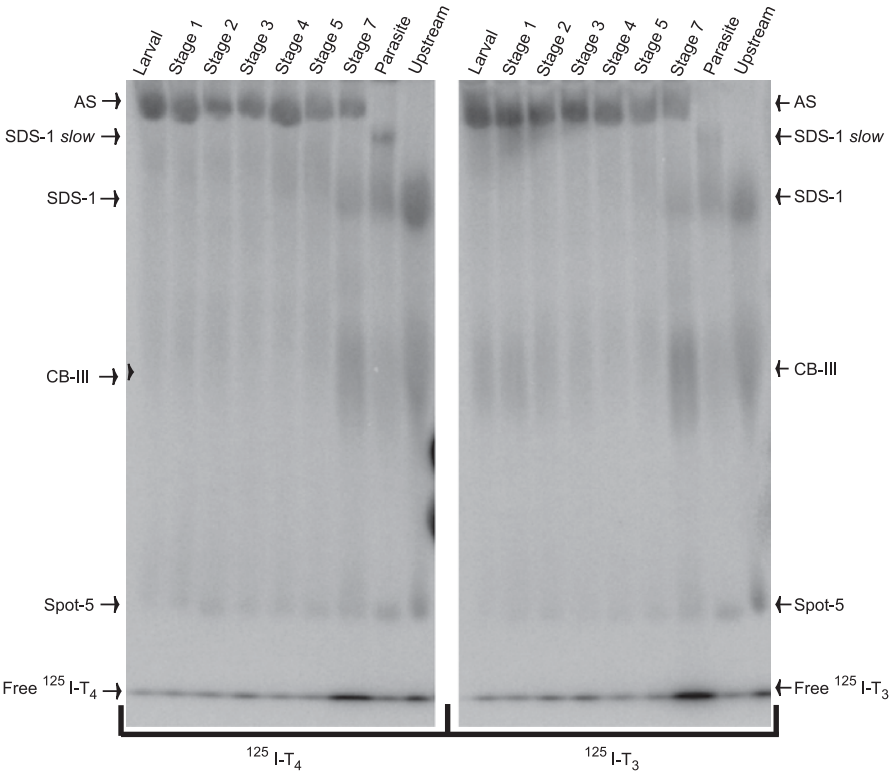

Fig. 4.5 Autoradiogram showing the general profile and overall thyroid hormone (*TH*) binding of sea lamprey serum TH distributor proteins (*THDP*) throughout the sea lamprey life cycle. For all stages 10 μl serum samples were incubated with either ^{125}I-T_4 or ^{125}I-T_3 for 2 h followed by non-denaturing polyacrylamide gel electrophoresis (*PAGE*) and autoradiography. The autoradiogram clearly shows the four developmentally regulated THDPs: AS, SDS-1, CB-III and Spot-5 as well as a slower migrating SDS-1 (SDS-1 *slow*) protein band which is often present in non-denaturing PAGE. (This figure was reprinted from Gross and Manzon (2011), with permission from Elsevier)

to an adult albumin than to the transient appearance of additional THDPs to facilitate a larger serum TH reservoir.

Peripheral TH deiodination by TH deiodinases represents a critical regulatory point in the thyroid system. It is notable that in fishes, unlike in most mammals, peripheral regulation via deiodinases is more important in maintaining homeostasis and modulating TH action than the central HP axis (Eales 1985; Eales and Brown 1993; Orozco and Valverde 2005). Moreover, in anurans, deiodinases, along with nuclear receptors (discussed below), are essential to ensure correct timing of differential tissue morphogenesis throughout metamorphosis despite all tissues being exposed to the same TH concentrations (Becker et al. 1997; Brown 2005; Buchholz et al. 2006). In vertebrates, three different deiodinases have been identified and named deiodinase type 1, 2, or 3. Although they were initially classed based on their

biochemical properties relative to the three mammalian deiodinases, more recent data rely on sequence homology rather than biochemical properties (reviewed in Bianco et al. 2002; Bianco and Kim 2006). Deiodination reactions can be broadly classed as inner- (IRD; inactivation) or outer- (ORD; activation) ring deiodinations. ORDs convert T_4 to the more biologically active T_3 and IRDs convert T_4 and T_3 to the inactive rT_3 or T_2 (Fig. 4.6). Thus, ORD and IRD can be considered activation and inactivation reactions, respectively. In mammals and most other vertebrates, deiodinase type 2 (D2) and type 3 (D3) are outer- and inner-ring deiodinases, respectively, whereas D1 is capable of both IRD and ORD reactions.

Given the important regulatory role of TH deiodinases in fishes and other vertebrates, one might predict that TH deiodinases either contribute to the decline in serum TH concentrations (i.e., IRD activity is elevated) at the onset of lamprey metamorphosis or alternatively that they function to maintain elevated cellular levels of the more biologically active T_3 (i.e., ORD activity is elevated) in the face of a reduced serum pool of TH during metamorphosis. In sea lamprey, the primary site of deiodinase activity is the intestine; this is consistent with the notion that a significant portion of TH produced by the endostyle reaches the bloodstream via intestinal absorption (Eales et al. 1997). The larval endostyle, in addition to TH synthesis, functions as a filter feeding apparatus, secreting mucopolysaccharides which trap and transport food particles, along with secreted THs, to the intestine for absorption (Barrington and Sage 1972). T_4 ORD was detected in larval sea lamprey liver, kidney, and muscle with highest activity levels reported for the intestine (Eales et al. 1997); however, T_4 IRD, T_3 ORD, and T_3 IRD activities were below the detection limit in all tissues examined. The intestines from upstream migrants contained appreciable levels of T_4 ORD, T_4 IRD, and T_3 IRD activity (Eales et al. 1997). When intestinal deiodinase levels were quantified throughout the life cycle of sea lamprey, a reciprocal relationship between ORD and IRD activities was observed. Intestinal T_4 ORD activities were low in small larval sea lamprey, increased to peak levels in immediately premetamorphic larvae and the first stages of metamorphosis, decreased dramatically to very low levels for the remainder of metamorphosis, and finally returned to modest levels in the adults (Eales et al. 2000). In contrast, IRD activities displayed the opposite pattern, with T_4 IRD negligible until stage 3 of metamorphosis, after which it increased steadily to peak levels at stage 7 followed by a decline to modest levels in adults (Eales et al. 2000). For those stages that it was measured, T_3 IRD closely followed T_4 IRD activities (Eales et al. 2000). Finally, the cDNA for sea lamprey D2 has recently been cloned and real-time PCR gene expression data for the intestine, liver, and kidney are consistent with T4 ORD activities (Stilborn et al. 2009). In summary, deiodinase data suggest that just prior to the initiation of metamorphosis, there may be a surge in T_3 production as measured by an increase in T_4 ORD followed by a shutdown in T_4 ORD and surge in T_4 and T_3 inactivation by IRD. These data lead to the conclusion that deiodinase activities function to minimize the availability of active hormone to tissues during sea lamprey metamorphosis and are consistent with goitrogen ablation and replacement experiments which suggest a decline in TH action is required for normal metamorphosis.

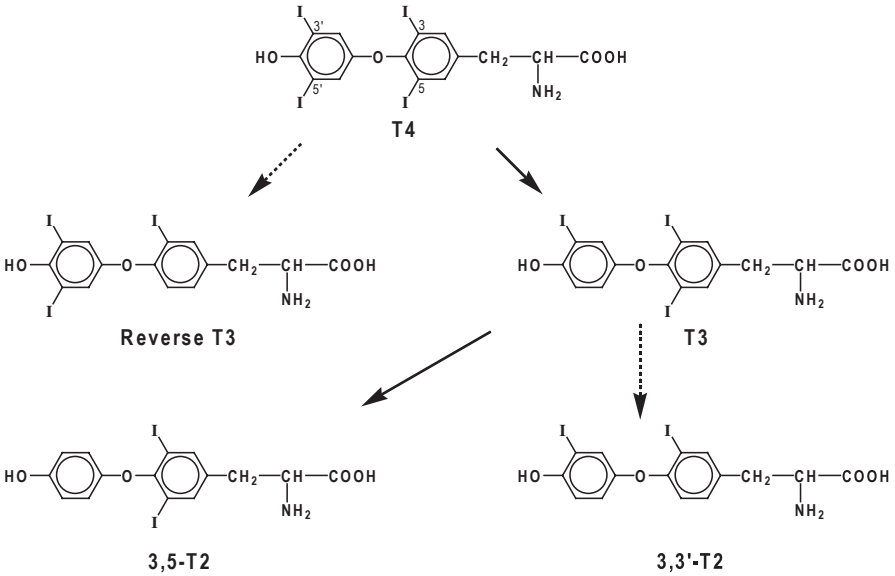

Fig. 4.6 Graphical representation of the major deiodination pathways of thyroid hormone metabolism. *Outer-ring* deiodination reactions (ORD ~ activation) which convert T_4 to the more biologically active T_3 are indicated by *solid arrows*. *Inner-ring* deiodination reactions (IRD ~ inactivation) which inactivate both T_4 and T_3 are indicated by *broken arrows*. T_4 = 3,5,3′,5′-tetraiodothyronine or thyroxine; T_3 = 3,5,3′-triiodothyronine; reverse T_3 = 3,5,5′-triiodothyronine; T_2 = diiodothyronine. (This figure was originally published in Manzon (2011) and reproduced by permission of Oxford University Press)

Thus far, all data seem to suggest that declines in TH concentrations and activity are essential for metamorphosis. However, a closer examination of serum TH data suggests that TH concentrations during lamprey metamorphosis, when they are at their lowest, might actually be comparable to levels during anuran metamorphosis and sufficient to drive morphogenesis. T_4 and T_3 concentrations in larval lampreys peak at 1,550–3,153 μg/dl and c. 7,800 ng/dl, respectively, and subsequently decline during metamorphosis to their lowest levels of 98 μg/dl of T_4 and 61 ng/dl of T_3 at stage 6 of metamorphosis (see Manzon 2011). Although T_4 and T_3 concentrations at metamorphosis are only c. 5 and 0.8 %, respectively, of peak larval concentrations, these lower T_3 concentrations are comparable to the T_3 concentrations of 75–150 ng/dl observed during metamorphic climax in bullfrog and the African clawed frog *Xenopus laevis* (White and Nicoll 1981; Tata et al. 1993). Moreover, it has been shown that hepatic nuclear T_3 binding increases, albeit marginally, during metamorphosis in sea lamprey (Lintlop and Youson 1983a). When these hepatic T_3 binding data are considered in conjunction with the idea that low lamprey TH concentrations are comparable to concentrations in other vertebrates, it is conceivable that there is sufficient TH to alter gene expression cascades during lamprey metamorphosis in a fashion similar to the metamorphoses in anurans and other fishes. However, the aforementioned data must be interpreted with some caution as the

hepatic T_3 binding data are limited and comparisons between different studies and species are not always precisely reliable.

The primary mode of TH action is via modulation of TH responsive genes through TRs and RXRs. As with all other vertebrates studied to date, lampreys (at least the sea lamprey) contain two TRs and three RXRs. However, phylogenetic analysis indicates that the lampreys diverged from the vertebrate lineage prior to the appearance of the events that gave rise to TRα and TRβ, and RXRα, RXRβ and RXRγ receptors found in all other vertebrates (Manzon 2006). Thus, the sea lamprey receptors have been designated PmTR1, PmTR2, PmRXR1, PmRXR2, and PmRXR3 (Manzon et al. 2014). Sea lamprey TRs are more similar to each other than either is to the TRα or TRβ found in other vertebrates, and PmRXRs, which are likely variants of the same gene, are more similar to each other than they are to any of the vertebrate RXRs (Escriva et al. 2002; Manzon et al. 2014). Despite having arisen from a lamprey-specific duplication, these lamprey receptors function in a similar manner to other vertebrate receptors in that they can activate reporter genes upon stimulation with T_3 (Manzon et al. 2014). Given that serum TH concentrations suggest that there might be sufficient hormone during metamorphosis, nuclear receptor availability might be critical in TH action.

In anurans, TRβ expression levels parallel TH concentrations and are upregulated in specific tissues at the time of morphogenesis, while TRα appears to be expressed constitutively throughout metamorphosis (reviewed in Shi 2000). Flatfishes display a similar trend; however, it is TRα expression that correlates with tissue morphogenesis and TRβ expression that is constitutively expressed throughout metamorphosis (Yamano and Miwa 1998). Developmental expression analyses of lamprey nuclear receptors suggest that PmTR1 expression fluctuates in a tissue-specific manner whereas PmTR2 and PmRXRs are constitutively expressed throughout metamorphosis (Manzon et al. 2014). More specifically, PmTR1 in the liver and intestine increase steadily from larval values to peak levels at stage 5 and 6, respectively, and then return to premetamorphic levels in the adult. In contrast, PmTR1 levels in kidney-gonad were highest in larvae and decreased 5-fold by stage 3/4 (Manzon el al. 2014); the elevation of TR expression in kidney-gonad might be related to the role of these tissues as sites of lipid deposition. Although these data are not definitive, they certainly support the hypothesis that TH and TRs function in lamprey development and the notion that perhaps all the components required, namely TH and TRs, are present at sufficient levels to drive metamorphosis in a fashion similar to that observed in other metamorphosing vertebrates.

When all the aforementioned data on the lamprey thyroid system are considered simultaneously, these data suggest that TH could have a dual role in lamprey development (Manzon 2011). High TH levels during the larval period promote feeding, growth, and lipid accumulation in larvae while simultaneously inhibiting metamorphosis (Manzon 2011). Youson (1997) has suggested that TH in lampreys might be analogous to juvenile hormone in insects and functions as a “juvenilizing hormone” in larval lampreys. Subsequently, following some unknown signal, metamorphosis begins and much lower TH levels are necessary to drive the morphogenetic processes associated with metamorphosis in a manner similar to that observed in other vertebrates (Manzon 2011).

Hypothalamic-Pituitary Axis

As described above, early attempts at showing that the hypothalamic-pituitary (HP) axis is involved in lamprey metamorphosis were met with little success. The first study to suggest that the HP axis was involved in lamprey metamorphosis showed that the removal of the larval rostral pars distalis (RPD) results in complete metamorphic stasis in the pouched lamprey (Joss 1985). In contrast, metamorphosis was initiated but arrested at stage 3 of metamorphosis following removal of the caudal pars distalis (CPD, also known as the proximal pars distalis; see Chap. 7) (Joss 1985). Several studies have also shown that hypothalamic gonadotropin-releasing hormone (GnRH) levels correlate with metamorphosis in three species of lamprey (Youson and Sower 1991; Youson et al. 1995a, 2006) and that GnRH might be involved or connected to the regulation of the thyroid axis (Youson and Sower 2001). Over the past two decades, great strides have been made towards the identification and characterization of the various hormones of the HP axis and these data have provided the requisite knowledge and framework for more detailed studies designed to investigate the role of these molecules in metamorphosis and their regulation of various endocrine glands. What follows is a brief overview of these hormones as it pertains to their potential involvement in lamprey metamorphosis. For in-depth treatment of the HP axis, their hormones, and evolutionary significance, the reader is referred to other essays on the topic (Kawauchi and Sower 2006; Sower et al. 2009; see Chap. 7).

In mammals the HP-gonadal, HP-thyroid and HP-adrenal axes function independently with little to no cross-regulation between the axes (Fort et al. 2007). However, in lower vertebrates, cross-regulation is not uncommon. For instance, hypothalamic corticotropin releasing hormone, which regulates the mammalian adrenal axis, has been shown to be a stimulator of pituitary thyroid stimulating hormone (thyrotropin; TSH) in representatives of all non-mammalian gnathostomes (De Groef et al. 2006). Likewise, numerous studies have shown that cross-regulation exists between the thyroid and reproductive axes (see Blanton and Specker 2007; Fort et al. 2007; McNabb 2007; see Chap. 7).

Lampreys contain three distinct hypothalamic GnRHs (GnRH-I, GnRH-II, and GnRH-III), three GnRH receptors, one pituitary gonadotropin (GTH), one gonadal glycoprotein hormone (GpH) receptor, and one thyroidal GpH receptor (see Chap. 7). This is in contrast to mammals that have only one or two GnRHs and two GnRH receptors regulating two pituitary GpH gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), which act via two gonadal GpH receptors. Other mammalian GpH family members include TSH, thyrostimulin (TSM), and chorionic gonadotropin (CG). Each of the five mammalian heterodimeric GpH family members consist of distinct β subunits; although TSM has a distinct and likely ancestral α-subunit (TSM-α; GPA2) (Nakabayashi et al. 2002; Sudo et al. 2005), the other four share the same α subunit (GPA1). Thus far, preliminary data suggest that lampreys have a single α-subunit (the GPA2) and two β-subunits, one (which may be similar to the ancestral β-subunit) is expressed in the pituitary

gland, while the other (lGpHB5), with similarity to TSM-β (GPB5), is mainly expressed in the gonads (Sower et al. 2009).

Two of the three GnRHs (GnRH-I and GnRH-III) have been studied extensively in multiple lamprey species (see Chap. 7). The third and most recently identified GnRH-II has been localized to the hypothalamus and shown to stimulate the pituitary-gonadal axis, but data on developmental expression or regulation are currently lacking (Kavanaugh et al. 2008). Several studies have shown that hypothalamic GnRH-I and -III levels are low throughout the larval period, tend to show a modest increase at stages 1/2 or 3/4 of metamorphosis in non-parasitic (western and American brook lampreys) and parasitic (sea lamprey) species, respectively, and a sharp rise to peak levels by stage 7 of metamorphosis with levels remaining elevated throughout the feeding and/or upstream migrant phases of the life cycle (Youson and Sower 1991; Youson et al. 1995a, 2006). The earlier onset of the GnRH increase in non-parasitic, metamorphosing lampreys is consistent with the fact that sexual maturation in these species begins shortly after the onset of metamorphosis with gonadal development being significantly advanced relative to parasitic species (see Docker 2009). These findings are also supported by, and consistent with, studies that have examined the distribution of GnRH and GnRH messenger RNA (mRNA) in the brain and hypothalamus of sea lamprey using immunocytochemistry and in situ hybridization, respectively (Wright et al. 1994; Tobet et al. 1995, 1996, 1997; Root et al. 2005).

Although the rise in GnRH is a feature of lamprey metamorphosis, these correlative data do not indicate that GnRH is directly involved in metamorphosis and the regulation of the thyroid axis in addition to its role in gonadal development. To clarify a role in metamorphosis and regulation of the thyroid axis, Youson and Sower (2001) examined GnRH levels during $KClO_4$-induced metamorphosis. Although a rise in GnRH-I and -III was observed following $KClO_4$-induced metamorphosis, this rise appeared slightly earlier than in spontaneous metamorphosis. Moreover, these data are difficult to interpret because of the asynchronous development associated with induced metamorphosis (Holmes and Youson 1993; Manzon and Youson 1997; Manzon et al. 1998, 2001; see Sect. above). It has been proposed that this asynchrony could be related in part to the timing and size of the GnRH peaks during induced metamorphosis (Youson and Sower 2001). An equally plausible hypothesis is that the size and timing of the GnRH peak deviates from spontaneous metamorphosis because some positive or negative feedback loop is absent during induced metamorphosis. Although not definitive, these and the aforementioned data suggest that a potential role of GnRH in metamorphosis warrants further investigation, as does the possible cross-regulation between the HP-gonadal and HP-thyroid axes.

Observations that salmon GTH or a GnRH analog produce significant elevations in serum T_4 in adult sea lamprey (Sower et al. 1985) suggest cross-regulation between the gonadal HP axis and the thyroid axis. When these data are considered in conjunction with the findings that lampreys contain gonad- and thyroid-specific GnRH receptors (Freamat et al. 2006) and that lamprey GpH-β is an outgroup of the FSH-β, LH-β and TSH-β subunits and may represent an ancestral form of this lineage, it seems very plausible that there is significant functional overlap between

Table 4.4 Summary of the features of lipid metabolism in the liver and kidney of sea lamprey during spontaneous and $KClO_4$-induced metamorphosis and following the blocking of $KClO_4$-induced metamorphosis with exogenous thyroid hormones (TH-blocked). (From Youson 2003)

	Spontaneous		$KClO_4$-induced		TH-blocked	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Total lipid	↓	↓	↓	↓	↑	↑
Lipolysis	↑	↑	↑	↑	↓	↓
Lipogenesis						
ACC	↓	↓	↓	n/d	↑	n/d
DGAT	↓	n/d	↓	↓	n/d	↑

ACC acetyl-CoA carboxylase, *DGAT* diacylglycerol acyltransferase, ↑ increase, ↓ decrease, *n/d* not determined

the reproductive and thyroid HP axes in lampreys (Sower et al. 2009). Thus, lampreys may represent our closest approximation of the ancestral HP axes. Future work should be aimed at characterizing the components of these axes as well as the HP-adrenal axis throughout the lamprey life cycle.

Lipids, Lipogenesis, and Lipolysis

Lamprey metamorphosis is characterized by a two-phase lipid metabolic cycle. In the fall prior to metamorphosis, sea lamprey enter into a lipogenic phase characterized by an increase in acetyl CoA carboxylase (ACC) and diacylglycerol acetyl transferase (DGAT) which results in the accumulation of triacylglycerol stores in the kidney and liver (Table 4.4; Kao et al. 1997a, b). Following this lipogenic phase where total body fat content increases from 4 to 14% (Lowe et al. 1973; O'Boyle and Beamish 1977), sea lamprey transition into a period of elevated lipolysis at stage 4/5 of metamorphosis, whereby lipid reserves fuel the remainder of the protracted, non-trophic metamorphosis. This lipolytic phase is characterized by elevations in lipase activity and suppression of the aforementioned lipogenic enzymes (Table 4.4; Kao et al. 1997a, b). The increases in insulin and somatostatin (SST) that coincide with the lipolytic phase of the cycle suggest that this shift in lipid metabolism is regulated and under hormonal control. Pancreatic-intestinal levels of somatostatin begin to increase at stage 4 of metamorphosis and peak at stage 7 (Elliott and Youson 1991); likewise, serum insulin levels are significantly elevated in stages 6 and 7 of metamorphosis (Youson et al. 1994). Although these elevations are in part related to the expansion and development of the endocrine pancreas (see Sect. 4.5.1.3), there is little doubt that they also play an important role in lipid mobilization. For instance, free fatty acid levels in larval and stage 6 metamorphosing lamprey are significantly elevated following intraperitoneal injections of SST-14 (Kao et al. 1998). Moreover, treatment of lamprey with insulin or alloxan resulted in plasma fatty acid levels that were lower and higher, respectively, than those in controls (Kao et al. 1999a). These changes in total plasma fatty acid levels coincided with appropriate changes in lipogenic and lipolytic enzymes, suggesting that

hormones modulate, either directly or indirectly, the activity of these lipolytic and lipogenic enzymes.

The hypothesis that THs have a dual role in metamorphosis (Manzon 2011), as discussed above, is consistent with the two-phase lipid metabolic cycle observed in lampreys and supported by data that suggest that TH can modulate lipid metabolism in lampreys as is observed in higher vertebrates. Treatment with $KClO_4$ results in changes in lipolytic and lipogenic patterns which closely approximate those observed during metamorphosis and these patterns can be reversed with simultaneous treatment by exogenous TH (Kao et al. 1999b). Perhaps the high TH levels observed late in larval life function to upregulate lipogenic enzymes and processes and promote lipid accumulation, ultimately preparing the animal for the non-trophic metamorphosis. Collectively these TH and lipogenic signals might be key factors in establishing the metamorphic program long before any external morphogenic changes.

In addition to their function as an essential energy source during metamorphosis, fat stores generated during the lipogenic phase may function more directly in the modulation of metabolism during metamorphosis and more specifically the metamorphic process itself. Leptin is an important regulator of food consumption, metabolism, and body mass and can influence growth, reproduction, and thyroid function in most mammals studied to date (Harvey and Ashford 2003; Ahima and Osei 2004, 2008). Leptin and its receptor are also expressed during embryonic development and have been shown to correlate with birth weight and fat accumulation prior to birth (Cetin et al. 2000; Lepercq et al. 2001). These findings prompted the search for a lamprey leptin and its potential function in lamprey metamorphosis. Using a polyclonal antibody against the C-terminal end of human leptin, four immunoreactive proteins were identified in lamprey tissue including a 65 kDa protein in serum, 100 and 50 kDa proteins in muscle and the fat column, and 50 and 16 kDa proteins in the nephric fold (Yaghoubian et al. 2001). Of particular interest is the presence of an immunoreactive protein, similar in size to the mammalian leptin (16 kDa), in the nephric fold early in metamorphosis (Yaghoubian et al. 2001). The nephric fold is the primary site of fat storage in larval and early metamorphic sea lamprey (Youson et al. 1979) and, therefore, it is feasible this leptin-like immunoreactivity is reflecting important functions of a leptin-like protein in metamorphosis of this species. Although definitive identification of lamprey leptin is still pending, recent findings in other non-amniotes (e.g., Crespi and Denver 2006; Copeland et al. 2011) provide good rationale to continue the search for lamprey leptin and the investigation into its possible function in lamprey metabolism and development.

4.5 Internal Changes: Morphology, Physiology, Biochemistry and Molecular Biology of Metamorphosis

4.5.1 Endocrine Systems

4.5.1.1 Hypothalamic-Pituitary (HP) Axis

The potential function of the HP axis in the modulation of lamprey metamorphosis has been discussed above (Sect. 4.4.2.2) and its role in reproduction is reviewed in this volume by Sower (see Chap. 7). What follows is a brief description of the changes in proopiocortin (POC) and proopiomelanotropin (POM) throughout the metamorphic period. In gnathostomes, members of the proopiomelanocortin (POMC) family of peptides are derived from the preprohormone, POMC, which is encoded for by a single gene. POMC is post-translationally processed, in a cell-specific manner, to generate the following bioactive hormones: adrenocorticotropin (ACTH), melanotropins (MSH; γ-MSH and β-MSH), endorphin (β-END), and lipotropin (LPH) (Smith and Funder 1988). In contrast to the jawed vertebrates, lamprey POMC family members are encoded for by two distinct genes, namely POC and POM (Heinig et al. 1995; Takahashi et al. 1995; Youson et al. 2006). POC encodes for ACTH, one MSH, β-END, and nasohypophysial factor (NHF), and POM encodes for MSH-A, MSH-B, and a different β-END (Takahashi et al. 1995). Although POMC expression is widely distributed in the brain and pituitary of most vertebrates, in lampreys POM and POC expression are restricted to the pars intermedia (PI) and the pars distalis (PD), respectively (Ficele et al. 1998). Likewise, immunohistochemical data for MSH-like and ACTH peptides indicate that they are restricted to the PI and PD, respectively (Nozaki et al. 1995, 2008). Thus, the spatial distribution of POM and POC in the lamprey adenohypophysis is consistent with the localization of POMC-derived peptides in gnathostomes where MSH and ACTH peptides are restricted to the melanotropes of the PI and the corticotropes of the PD, respectively (see Sower 1998).

The expression of both POM and POC are temporally and spatially regulated in the adenohypophysis of sea lamprey. Messenger RNA levels of both transcripts increase following the completion of metamorphosis, and are elevated in parasitic juveniles and prespawning adults (Heinig et al. 1999; Youson et al. 2006). Transcript levels in larvae are low, but increase later in metamorphosis. A similar temporal expression pattern was observed in the non-parasitic American brook lamprey, although the rise in expression begins slightly earlier than in sea lamprey, which may be a function of the earlier onset of gonadal maturation in the non-parasitic brook lamprey (Youson et al. 2006).

Detailed spatial and temporal analysis of POC expression, using in situ hybridization, shows it is expressed uniformly in the rostral pars distalis (RPD) of larvae, metamorphosing lamprey, and spawning adults (Ficele et al. 1998). POC-expressing cells are also scattered throughout the caudal (or proximal) pars distalis (CPD

or PPD) beginning at stage 5 of metamorphosis with expression becoming restricted to dorsally located cells of the CPD in prespawning adults. Quantitative analysis of signal density and volume in these in situ experiments are consistent with the aforementioned gene expression studies. The increase in POC and ACTH immunoreactivity late in metamorphosis (Nozaki et al. 2008) suggest that ACTH may be important in lamprey metamorphosis. In amphibians, glucocorticoids, whose synthesis is regulated by ACTH, modulate TH action in temporal and tissue specific manners (Denver et al. 2002; Glennemeier and Denver 2002) and are essential for the completion of metamorphosis (Rémy and Bounhiol 1971). This role for POC and ACTH in lamprey metamorphosis is further corroborated by the fact that, in pouched lamprey, removal of the RPD prevented metamorphosis and removal of the CPD resulted in metamorphic stasis at stage 3 (Joss 1985).

POM expression was detected in most cells of the PI throughout all life cycle stages with signal density being higher in immediately premetamorphic animals than all other stages except spawning adults. Signal density decreased during stages 1–5 of metamorphosis, then gradually increased to high levels in spawning adults (Ficele et al. 1998). Despite the decrease in signal density, the number of POM-expressing cells and volumetric data indicate an overall increase in POM expression with the decrease in density likely related to an increase in the size of the PI (Ficele et al. 1998). This latter conclusion is consistent with earlier northern blotting data (Heinig et al. 1999; Youson et al. 2006). The maximal expression of POM just prior to metamorphosis suggests that it may be involved in preparing larvae for metamorphosis (Ficele et al. 1998). Similarly, the elevated levels of POM late in metamorphosis coincide with, and likely function to elicit, the changes in pigmentation observed at stages 5 and 6 of metamorphosis.

4.5.1.2 Thyroid Axis

In addition to functioning in lamprey metamorphosis, the thyroid hormone-producing tissue itself also undergoes profound changes during metamorphosis. Larval lampreys are unique among vertebrates in that they are the only members of the subphylum that does not use follicles or tubules to synthesize TH (Leatherland 1994). Instead, TH synthesis in larval lampreys occurs in the subpharyngeal endostyle, which is similar in form and function to the endostyle of protochordates (Barrington and Sage 1972). The larval endostyle undergoes transformation during metamorphosis, giving rise to the typical vertebrate thyroid follicles found in post-metamorphic lampreys. The transformation of the endostyle into follicular thyroid tissues has been studied extensively over the years (e.g., Wright and Youson 1976, 1980; Wright et al. 1978, 1980) as has its homology to the protochordate endostyle (Ogasawara et al. 1999). Many of the details associated with the changes of the thyroid axis during lamprey metamorphosis have been addressed throughout this chapter (see Sect. 4.4.2.2) and in other recent reviews on the topic (Youson 2007; Manzon 2011).

4.5.1.3 Entero-Pancreatic Endocrine System

At the time of the last major review of the morphology and physiology of lamprey metamorphosis (Youson 1980), information on the endocrine pancreatic tissue at this interval of the life cycle was based on studies that took place in the 1920s and 1930s. Youson (1981a, c), in reviews of the morphology and physiology of the alimentary canal and the liver, respectively, throughout the lamprey life cycle, emphasized the relationship of changes in these organs during metamorphosis to alterations in the entero-pancreatic tissue. Youson (1981a) also emphasized, and supported with new data, earlier literature that endocrine cells are present within the intestinal epithelium of both larvae and adults. These latter cells were, at that time, referred to as members of the amino precursor uptake and decarboxylation (APUD) series. Since that time, it has become more common to refer to the endocrine cells of the vertebrate alimentary canal and cells of the endocrine pancreas as having a common ontogeny (Falkmer 1985) and being part of the gastro-entero-pancreatic system (GEP) and hence, as GEP cells liberating GEP peptides. Since the lamprey has no stomach, it seems more correct to use the term entero-pancreatic (EP) endocrine system (Youson and Al-Mahrouki 1999; Youson 2000, 2007). Furthermore, Youson and Al-Mahrouki (1999) provide a case for using the term islet organ in lampreys; this term was used by Epple and Brinn (1986) to describe the islet aggregates in larval lampreys. In addition, since the islet tissue in adult lampreys is compacted into one or two large bodies or lobules without intervening exocrine acini, the term principal islet is recommended for each large body (Youson and Al-Mahrouki 1999).

The islet organ of larval lampreys consists only of insulin-containing (B) cells but the degree of immunoreactivity (IR) is variable among holarctic and some Southern Hemisphere species (Youson and Potter 1993b). All larval lampreys have endocrine cells in the intestine IR to antisera against several peptides of the neuropeptide Y (NPY)/pancreatic polypeptide (PP) and somatostatin (SST) families. The islet organs of adults of the two Southern Hemisphere species examined to date have only a cranial principal islet (Hilliard and Potter 1988; Youson and Potter 1993b), as a result of a difference in EP morphogenesis at metamorphosis from that which takes place in holarctic lampreys; the latter have both a cranial and a caudal islet. Immunohistochemistry reveals that the principal islets of adult lampreys are primarily IR for insulin and SST antisera, with some IR with NPY family antisera (Cheung et al. 1990, 1991a; Youson and Potter 1993a). The intestine has cells IR with antisera for the glucagon family of peptides, for the NPY family of peptides, and a cell type that co-localizes both family peptides (Cheung et al. 1991a).

The morphogenesis of the lamprey EP system, and mainly the islet organ, has been reported and reviewed in great detail over the past 20 years (Youson and Elliott 1989; Youson and Cheung 1990; Youson and Al-Mahrouki 1999; Youson 2000, 2007) and the reader is referred to these publications for an expansive report on both the ontogeny and the phylogenetic significance. Here we provide only the highlights of the findings during this very active period of investigation.

The extrahepatic common bile duct (ECBD) of larvae has a variable role in the production of the adult islet organ; this was demonstrated in the 1920s and 1930s

from serial sections of metamorphosing European brook and river lampreys (Keibel 1927; Boenig 1928, 1929) and observations on the gut diverticulum during metamorphosis in pouched lamprey (Maskell 1931), and was later confirmed in both sea lamprey (Elliott and Youson 1987) with immunohistochemistry and in pouched lamprey (Hilliard and Potter 1988) with more conventional staining methods. As noted below in the descriptions of the liver at metamorphosis (Sect. 4.5.2.2), there is no contribution of the larval ECBD to the formation of the islet organ in adult pouched lamprey which consists of only a cranial principal islet (Hilliard et al. 1985; Hilliard and Potter 1988). In contrast, in sea lamprey (Elliott and Youson 1988, 1993a, b) and most of the other holarctic lampreys (Youson et al. 1988), there are both cranial and caudal principal islets with a narrow band of islets (the intermediate cord) between the two principal islets (Fig. 4.7). In holarctic species, the larval ECBD cells do not completely regress but the epithelium undergoes mitosis and transforms into endocrine cells (Fig. 4.8) that eventually are IR to either insulin or somatostatin antisera and make up the caudal principal islet (Elliott and Youson 1987). Autoradiography with ^{3}H-thymidine shows that some proliferation of the epithelium of the ECBD occurs at stages 1 and 2 in metamorphosis of sea lamprey but by stage 3, there is a multi-layered ECBD with a reduced lumen (Elliott and Youson 1993a). At this stage, and stage 4, extensive labeling for DNA synthesis is evident over many mitotic figures with clusters of cells budding from the epithelium. The ECBD appears as a remnant with a small lumen in stage 5 and the islet mass appears much like a small caudal principal islet but still showing extensive DNA synthesis in this stage and stage 6 (Fig. 4.8). Stage 7 shows decreased labeling with ^{3}H-thymidine from earlier stages and the caudal principal islet has the form and position of the juvenile. Details of the anatomical repositioning of the caudal principal islet relative to other components of the digestive system and the cranial principal islet are provided in Elliott and Youson (1993a) and also illustrated in Fig. 4.7. Fine structural observations and immunocytochemistry with insulin and somatostatin antisera show that transforming bile duct cells eventually possess insulin-IR granules by stage 4 but cells with somatostatin-IR granules are not present in the cranial principal islet until stage 7 (Elliott and Youson 1993b).

As was suspected for the pouched lamprey (Hilliard and Potter 1988), the insulin-IR cells that make up the cranial principal islet of the sea lamprey were initially believed to originate from the islet organ of larvae (Elliott and Youson 1987), which is made up exclusively of insulin (B) cells (Elliott and Youson 1986, 1987; Cheung et al. 1991b). Somatostatin-IR cells or D cells and cells containing IR for peptides of the NPY family are restricted to the larval intestine (Elliott and Youson 1986; Cheung et al. 1991b). There are only insulin-IR cells in the developing cranial principal islet from stages 1 to 5 in metamorphosing sea lamprey but somatostatin-IR cells appear late in metamorphosis as islets budding from epithelium of the newly developed diverticulum at the esophageal-intestinal junction (Elliott and Youson 1987). Sea lamprey anti-insulin permitted the development of an homologous radioimmunoassay for measurement of the changing profile of serum levels of insulin that corresponded with the development at metamorphosis of the cranial and caudal principal islets in sea lamprey (Youson et al. 1994). The late appearance

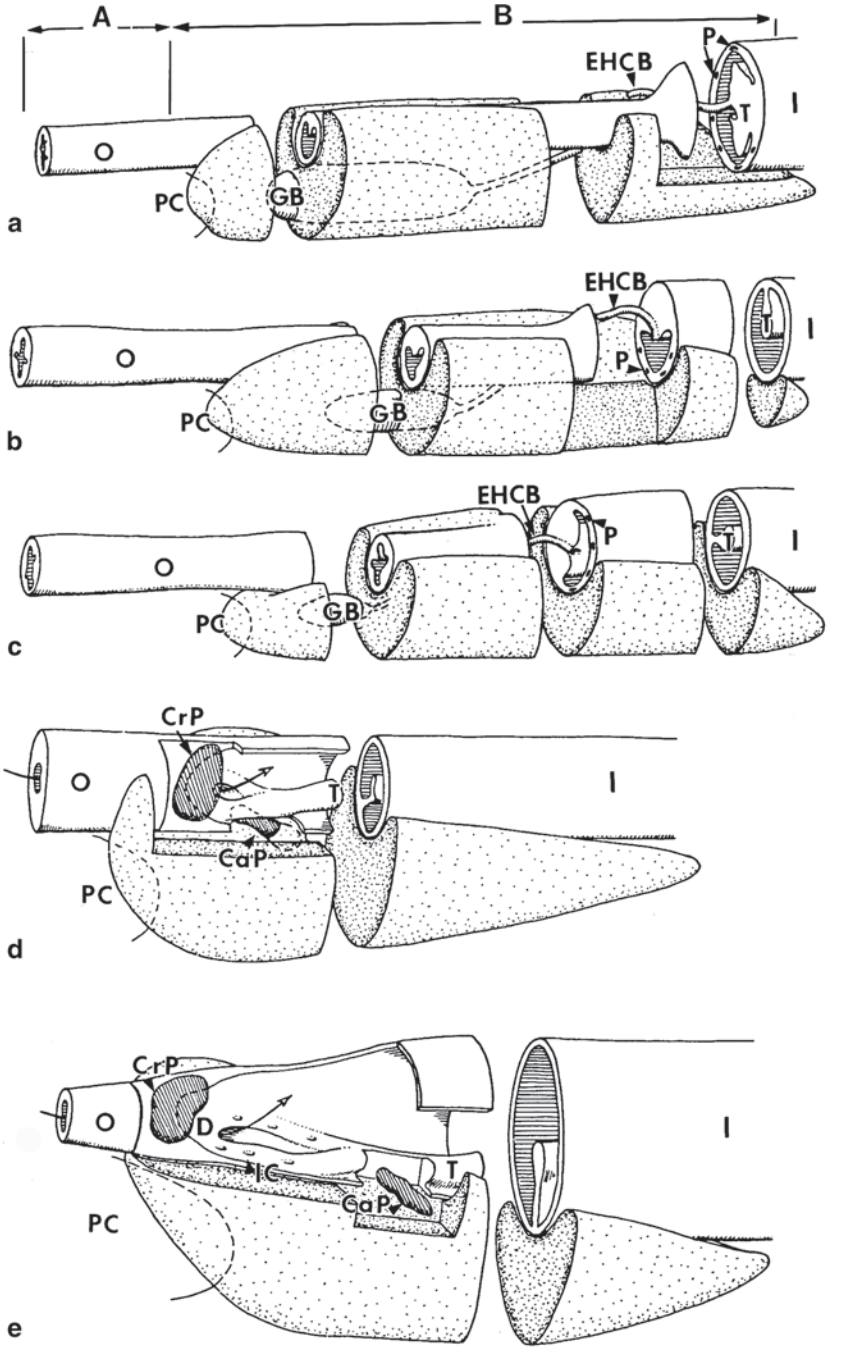

Fig. 4.7 Diagrammatic representation of the larval esophagus (*O*), and anterior intestine (*I*) during sea lamprey metamorphosis. The larval esophagus is separated into A and B regions in the larva

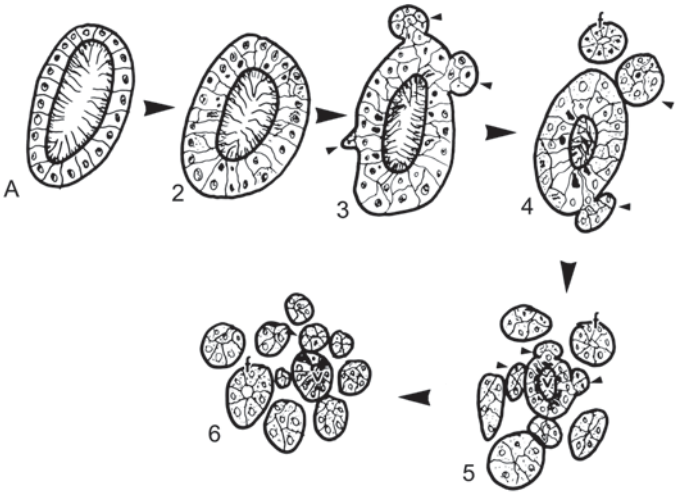

Fig. 4.8 Diagrammatic representation of the transformation of the extrahepatic common bile duct into the caudal principal islet during stages 2–6 of metamorphosis in sea lamprey. In larvae (*A*), the bile duct is composed of a simple ciliated columnar epithelium. In stage 2, the epithelium becomes bilayered in places. In stage 3, small islets (*small arrowheads*) have formed in the base of the epithelium. In stage 4, the islets continue to form but newly formed islets (*f*) have migrated into the surrounding connective tissue (*small arrowheads*) and become located near the bile duct. The bile duct (*v*) becomes progressively smaller in stage 5 and by stage 6 it is very small or residual. This figure was originally published in Elliott and Youson (1993a) and reproduced with permission of John Wiley & Sons, Inc

of the somatostatin-producing D cells, relative to the B cells, is consistent with that of the caudal principal islet and is also consistent with data showing increased levels of somatostatin in intestinal/islet organ extracts during metamorphosis in sea lamprey (Elliott and Youson 1991). Autoradiography and ^{3}H-thymidine reveal that much of the islet tissue of the cranial principal islet arises from the budding of cell clusters from the diverticular epithelium in metamorphosing sea lamprey (Elliott and Youson 1993a). Islet tissue attached to, or within, the esophagus and intestinal epithelia shows isotope label but there is no indication of DNA synthesis in the isolated larval islets (Elliott and Youson 1993a). It is not until about stages 4 and 5 that DNA synthesis is evident in any significant amount in the developing cranial islet tissues; substantive labeling with ^{3}H-thymidine is evident in the diverticular

(**a**) to show the contributions of this structure to the changing alimentary canal at stage 2 (**b**), stage 3 (**c**), stage 4 (**d**), and in the adult (**e**). The diagrams also show the larval liver with intrahepatic gall bladder (*GB*), the extrahepatic common bile duct (*EHCB*), and the location of the pancreatic islets (*P*). The cranial principal islet (*CrP*) appears and moves towards the developing pericardial cavity (*PC*) with the forward movement of the larval esophagus, whereas the caudal principal islet (*CaP*) appears at the site where the EHCB entered the larval esophagus-intestinal junction. Note the disappearance of the gall bladder and EHCB and the development of a diverticulum (*D*). (This figure was originally published in Elliott (1989) and reproduced with permission of W. M. Elliott)

epithelium at this time. By stage 6, the cranial principal islet is adult in form with reduced DNA synthesis but it is not until stage 7 that it resembles the size observed in juveniles. It is noteworthy that this recruitment of endocrine cells from the diverticular epithelium to the cranial principal islet continues through adult life, even during the upstream, spawning migrations (Cheung et al. 1990). Sometime during metamorphosis, cells with peptides IR to the NPY family antisera must be recruited from the intestinal epithelium because both the cranial and caudal principal islets in adult sea lamprey have cells with content that is NPY family-IR. The events of such recruitment or any other source of this new anti-NPY immunoreactive F cell (see Youson and Al-Mahrouki 1999) for the adult islet organ has not been studied. Cheung et al. (1991a) have speculated, based on observations of co-localization of peptides, that there may be a precursor cell from the intestine that gives rise to both insulin- and NPY-containing cells in the cranial principal islet. Another consequence of metamorphosis was the co-localization of a glucagon-like peptide in the same cells in the intestine that contain NPY-IR peptides and other cells that contain only glucagon family peptides (Cheung et al. 1991a).

4.5.2 Digestive System

Changes to the digestive system during lamprey metamorphosis have been of interest to morphologists for at least 125 years (see reviews by Youson 1980, 1981a, c, 1985). In adult lampreys, the term digestive system encompasses the mouth, teeth, and tongue, the digestive tube or the alimentary canal, the liver, and the exocrine pancreas. These adult structures arise as either modifications of similar larval structures or develop from undifferentiated larval tissues that were likely present since the completion of embryogenesis. In all species of lampreys, the changes in the digestive system are so dramatic that feeding is not possible (i.e., this is a non-trophic interval of the life cycle regardless of adult feeding type); during this time, lampreys rely on internal stores for the energy required for the developmental processes. The limits of space for this chapter prevent a discussion of all parts of the digestive system during metamorphosis. Since the earlier reviews (referenced above), there have been extensive studies on alterations to the alimentary canal and liver during lamprey metamorphosis. The following is a summary of these studies.

4.5.2.1 Alimentary Canal

Esophagus

For extensive treatment of the research in this subject area up to and including 1981, the reader is referred to Youson (1981a). In summary, all changes of the alimentary canal occur in both parasitic and non-parasitic species, even though the latter will never feed in adult life. To permit tidal ventilation of the adult gills while the animal is attached by the oral disc, a new esophagus develops that is independent of the

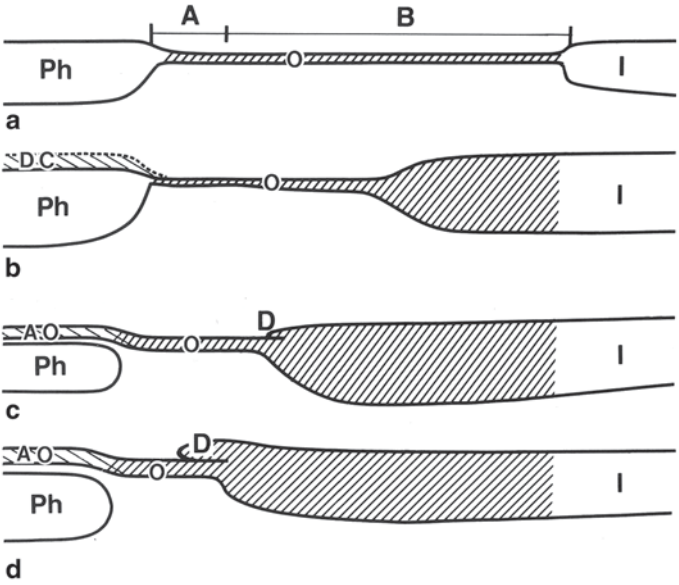

Fig. 4.9 Diagrammatic representation showing the forward movement of the esophagus-intestinal junction and the development of the adult esophagus (*AO*) from a dorsal cord (*DC*) of tissue during metamorphosis in sea lamprey: **a** larva, **b** metamorphosing stage 3, **c** metamorphosing stage 4, **d** juvenile. Whereas the larval esophagus (*O*) leads from the caudal end of the pharynx (*Ph*), the adult esophagus is independent of the pharynx. *A* and *B* denote regions of the larval esophagus and how it contributes to development of the adult esophagus and intestine (*I*) and a small diverticulum (*D*). (This figure was originally published in Elliott (1989) and reproduced with permission of W. M. Elliott)

pharynx but connects the oral cavity with the intestine. The new esophagus appears from the dorsal wall of the larval pharynx as a cord of cells that undergoes proliferation and vacuolation to produce a lumen for the tube in an anterior to posterior progression (Fig. 4.9). The timing of the patency of the esophageal lumen seems to be variable both among and within species and, in anadromous species, is important for the initiation of saltwater acclimation (e.g., Richards and Beamish 1981; Beamish and Youson 1987).

There has been little attention to the events of metamorphosis surrounding what happens to the larval esophagus. In sea lamprey, the larval esophagus leads from the caudal end of the pharynx and extends beneath the liver to unite with the intestine at the caudal tip of the liver (Figs. 4.7 and 4.9). It is at the esophageal-intestinal junction where the islet organ of the larva is located within the submucosa. In the adult, the esophageal-intestinal junction is at the caudal end of the cardiac region at the top of the liver and there is an associated cranial islet tissue. The question of whether the larval esophagus contributes to the formation of either the adult esophagus or the adult anterior intestine was answered through the extensive investigation of Elliott (1989) during metamorphosis in the sea lamprey (Figs. 4.7 and 4.9). The anterior-most portion of the larval esophagus develops into the posterior portion of the adult esophagus (Fig. 4.9). By stage 2–3 of metamorphosis, the anterior

(cranial) portion of the larval esophagus within the developing pericardial cavity is collapsed with a narrow lumen, whereas the more caudal part still retains the larval form. The anterior portion eventually collapses into a solid cord of epithelial cells that becomes continuous with the new cord of cells appearing more cranially from the dorsal wall of the pharynx. A small lumen appears by stage 5 in the most caudal part of the cord (i.e., the part produced from the larval anterior esophagus) as the result of the merging of intercellular spaces, similar to the development of the adult esophageal lumen more cranially. Longitudinal mucosal folds are evident in stage 6 and reach the adult state at stage 7. At this latter stage, the esophageal-intestinal junction is now situated at the caudal end of the pericardial cavity (Fig. 4.7). Elliott (1989) suggested that the movement of this junction in metamorphosis is related to the developing pericardial cartilage since it is attached to the cartilage from the first appearance of this developing structure. This interpretation was also expressed for the anterior movement of the esophageal-intestinal junction in the pouched lamprey (Hilliard and Potter 1988). However, there are likely many other factors involved in this junctional repositioning, such as changes in the epithelial lining of the larval esophagus, the counter-clockwise rotation of this region of the alimentary canal (Hilliard and Potter 1988; Elliott 1989), changes/contractions to the sub-epithelial layers in the gut wall (Elliott 1989), and changes to the vasculature of the liver and intestine, namely to the anterior mesenteric artery and the hepatic portal vein (Hilliard and Potter 1988). Other contributing factors are loss of the extrahepatic common bile duct (ECBD), the intestinal diverticula, and even a lobe of the liver in the case of the pouched lamprey (Hilliard and Potter 1988). Regardless of which of these are the most important factors in the repositioning of the esophageal-intestinal junction during lamprey metamorphosis, it is clear that any disruption to, or delay in, the process can result in a different result. The potentially parasitic western brook lamprey morphotype ("marifuga") in Morrison Creek on Vancouver Island (see Sect. 4.2.2) shows many features of a delayed or interrupted metamorphosis, including a much more caudal positioning of the esophageal-intestinal junction and no cranial portion of the islet organ compared to non-parasitic western brook lamprey after metamorphosis (Youson and Beamish 1991).

The remaining portion of the larval esophagus transforms into a portion of the adult anterior intestine, thereby lengthening the intestinal portion of the alimentary canal. This development may be necessary to increase the surface area for absorption and for temporary storage of ingested material for an animal about to embark on a new feeding regime high in protein and fat. Elliott (1989) provides a detailed ultrastructural analysis of the transformation of the larval esophageal epithelium into adult intestinal epithelium in the sea lamprey. The details of this transformation will be summarized in the section below.

Intestine

Since there is no stomach in lampreys, the esophagus leads directly into the anterior intestine. Youson (1985) describes regional functional specialization of the adult lamprey intestine. The anterior intestine is specialized for ion transport during

osmoregulation, the release of digestive enzymes, and the majority of fat absorption. There is a short transition region between the anterior and posterior regions of the intestine, with the latter region and the hindgut (leading into the cloaca) specialized for protein absorption and mucus secretion. All three areas (the anterior intestine, posterior intestine, and hindgut) are involved in the accumulation and release of the bile pigment, biliverdin, and iron into the intestinal lumen. All of the regional functional specializations are a consequence of modifications to the larval alimentary canal that take place at metamorphosis.

As indicated above, the majority of the larval esophagus transforms or remodels into the most cranial segment of the adult anterior intestine (Elliott 1989). This remodeling involves extrusion of ciliated, secretory (zymogen), and mucous cells into the lumen (which remains patent throughout metamorphosis) and infiltration of the transforming esophageal epithelium by macrophages and extravasated blood cells (i.e., blood cells that have exited the capillaries). The presence of the latter cells may imply that apoptosis occurs in the epithelium at this time. Unlike the development of the adult intestinal epithelium in anuran amphibians, where there are cell nests or a secondary epithelium in the larva to generate the definitive epithelium at metamorphosis (Bonneville 1963; Shi and Oshizuya-Oka 1996), the adult intestinal epithelium in lampreys arises through histogenesis and histolysis of existing larval cells. However, autoradiography with ^{3}H-thymidine indicates that basal columnar cells may serve as stem cells during early stages of metamorphosis in sea lamprey (Elliott 1989). Ciliated, ion-transporting, and secretory (zymogen) cells differentiate from absorptive cells during stages 6 and 7 of metamorphosis in sea lamprey. The loss of larval secretory cells and their replacement with adult secretory cells is necessitated by the changing diet and the elaboration of a different set of digestive enzymes in adults (Hilliard and Potter 1988).

Development of longitudinal mucosal folds is a primary feature of metamorphosis of the alimentary canal, particularly the intestine (Youson and Connelly 1978; Youson and Horbert 1982). Autophagy, heterophagy, apoptosis, and cell proliferation are key events in this process. In addition, Elliott and Youson (1994) studied the transforming larval esophagus to show the interaction of dedifferentiated, migrating smooth muscles in the sub-epithelial layers with the mucosal epithelium as a critical process in the development of the folds. Cells extruded from the transforming anterior regions of the intestine are phagocytosed by mucosal cells in the posterior intestine (Youson and Horbert 1982).

4.5.2.2 Liver

The changes to the liver during metamorphosis were recognized by Bujor (1891) and since that time have received wide attention (Youson 1981c). The attention has primarily been driven by curiosity regarding the loss of the biliary tree and the gall bladder during metamorphosis and how post-metamorphic lampreys survive without these structures for both the storage and elimination of bile products. The review by Youson (1993) highlights and provides the references for numerous publications that describe most features of the processes of bile duct and gall bladder loss and

the changes to hepatocytes, and discusses both the consequences of these events and compensatory mechanisms for adult life. These include changes in hepatocyte cell junctions, periductal fibrosis, an apparent cholestasis, and alternation in bile pigments. A disruption to this programmed loss of bile ducts has been reported in the potentially parasitic morph of the western brook lamprey (see Sect. 4.2.2), presumably due to alterations in the timing of metamorphosis (Youson and Beamish 1991). This biliary atresia in lampreys has been of interest to the medical community, for biliary atresia in human infants usually results in death unless a liver transplant is available (see Youson 1993; Morii et al. 2012; see Chap. 1). Recently, apoptosis has been identified as an early event in bile duct loss in both induced (Boomer et al. 2010) and spontaneous (Morii et al. 2010) metamorphosis in sea lamprey and Far Eastern brook lamprey, respectively.

The other curious event about the loss of the biliary tree that was recognized by early anatomists was the close relationship of the degenerating extrahepatic common bile duct (ECBD) with the development of a caudal portion of the islet organ in some, but not all, lamprey species. In fact, the presence or absence of a caudal portion of the islet organ in a lamprey species is related to the site at which the larval ECBD enters the alimentary canal (Youson 1985; Youson and Elliott 1989; Youson and Cheung 1990; Potter and Gill 2003). Thus, in the pouched and short-headed lampreys from the Southern Hemisphere, the ECBD enters the cranial end of the left diverticulum and this ECBD does not contribute to the formation of a caudal portion of the islet organ or any islet tissue (see Sect. 4.5.1.3). In contrast, adults of all Northern Hemisphere species of lampreys have a caudal portion of their islet organ, for the larval ECBD enters the alimentary canal at the esophageal-intestinal junction (Fig. 4.7) and the ECBD epithelium undergoes a transdifferentiation (dedifferentiation/redifferentiation) at metamorphosis (Elliott and Youson 1993a, b). Ultimately, these former ECBD cells contain immunoreactivity for insulin and somatostatin, proliferate and migrate, and form small islets within the caudal islet organ. That biliary atresia is an important event of metamorphosis in all species is illustrated by the abnormal development that takes place when the intrahepatic bile ducts and the ECBD are prevented from atresia due to the presence of nematodes in American brook lamprey (Eng and Youson 1992a, b).

One other feature of the metamorphosing liver that attracted much research activity over the past two decades relates to changes in iron metabolism. The prominent literature on this subject matter has been reviewed (Youson 1993, 2003) but it is important to emphasize and summarize the events here, for without such a report, the full story of the metamorphosing liver would be incomplete. For a more detailed summary of the extent of the changes in iron metabolism and deposition during the life cycle, the reader is referred to Youson (2003). In larval lampreys, non-heme iron is bound to ferritin in the plasma at very high concentrations in all lamprey species, with the highest recorded for short-headed lamprey at 27,000 μg/100 mL (Macey et al. 1982). Even the blood values in larvae of other lamprey species are 250–500 times that recorded for humans (Macey and Potter 1986; Youson et al. 1987).

There are many other larval tissues, mainly those that have fat deposits, that have high concentrations of iron (Macey and Youson 1990; Youson 2003). Staining for elemental iron shows a gradual accumulation of this metal in the hepatocytes

of the liver during lamprey metamorphosis (Youson et al. 1983), and this morphology is reflected in increased concentrations of hepatic iron in assays (Sargent and Youson 1986; Smalley et al. 1986; Harris et al. 1990). This increase in hepatic iron is coincident with the decline in levels of serum iron (Youson et al. 1987) and the change from ferritin to a transferrin as the iron-binding plasma protein (Macey et al. 1982). Youson (1993) provides evidence to support the view that iron overload in the liver is a consequence of alterations to the alimentary canal and to the biliary system during lamprey metamorphosis that prevent the elimination of excess iron. However, the lamprey seems to have the means to deal with the potential toxicity of the iron (i.e., the interaction of iron with hydrogen peroxide and a superoxide radical to produce hydroxyl radicals) through the increased activity towards the end of metamorphosis of the key enzyme superoxide dismutase (Harris et al. 1990). As with the study of biliary atresia, how lampreys avoid the harmful effects associated with such high concentrations of non-heme iron is of biomedical interest (see Macey et al. 1988; Harris et al. 1995; see Chap. 1).

4.5.3 Renal System

The changes to the kidneys of lampreys is a dramatic event at metamorphosis and has received considerable discussion in past reviews (Youson 1980, 1981b). In summary, the larval renal tissue consists of a pronephros, an opisthonephros, and undifferentiated nephrogenic tissue extending to the cloaca (Fig. 4.10). Ellis (1993) was the first to describe the appearance of the pronephros throughout the lamprey life cycle. This kidney shows degenerative changes throughout larval life (Ellis and Youson 1990), and during metamorphosis there is gradual compacting of the paired pronephroi, relative to the length of each kidney in larvae, as this kidney becomes isolated from the coelomic cavity with development of a cartilage casing around the heart (Ellis 1993). The cells of presumptive adrenocortical tissue within the pronephros undergo changes during metamorphosis that suggest involvement in steroidogenesis (Ellis 1993). The healthy state of these cells was noted previously in the regressing larval opisthonephros (Youson 1980). At metamorphosis, the larval opisthonephros undergoes a complete regression and is replaced by a more posterior adult opisthonephros that arises from the nephrogenic tissue that has been present since embryogenesis. Youson (1984) has provided a detailed ultrastructural description of tubulogenesis during metamorphosis in sea lamprey. There are some fascinating developmental events during this process; one example is the method of producing cilia for the neck segment portion of the most proximal portion of the tubule (Youson 1982). The complete regression of the larval opisthonephros during metamorphosis (Fig. 4.10) is an event that would parallel tail reabsorption in amphibian metamorphosis. In the latter process, there is a detailed understanding and profile of endocrine involvement and the events of regression (see Shi 2000). Although it has been many years since the morphology was described for renal regression in lampreys (Ooi and Youson 1979), no one has taken up the challenge

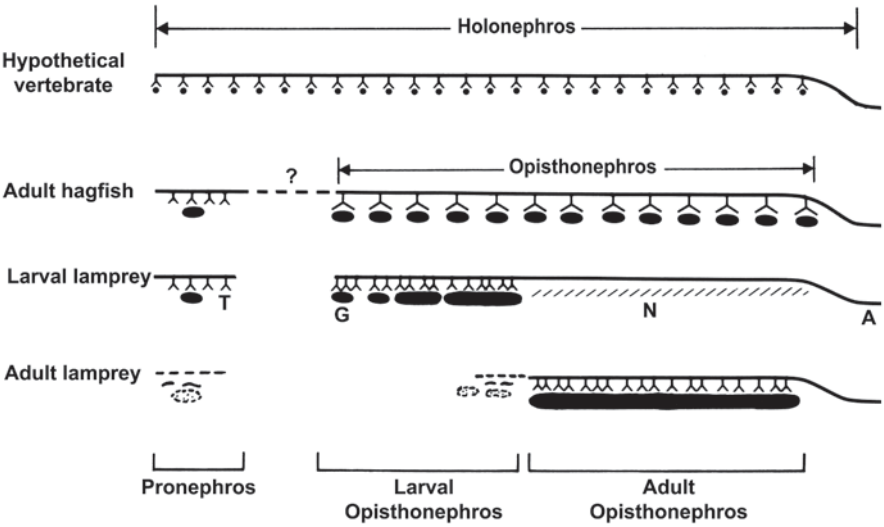

Fig. 4.10 Schematic interpretation of the relationship of the kidneys of larval and adult lampreys on one side of the body at each stage of the life cycle (i.e., the kidneys are paired). The larval lamprey renal tissue consists of a functioning pronephros with renal tubules (*T*) and a single glomus (*G*). The more caudal larval opisthonephros, situated about mid-position in the abdomen, has several end-to-end glomera with draining tubules. There is undifferentiated nephrogenic tissue (*N*) extending to the cloaca near the archinephric duct (*A*) which is continuous between the pronephros and opisthonephros (not shown). Throughout life, the pronephric tubules gradually degenerate whereas at metamorphosis the larval opisthonephros undergoes a rapid degeneration (with remnants still present in the adult) and the adult opisthonephros develops behind from the nephrogenic tissue. The adult kidney has a single glomus and many draining tubules. (Modified from Youson 1985)

to explain the regressive events in the context of hormonal and/or genetic control. Given that the specific triggers for lamprey metamorphosis are still in question (Gross and Manzon 2011), it would seem that regression of the larval opisthonephros, and even the development of the adult opisthonephros, would serve as an excellent system for study of the genes and hormone interaction of lamprey metamorphosis. The success at inducing lamprey metamorphosis (see Sect. 4.4.2.2) and both the regressive and growth processes (Boomer et al. 2010) only adds to the value of this potential model.

4.5.4 Respiratory System

Metamorphosis marks the shift from the unidirectional, flow-through ventilation of the larval branchial basket to the tidal, pumping ventilation of the adult. It involves not only modifications to the gill pouches and their connecting tube, but also enlargement of the heart and development of the pericardial cartilage, modifications

to the circulatory system, and appearance of an adult-type hemoglobin. Hardisty (2006) suggested that the rate at which such changes to the "breathing system" take place are particularly critical to the survival of metamorphosing lampreys, and that—given that these changes must be such an insult to the normal pattern of oxygen exchange at the gills—some oxygen must be absorbed across the skin during this process. Reviews of the development of the gill pouches during lamprey metamorphosis are given by Lewis (1980) and Youson (1980). Lewis and Potter (1977) showed how the rate of oxygen consumption increases during metamorphosis in European river and brook lampreys, and Galloway et al. (1987) showed a similar increased rate of oxygen consumption between stages 3 and 5 in metamorphosis of the pouched lamprey, followed by a dramatic increase by stage 6. There is likely a correlation between this dramatic increase in oxygen consumption and both the completion of gill pouch modification and the appearance of adult hemoglobin. Changes to the pericardial cartilage are summarized in Sect. 4.5.5.2; the shift to the adult-type hemoglobin is reviewed in Sect. 4.5.6.1.

The other important change taking place in the gills of lampreys during their metamorphosis is that the gill epithelium undergoes modification (Peek and Youson 1979a; Morris 1980; Youson 1980). The primary modification is the proliferation of undifferentiated interlamellar cells and their differentiation into chloride cells that typify the interlamellar epithelium of adult lampreys (Peek and Youson 1979b). The importance of these chloride cells to marine osmoregulation in adult lampreys, namely the secretion of excess Na^+ and Cl^-, has been emphasized (Bartels and Potter 1991, 2004), and is further supported by the lack of these cells in silver lamprey *Ichthyomyzon unicuspis*, which are parasitic but entirely freshwater resident (Bartels et al. 2012). Interestingly, adults of the non-parasitic and freshwater American brook lamprey do possess chloride cells in early post-metamorphic life (Bartels et al. 2011). Presumably, the chloride cells in this species have been retained from a relatively recent anadromous ancestor (see Docker and Potter in press), whereas all evidence suggests that silver lamprey have been confined to fresh water for a long period of time (Bartels et al. 2012).

Morris (1972) reported that sometime during metamorphosis in non-parasitic European brook lamprey, there was a limited ability to osmoregulate in hypertonic water. However, Mathers and Beamish (1974) indicated that in sea lamprey, acclimation to even dilute sea water was only possible when metamorphosis was close to completion. There has been a recent study that focused on the activity of key ion-transporting proteins (Na^+/K^+-ATPase, vacuolar [V]-type H^+-ATPase, and carbonic anhydrase) in the gill epithelium of metamorphosing sea lamprey subjected to varied concentrations of sea water (Reis-Santos et al. 2008). Increased rates of survival at 25–35‰ salinity (i.e., 70–100 % sea water) as metamorphosis progressed are correlated with increased concentrations of Na^+/K^+-ATPase in metamorphosing lampreys compared to larvae. Immunohistochemistry shows that the Na^+/K^+-ATPase activity is localized within the interlamellar area where the mitochondria-rich, chloride cells are developing. Conversely, H^+-ATPase activity is downregulated in metamorphosing animals with changes in salinity. Thus it seems, at least in parasitic sea lamprey and in non-parasitic European brook lamprey, that osmoregulation in

hypertonic environments is possible during some interval of metamorphosis (see Chap. 3). The recent evidence implies that it has as much to do with increased activity of key ion-transporting enzymes as with the state of differentiation of chloride cells (Reis-Santos et al. 2008).

4.5.5 Skeletal System

As has been emphasized in past reviews (Youson 1980; Hardisty 1981), the changing skeleton of lampreys during metamorphosis attracted much attention from a wide group of zoologists and paleontologists from about 1880 to the mid-point of the twentieth century. In particular, a detailed analysis of the conflicts and issues between two of the most prominent investigators, Damas (1935, 1944) and Johnels (1948), was provided by Hardisty (1981), who emphasized the importance of the description of development of the cranial skeleton in lampreys to investigations and views of gnathostome and agnathan relationships that existed at that time. The skeleton of both larval and adult lampreys consists of axial and cranial portions, with the former consisting primarily of the notochord and what some (e.g., Hardisty 1981) have referred to as vertebral rudiments, the arcualia.

4.5.5.1 Axial Skeleton

Relatively little is known about changes to the axial skeleton of lampreys during metamorphosis. Potter and Welsch (1992) implied that the arcualia develop from sclerotome rudiments sometime during metamorphosis, while Zhang (2009) argued, based on recent gene expression data, that the origin of arcualia could be either sclerotome or notochord. It is noteworthy that cultured lamprey cartilage is capable of calcification (Langille and Hall 1993) and histological sections reveal what appear as bony, wedge-shaped structures around the spinal cord of spawning-phase sea lamprey (Youson unpublished data).

As far as we know, no changes take place in the notochord during lamprey metamorphosis, but no investigations have been undertaken to specifically study this axial structure during this event. Investigation seems to be justified, however, since the lamprey notochord has become an important tool for studies of vertebrate collagens (Koob and Long 2000). Specifically, the major collagen in lamprey notochordal sheath is type II collagen (Kimura and Kamimura 1982; Sheren et al. 1986) with a crystalline fibril structure (Eikenberry et al. 1984). The crystalline nature of lamprey type II collagen fibrils lends itself for study by X-ray diffraction (Eikenberry et al. 1984), and analysis of the cross-linked organization which is critical for fibrillogenesis has been suggested as an important tool for studying tissue assembly and degradation in human osteo- and rheumatoid arthritis (Antipova and Orgel 2010). Other aspects of the notochord that have been studied in the context of functional flexibility are its proteoglycan (Welsch et al. 1991) and elastic (Schinko et al. 1992) nature and cell junctions (Bartels and Potter 1998).

4.5.5.2 Cranial Skeleton

It is the cranial skeleton that has generated most research interest, for it changes during metamorphosis to accommodate the dramatic changes in feeding and respiration in the larval versus adult stages. Furthermore, the relationship of the lamprey cranial skeleton and its head segmentation during embryogenesis to that of gnathostomes has long been of interest to developmental and evolutionary biologists (Richardson et al. 2010; Lee and McCauley in press). Before discussing the cranial skeleton in metamorphosis, it is important to outline some of the key events during lamprey embryogenesis that lead to the larval skeleton. After all, metamorphosis is just a continuation of this earlier ontogeny that is interrupted by a larval interval (Youson 1988). It has been suggested that mesoderm development during lamprey embryogenesis may signify the ancestral state of gene regulation from which the complex body plan of gnathostomes evolved (Kusakabe and Kuratani 2007). In a recent review on this subject, Holland et al. (2008) concluded, based on the expression of the *engrailed* and *tbx1* genes, that the cranial paraxial mesoderm of lampreys (and sharks) evolved from the anterior somites of an amphioxus-like ancestor and is responsible for the lamprey head cavities. Remnants of these mesodermal head segments persist in the muscles of the eye and jaw of higher vertebrates but the segments were lost during evolution. Another important feature of lamprey embryogenesis that influences the larval and adult cranial skeleton involves the neural crest (Langille and Hall 1988; Hall 1999). For example, migration of neural crest cells is important to development of specific branchial arches (Langille and Hall 1988; McCauley and Bronner-Fraser 2003). This process involves lamprey *SoxE* genes that, although the chondrogenic function of its regulators seem to be from a common vertebrate ancestor, in lampreys may have undergone independent duplication leading to their distinct functions in different parts of the pharynx (McCauley and Bronner-Fraser 2006). In fact, the lamprey has been described as a model system for studying neural crest gene regulatory networks (Nikitina et al. 2008; Sauka-Spengler and Bronner-Fraser 2008). This is just one of the many features of lamprey development that make it an ideal model for evolutionary studies (Osório and Rétaux 2008; see Chap. 1; Lee and McCauley in press). The use of the lamprey model, however, has required some better definition of developmental events and processes, such as in the embryogenesis of the visceral skeleton leading to the production of the branchial basket (Martin et al. 2009).

As a consequence of chondrogenesis and matrix deposition in embryogenesis between days 13 and 19 post-fertilization (McBurney and Wright 1996; McBurney et al. 1996; Morrison et al. 2000), the cranial skeleton of larval lampreys is produced to suit the sedentary life of a filter feeder (Martin et al. 2009). Subsequently, this skeleton and the surrounding tissues are the site and foundation for changes at metamorphosis that will result in the definitive skeleton of a post-metamorphic lamprey suited for a parasitic (predatory) life. Figure 4.11 shows the endoskeleton of cartilage in the head and branchial regions of larval and adult lampreys, with the latter having the greater complexity due to modifications and additions that are part of metamorphosis.

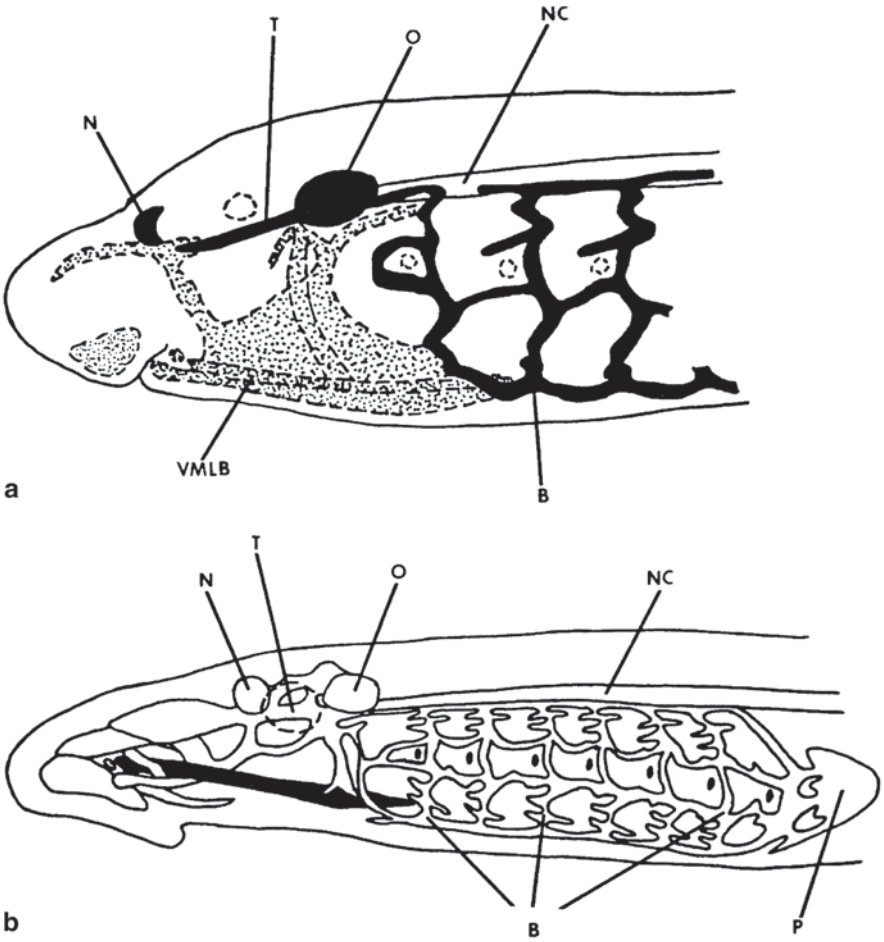

Fig. 4.11 Diagrammatic representations of the elements of the skeleton of the larval (**a**) and (**b**) adult lamprey. In the larva, the cartilaginous structures are shaded *black*. The neurocranium consists of the nasal capsule (*N*), trabeculae (*T*), and otic capsule (*O*). The branchial cartilages (*B*) compose the branchial arches (basket). A special connective tissue, mucocartilage, appears *stippled*. A region of mucocartilage, the ventromedial longitudinal bar (*VMLB*), develops into the piston cartilage (see adult below). The same neurocranial cartilages are present in the adult, but there are more cartilages supporting the oral disc. The piston cartilage (seen in *black*) develops from the larval VMLB, the branchial cartilages are more elaborate, and there is a new pericardial cartilage (*P*). *NC* notochord. (This figure was originally published in Armstrong et al. 1987)

The larval neurocranium and branchial arches are composed of cartilage, and the cranium also contains a unique skeletal structure called mucocartilage with one special region called the ventromedial longitudinal bar. At least part of the neurocranium, and some of the branchial arches, but apparently not the mucocartilage, show contributions from the neural crest during embryogenesis (Langille and Hall 1988; Martin et al. 2009). The neurocranium in both stages consists of nasal

capsule, trabeculae, and otic capsules, but in the adult there are more cartilages supporting the oral disc (annular cartilage) and tongue (piston cartilage) and the branchial cartilages are more complex (Fig. 4.11). A new pericardial cartilage is present in the adult. Fine structural studies show that the fibrous component of the extracellular matrix (ECM) of annular, piston, cranial, and dorsal plate cartilages is not collagen. Instead, the ECM is composed of a network of 15–40 nm diameter, randomly arranged, branched fibrils (Wright and Youson 1983). Nasal, branchial, and pericardial cartilages, however, are more cellular and the ECM composition is slightly different (Wright et al. 1988). Verhoeff's stain implies an elastic nature to the lamprey ECM but the amino acid composition varies from elastin protein, leading to the name of lamprin for the major ECM protein of lamprey cartilage (Wright and Youson 1983). On the other hand, nasal, branchial, and pericardial cartilages also show elastic-like fibers and elastin-like immunoreactivity in a portion of their ECM (Wright et al. 1988). Three lamprin cDNAs were isolated and their derived protein sequences show repeated amino acid sequences similar to those found in mammalian and avian elastin and spider dragline silk (Robson et al. 1993). Multiple genes for lamprin have been identified in sea lamprey and western brook lamprey (Robson et al. 2000). One might expect that elastic-like properties would be important in cartilages, such as pericardial and branchial cartilages, that surround or support structures that change their shape. The major ECM protein in these two cartilages is not lamprin, but a related protein containing hydroxyproline (Robson et al. 1997). Subsequently, it was shown that in branchial cartilage, and not annular cartilage, the elastin-like protein matrix is cross-linked by mainly lysyl pyridinoline at a ratio of 7:1 to hydroxylysyl pyridinoline (Fernandes and Eyre 1999). Since pyridinoline cross-links characteristic of collagen and desmosine cross-links characteristic of vertebrate elastin are absent, this poses some interesting mechanical and evolutionary questions. The evolutionary question revolves around whether fibrillar collagen type II exists in all types of lamprey cartilage based on the evidence that they possess two type II collagen genes (*Col2α1*, a clade A fibrillar collagen gene) and its transcriptional regulator (*Sox9*, a group E Sox gene) that are expressed in the branchial cartilage during sea lamprey development (Zhang et al. 2006). Ohtani et al. (2008), using their whole-mount procedure (Yao et al. 2008), studied the *Sox–Col2α1* genetic cascade during the development of both trabecular and branchial cartilage in Arctic lamprey and did not detect expression of the clade A fibrillar collagen genes in trabecular cartilage and only one of two clade A orthologs expressed in the pharyngeal chondrocytes. *SoxD* and *SoxE* are expressed in both types of cartilage in developing Arctic lamprey and the conservation of these transcription regulators implies homology among lamprey and gnathostome cartilages. On the other hand, Ohtani et al. (2008) proposed from their overall results that lampreys possess an ancestral, elastin-like cartilage similar to that of amphioxus and that the fibrillar type II collagen cartilage of gnathostomes was derived after the loss of the elastin-like protein from ancestral cartilage.

Mucocartilage of lamprey larvae is not a definitive cartilage for it lacks the ECM protein lamprin, it does not stain for elastin, and it consists primarily of a proteoglycan aggregate, microfibrils, and a few fibroblasts (Wright and Youson 1982).

It is a form of loose, embryonic connective tissue. During metamorphosis there is differential expression of the genes for lamprin (Robson 1998), as certain areas occupied by mucocartilage in larvae transform into lamprin-containing cartilage. This process has been described in detail in metamorphosis of sea lamprey where the ventromedial longitudinal bar (VMLB) of mucocartilage transforms into the piston cartilage supporting the rasping tongue of adults (Armstrong et al. 1987). Mucocartilage at metamorphosis is awakened from its resting embryonic status to differentiate into a mesenchyme-like tissue. This tissue in turn redifferentiates into chondroblasts that synthesize and secrete the lamprin-containing ECM cartilage (Armstrong et al. 1987). By the end of stage 2 of metamorphosis, the region previously occupied by mucocartilage appears like typical vertebrate mesenchymal tissue and is a consequence of cell migration and degradation of mucocartilage ECM by these cells. Stage 3 shows a blastema of precartilage cells in the center of the VMLB undergoing mitosis and liberating an ECM of sulfated proteoglycan. The differentiation and proliferation of chondroblasts by stage 4 is coincident with further elaboration of the lamprin-containing ECM but there is still some cellular degeneration. Subsequent stages show the increase in cellularity and ECM density of the piston cartilage and a decline in mitosis and cell degeneration (Armstrong et al. 1987). It would be of interest to study these processes in the piston cartilage, and in all the other cranial cartilages, with all the modern tools such as lamprin isoforms and the probes for fibrillar collagen genes that were described above. Since metamorphosis is a delayed stage of chondrogenesis that commenced during embryogenesis, we will not have a full picture of the events of lamprey cartilage development until such studies take place.

4.5.6 Other Biochemical or Physiological Changes

4.5.6.1 Blood Protein Profiles

Like most other tissues, the blood undergoes numerous changes during lamprey metamorphosis. Included among these are changes in the abundance and type of iron-binding protein from ferritin to transferritin (Macey et al. 1982), a shift from the larval to the adult hemoglobin (Adinolfi and Chieffi 1958; Potter and Brown 1975; Lanfranchi et al. 1994), and a shift in the dominant serum proteins and serum albumins. Changes in the abundance and type of iron-binding protein were reviewed in Sect. 4.5.2.2, and Potter and Brown (1975) nicely summarized the shift from larval to adult hemoglobins in the paired European river and brook lampreys. Despite the more rapid onset of sexual maturity in the non-parasitic brook lamprey relative to the parasitic river lamprey (see Sect. 4.2.2), the brook lamprey showed a slower rate of change from larval to adult hemoglobins. These different rates are likely related to differences in the ecology of the two species during metamorphosis; whereas the European brook lamprey remains in silted areas typical of the larvae until just prior to spawning, the river lamprey may move into faster-flowing areas with more

oxygenated sediments sometime during metamorphosis (Potter and Brown 1975). The remainder of this section will provide a brief overview of the shift in the serum protein profiles and serum albumins during lamprey metamorphosis.

Lamprey serum contains several dominant proteins including the serum albumins AS (Filosa et al. 1986), SDS-1 (Filosa et al. 1982), and LAS (for *Lampetra* AS; Danis et al. 2000), as well as the glycolipoprotein CB-III (Filosa et al. 1986). Each of these proteins constitutes a major component of the total serum protein complement at some point in the life cycle (see Fig. 4.5). Initially, AS and SDS-1 were referred to as albumin-like proteins because they lacked many of the classical biochemical properties characteristic of vertebrate albumins. Confirmation that SDS-1 (Gray and Doolittle 1992) and AS (Filosa et al. 1998) are in fact vertebrate albumins was ultimately provided by using sequence data to show they were homologous to other vertebrate albumins. Curiously, lamprey albumins are unique in that they are glycosylated and much larger than other vertebrate albumins. Unlike human albumin which is 68 kDa, AS and SDS-1 are approximately 164 kDa and 156 kDa in size, respectively. Amino acid sequence information clarified that this size discrepancy is related to the glycosylation and the fact that lamprey albumins consist of seven 190-amino acid repeats (albumin domain) rather than the three repeats typical of other vertebrate albumins.

In sea lamprey, AS synthesized by the liver makes up approximately 70% of all serum proteins in the larval and metamorphic phases, up to 40% in juveniles, and 0.5% in upstream migrant adults (Filosa et al. 1992). In contrast, SDS-1 makes up less than 2% of total serum proteins in the larval stage. The rapid decline in AS concentrations at stage 7 of metamorphosis coincides with an increase in serum SDS-1 concentrations, which now represent 20% of the total protein pool. By the juvenile and upstream migrant adult periods, SDS-1 represents 30% and 40%, respectively, of the total serum proteins (Filosa et al. 1982, 1986, 1992; Ito et al. 1988). This shift from AS to SDS-1 may be analogous to, or an ancestral example of, the embryonic shift from α-fetoprotein to adult albumin in mammals. Furthermore, there is an up-regulation of serum CB-III concentration associated with this transition from AS to SDS-1. CB-III makes up less than 10% of total serum proteins in the larval stage, 20% in the late metamorphic period, and 50% in upstream migrating adults (Filosa et al. 1982, 1986; Ito et al. 1988).

It is noteworthy that although American brook lamprey have an albumin (LAS) as a major serum protein during the larval phase, as a juvenile it completely lacks any albumin or albumin-like molecule (Danis et al. 2000). These data raise the question as to whether life history plays a role in the pattern of major serum proteins observed throughout metamorphosis. For instance, is the lack of an albumin in juvenile and adult American brook lamprey related to a completely freshwater existence or non-parasitic life history? To address this question, Eng (2004) used antisera directed against AS, LAS, and SDS-1 to investigate serum proteins in the paired chestnut lamprey *Ichthyomyzon castaneus* (which is parasitic in fresh water) and southern brook lamprey *I. gagei* (which is non-parasitic in fresh water), as well as the western brook lamprey (freshwater, non-parasitic) and Pacific lamprey (anadromous, parasitic); the latter two species are not paired species, but specimens

of the North American river lamprey, the western brook lamprey's parasitic counterpart, were not accessible (Eng 2004). Like sea lamprey, the two parasitic species (whether freshwater-resident or anadromous) contain two albumin molecules, with the larval albumin and the juvenile/adult albumins containing immunogenic determinants similar to AS and SDS-1, respectively (Eng 2004). These findings suggest an association between the parasitic life history and a second albumin, as the non-parasitic American brook lamprey lacks an adult albumin. However, the southern brook lamprey, although also non-parasitic, did contain an adult type albumin with immunogenic determinants characteristic of both AS/LAS and SDS-1. The presence of an albumin with determinants of both AS/LAS and SDS-1 could represent an intermediate in the transition to the complete loss of an adult albumin in non-parasitic lampreys (Eng 2004). Consistent with this, molecular data do suggest a more recent origin of southern brook lamprey (from chestnut lamprey) than of American brook lamprey (from its presumed ancestor, Arctic lamprey; see Docker and Potter in press). This hypothesis requires further evidence, and future work should be aimed at investigating the serum albumin profiles of additional non-parasitic lampreys.

4.6 Conclusions

Metamorphosis as a developmental strategy is rare among vertebrates; however, it has likely been a component of the lamprey life cycle for most of their long evolutionary history. So indispensable is metamorphosis to successful reproduction, and thus species survival, that even non-parasitic lampreys must undergo a complete metamorphosis despite the fact they do not feed as juveniles. The different life history strategies (i.e., parasitic and non-parasitic) seen in lampreys also highlights the plasticity offered by an indirect development; it has been suggested that the non-parasitic life history type is the result of a delay in the timing of metamorphosis relative to sexual maturity. A polymorphic population of western brook lamprey on Vancouver Island, producing both non-parasitic and potentially parasitic individuals, certainly warrants further study as these alternate morphotypes offer the opportunity to better understand the possible role of developmental plasticity in the evolution of lampreys and lamprey life history.

Numerous factors are known to affect the rate and incidence of metamorphosis in lampreys, and these have been summarized in Table 4.1. Among the exogenous (environmental) factors that can affect lamprey metamorphosis, water temperature is the most important and has the greatest impact. Numerous studies have clearly shown that the rise from cool winter to warm spring water temperatures is the single most critical factor affecting the incidence and rate of metamorphosis in lampreys, and that the water temperature in the month immediately prior to the onset of metamorphosis is particularly important. It has also been suggested that warmer spring water temperatures (up to approximately 21 °C) can result in an increased rate of metamorphosis, with juveniles potentially emerging to migrate downstream

to begin feeding in the late fall rather than the following spring. Future work should be aimed at understanding the role of water temperature in the early recruitment of parasitic phase sea lamprey to the Great Lakes, the impact on its host species populations, and the effect on the size and fecundity of the resultant adult sea lamprey.

Of the many endogenous factors examined, size (length, mass, and condition factor, CF) and thyroid hormones (THs) are the two that have been shown to be critical for metamorphosis. There is strong empirical evidence that indicates that larvae must attain a minimum size for metamorphosis. Within the Great Lakes basin, the minimum length required for sea lamprey metamorphosis is 120 mm, but attaining the minimum length is not alone sufficient for metamorphosis. In many instances, lampreys must also undergo a period of physiological conditioning (an "arrested growth phase") whereby there is an increase in weight and lipid content, but not length, in preparation for the pending non-trophic metamorphosis. This physiological conditioning is readily measured as an increase in CF in the months prior to the start of metamorphosis. Consistent with the requirement of sufficient lipid reserves and an increase in total body lipid content are observations that lipogenic enzymes are elevated prior to and in the early stages of metamorphosis. This phase of lipogenesis is followed by a phase of lipolysis evidenced by an increase in lipolytic enzyme activities at mid-metamorphosis.

The potential role of the thyroid in lamprey metamorphosis has been investigated for almost a century. Despite this effort, the picture is not as clear as it is for amphibians (or teleost fishes), where THs are the most critical morphogen driving metamorphosis. A precise causal function may be lacking, but there is little doubt that THs function in lamprey growth and metamorphosis. Numerous lines of evidence indicate that THs are inhibitory to lamprey metamorphosis: serum TH levels peak prior to metamorphosis and decline at the onset of metamorphosis, goitrogens can induce precocious metamorphosis, elevated TH levels block and disrupt goitrogen-induced and natural metamorphosis, respectively, and TH inactivation is highest during metamorphosis. This evidence strongly supports the notion that elevated TH levels are required for larval growth and development and that a decline in TH levels is required for metamorphosis. However, it is important to note that when TH levels are at their lowest during lamprey metamorphosis, their levels may be similar to peak concentrations during amphibian metamorphosis and preliminary data suggest that thyroid hormone receptor (TR) gene expression is upregulated during tissue morphogenesis. Collectively, these findings led to the dual role hypothesis (Manzon 2011), which suggests that elevated TH levels are required for larval feeding, growth and premetamorphic conditioning while simultaneously inhibiting natural metamorphosis. Following some yet to be identified endogenous or exogenous signals, THs must decline dramatically for metamorphosis to proceed normally and for THs to drive metamorphic events. It is unlikely that THs are the sole endogenous modulator for lamprey metamorphosis, and there is sufficient evidence to suggest that other endogenous factors might be involved in metamorphosis. Likely candidates include gonadotropin-releasing hormones (GnRH) and glycoprotein hormones (GpH) from the hypothalamus and pituitary gland, respectively, as well as molecules involved in the regulation of lipid metabolism and/or feeding such as insulin, somatostatin and leptin-like proteins.

The dramatic transformation from a sedentary, larval filter feeder to a free swimming parasitic or non-parasitic juvenile lamprey involves an extensive suite of external and internal changes resulting in some degree of morphological, cellular, or biochemical transformation in every organ and tissue. The most notable external changes include the appearance of eyes and oral suctorial disc, the transformation of the branchiopores and branchial region, the growth and differentiation of fins, and change in body coloration. Although the internal changes may be less visible, they are no less dramatic as they include substantial changes to all systems.

Major changes to the endocrine tissues include changes in gene expression in the hypothalamic-pituitary axis, the transformation of the larval endostyle to follicular thyroid tissue, and restructuring of the numerous aspects of the entero-pancreatic system. The changes in the entero-pancreatic system are associated with the transformation of the digestive system which, among other changes, includes the appearance of the oral disc complete with teeth and piston-like tongue, the transformation of the larval esophagus into the anterior portion of the juvenile alimentary canal, and the appearance of a new juvenile esophagus from dorsal tissue of the pharynx. These changes ultimately result in a blind-ended pharynx necessary for tidal ventilation when the animal is attached by its oral disc. The larval intestine also undergoes a dramatic morphogenesis, developing longitudinal mucosal folds and regional specialization along the anterior posterior axis. Intestinal transformation involves autophagy, heterophagy, apoptosis, and proliferation of existing larval intestinal cells to give rise to the juvenile intestine. The other curious change in the digestive system includes the loss of the hepatic biliary tree (biliary atresia), gall bladder, and extrahepatic common bile duct, the latter of which contributes to the formation of the islet organ in Northern, but not Southern, Hemisphere lampreys.

As described above, all systems undergo some degree of transformation during lamprey metamorphosis and the renal, respiratory, and skeletal systems are no exception. In the renal system, the larval pronephros continues to degenerate while the larval opisthonephros completely regresses and is replaced with the more caudally located juvenile opisthonephros that develops from the nephrogenic cord tissue. Changes to the respiratory system include the shift from unidirectional to tidal ventilation of the branchial basket. There is an enlargement of the heart, remodeling of the circulatory system, and the appearance of the adult hemoglobin. Other changes include remodeling of the gills and the appearance of the chloride cells of the interlamellar region which are responsible for Na^+ and Cl^- excretion (efflux) in hypertonic (marine) environments. The lamprey skeleton consists of axial and cranial portions, with the latter being a subject of great interest due to its importance in understanding the relationship between agnathans and gnathostomes. The larval cranial skeleton is suited to a life of sedentary filter feeding, but undergoes substantial morphogenesis and increased complexity to accommodate the tidal ventilation and free swimming parasitic life of the juvenile/adult. This increased complexity also includes a change in the protein composition and an increase in the cellular content of the cartilage of the cranial skeleton.

Lamprey metamorphosis is clearly a fascinating developmental event that has spurred the curiosity of scientists for over a century. Lamprey metamorphosis

represents a unique and fruitful model system to study numerous aspects of biology with potentially great implications towards our understanding of evolution, due to the lamprey's ancient origins. Lamprey metamorphosis is an excellent subject for "evo-devo" studies ranging from the ecology and evolution of life history transitions through to the cellular and molecular mechanisms of development and the evolution of these processes. Despite extensive study over the past 40–50 years, we are just beginning to understand the processes associated with lamprey metamorphosis. Advances in molecular technology and the sequencing of the lamprey genome will greatly facilitate the next 10–15 years of research, allowing us to make great strides in our understanding of the complex process of lamprey metamorphosis.

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Abbreviations and Acronyms

ACC	Acetyl CoA carboxylase
ACTH	Adrenocorticotropin
APUD	Amino precursor uptake and decarboxylation
AS	Lamprey-specific albumin, named for ammocoete spot
CB-III	Glycolipoprotein band, named for Cibacron Blue
cDNA	Complementary DNA
CF	Condition factor ($W/L^3 \times 10^6$); W is weight (g), L is length (mm)
CG	Chorionic gonadotropin
ClO_4^-	Perchlorate anion
CPD	Caudal pars distalis = proximal pars distalis
DGAT	Diacylglycerol acetyl transferase
ECBD	Extrahepatic common bile duct
ECM	Extracellular matrix
END	Endorphin
EP	Entero-pancreatic
GEP	Gastro-entero-pancreatic
GnRH	Gonadotropin-releasing hormone
GPA	Glycoprotein alpha subunit
GpH	Glycoprotein hormone
GpHB, GPB	Glycoprotein hormone beta subunit
GTH	Gonadotropin
HP	Hypothalamic-pituitary
IR	Immunoreactive or immunoreactivity

IRD	Inner-ring deiodination
$KClO_4$	Potassium perchlorate
LAS	American brook lamprey AS
LPH	Lipotropin
MMI	Methimazole
mRNA	Messenger RNA
MSH	Melanotropin
$NaClO_4$	Sodium perchlorate
NHF	Nasohypophysial factor
NPY	Neuropeptide Y
ORD	Outer-ring deiodination
PD	Pars distalis
PI	Pars intermedia
PmRXR1, PmRXR2, PmRXR3	Sea lamprey retinoid-X-receptors
POC	Proopiocortin
POM	Proopiomelanotropin
POMC	Proopiomelanocortin
PP	Pancreatic polypeptide
RPD	Rostral pars distalis
RXRs	Retinoid-X-receptors
SDS-1	Albumin, named for sodium dodecyl sulfate fraction I
SST	Somatostatin
T_3	3,5,3′-triiodothyronine
T_4	Thyroxine (3,5,3′,5′-tetraiodothyronine)
TH	Thyroid hormone
THDP	Thyroid hormone distributor protein
TRs	TH nuclear receptors
TSH	Thyroid stimulating hormone = thyrotropin
ΔT	Temperature change
TSM	Thyrostimulin
TTR	Transthyretin
VMLB	Ventromedial longitudinal bar

References

Adinolfi M, Chieffi G (1958) Larval and adult haemoglobins of the cyclostome *Petromyzon planeri.* Nature 182:730

Ahima RS, Osei SY (2004) Leptin signaling. Physiol Behav 81:223–241

Ahima RS, Osei SY (2008) Adipokines in obesity. Obes Metab 36:182–197

Antipova O, Orgel JPRO (2010) *In situ* D-periodic molecular structure of type II collagen. J Biol Chem 285:7087–7096

Applegate VC (1950) Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. US Fish and Wildlife Service Special Scientific Report 55, Washington, DC

Applegate VC, Thomas MLH (1965) Sex ratios and sexual dimorphism among recently transformed sea lampreys, *Petromyzon marinus* Linnaeus. J Fish Res Board Can 22:695–711
Armstrong LA, Wright GM, Youson JH (1987) Transformation of mucocartilage to a definitive cartilage during metamorphosis in the sea lamprey, *Petromyzon marinus*. J Morphol 194:1–21
Barrington EJW, Sage M (1963a) On the responses of iodine-binding regions of the endostyle of the larval lamprey to goitrogens and thyroxine. Gen Comp Endocrinol 3:669–679
Barrington EJW, Sage M (1963b) On the responses of the glandular tracts and associated regions of the endostyle of the larval lamprey to goitrogens and thyroxine. Gen Comp Endocrinol 3:153–165
Barrington EJW, Sage M (1972) The endosytle and thyroid gland. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 2. Academic Press, New York, pp 105–134
Bartels H, Potter IC (1991) Structural changes in the zonular occludentes of the chloride cells of young adult lampreys following acclimation to seawater. Cell Tissue Res 265:447–457
Bartels H, Potter IC (1998) Membrane structure of the cells of the lamprey notochord. J Electron Microsc 47:627–636
Bartels H, Potter IC (2004) Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys: implications for osmoregulation in fresh and seawater. J Exp Biol 207:3447–3462
Bartels H, Fazekas U, Youson JH, Potter IC (2011) Changes in the cellular composition of the gill epithelium during the life cycle of a nonparasitic lamprey: functional and evolutionary implications. Can J Zool 89:538–545
Bartels H, Docker MF, Fazekas U, Potter IC (2012) Functional and evolutionary implications of the cellular composition of the gill epithelium of feeding adults of a freshwater parasitic species of lamprey, *Ichthyomyzon unicuspis*. Can J Zool 90:1278–1283
Beamish FWH (1980) Osmoregulation in juvenile and adult lampreys. Can J Fish Aquat Sci 37:1735–1750
Beamish FWH, Austin LS (1985) Growth of the mountain brook lamprey *Ichthyomyzon greeleyi* Hubbs and Trautman. Copeia 4:881–890
Beamish FWH, Hanson LH (1987) Summary of evaluation methods and population studies of parasitic phase sea lamprey, Section B. In: Johnson BGH (ed) Workshop to evaluate sea lamprey populations "WESLP." Great Lakes Fishery Commission, Special Publication 87-2, Ann Arbor
Beamish FWH, Medland TE (1988) Metamorphosis of the mountain brook lamprey *Ichthyomyzon greeleyi*. Environ Biol Fish 23:45–54
Beamish FWH, Thomas EJ (1984) Metamorphosis of the southern brook lamprey, *Ichthyomyzon gagei*. Copeia 1984:502–515
Beamish FWH, Strachan PD, Thomas E (1978) Osmotic and ionic performance of the anadromous sea lamprey, *Petromyzon marinus*. Comp Biochem Physiol 60A:435–443
Beamish RJ (1985) Freshwater parasitic lamprey on Vancouver island and a theory of the evolution of the freshwater parasitic and nonparasitic life histories. In: Foreman RE, Gorbman A, Dodd JM, Olsson R (eds) Evolutionary biology of primitive fishes. Plenum, New York, pp 123–140
Beamish RJ (1987) Evidence that parasitic and nonparasitic life-history types are produced by one population of lamprey. Can J Fish Aquat Sci 44:1779–1782
Beamish RJ, Youson JH (1987) Life history and abundance of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the Strait of Georgia. Can J Fish Aquat Sci 44:525–537
Becker KB, Stephens KC, Davey JC, Schneider MJ, Galton VA (1997) The type 2 and type 3 iodothyronine deiodinases play important roles in coordinating development in *Rana catesbeiana* tadpoles. Endocrinology 138:2989–2997
Berry DL, Rose CS, Remo BF, Brown DD (1998) The expression pattern of thyroid hormone response genes in remodeling tadpole tissues defines distinct growth and resorption gene expression programs. Dev Biol 203:24–35
Bianco PG (1986) *Lethenteron zanandreai* (Vladykov 1955). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 237–246

Bianco AC, Kim BW (2006) Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest 116:2571–2579
Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 23:38–89
Bird DJ, Potter IC (1979) Metamorphosis in the paired species of lampreys, *Lampetra fluviatilis* (L) and *Lampetra planeri* (Bloch). 1. Description of the timing and stages. Zool J Linn Soc 65:127–143
Blanton ML, Specker JL (2007) The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. Crit Rev Toxicol 37:97–115
Boenig H (1928) Studien zur Morphologie und Entsicklungsgeschichte des Pankreas beim Nachneunauge (*Lampetra (Petromyzon) planeri)*. Teil II. Z Mikrosk-anat Forsch 12:537–594
Boenig H (1929) Studien zur Morphologie und Entsicklungsgeschichte des Pankreas beim Bachneunauge (*Lampetra [Petromyzon] planeri*). Teil III. Die histologie und histogenese des pankreas. Z Mikrosk-anat Forsch 17:124–184
Bonneville MA (1963) Fine structural changes in the intestinal epithelium of the bullfrog during metamorphosis. J Cell Biol 18:579–597
Boomer LA, Bellister SA, Stephenson LL et al (2010) Cholangiocyte apoptosis is an early event during induced metamorphosis in the sea lamprey, *Petromyzon marinus* L. J Pediatr Surg 45:114–120
Brown DD (2005) The role of deiodinases in amphibian metamorphosis. Thyroid 15:815–821
Brown DD, Cai LQ (2007) Amphibian metamorphosis. Dev Biol 306:20–33
Buchholz DR, Paul BD, Fu LZ, Shi YB (2006) Molecular and developmental analyses of thyroid hormone receptor function in *Xenopus laevis*, the African clawed frog. Gen Comp Endocrinol 145:1–19
Buchholz DR, Moskalik C, Kulkarni S, Hollar A, Ng A (2011) Hormone regulation and the evolution of frog metamorphic diversity. In: Flatt T, Heyland A (eds) Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University, Oxford, pp 87–98
Bujor MP (1891) Contribution à l'étude métamorphose de l'ammocoetes branchialis en *Petromyzon planeri*. Rev Biol N Fr 12:475–486
Callery EM, Fang H, Elinson RP (2001) Frogs without polliwogs: evolution of anural direct development. Bioessays 23:233–241
Cetin I, Morpurgo PS, Radaelli T et al (2000) Fetal plasma leptin concentrations: relationship with different intrauterine growth patterns from 19 weeks to term. Pediatr Res 48:646–651
Cheung R, Plisetskaya EM, Youson JH (1990) Distribution of two forms of somatostatin in the brain, anterior intestine, and pancreas of adult lampreys (*Petromyzon marinus*). Cell Tissue Res 262:283–292
Cheung R, Andrews PC, Plisetskaya EM, Youson JH (1991a) Immunoreactivity to peptides belonging to the pancreatic polypeptide family (NPY, aPY, PP, PYY) and to glucagon-like peptide in the endocrine pancreas and anterior intestine of adult lampreys, *Petromyzon marinus*: an immunohistochemical study. Gen Comp Endocrinol 81:51–63
Cheung R, Ferreira LCG, Youson JH (1991b) Distribution of two forms of somatostatin and peptides belonging to the pancreatic polypeptide family in tissues of larval lampreys, *Petromyzon marinus* L.: an immunohistochemical study. Gen Comp Endocrinol 82:93–102
Clements-Merlini M (1962) Altered metabolism of I^{131} by the endostyle and notochord of ammocoetes larvae. II. Effects of treatment with thiourea or potassium thiocyanate. Gen Comp Endocrinol 2:361–368
Cochran PA, Lyons J, Merino-Namob E (1996) Notes on the biology of the Mexican lampreys *Lampetra spadicea* and *L. geminis* (Agnatha: Petromyzontidae). Ichthyol Explor Freshw 7:173–180
Cole WC, Youson JH (1981) The effect of pinealectomy, continuous light, and continuous darkness on metamorphosis of anadromous sea lampreys, *Petromyzon marinus* L. J Exp Zool 218:397–404

Copeland DL, Duff RJ, Liu Q, Prokop J, Londraville RL (2011) Leptin in teleost fishes: an argument for comparative study. Front Physiol 2:1–11

Crespi EJ, Denver RJ (2006) Leptin (ob gene) of the South African clawed frog *Xenopus laevis*. Proc Natl Acad Sci U S A 103:10092–10097

Damas H (1935) Contribution à l'étude de la métamorphose de la tête de la lamproie. Arch Biol Paris 46:171–227

Damas H (1944) Recherches sur le développement de *Lampetra fluviatilis* L.: contribution à l'étude de la céphalogenèse des Vertebrés. Arch Biol Paris 55:1–284

Danis MH, Filosa MF, Youson JH (2000) An albumin-like protein in the serum of non-parasitic brook lamprey (*Lampetra appendix*) is restricted to preadult phases of the life cycle in contrast to the parasitic species *Petromyzon marinus*. Comp Biochem Physiol B Biochem Mol Biol 127:251–260

De Groef B, Van derGS, Darras VM, Kuhn ER (2006) Role of corticotropin-releasing hormone as a thyrotropin-releasing factor in non-mammalian vertebrates. Gen Comp Endocrinol 146:62–68

Delarbre C, Gallut C, Barriel V, Janvier P, Gachelin G (2002) Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. Mol Phylogenet Evol 22:184–192

Denver RJ (1998) Hormonal correlates of environmentally induced metamorphosis in the western spadefoot toad, *Scaphiopus hammondii*. Gen Comp Endocrinol 110:326–336

Denver RJ, Glennemeier KA, Boorse GC (2002) Endocrinology of complex life cycles: amphibians. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Rubin R (eds) Hormones, brain and behavior, vol 2. Academic Press, San Diego, pp 469–513

Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 71–114

Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Eales JG (1985) The peripheral metabolism of thyroid hormones and regulation of thyroidal status in poikilotherms. Can J Zool 63:1217–1231

Eales JG (1997) Iodine metabolism and thyroid-related functions in organisms lacking thyroid follicles: are thyroid hormones also vitamins? Proc Soc Exp Biol Med 214:302–317

Eales JG, Brown SB (1993) Measurement and regulation of thyroidal status in teleost fish. Rev Fish Biol Fish 3:299–347

Eales JG, Holmes JA, McLeese JM, Youson JH (1997) Thyroid hormone deiodination in various tissues of larval and upstream-migrant sea lampreys, *Petromyzon marinus*. Gen Comp Endocrinol 106:202–210

Eales JG, McLeese JM, Holmes JA, Youson JH (2000) Changes in intestinal and hepatic thyroid hormone deiodination during spontaneous metamorphosis of the sea lamprey, *Petromyzon marinus*. J Exp Zool 286:305–312

Eddy JMP (1969) Metamorphosis and pineal complex in brook lamprey, *Lampetra planeri*. J Endocrinol 44:451–452

Eikenberry EF, Childs B, Sheren SB et al (1984) Crystalline fibril structure of type II collagen in lamprey notochord sheath. J Mol Biol 176:261–277

Elliott WM (1989) Development of the endocrine pancreas and related parts of the alimentary canal during metamorphosis in the lamprey, *Petromyzon marinus* L. PhD thesis, University of Toronto, Toronto

Elliott WM, Youson JH (1986) Immunocytochemical localization of insulin and somatostatin in the endocrine pancreas of the sea lamprey, *Petromyzon marinus* L., at various stages of its life cycle. Cell Tissue Res 243:629–634

Elliott WM, Youson JH (1987) Immunohistochemical observations of the endocrine pancreas during metamorphosis of the sea lamprey, *Petromyzon marinus* L. Cell Tissue Res 247:351–357

Elliott WM, Youson JH (1988) Fine structure and immunocytochemistry of cells within the endocrine pancreas of larval and adult sea lampreys, *Petromyzon marinus* L. Am J Anat 182:73–83
Elliott WM, Youson JH (1991) Somatostatin concentrations in the pancreatic-intestinal tissues of the sea lamprey, *Petromyzon marinus* L., at various periods of its life cycle. Comp Biochem Physiol A Physiol 99:357–360
Elliott WM, Youson JH (1993a) Development of the adult endocrine pancreas during metamorphosis in the sea lamprey, *Petromyzon marinus* L. I. Light microscopy and autoradiography. Anat Rec 237:259–270
Elliott WM, Youson JH (1993b) Development of the adult endocrine pancreas during metamorphosis in the sea lamprey, *Petromyzon marinus* L. II. Electron microscopy and immunocytochemistry. Anat Rec 237:271–290
Elliott WM, Youson JH (1994) Alterations to the subepithelial layers of the larval alimentary tract during metamorphosis in the sea lamprey *Petromyzon marinus*, with emphasis on tissue interactions. Cell Tissue Res 276:503–513
Ellis LC (1993) An ultrastructural investigation of the pronephric kidney of the sea lamprey, *Petromyzon marinus* L., throughout the life cycle. PhD thesis, University of Toronto, Toronto
Ellis LC, Youson JH (1990) Pronephric regression during larval life in the sea lamprey, *Petromyzon marinus* L.: a histochemical and ultrastructural study. Anat Embryol 182:41–52
Eng EWY (2004) Investigation of albumin-like serum proteins associated with lamprey life histories. MSc thesis, University of Toronto, Toronto
Eng F, Youson JH (1992a) Morphology of the bile ducts of the brook lamprey, *Lampetra lamottenii* (Le Sueur) before and during infection with the nematode, *Truttaedacnitis stelmioides* (Vessichelli, 1910) (Nematoda, Cucullanidae). Anat Rec 234:201–214
Eng F, Youson JH (1992b) Morphology of the liver of the brook lamprey, *Lampetra lamottenii* before and during infection with the nematode, *Truttaedacnitis stelmioides*, hepatocytes, sinusoids, and perisinusoidal cells. Tissue Cell 24:575–592
Epple A, Brinn JE (1986) Pancreatic islets. In: Pang PKT, Schreibman M (eds) Vetebrate endocrinology: funadmentals and biochemical implications. Academic Press, New York, pp 279–317
Escriva H, Manzon L, Youson J, Laudet V (2002) Analysis of lamprey and hagfish genes reveals a complex history of gene duplications during early vertebrate evolution. Mol Biol Evol 19:1440–1450
Etkin W (1935) The mechanism of anuran metamorphosis. I. Thyroxine concentrations and the metamorphic pattern. J Exp Zool 71:317–340
Etkin W (1964) Metamorphosis. In: Moore JA (ed) Physiology of the amphibia. Academic, London, pp 371–410
Etkin W (1968) Hormonal control of amphibian metamorphosis. In: Etkin W, Gilbert LI (eds) Metamorphosis: a problem in developmental biology. Meredith Corporation, New York, pp 313–348
Falkmer S (1985) Comparative morphology of pancreatic islets in animals. In: Vok BW, Arquilla ER (eds) The diabetic pancreas. Plenum, New York, pp 17–52
Fernandes RJ, Eyre DR (1999) The elastin-like protein matrix of lamprey branchial cartilage is cross-linked by lysyl pyridinoline. Biochem Biophys Res Commun 261:635–640
Ficele G, Heinig JA, Kawauchi H et al (1998) Spatial and temporal distribution of proopiomelanotropin and proopiocortin mRNA during the life cycle of the sea lamprey: a qualitative and quantitative in situ hybridization study. Gen Comp Endocrinol 110:212–225
Filosa MF, Sargent PA, Fisher MM, Youson JH (1982) An electrophoretic and immunoelectrophoretic characterization of the serum proteins of the adult lamprey, *Petromyzon marinus* L. Comp Biochem Physiol B Biochem Mol Biol 72:521–530
Filosa MF, Sargent PA, Youson JH (1986) An electrophoretic and immunoelectrophoretic study of serum proteins during the life cycle of the lamprey, *Petromyzon marinus* L. Comp Biochem Physiol B Biochem Mol Biol 83:143–149
Filosa MF, Ito MA, Youson JH (1992) Quantitative changes in a serum protein, AS, during the life cycle of the lamprey, *Petromyzon marinus* L. Fish Physiol Biochem 9:417–426

Filosa MF, Adam I, Robson P et al (1998) Partial clone of the gene for AS protein of the lamprey *Petromyzon marinus*, a member of the albumin supergene family whose expression is restricted to the larval and metamorphic phases of the life cycle. J Exp Zool 282:301–309
Forey P, Janvier P (1993) Agnathans and the origin of jawed vertebrates. Nature 361:129–134
Forey P, Janvier P (1994) Evolution of the early vertebrates. Am Sci 82:554–565
Fort DJ, Degitz S, Tietge J, Touart LW (2007) The hypothalamic-pituitary-thyroid (HPT) axis in frogs and its role in frog development and reproduction. Crit Rev Toxicol 37:117–161
Freamat M, Kawauchi H, Nozaki M, Sower SA (2006) Identification and cloning of a glycoprotein hormone receptor from sea lamprey, *Petromyzon marinus*. J Mol Endocrinol 37:135–146
Galloway R, Potter IC, Macey DJ, Hilliard RW (1987) Oxygen consumption and responses to hypoxia of ammocoetes of the southern hemisphere lamprey *Geotria australis*. Fish Physiol Biochem 4:63–72
Gess RW, Coates MI, Rubidge BS (2006) A lamprey from the Devonian period of South Africa. Nature 443:981–984
Glennemeier KA, Denver RJ (2002) Role for corticoids in mediating the response of *Rana pipiens* tadpoles to intraspecific competition. J Exp Zool 292:32–40
Gray JE, Doolittle RF (1992) Characterization, primary structure, and evolution of lamprey plasma albumin. Protein Sci 1:289–302
Griffith RW, Beamish FWH, Morrison BJ, Barker LA (2001) Factors affecting larval sea lamprey growth and length at metamorphosis in lampricide-treated streams. Trans Am Fish Soc 130:289–306
Gross TN, Manzon RG (2011) Sea lamprey (*Petromyzon marinus*) contain four developmentally regulated serum thyroid hormone distributor proteins. Gen Comp Endocrinol 170:640–649
Gudernatsch JF (1912) Feeding experiments on tadpoles. I. The influence of specific organs given as food on growth and differentiation. A contribution to the knowledge of organs with internal secretion. Arch Entwicklungsmech Org 35:457–483
Hall BK (1999) The neural crest in development and evolution. Springer, New York
Hardisty MW (1979) Biology of cyclostomes. Chapman and Hall, London
Hardisty MW (1981) The skeleton. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 3. Academic Press, London, pp 333–376
Hardisty MW (1986a) *Lampetra fluviatilis* (Linnaeus, 1758). In: Holčík J (ed), The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA-Verlag, Wiesbaden, pp 249–278
Hardisty MW (1986b) *Lampetra planeri* (Bloch, 1784). In: Holčík J (ed), The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA-Verlag, Wiesbaden, pp 279–304
Hardisty MW (2006) Lampreys life without jaws. Forrest Text, Tresaith
Hardisty MW, Potter IC (1971a) Paired species. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 249–277
Hardisty MW, Potter IC (1971b) The behaviour, ecology and growth of larval lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic, London, pp 88–125
Harris LR, Cake MH, Macey DJ, Potter IC (1990) An increase in the concentration of hepatic iron during the metamorphosis of the lamprey *Geotria australis* is accompanied by increased superoxide dismutase activity. Fish Physiol Biochem 8:451–457
Harris LR, Macey DJ, Potter IC, Cake MH (1995) Hepatic molecular conversion and detoxification of ferritin iron in adult lampreys (*Geotria australis*) following natural and induced iron loading. Biochem J 305:975–980
Hart JL (1973) Pacific fishes of Canada. Bulletin Fisheries Research Board of Canada 180, Ottawa
Harvey J, Ashford MLJ (2003) Leptin in the CNS: much more than a satiety signal. Neuropharmacology 44:845–854
Heard WR (1966) Observations on lampreys in the Naknek River system of southwest Alaska. Copeia 1966:332–339
Heimberg AM, Cowper-Sal·lari R, Sémon M, Donoghue PCJ, Peterson KJ (2010) microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. Proc Natl Acad Sci U S A 107:19379–19383

Heinig JA, Keeley FW, Robson P, Sower SA, Youson JH (1995) The appearance of proopiomelanocortin early in vertebrate evolution: cloning and sequencing of POMC from a lamprey pituitary cDNA library. Gen Comp Endocrinol 99:137–144

Heinig JA, Keeley FW, Kawauchi H, Youson JH (1999) Expression of proopiocortin and proopiomelanotropin during the life cycle of the sea lamprey (*Petromyzon marinus*). J Exp Zool 283:95–101

Henson MP, Bergstedt RA, Adams JV (2003) Comparison of spring measures of length, weight, and condition factor for predicting metamorphosis in two populations of sea lamprey (*Petromyzon marinus*) larvae. J Great Lakes Res 29(Suppl 1):204–213

Hilliard RW, Potter IC (1988) Morphology of the exocrine pancreas of the southern hemisphere lamprey, *Geotria australis*, and changes during metamorphosis. J Morphol 197:33–52

Hilliard RW, Epple A, Potter IC (1985) The morphology and histology of the endocrine pancreas of the Southern Hemisphere lamprey, *Geotria australis* Gray. J Morphol 184:253–261

Hoff JG (1988) Some aspects of the ecology of the American brook lamprey, *Lampetra appendix*, in the Mashpee River, Cape Cod, Massachusetts. Can Field-Nat 102:735–737

Hoheisel G, Sterba G (1963) Uber die Wirkung von Kaliumperchlorat ($KClO_4$) auf Ammocoeten von *Lampetra Planeri* Bloch. Z Mikroskop Anat Forsch 70:490–516

Holčík J (1986a) *Lethenteron japonicum* (Martens, 1868). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 198–219

Holčík J (1986b) *Caspiomyzon wagneri* (Kessler, 1870). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 119–142

Holčík J, Renaud CB (1986) *Eudontomyzon mariae* (Berg, 1931). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1: Petromyzontiformes. AULA, Wiesbaden, pp 165–185

Holland LZ, Holland ND, Gilland E (2008) Amphioxus and the evolution of head segmentation. Integr Comp Biol 48:630–646

Hollett AK (1998) Condition factor and statolith aging in assessment of metamorphosis in sea lampreys (*Petromymzon marinus*), in the Great Lakes. MSc thesis, University of Toronto, Toronto

Holmes JA, Lin P (1994) Thermal niche of larval sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 51:253–262

Holmes JA, Youson JH (1993) Induction of metamorphosis in landlocked sea lampreys, *Petromyzon marinus*. J Exp Zool 267:598–604

Holmes JA, Youson JH (1994) Fall condition factor and temperature influence the incidence of metamorphosis in sea lampreys, *Petromyzon marinus*. Can J Zool 72:1134–1140

Holmes JA, Youson JH (1997) Laboratory study of the effects of spring warming and larval density on the metamorphosis of sea lampreys. Trans Am Fish Soc 126:647–657

Holmes JA, Youson JH (1998) Extreme and optimal temperatures for metamorphosis in sea lampreys. Trans Am Fish Soc 127:206–211

Holmes JA, Beamish FWH, Seelye JG, Sower SA, Youson JH (1994) Long-term influence of water temperature, photoperiod, and food deprivation on metamorphosis of sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 51:2045–2051

Holmes JA, Chu H, Khanam SA, Manzon RG, Youson JH (1999) Spontaneous and induced metamorphosis in the American brook lamprey, *Lampetra appendix*. Can J Zool 77:959–971

Horton FM (1934) On the relation of the thyroid gland to metamorphosis in the lamprey. J Exp Biol 11:257–261

Hubbs CL, Potter IC (1971) Distribution, phylogeny and taxonomy. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 1–65

Ito MA, Filosa MF, Youson JH (1988) In vitro study of serum protein synthesis in the livers of larvae and adults of the lamprey, *Petromyzon marinus* L. J Exp Zool 245:256–263

Janvier P (2010) microRNAs revive old views about jawless vertebrate divergence and evolution. Proc Natl Acad Sci U S A 107:19137–19138

Johnels AG (1948) On the development and morphology of the skeleton of the head of *Petromyzon*. Acta Zool 29:139–279

Johnson CK, Voss SR (2013) Salamander paedomorphosis: linking thyroid hormone to life history and life cycle evolution. Curr Top Dev Biol 103:229–258

Jones RP (1947) Effect of thiourea on the endostyle of ammocoetes. Nature 160:638–639
Joss JMP (1985) Pituitary control of metamorphosis in the southern hemisphere lamprey, *Geotria australis*. Gen Comp Endocrinol 60:58–62
Kao YH, Youson JH, Holmes JA, Sheridan MA (1997a) Changes in lipolysis and lipogenesis in selected tissues of the landlocked lamprey, *Petromyzon marinus*, during metamorphosis. J Exp Zool 277:301–312
Kao YH, Youson JH, Sheridan MA (1997b) Differences in the total lipid and lipid class composition of larvae and metamorphosing sea lampreys, *Petromyzon marinus*. Fish Physiol Biochem 16:281–290
Kao YH, Youson JH, Holmes JA, Sheridan MA (1998) Effects of somatostatin on lipid metabolism of larvae and metamorphosing landlocked sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 111:177–185
Kao Y, Youson JH, Holmes JA, Al-Mahrouki A, Sheridan MA (1999a) Effects of insulin on lipid metabolism of larvae and metamorphosing landlocked sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 114:405–414
Kao YH, Manzon RG, Sheridan MA, Youson JH (1999b) Study of the relationship between thyroid hormones and lipid metabolism during $KClO_4$-induced metamorphosis of landlocked lamprey *Petromyzon marinus*. Comp Biochem Physiol C Toxicol Pharmacol 122:363–373
Kavanaugh SI, Nozaki M, Sower SA (2008) Origins of gonadotropin-releasing hormone (GnRH) in vertebrates: identification of a novel GnRH in a basal vertebrate, the sea lamprey. Endocrinology 149:3860–3869
Kawauchi H, Sower SA (2006) The dawn and evolution of hormones in the adenohypophysis. Gen Comp Endocrinol 148:3–14
Keibel F (1927) Zur Entwicklungsgeschichte des Vorderdarmes und das Pankreas beim Bachneunauge (*Lampetra* (*Petromyzon*) *planeri*) und beim Flussneunauge (*Lampetra* (*Petromyzon*) *fluviatilis*). Z Mikrosk Anat Forsch 8:408–476
Kimura S, Kamimura T (1982) The characterization of lamprey notochord collagen with special reference to its skin collagen. Comp Biochem Physiol B Biochem Mol Biol 73:335–339
Klenner JJ, Schipper AL (1954) The response of the endostyle of *Lampetra lamottenii* to itrumil, thiouracil, thiourea, thiocyanate and thyroxin. Anat Rec 120:790
Knowles FGW (1941) Duration of larval life in ammocoetes. Proc Zool Soc Lond 111:101–109
Koob TJ, Long JH (2000) The vertebrate body axis: evolution and mechanical function. Am Zool 40:1–18
Kusakabe R, Kuratani S (2007) Evolutionary perspectives from development of mesodermal components in the lamprey. Dev Dynam 236:2410–2420
Lanfranchi G, Pallavicini A, Laveder P, Valle G (1994) Ancestral hemoglobin switching in lampreys. Dev Biol 164:402–408
Langille RM, Hall BK (1988) Role of the neural crest in development of the trabeculae and branchial arches in embryonic sea lamprey, *Petromyzon marinus* (L). Development 102:301–310
Langille RM, Hall BK (1993) Calcification of cartilage from the lamprey *Petromyzon marinus* (L) *in vitro*. Acta Zool 74:31–41
Laudet V (2011) The origins and evolution of vertebrate metamorphosis. Curr Biol 21:R726–R737
Leach WJ (1940) Occurrence and life history of the northern brook lamprey, *Ichthyomyzon fossor*, in Indiana. Copeia 1940:21–34
Leach WJ (1946) Oxygen consumption of lampreys, with special reference to metamorphosis and phylogenetic position. Physiol Zool 19:365–374
Leatherland JF (1994) Reflections on the thyroidology of fishes: from molecules to humankind. Guelph Ichthyol Rev 2:1–67
Leatherland JF, Hilliard RW, Macey DJ, Potter IC (1990) Changes in serum thyroxine and triiodothyronine concentrations during metamorphosis of the southern hemisphere lamprey *Geotria australis*, and the effect of propylthiouracil, triiodothyronine and environmental temperature on serum thyroid hormone concentrations of ammocoetes. Fish Physiol Biochem 8:167–177

Lee M-J, McCauley DW (in press) Lampreys as model vertebrates in evolutionary developmental biology. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Lepercq J, Challier JC, Guerre-Millo M et al (2001) Prenatal leptin production: evidence that fetal adipose tissue produces leptin. J Clin Endocrinol Metab 86:2409–2413

Lewis SV (1980) Respiration of lampreys. Can J Fish Aquat Sci 37:1711–1722

Lewis SV, Potter IC (1977) Oxygen consumption during metamorphosis of parasitic lamprey, *Lampetra fluviatilis* (L) and its non-parasitic derivative, *Lampetra planeri* (Bloch). J Exp Biol 69:187–198

Lintlop SP, Youson JH (1983a) Binding of triiodothyronine to hepatocyte nuclei from sea lampreys, *Petromyzon marinus* L, at various stages of the life cycle. Gen Comp Endocrinol 49:428–436

Lintlop SP, Youson JH (1983b) Concentration of triiodothyronine in the sera of the sea lamprey, *Petromyzon marinus*, and the brook lamprey, *Lampetra lamottenii*, at various phases of the life cycle. Gen Comp Endocrinol 49:187–194

Lowe DR, Beamish FWH, Potter IC (1973) Changes in proximate body composition of landlocked sea lamprey *Petromyzon marinus* (L) during larval life and metamorphosis. J Fish Biol 5:673–682

Macey DJ, Potter IC (1986) Concentrations of non-heme iron in ammocoetes of species representing the three extant lamprey families. Comp Biochem Physiol A Physiol 84:77–79

Macey DJ, Youson JH (1990) Occurrence and structure of iron inclusions in adipocytes of larval lampreys. Acta Zool 71:69–76

Macey DJ, Webb J, Potter IC (1982) Iron levels and major iron-binding proteins in the plasma of ammocoetes and adults of the southern hemisphere lamprey *Geotria australis* Gray. Comp Biochem Physiol A Physiol 72:307–312

Macey DJ, Cake MH, Potter IC (1988) Exceptional iron concentrations in larval lampreys (*Geotria australis*) and the activities of superoxide radical detoxifying enzymes. Biochem J 252:167–172

Maitland PS (1980) Review of the ecology of lampreys in northern Europe. Can J Fish Aquat Sci 37:1944–1952

Mallatt J (1983) Laboratory growth of larval lampreys (*Lampetra (Entosphenus) tridentata* Richardson) at different food concentrations and animal densities. J Fish Biol 22:293–301

Malmqvist B (1983) Growth, dynamics, and distribution of a population of the brook lamprey *Lampetra planeri* in a south Swedish stream. Holarct Ecol 6:404–412

Manion PJ, Stauffer TM (1970) Metamorphosis of the landlocked sea lamprey, *Petromyzon marinus*. J Fish Res Board Can 27:1735–1746

Manzon LA (2006) Cloning and developmental expression of sea lamprey (*Petromyzon marinus*) thyroid hormone and retinoid X receptors. PhD thesis, University of Toronto, Toronto

Manzon LA, Youson JH, Holzer G, Staiano L, Laudet V, Manzon RG (2014) Thyroid hormone and retinoid X receptor function and expression during sea lamprey (*Petromyzon marinus*) metamorphosis. Gen Comp Endocrinol 204:211–222

Manzon RG (2011) Thyroidal regulation of life history transitions in fish. In: Flatt T, Heyland A (eds) Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University, Oxford, pp 72–86

Manzon RG, Youson JH (1997) The effects of exogenous thyroxine (T4) or triiodothyronine (T3), in the presence and absence of potassium perchlorate, on the incidence of metamorphosis and on serum T4 and T3 concentrations in larval sea lamrpey (*Petromyzon marinus*). Gen Comp Endocrinol 106:211–220

Manzon RG, Youson JH (2002) $KClO_4$ inhibits thyroidal activity in the larval endostyle in vitro. Gen Comp Endocrinol 128:214–223

Manzon RG, Eales JG, Youson JH (1998) Blocking of $KClO_4$-induced metamorphosis in premetamorphic sea lampreys by exogenous thyroid hormones (TH): effects of $KClO_4$ and TH on serum TH concentrations and intestinal thyroxine outer-ring deiodination. Gen Comp Endocrinol 112:54–62

Manzon RG, Holmes JA, Youson JH (2001) Variable effects of goitrogens in inducing precocious metamorphosis in sea lampreys (*Petromyzon marinus*). J Exp Zool 289:290–303
Manzon RG, Neuls TM, Manzon LA (2007) Molecular cloning, tissue distribution, and developmental expression of lamprey transthyretins. Gen Comp Endocrinol 151:55–65
Marsden JE, Siefkes MJ (in press) Sea lamprey control in the Great Lakes, Finger Lakes, and Lake Champlain. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Martin WM, Bumm LA, McCauley DW (2009) Development of the viscerocranial skeleton during embryogenesis of the sea lamprey, *Petromyzon marinus*. Dev Dynam 238:3126–3138
Maskell FG (1931) On the New Zealand lamprey, *Geotria australis* Gray. Part 3. The loss of the mid-gut diverticula of the ammocoetes stage at metamorphosis. Trans Proc N Z Inst 62:120–128
Mathers JS, Beamish FWH (1974) Changes in serum osmotic and ionic concentration in landlocked *Petromyzon marinus*. Comp Biochem Physiol 49:677–688
McBurney KM, Wright GM (1996) Chondrogenesis of a non-collagen-based cartilage in the sea lamprey, *Petromyzon marinus*. Can J Zool 74:2118–2130
McBurney KM, Keeley FW, Kibenge FSB, Wright GM (1996) Spatial and temporal distribution of lamprin mRNA during chondrogenesis of trabecular cartilage in the sea lamprey. Anat Embryol 193:419–426
McCauley DW, Bronner-Fraser M (2003) Neural crest contributions to the lamprey head. Development 130:2317–2327
McCauley DW, Bronner-Fraser M (2006) Importance of SoxE in neural crest development and the evolution of the pharynx. Nature 441:750–752
McGree M, Whitesel TA, Stone J (2008) Larval metamorphosis of individual Pacific lampreys reared in captivity. Trans Am Fish Soc 137:1866–1878
McNabb FMA (2007) The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. Crit Rev Toxicol 37:163–193
Moore JW, Beamish FWH (1973) Food of larval sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). J Fish Res Board Can 30:7–15
Moore JW, Mallatt J (1980) Feeding of larval lamprey. Can J Fish Aquat Sci 37:1658–1664
Morgado I, Santos CRA, Jacinto R, Power DM (2007) Regulation of transthyretin by thyroid hormones in fish. Gen Comp Endocrinol 152:189–197
Morii M, Mezaki Y, Yamaguchi N et al (2010) Onset of apoptosis in the cystic duct during metamorphosis of a Japanese lamprey, *Lethenteron reissneri*. Anat Rec 293:1155–1166
Morii M, Mezaki Y, Yoshikawa K et al (2012) How do lampreys avoid cholestasis after bile duct degeneration? In: Tripodi V (ed) Cholestasis. InTech, Shanghai, pp 81–98
Morkert SB, Swink WD, Seelye JG (1998) Evidence for early metamorphosis of sea lampreys in the Chippewa River, Michigan. N Am J Fish Manag 18:966–971
Morman RH (1987) Relationship of density to growth and metamorphosis of caged larval sea lampreys, *Petromyzon marinus* Linnaeus, in Michigan streams. J Fish Biol 30:173–181
Morris R (1972) Osmoregulation. In: Hardisty MW, Potter IC (eds) Biology of lampreys, vol 2. Academic Press, London, pp 193–239
Morris R (1980) Blood composition and osmoregulation in ammocoete larvae. Can J Fish Aquat Sci 37:1665–1679
Morrison SL, Campbell CK, Wright GM (2000) Chondrogenesis of the branchial skeleton in embryonic sea lamprey, *Petromyzon marinus*. Anat Rec 260:252–267
Murdoch SP, Docker MF, Beamish FWH (1992) Effect of density and individual variation on growth of sea lamprey (*Petromyzon marinus*) larvae in the laboratory. Can J Zool 70:184–188
Nakabayashi K, Matsumi H, Bhalla A et al (2002) Thyrostimulin, a heterodimer of two new human glycoprotein hormone subunits, activates the thyroid-stimulating hormone receptor. J Clin Invest 109:1445–1452
Nelson JS (2006) Fishes of the world, 4th edn. Wiley, Hoboken
Nikitina N, Sauka-Spengler T, Bronner-Fraser M (2008) Dissecting early regulatory relationships in the lamprey neural crest gene network. Proc Natl Acad Sci U S A 105:20083–20088

Nozaki M, Takahashi A, Amemiya Y, Kawauchi H, Sower SA (1995) Distribution of lamprey adrenocorticotropin and melanotropins in the pituitary of the adult sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 98:147–156

Nozaki M, Ominato K, Shimotani T et al (2008) Identity and distribution of immunoreactive adenohypophysial cells in the pituitary during the life cycle of sea lampreys, *Petromyzon marinus*. Gen Comp Endocrinol 155:403–412

O'Boyle RN, Beamish FWH (1977) Growth and intermediary metabolism of larval and metamorphosing stages of the landlocked sea lamprey, *Petromyzon marinus* L. Environ Biol Fish 2:103–120

Ogasawara M, Di Lauro R, Satoh N (1999) Ascidian homologs of mammalian thyroid peroxidase genes are expressed in the thyroid-equivalent region of the endostyle. J Exp Zool 285:158–169

Ohtani K, Yao TY, Kobayashi M et al (2008) Expression of Sox and fibrillar collagen genes in lamprey larval chondrogenesis with implications for the evolution of vertebrate cartilage. J Exp Zool B Mol Dev E 310:596–607

Ooi EC, Youson JH (1979) Regression of larval opisthonephros during metamorphosis of sea lamprey, *Petromyzon marinus* L. Am J Anat 154:57–79

Orozco A, Valverde R (2005) Thyroid hormone deiodination in fish. Thyroid 15:799–813

Osório J, Rétaux S (2008) The lamprey in evolutionary studies. Dev Genes Evol 218:221–235

Paris M, Escriva H, Schubert M et al (2008) Amphioxus postembryonic development reveals the homology of chordate metamorphosis. Curr Biol 18:825–830

Parker PS, Lennon RE (1956) Biology of the sea lamprey in its parasitic phase. US Fish Wildl Serv Res Rep 44:1–32

Peek WD, Youson JH (1979a) Transformation of the interlamellar epithelium of the gills of the anadromous sea lamprey, *Petromyzon marinus* L, during metamorphosis. Can J Zool 57:1318–1332

Peek WD, Youson JH (1979b) Ultrastructure of chloride cells in young adults of the anadromous sea lamprey, *Petromyzon marinus* L, in fresh water and during adaptation to sea water. J Morphol 160:143–163

Piavis GW (1961) Embryological stages in the sea lamprey and effects of temperature on development. US Fish Wildl Serv Fish Bull 61:111–143

Potter IC (1970) Life cycles and ecology of Australian lampreys of genus *Mordacia*. J Zool 161:487–511

Potter IC (1980) Ecology of larval and metamorphosing lampreys. Can J Fish Aquat Sci 37:1641–1657

Potter IC, Beamish FWH (1975) Lethal temperatures in ammocoetes of four species of lampreys. Acta Zool 56:85–91

Potter IC, Brown ID (1975) Changes in haemoglobin electorpherograms during the life cycle of two closely related lampreys. Comp Biochem Physiol 51B:517–519

Potter IC, Gill HS (2003) Adaptive radiation of lampreys. J Great Lakes Res 29:95–112

Potter IC, Welsch U (1992) Arrangement, histochemistry and fine structure of the connective tissue architecture of lampreys. J Zool 226:1–30

Potter IC, Wright GM, Youson JH (1978) Metamorphosis in the anadromous sea lamprey, *Petromyzon marinus* L. Can J Zool 56:561–570

Potter IC, Hilliard RW, Bird DJ (1980) Metamorphosis in the southern hemisphere lamprey, *Geotria australis*. J Zool 190:405–430

Potter IC, Hilliard RW, Bird DJ (1982) Stages in metamorphosis. In: Hardisty MW, Potter IC (eds) Biology of lampreys, vol 4b. Academic Press, London, pp 137–164

Pough FH, Janis CM, Heiser JB (2013) Vertebrate Life, 9th edn. Pearson, Boston

Power DM, Einarsdottir IE, Pittman K et al (2008) The molecular and endocrine basis of flatfish metamorphosis. Rev Fish Sci 16:95–111

Purvis HA (1970) Growth, age at metamorphosis, and sex ratio of northern brook lamprey in a tributary of southern Lake Superior. Copeia 1970:326–332

Purvis HA (1979) Variations in growth age at transformation and sex ratio of sea lampreys *Petromyzon marinus* reestablished in chemically treated tributaries of the Upper Great Lakes USA. Great Lakes Fish Comm Tech Rep 35:1–36

Purvis HA (1980) Effects of temperature on metamorphosis and the age and length at metamorphosis in sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:1827–1834

Reis-Santos P, McCormick SD, Wilson JM (2008) Ionoregulatory changes during metamorphosis and salinity exposure of juvenile sea lamprey (*Petromyzon marinus* L.). J Exp Biol 211:978–988

Rémy C, Bounhiol JJ (1971) Normalized metamorphosis achieved by adrenocorticotropic hormone in hypophysectomized and thyroxined *Alytes* tadpoles. C R Acad Sci Hebd Seances Acad Sci D 272:455–458

Renaud CB (1986) *Eudontomyzon hellenicus* Vladykov, Renaud, Kott, and Economidis, 1982. In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 186–195

Renaud CB, Cochran PA (in press) Post-metamorphic feeding in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Renaud CB, Holčík J (1986) *Eudontomyzon danfordi Regan,* 1911. In: J Holčík (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA-Verlag, Wiesbaden, Germany, pp 146–164

Renaud CB, Docker MF, Mandrak NE (2009) Taxonomy, distribution, and conservation of lampreys in Canada. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 293–309

Richards JE, Beamish FWH (1981) Initiation of feeding and salinity tolerance in the Pacific lamprey *Lampetra tridentata*. Mar Biol 63:73–77

Richardson SJ (2008) Marsupial models for understanding evolution of thyroid hormone distributor proteins. Mol Cell Endocrinol 293:32–42

Richardson SJ, Monk JA, Shepherdley CA et al (2005) Developmentally regulated thyroid hormone distributor proteins in marsupials, a reptile, and fish. Am J Physiol Regul Integr Comp Physiol 288:R1264–R1272

Richardson MK, Admiraal J, Wright GM (2010) Developmental anatomy of lampreys. Biol Rev 85:1–33

Ricker WE (1975) Computation and interpretation of biological statistics of fish populations. Bulletin Fisheries Research Board of Canada 191, Ottawa

Robson P (1998) Biochemical and molecular studies of the cartilaginous endoskeleton of adult lampreys and hagfish. PhD thesis, University of Toronto, Toronto

Robson P, Wright GM, Sitarz E et al (1993) Characterization of lamprin, an unusual matrix protein from lamprey cartilage. Implications for evolution, structure, and assembly of elastin and other fibrillar proteins. J Biol Chem 268:1440–1447

Robson P, Wright GM, Youson JH, Keeley FW (1997) A family of non-collagen-based cartilages in the skeleton of the sea lamprey, *Petromyzon marinus*. Comp Biochem Physiol B Biochem Mol Biol 118:71–78

Robson P, Wright GM, Youson JH, Keeley FW (2000) The structure and organization of lamprin genes: multiple-copy genes with alternative splicing and convergent evolution with insect structural proteins. Mol Biol Evol 17:1739–1752

Rodríguez-Muñoz R, Nicieza AG, Braña F (2003) Density-dependent growth of sea lamprey larave: evidence for chemical interference. Funct Ecol 17:403–408

Root AR, Nucci NV, Sanford JD et al (2005) *In situ* characterization of gonadotropin-releasing hormone-I, -III, and glutamic acid decarboxylase expression in the brain of the sea lamprey, *Petromyzon marinus*. Brain Behav Evol 65:60–70

Sargent PA, Youson JH (1986) Quantification of iron deposits in several body tissues of lampreys (*Petromyzon marinus* L.) throughout the life cycle. Comp Biochem Physiol A Physiol 83:573–577

Sauka-Spengler T, Bronner-Fraser M (2008) Insights from a sea lamprey into the evolution of neural crest gene regulatory network. Biol Bull 214:303–314

Schinko I, Potter IC, Welsch U, Debbage P (1992) Structure and development of the notochord elastica externa and nearby components of the elastic fiber system of agnathans. Acta Zool 73:57–66

Sheren SB, Eikenberry EF, Broek DL et al (1986) Type-II collagen of lamprey. Comp Biochem Physiol B Biochem Mol Biol 85:5–14

Shi YB (2000) Amphibian metamorphosis: from morphology to molecular biology. Wiley, New York

Shi YB, Ishizuya-Oka A (1996) Biphasic intestinal development in amphibians: embryogenesis and remodeling during metamorphosis. Curr Top Dev Biol 32:205–235

Smalley SR, Macey DJ, Potter IC (1986) Changes in the amount of non-heme iron in the plasma, whole body, and selected organs during the postlarval life of the lamprey *Geotria australis*. J Exp Zool 237:149–157

Smith AI, Funder JW (1988) Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. Endocr Rev 9:159–179

Smith BR, Tibbles JJ (1980) Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936-78. Can J Fish Aquat Sci 37:1780–1801

Sower SA (1998) Brain and pituitary hormones of lampreys, recent findings and their evolutionary significance. Am Zool 38:15–38

Sower SA, Plisetskaya E, Gorbman A (1985) Steroid and thyroid hormone profiles following a single injection of partly purified salmon gonadotropin or GnRH analogs in male and female sea lamprey. J Exp Zool 235:403–408

Sower SA, Freamat M, Kavanaugh SI (2009) The origins of the vertebrate hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) endocrine systems: new insights from lampreys. Gen Comp Endocrinol 161:20–29

Stilborn SSM, Manzon LA, Schauenberg JD, Manzon RG (2009) Expression of sea lamprey, *Petromyzon marinus*, deiodinase type II throughout metamorphosis and following a thyroid challenge. Integr Comp Biol 49:E163

Sudo S, Kuwabara Y, Park JI, Hsu SY, Hsueh AJW (2005) Heterodimeric fly glycoprotein hormone-α2 (GPA2) and glycoprotein hormone-β5 (GPB5) activate fly leucine-rich repeat-containing G protein-coupled receptor-1 (DLGR1) and stimulation of human thyrotropin receptors by chimeric fly GPA2 and human GPB5. Endocrinology 146:3596–3604

Suzuki S (1986) Induction of metamorphosis and thyroid function in the larval lamprey. Front Thyroidol 1:667–670

Suzuki S (1987) Induction of metamorphosis and thyroid function in the larval lamprey. In: Ohnishi E, Nagahama Y, Ishizaki H (eds) Proceedings of the 1st Congress of the Asia Oceania Society for Comparative Endocrinology, Nagoya University Press, Nagoya, Japan, pp 220–221

Suzuki S (1989) Why goitrogens are chemical triggers of metamorphosis in the lamprey relationship between thyroid-function and metamorphosis. Gen Comp Endocrinol 74:277

Swink WD (1995) Growth and survival of newly parasitic sea lampreys at representative winter temperatures. Trans Am Fish Soc 124:380–386

Swink WD, Johnson NS (2014) Growth and survival of sea lampreys from metamorphosis to spawning in Lake Huron. Trans Am Fish Soc 143:380–386

Takahashi A, Amemiya Y, Sarashi M, Sower SA, Kawauchi H (1995) Melanotropin and corticotropin are encoded on two distinct genes in the lamprey, the earliest evolved extant vertebrate. Biochem Biophys Res Commun 213:490–498

Tata JR (2006) Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. Mol Cell Endocrinol 246:10–20

Tata JR, Baker BS, Machuca I, Rabelo EML, Yamauchi K (1993) Autoinduction of nuclear receptor genes and its significance. J Steroid Biochem Mol Biol 46:105–119

Tobet SA, Nozaki M, Youson JH, Sower SA (1995) Distribution of lamprey gonadotropin-releasing hormone-III (GnRH-III) in brains of larval lampreys (*Petromyzon marinus*). Cell Tissue Res 279:261–270

Tobet SA, Chickering TW, Sower SA (1996) Relationship of gonadotropin-releasing hormone (GnRH) neurons to the olfactory system in developing lamprey (*Petromyzon marinus*). J Comp Neurol 376:97–111

Tobet SA, Sower SA, Schwarting GA (1997) Gonadotropin-releasing hormone containing neurons and olfactory fibers during development: from lamprey to mammals. Brain Res Bull 44:479–486

Treble AJ, Jones ML, Steeves TB (2008) Development and evaluation of a new predictive model for metamorphosis of Great Lakes larval sea lamprey (*Petromyzon marinus*) populations. J Great Lakes Res 34:404–417

Tsuneki K, Ouji M (1984) Morphometric changes during growth of the brook lamprey *Lampetra reissneri*. Jpn J Ichthyol 31:38–46

Vladykov VD (1985) Does neoteny occur in holarctic lampreys (Petromyzontidae)? Syllogeus 57:1–13

Walsh SR, Burr BM (1981) Distribution, morphology and life history of the least brook lamprey, *Lampetra aepyptera* (Pisces: Petromyzontidae) in Kentucky. Brimleyana 6:83–100

Welsch U, Erlinger R, Potter IC (1991) Proteoglycans in the notochord sheath of lampreys. Acta Histochem 91:59–65

White B, Nicoll CS (1981) Hormonal control of amphibian metamorphosis. In: Gilbert LA, Frieden E (eds) Metamorphosis: a problem in developmental biology. Plenum, New York, pp 263–396

Wright GM, Youson JH (1976) Transformation of the endostyle of anadromous sea lamprey, *Petromyzon marinus* L, during metamorphosis. 1. Light microscopy and autoradiography with ^{125}I. Gen Comp Endocrinol 30:243–257

Wright GM, Youson JH (1977) Serum thyroxine concentrations in larval and metamorphosing anadromous sea lamprey, *Petromyzon marinus* L. J Exp Zool 202:27–32

Wright GM, Youson JH (1980) Transformation of the endostyle of the anadromous sea lamprey, *Petromyzon marinus* L, during metamorphosis. 2. Electron microscopy. J Morphol 166:231–257

Wright GM, Youson JH (1982) Ultrastructure of mucocartilage in the larval anadromous sea lamprey, *Petromyzon marinus* L. Am J Anat 165:39–51

Wright GM, Youson JH (1983) Ultrastructure of cartilage from young adult sea lamprey, *Petromyzon marinus* L: a new type of vertebrate cartilage. Am J Anat 167:59–70

Wright GM, Filosa MF, Youson JH (1978) Immunocytochemical localization of thyroglobulin in endostyle of anadromous sea lamprey, *Petromyzon marinus* L. Am J Anat 152:263–268

Wright GM, Filosa MF, Youson JH (1980) Immunocytochemical localization of thyroglobulin in the transforming endostyle of anadromous sea lampreys, *Petromyzon marinus* L, during metamorphosis. Gen Comp Endocrinol 42:187–194

Wright GM, Armstrong LA, Jacques AM, Youson JH (1988) Trabecular, nasal, branchial, and pericardial cartilages in the sea lamprey, *Petromyzon marinus*: fine structure and immunohistochemical detection of elastin. Am J Anat 182:1–15

Wright GM, Mcburney KM, Youson JH, Sower SA (1994) Distribution of lamprey gonadotropin-releasing-hormone in the brain and pituitary gland of larval, metamorphic, and adult sea lampreys, *Petromyzon marinus*. Can J Zool 72:48–53

Yaghoubian S, Filosa MF, Youson JH (2001) Proteins immunoreactive with antibody against a human leptin fragment are found in serum and tissues of the sea lamprey, *Petromyzon marinus* L. Comp Biochem Physiol B Biochem Mol Biol 129:777–785

Yamano K (2005) The role of thyroid hormone in fish development with reference to aquaculture. Jpn Agric Res Q 39:161–168

Yamano K, Miwa S (1998) Differential gene expression of thyroid hormone receptor α and β in fish development. Gen Comp Endocrinol 109:75–85

Yamauchi K, Kasahara T, Hayashi H, Horiuchi R (1993) Purification and characterization of a 3,5,3′-L-triiodothyronine-specific binding protein from bullfrog tadpole plasma: a homolog of mammalian transthyretin. Endocrinology 132:2254–2261

Yao T, Ohtani K, Wada H (2008) Whole mount observation of pharyngeal and trabecular cartilage development in lampreys. Zool Sci 25:976–981

Young JZ, Bellerby CW (1935) The response of the lamprey to injection of anterior lobe pituitary extract. J Exp Biol 12:246–253
Youson JH (1980) Morphology and physiology of lamprey metamorphosis. Can J Fish Aquat Sci 37:1687–1710
Youson JH (1981a) The alimentary canal. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 3. Academic Press, London, pp 95–189
Youson JH (1981b) The kidneys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 3. Academic Press, London, pp 192–261
Youson JH (1981c) The liver. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 3. Academic Press, New York, pp 262–332
Youson JH (1982) Replication of basal bodies and ciliogenesis in a ciliated epithelium of the lamprey. Cell Tissue Res 223:255–266
Youson JH (1984) Differentiation of the segmented tubular nephron and excretory duct during lamprey metamorphosis. Anat Embryol 169:275–292
Youson J (1985) Organ development and specialization in lamprey species. In: Foreman RE, Gorbman A, Dodd JM, Olsson R (eds) The evolutionary biology of primitive fishes. Plenum, New York, pp 141–156
Youson JH (1988) First metamorphosis. In: Hoar WS, Randall DJ (eds) Fish physiology: the physiology of developing fish, vol 11A. Academic Press, New York, pp 135–196
Youson JH (1993) Biliary atresia in lampreys. In: Cornelius CE (ed) Advances in veterinary science and comparative medicine, vol 37, animal models in liver research. Academic Press, San Diego, pp 197–255
Youson (1994) Environmental and hormonal cues and endocrine glands during lamprey metamorphosis. In: Davey KG, Peter RE, Tobe SS (eds) Perspectives in comparative endocrinology. National Research Council of Canada, Ottawa, pp 400–408
Youson JH (1997) Is lamprey metamorphosis regulated by thyroid hormones? Am Zool 37:441–460
Youson JH (2000) The agnathan enteropancreatic endocrine system: phylogenetic and ontogenetic histories, structure, and function. Am Zool 40:179–199
Youson JH (2003) The biology of metamorphosis in sea lampreys: endocrine, environmental, and physiological cues and events, and their potential application to lamprey control. J Great Lakes Res 29(Suppl 1):26–49
Youson JH (2004) The impact of environmental and hormonal cues on the evolution of fish metamorphosis. In: Hall BK, Pearson RD, Muller GB (eds) Environment, development, and evolution: toward a synthesis. Massachusetts Institute of Technology Press, Cambridge, pp 239–278
Youson JH (2007) Peripheral endocrine glands. I. The gastroenteropancreatic endocrine system and the thyroid gland. In: McKenzie DJ, Farrell AP, Brauner CJ (eds) Primitive fishes. Academic Press, New York, pp 381–455
Youson JH, Al-Mahrouki AA (1999) Ontogenetic and phylogenetic development of the endocrine pancreas (islet organ) in fishes. Gen Comp Endocrinol 116:303–335
Youson JH, Beamish RJ (1991) Comparison of the internal morphology of adults of a population of lampreys that contains a nonparasitic life-history type, *Lampetra richardsoni*, and a potentially parasitic form, *L. richardsoni* var. *marifuga*. Can J Zool 69:628–637
Youson JH, Cheung R (1990) Morphogenesis of somatostatin- and insulin-secreting cells in the lamprey endocrine pancreas. Fish Physiol Biochem 8:389–397
Youson JH, Connelly KL (1978) Development of longitudinal mucosal folds in intestine of the anadromous sea lamprey, *Petromyzon marinus* L, during metamorphosis. Can J Zool 56:2364–2371
Youson JH, Elliott WM (1989) Morphogenesis and distribution of the endocrine pancreas in adult lampreys. Fish Physiol Biochem 7:125–131
Youson JH, Horbert WR (1982) Transformation of the intestinal epithelium of the larval anadromous sea lamprey, *Petromyzon marinus* L., during metamorphosis. J Morphol 171:89–117
Youson JH, Manzon RG (2012) Lamprey metamorphosis. In: Dufour S, Rousseau K, Kapoor BG (eds) Metamorphosis in fish. Science Publishers, Enfield, pp 12–75

Youson JH, Potter IC (1979) Description of the stages in the metamorphosis of the anadromous sea lamprey, *Petromyzon marinus* L. Can J Zool 57:1808–1817

Youson JH, Potter IC (1993a) An immunohistochemical study of enteropancreatic endocrine cells in larvae and juveniles of the southern-hemisphere lampreys *Geotria australis* and *Mordacia mordax*. Gen Comp Endocrinol 92:151–167

Youson JH, Potter IC (1993b) An immunohistochemical study of enteropancreatic endocrine cells in larvae and juveniles of the Southern-Hemisphere lampreys *Geotria australis* and *Mordacia mordax*. Gen Comp Endocrinol 92:151–167

Youson JH, Sower SA (1991) Concentration of gonadotropin-releasing-hormone in the brain during metamorphosis in the lamprey, *Petromyzon marinus*. J Exp Zool 259:399–404

Youson JH, Sower SA (2001) Theory on the evolutionary history of lamprey metamorphosis: role of reproductive and thyroid axes. Comp Biochem Physiol B Biochem Mol Biol 129:337–345

Youson JH, Lee J, Potter IC (1979) Distribution of fat in larval, metamorphosing, and young adult anadromous sea lampreys, *Petromyzon marinus* L. Can J Zool 57:237–246

Youson JH, Sargent PA, Sidon EW (1983) Iron loading in the livers of metamorphosing lampreys, *Petromyzon marinus* L. Cell Tissue Res 234:109–124

Youson JH, Sargent PA, Barrett A (1987) Serum iron concentration and other blood parameters during the life cycle of the sea lamprey, *Petromyzon marinus* L. Comp Biochem Physiol A Physiol 88:325–330

Youson JH, Elliott WM, Beamish RJ, Wang DW (1988) A comparison of endocrine pancreatic tissue in adults of four species of lampreys in British Columbia: a morphological and immunohistochemical study. Gen Comp Endocrinol 70:247–261

Youson JH, Holmes JA, Guchardi JA et al (1993) Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 50:2448–2456

Youson JH, Plisetskaya EM, Leatherland JF (1994) Concentrations of insulin and thyroid hormones in the serum of landlocked sea lampreys (*Petromyzon marinus*) of three larval year classes, in larvae exposed to two temperature regimes, and in individuals during and after metamorphosis. Gen Comp Endocrinol 94:294–304

Youson JH, Docker M, Sower SA (1995a) Concentration of gonadotropin-releasing hormones in brain of larval and metamorphosing lampreys of two species with different adult life histories. In: Goetz FW, Thomas P (eds) Proceedings of the 5th Internatinal Symposium Reproductive Physiology of Fish. University of Texas Press, Austin, p 83

Youson JH, Holmes JA, Leatherland JF (1995b) Serum concentrations of thyroid hormones in $KClO_4$-treated larval sea lampreys (*Petromyzon marinus* L). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 111:265–270

Youson JH, Manzon RG, Peck BJ, Holmes JA (1997) Effects of exogenous thyroxine (T4) and triiodothyronine (T3) on spontaneous metamorphosis and serum T4 and T3 levels in immediately premetamorphic sea lampreys, *Petromyzon marinus*. J Exp Zool 279:145–155

Youson JH, Heinig JA, Khanam SF et al (2006) Patterns of proopiomelanotropin and prooplocortin gene expression and of immunohistochemistry for gonadotropin-releasing hormones (GnRH-I and III) during the life cycle of a nonparasitic lamprey: relationship to this adult life history type. Gen Comp Endocrinol 148:54–71

Zanandrea G (1957) Neoteny in a lamprey. Nature 179:925–926

Zerrenner A, Marsden JE (2005) Influence of larval sea lamprey density on transformer life history characteristics in Lewis Creek, Vermont. Trans Am Fish Soc 134:687–696

Zerrenner A, Marsden JE (2006) Comparison of larval sea lamprey life history characteristics in a lampricide-treated tributary and untreated tributary system of Lake Champlain. Trans Am Fish Soc 134:1301–1311

Zhang G (2009) An evo-devo view on the origin of the backbone: evolutionary development of the vertebrae. Integr Comp Biol 49:178–186

Zhang G, Miyamoto MM, Cohn MJ (2006) Lamprey type II collagen and Sox9 reveal an ancient origin of the vertebrate collagenous skeleton. Proc Natl Acad Sci U S A 103:3180–3185

Chapter 5
Lamprey Spawning Migration

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Abstract During recent decades, new insights regarding the spawning migration of lampreys have been gained due to advances in technology and growing interest in this key life history phase. The development of miniaturized active and passive transmitters has led to detailed information on the timing and extent of lamprey migrations. These tools, together with sophisticated laboratory experiments, have provided fertile ground for studies of lamprey migratory physiology and behavior. New molecular tools have been applied to questions of population structure and philopatry, while the identification of lamprey pheromones has illuminated heretofore unimagined mechanisms of migration and orientation. Interest in spawning migration has been spurred by the growing need to restore native lamprey populations and the equally pressing need to control invasive sea lamprey in the Laurentian Great Lakes. While important advances in anadromous lamprey biology have been achieved, gaps remain in our understanding of marine movements, species-specific differences, mechanisms of orientation, and the factors controlling passage success. Moreover, with the exception of the landlocked sea lamprey in the Great Lakes, research on the spawning migrations of the strictly potamodromous species (i.e., those that are parasitic in fresh water and the non-parasitic "brook" lampreys)

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is sorely lacking, seriously compromising our ability to assess what constitutes barriers to their migration.

Keywords Adfluvial · Anadromous · Behavior · Dams · Orientation · Passage · Pheromones · Potamodromous · Swimming performance

5.1 Introduction

For centuries, the natural history of migration has been described in terms of function (e.g., to reach spawning or feeding grounds), route, timing, and rate of movement. More recently, the physiological condition of migrating animals and the cues that they use to orient and navigate have been considered, and advances have been made regarding these mechanisms of migration. Here we examine the timing and extent of lamprey spawning migrations, the swimming performance of upstream migrants (particularly at barriers), the physiological and behavioral changes that accompany this life stage, the cues lampreys use to orient, and the management implications related to this critical phase of the lamprey life history.

There are currently between 41 and 44 recognized lamprey species worldwide (see Chaps. 1 and 2); 18 species are parasitic and 23–26 species are non-parasitic (see Docker 2009; Docker and Potter in press). Because some parasitic forms can be displaced great distances while attached to their hosts, the adult stages of these species have evolved a migratory life history that brings them into streams suitable for spawning. Five of the parasitic species are exclusively or almost exclusively anadromous (i.e., they move from the ocean or estuary to fresh water for spawning), which presents great challenges with respect to the migration distance (hundreds of kilometers in some species; see Sect. 5.2.1) and the physiological changes needed to cope with osmoregulation (Sect. 5.3.1). Nine of the parasitic species are potamodromous (i.e., showing directed movement within fresh water), and the remaining four parasitic species have both anadromous and potamodromous populations (see Docker and Potter in press). The 23–26 non-parasitic (resident or "brook lamprey") species are exclusively potamodromous. Although typically less extensive, potamodromous lampreys also exhibit discrete spawning migrations and the attendant changes in physiology and behavior.

Spawning migration in lampreys has been reviewed in the past (Hardisty and Potter 1971; Larsen 1980); however, recent discoveries warrant a renewed look at this fascinating phase of the lamprey life history. With the development of miniaturized transmitters and passive integrated transponder (PIT) technology, new insights have been gained regarding the timing, rate, and extent of lamprey migration. In addition, exciting new research has revealed the role of pheromones in lamprey orientation during pre-spawning movements (see Sects. 5.4.1 and 5.6.1). In this chapter, we review recent advances in our understanding of upstream migration by adult lampreys. Basic data regarding the timing and extent of spawning migration are available for many anadromous and potamodromous species. However, there is a dearth of information on swimming performance, physiology, orientation,

and behavior for most potamodromous species. Hence, for these topics, our review focused primarily on the anadromous species and, where possible, the landlocked sea lamprey *Petromyzon marinus* in the Laurentian Great Lakes. The downstream migration of juveniles (i.e., after metamorphosis) is covered elsewhere in this volume (see Chap. 3).

Many recent advances have resulted from the need either to conserve native lampreys (see Chap. 8) or to develop methods for controlling the invasive sea lamprey in the Great Lakes (see Marsden and Siefkes in press). Native lamprey species are in decline in many regions of the world, and disruption of the spawning migration with the attendant loss of habitat availability is frequently cited as the primary cause for population declines (Renaud 1997; Close et al. 2002). Consequently, recent research has been directed toward identification of passage impediments encountered by lampreys during their spawning migration and methods to promote passage. Information regarding the mechanisms of migration is equally important for development of effective sea lamprey control. Regular funding of lamprey research in support of integrated pest management schemes has been the source of a large volume of data on the spawning migration of potamodromous ("landlocked") sea lamprey in the Great Lakes. Thus, while advancing our knowledge of basic lamprey biology, the research reviewed here also has myriad applications for management of lampreys and the ecosystems where they occur.

5.2 Timing and Extent of Spawning Migration

The timing and extent of the spawning migration varies widely both among and within lamprey genera and species. This is particularly obvious when comparing parasitic and non-parasitic species, as parasitic lampreys often range great distances from their spawning and rearing areas (i.e., while attached to their hosts) and non-parasitic resident forms apparently remain within their natal streams. The timing and extent of spawning migrations can also vary substantially within species. For example, the anadromous sea lamprey has access to larger river systems and migrates over greater distances and longer time periods than the landlocked form of this species in the Great Lakes (Clemens et al. 2010). The environmental factors encountered during spawning migration thus vary widely among species and populations, demanding both physiological and behavioral plasticity.

For the purpose of this review, we define spawning migration as that part of the lamprey life history from the end of the trophic phase to arrival on the spawning grounds. For information regarding spawning timing and fine-scale movement to find mates and select spawning sites, see Chap. 6. In brook lampreys, the temporal extent of the spawning migration is relatively short (i.e., the months between metamorphosis to spawning site selection; see Sect. 5.2.3.2). In contrast, some anadromous species exhibit spawning migrations of over 1.5 years (Sect. 5.2.1). The anadromous spawning migration can be divided into three stages: (1) migration from the ocean or estuary into rivers or streams; (2) pre-spawning holding in fresh water; and

(3) upstream movement within rivers and streams to spawning sites (Clemens et al. 2010). The physiological basis of this scheme has not been explicitly described and it is possible that these stages are transition points in a continuum. Sea lamprey in the Great Lakes appear to exhibit behaviors similar to those of the marine form (Vrieze et al. 2010, 2011), but generally undergo less extensive migrations; they are reviewed separately here. Parasitic and brook lampreys that exhibit only potamodromous migrations are also treated separately at the end of this section.

5.2.1 Timing and Migration Distance: Anadromous Lampreys

Anadromous lampreys move from the ocean or estuary into fresh water to spawn. There are only nine anadromous species, five that are exclusively or almost exclusively anadromous (pouched lamprey *Geotria australis*, short-headed lamprey *Mordacia mordax*, Chilean lamprey *M. lapicida*, Caspian lamprey *Caspiomyzon wagneri*, and North American or western river lamprey *Lampetra ayresii*, the latter of which is largely estuarine) and four that are also sometimes found in fresh water (sea lamprey, European river lamprey *Lampetra fluviatilis*, Arctic lamprey *Lethenteron camtschaticum*, and Pacific lamprey *Entosphenus tridentatus*) (see Chap. 2; Docker and Potter in press).

There is considerable inter- and intraspecific variation in the timing of freshwater entry and the duration of the freshwater stage of the migration. Although all lampreys spawn in the spring or summer, some may enter fresh water as much as 16 months before spawning (e.g., pouched lamprey, some Pacific lamprey; see below). Recent research shows that even within a species, duration of the freshwater migration can vary greatly (e.g., ranging from a few months to over two years in Pacific lamprey; Clemens 2011). These differences are often but not always correlated with migration distances in fresh water (see below).

Little is known about overall migration distances, which depend on the extent of free-swimming movement in the ocean and may be even greater than is currently known. Recent distribution data for Pacific lamprey in the North Pacific Ocean suggest that the marine phase of the spawning migration in this species is more extensive than previously thought (Orlov et al. 2008). Thus, the factors involved in the regulation of migration timing are not clear. Although one cannot exclude the possibility of genetic control (see Hess et al. 2013), we hypothesize that the marine phase of the anadromous lamprey life cycle plays an important role in both the timing and extent of spawning migration. Different oceanic feeding grounds with particular physical-chemical characteristics could influence the timing of migratory behavior.

The proximate cues for migration, however, appear linked to environmental conditions of the rivers in which spawning occurs. The upstream spawning migration of anadromous lampreys is triggered by flow variations and temperature (see Sect. 5.5.2). Studies on the anadromous sea lamprey (Almeida et al. 2002a; Andrade et al. 2007) and European river lamprey (Abou-Seedo and Potter 1979;

Masters et al. 2006) have demonstrated that migratory activity increases with increased stream discharge. This may be a mechanism to ensure that lamprey passage is facilitated through difficult areas and/or that access to sheltered headwater rearing sites is available (Binder et al. 2010). The timing and upstream progress of adult Pacific lamprey in the Columbia River system is also strongly influenced by river temperature and total discharge (Keefer et al. 2009a). In this species, migration timing is significantly later and migration rates are slower in cold, high-flow years. This adaptation ensures that Pacific lamprey migrate during periods of optimal metabolic scope while avoiding periods of highest current velocity.

In British Columbia and the northwestern United States, Pacific lamprey generally enter river basins during spring (April–June; R. J. Beamish 1980), and initiate upstream migration during summer (July–September) before overwintering between October and March (Scott and Crossman 1973; Keefer et al. 2009a) and spawning between April and May the following year (Clemens et al. 2009; see Chap. 6). In the Columbia and Snake rivers, this species is known to swim hundreds of kilometers to reach spawning grounds (Moser and Close 2003). Prior to the completion of the Grand Coulee Dam in 1941, spawning migrations of 800 km up to Kettle Falls, Washington, occurred (Renaud 2011). In contrast, short migration distances are common in coastal streams (Kostow 2002). An extreme example is the Lilliwaup River (a small Olympic Peninsula watershed draining to Puget Sound, Washington) where Pacific lamprey spawn downstream from an impassable waterfall located at river kilometer 0.7 km (Richard S. Endicott, Lilliwaup Hatchery, Long Live the Kings, Seattle, WA, personal communication, 2011).

North American river lamprey exhibit summer and fall freshwater migrations, with spawning in the following spring. In British Columbia, this species migrates from coastal waters and estuaries into fresh water (e.g., the Fraser River and its tributaries) by September (Beamish and Youson 1987). Migration distances may exceed 300 km in large watersheds such as the Sacramento River in California, where adults have been collected as high as Mill Creek (Scott and Crossman 1973).

Considerable variation has been observed in both the timing and distance of the upstream migration in anadromous Arctic lamprey. In northwest Russia, migration starts in late summer and the beginning of autumn, and distance to the spawning grounds ranges from approximately 200 km in the Onega River to over 600 km in the Northern Dvina River (Holčík 1986b). In the Utkholok River basin, Kamchatka, in eastern Russia, the spawning migration occurs between April and June (Kucheryavyi et al. 2007; Renaud 2011); migration distances in this system are less than 100 km (Kucheryavyi et al. 2007). In Japan, spawning adults ascend rivers between October and January (Renaud 2011). In the Yukon River, Alaska, where the migration distance can exceed 1,600 km, spawning migration occurs between late November and late April (Renaud 2011). Long migration distances have also been reported in the Mutantiang River (1,700 km), a tributary of the Amur River in China, and in the Tom River in Russia (where this species is known to migrate over 2,100 km upstream from the estuary of the Ob River); these long migrations in Asia may take as much as 1.5 years to complete (Holčík 1986a).

Anadromous sea lamprey likewise exhibit great variation in run timing, although in comparison with the species mentioned previously, freshwater entry generally occurs closer to the spawning time. Along the east coast of North America, spawning migrations occur between March and September, but this varies with latitude (F. W. H. Beamish 1980a). Migration occurs earlier in streams at lower latitudes (e.g., largely in March and April in North Carolina to Maryland and May to June or July in the northern part of their range), but some migratory activity can extend through to August or September (in North Carolina and New Brunswick, respectively), with migrants apparently arriving shortly before spawning (F. W. H. Beamish 1980a). In Portuguese rivers, the sea lamprey spawning migration begins in December and peaks between February and April (Alexandrino 1990; Machado-Cruz et al. 1990; Oliveira et al. 2004), with spawning usually occurring between May and June (Almeida et al. 2000). In Britain, sea lamprey migration begins in February and continues through May and June (Hardisty 1986a).

Migration distance in anadromous sea lamprey can range from tens to hundreds of kilometers, depending on the size of the river, location of suitable spawning areas, and the length of river stretches downstream from impassable barriers (Hardisty 1986a). In Portugal, sea lamprey spawning areas are located between 30 and 190 km from the mouth of the estuary. However, all major rivers used by this species now have impassable dams, so these obstructions represent the upstream limit of migration (Almeida et al. 2002b). In Britain, sea lamprey spawn 10–100 km from the tidal limit (Hardisty 1986a; Martyn C. Lucas, Durham University, Durham, U.K., personal communication, 2011). In the Delaware and Susquehanna river systems of North America, sea lamprey were known to migrate up to 320 km from the sea (Bigelow and Schroeder 1948), but construction of the Conowingo Dam near the mouth of the latter river system in 1928 has since obstructed sea lamprey migration (Waldman et al. 2009). Likewise, sea lamprey once spawned at least 240 km upstream from the estuary in the Savannah River in South Carolina (F. W. H. Beamish 1980a). Upstream migration in the St. John River, New Brunswick, is now restricted by a dam 140 km from the estuary, but sea lamprey once migrated farther upstream (F. W. H. Beamish 1980a). Although Daniels (2001) questions that anadromous sea lamprey could migrate 725 km to reach Lake Ontario through the St. Lawrence River (see Docker and Potter in press), there is evidence that historically, European runs could reach 850 km in the River Rhine (Hardisty 1986a).

The timing and extent of adult European river lamprey migrations is likewise highly variable, with the extent depending largely on obstructions that block upstream progress. Spawning areas are typically within 100 km of the estuary mouth (e.g., River Sorraia, Portugal: Almeida unpublished data; River Swale, England: Lucas et al. 2009; Oir River, France: Lasne et al. 2010). In the United Kingdom (U.K.), the spawning migration of European river lamprey can extend from as early as July (i.e., the summer prior to spawning) to as late as June the following year (Hardisty and Potter 1971; Abou-Seedo and Potter 1979); it appears, however, that most individuals migrate in the autumn and winter months (Hardisty 1986b; Martyn C. Lucas, Durham University, Durham, U.K., personal communication, 2011). River lamprey likewise enter rivers of the Baltic Sea (e.g., Perhonjoki and Kalajoki

rivers in Finland) in early autumn (Jukka Tuohino, North Ostrobothnian Centre for Economic Development, Transport and the Environment, Finland, personal communication, 2011). There are many examples reviewed by Hardisty (1986c) and confirmed by other authors (e.g., Winter and Van Densen 2001; Masters et al. 2006) that indicate this is the general migration season for river lamprey throughout Europe. However, overall freshwater entry periods are often protracted as a result of distinct modes of movement by early migrants in autumn and by later, more fully developed, migrants as the time for spawning nears in spring (Hardisty and Potter 1971; Abou-Seedo and Potter 1979). Lampreys that have shorter migration distances may be more likely to delay migration to the fall or winter before spawning.

Depending on meteorological conditions, the spawning migration of Caspian lamprey in the Shirud River, Iran, peaks in mid-March, although the species is first detected in this river basin during late September (Nazari and Abdoli 2010) and both fall and spring migrants have been reported in this basin (Ahmadi et al. 2011). The spawning run up the Kura River (starting in Azerbaijan) occurs between November and February, and in the Volga River between mid-September and March (Renaud 2011). Construction of barriers in these rivers appears to have altered the timing of upstream migration (e.g., delaying the peak of migration observed at Volgograd from the beginning of December to February; Holčík 1986b). Migration duration can last for several months, depending on distance to the spawning sites, swimming speed, and water velocity. In the relatively short Shirud River (36 km), upstream migration of the spring migrants lasts from the middle of March until late April. In contrast, Caspian lamprey that enter the Volga River estuary in mid-September reach Kazań (1,500 km upstream) six months later (Holčík 1986b). The observation that Arctic lamprey can take up to 18 months to travel similar distances (see above) highlights species-specific differences in lamprey swimming performance and behavior.

In the Southern Hemisphere, lampreys undergo long migrations and exhibit a very long period of freshwater maturation. On mainland Australia, pouched lamprey may migrate up to several hundred kilometers up the Murray River, and the spawning run of short-headed lamprey is known to reach over 1,600 km up this river system (Renaud 2011). In Australia, both species enter fresh water from January to July, with peaks of migration from September through November (Hardisty and Potter 1971; Fernholm 1990). In Chile, mature pouched lamprey adults reach continental waters during the austral summer months (January–March; Renaud 2011). Although the timing of freshwater entry relative to spawning time is unknown since it is not certain when spawning occurs in these species (Jellyman et al. 2002; see Chap. 6), radiotelemetry evidence has indicated that pouched lamprey do not spawn until their second spring in fresh water (Jellyman et al. 2002). Laboratory experiments further confirmed that this species requires over 16 months in fresh water to reach sexual maturity (Bird and Potter 1983; Potter et al. 1983; James 2008). Moreover, these lamprey may have already accomplished lengthy spawning migrations in the ocean. Potter et al. (1979) hypothesized that marine movements of the pouched lamprey were over thousands of kilometers.

5.2.2 Timing and Migration Distance: Landlocked Sea Lamprey

Landlocked sea lamprey in the Laurentian Great Lakes have been the subject of intensive study since the mid-1900s. Fundamental similarities in the upstream spawning migration of anadromous and landlocked sea lamprey appear to exist. However, these comparisons are complicated by the fact that landlocked sea lamprey are approximately half the size (40−50 cm in length) of the anadromous form (Beamish and Potter 1975; Clemens et al. 2010). Due to their smaller size, landlocked sea lamprey are less adept than anadromous lamprey at both swimming and climbing (McAuley 1996; Clemens et al. 2010). Moreover, tributaries to the Great Lakes are relatively small and many have been dammed for sea lamprey control (see Marsden and Siefkes in press). Early records from Applegate (1950) indicate that landlocked forms once traveled up to 100 km inland to spawn, although the extent of upstream migrations (i.e., the distance to lamprey barriers) in most Great Lakes tributaries is now considerably shorter, on the order of dozens of kilometers (Clemens et al. 2010).

Landlocked sea lamprey embark on their spawning migration after spending little more than a year feeding in open water (approximately 8–12 months less than anadromous sea lamprey; F. W. H. Beamish 1980a; Bergstedt and Seelye 1995), and generally begin their upstream migration later in the season than the anadromous form (Sect. 5.2.1). Upstream migration generally occurs in late April to early June, but can extend into July in the colder waters of the northern Great Lakes where spawning likewise occurs later in the season (see Chap. 6). Stream capture records indicate that landlocked sea lamprey accumulate at stream mouths in February and March until temperature rises above 10 °C (Applegate 1950; Vrieze 2008). They appear to gather there because of pheromonal odors while the temperature threshold apparently triggers odor-driven rheotaxis and the start of upstream movement (Sorensen and Hoye 2007; Vrieze 2008; see Sect. 5.5.2). This strategy presumably ensures arrival at the spawning grounds near the time that temperature is optimal for reproduction and embryonic development.

5.2.3 Timing and Migration Distance: Potamodromous Lampreys

We consider lamprey species that exist entirely in fresh water to be potamodromous, or "freshwater lampreys." These include both parasitic and non-parasitic species that move either within rivers or between larger rivers or lakes and smaller streams for the purposes of spawning. Most potamodromous species embark on their upstream migration a few weeks to a few months before spawning (consistent with a shorter migration) and are generally not well studied. While non-parasitic brook lampreys do not exhibit the dramatic spawning migration of their parasitic

counterparts, at least some species are known to participate in discrete, albeit short, upstream movements prior to spawning—counteracting the passive downstream movement experienced during the larval phase (Malmqvist 1980; Hardisty 1986d). For the most part, as illustrated below, information on the timing and extent of spawning migration is scarce for most potamodromous species.

5.2.3.1 Freshwater Parasitic Species

The general lack of information on spawning movements of freshwater parasitic forms is appalling, particularly in light of the fact that anthropogenic activities have almost certainly blocked or disrupted spawning migrations in some species. For example, native *Ichthyomyzon* species are regularly captured at barriers designed to limit spawning movements of invasive sea lamprey in the Great Lakes (Schuldt and Goold 1980; COSEWIC 2010). These and other man-made obstacles have likely contributed to the decline of silver lamprey *Ichthyomyzon unicuspis* in Great Lakes tributaries and elsewhere, and headwater dams in the Danube drainage of Europe are thought to be a threat to the Carpathian lamprey *Eudontomyzon danfordi* (see Chap. 8). The overall paucity of information on freshwater parasitic lampreys makes it difficult to generalize regarding the timing and extent of spawning migration. The following brief review of known life history modes emphasizes their incredible diversity.

Freshwater parasitic *Entosphenus* species exhibit adfluvial (i.e., migrating from lakes to rivers or streams), lentic, and/or lotic spawning movements. Prior to its near extirpation from the Miller Lake drainage in Oregon, the Miller Lake lamprey *E. minimus* was thought to be primarily lacustrine with lentic spawning and larval rearing in the lake (Kan and Bond 1981). This species metamorphosed in fall and apparently spawned in June or July of the following year (at a size smaller than that of late stage larvae) and presumably without migration into tributary streams. More recent collections from other parts of Oregon, however, suggest lotic spawning (Lorion et al. 2000). Klamath lamprey *E. similis* exhibits both wholly riverine and adfluvial life histories in Oregon (Kostow 2002), but few details are known regarding the spawning movements of this species. There is likewise little known about spawning migration in the Vancouver or Cowichan lamprey *E. macrostomus*. This appears to be a lacustrine species with lentic spawning, although the presence of larvae in the lower portions of some lake tributaries suggests that some spawners migrate into tributaries as well (Beamish 1987).

The parasitic species in the genus *Ichthyomyzon* (silver lamprey, chestnut lamprey *I. castaneus*, and Ohio lamprey *I. bdellium*) occur in eastern North America. The latter two species appear to have largely riverine life histories, although chestnut lamprey may sometimes feed in lakes (Scott and Crossman 1973; Renaud 2011). The spawning migration of these two species is therefore of short duration and extent; upstream migrating chestnut lamprey have been captured at dams in late May or June, shortly before spawning (COSEWIC 2010). Relatively little is known about the biology of

these species, however, despite the concern—based on the large number of collections made at dams and weirs—that these structures impede their upstream migration (COSEWIC 2010). The spawning migration of silver lamprey, which generally feeds in large rivers and lakes, appears to be more substantial; silver lamprey may travel over 100 km to reach spawning areas (COSEWIC 2011). Silver lamprey migration has been best studied in the Fox River, Wisconsin, where upstream migrants have been captured from early April to early June (Cochran and Marks 1995; Cochran and Lyons 2004). It has been postulated that lamprey attached to lake sturgeon *Acipenser fulvescens* in the early spring could be transported upstream toward their spawning grounds (Cochran et al. 2003). Thus, in addition to disruption of spawning migrations by physical or electric barriers, changes in host distribution and abundance could also interfere with upstream migration in this species.

The Mexican or Chapala lamprey *Tetrapleurodon spadiceus* begins its upstream migration to the spawning grounds in the upper reaches of the Celio River in late June and early July (Renaud 2011). This species inhabits the highly developed Lerma River, where eleven dams divert water and likely impede migrations of this adfluvial species (IUCN 2013).

The Carpathian lamprey is endemic to the Danube system and migrates upstream during spawning time in April–June (Kottelat and Freyhof 2007). This strictly riverine migration occurs to the upstream reaches of brooks, which may require migration to spawning locations at over 1,000 m elevation (Renaud and Holčík 1986). Virtually nothing is known about the biology of the Korean lamprey *Eudontomyzon morii* (Renaud 2011).

5.2.3.2 Freshwater Non-Parasitic Species

As is the case for freshwater parasitic lampreys, relatively little is known of the timing and extent of spawning migration in brook lampreys. Malmqvist (1980) used tag-recapture and trapping experiments in a small Swedish stream to elucidate the timing and migration cues used by European brook lamprey *Lampetra planeri*, which typically spawns from late March to June (see Chap. 6). As in some parasitic forms (see Sect. 5.5.2), increasing temperature and decreasing stream discharge were the most important factors associated with the onset of spawning migration in this brook lamprey. Temperature has been implicated as a primary cue in the onset of migration for other brook lampreys, such as the least and Ukrainian brook lampreys (*Lampetra aepyptera* and *Eudontomyzon mariae*, respectively; reviewed in Hardisty and Potter 1971).

For most brook lampreys, information is available regarding capture dates of adults in spawning condition (see Renaud 2011), but few studies have investigated the timing or extent of migration. However, all suggest that migration is of short duration. Malmqvist (1980)'s mark-recapture study showed that European brook lamprey moved up to 2 km upstream prior to spawning, over a compressed time period from late March to early May. Jacona or Mexican brook lamprey *Tetrapleurodon*

geminis spend the first 3–4 months after metamorphosis in the River Duero, after which they engage in a short (3 km) upstream migration to the upper reaches of the River Celio (Renaud 2011). F. W. H. Beamish 1982) documented pre-spawning movements of at least 1 km in southern brook lamprey *Ichthyomyzon gagei.*

5.3 Physiology of Migrants

The spawning migration phase of the lamprey life cycle represents a time of profound morphological and physiological change, particularly for anadromous forms. While non-parasitic lampreys undergo sexual maturation coincident with metamorphosis (Docker 2009) and perform only short spawning migrations, metamorphosis and sexual maturation are separate processes in parasitic lampreys, and both potamodromous and anadromous parasitic lampreys exhibit a discrete, free-swimming migration that may be of great extent and duration in some anadromous species (Sect. 5.2.1) and that often includes a gauntlet of obstacles (Sect. 5.6.2). One of the most dramatic physiological changes during the upstream migration, especially in anadromous lampreys, is a pronounced decrease in body size. Lampreys stop feeding at or prior to the onset of migration and must rely on the energy reserves accumulated during the parasitic phase to fuel the spawning migration (Larsen 1980; see Sect. 5.3.2).

The environmental or physiological cues that stimulate lampreys to detach from their host and embark on the free-swimming, non-trophic spawning migration are completely unknown. Do lampreys respond to physiological changes mediated by day length or other environmental factors? Can lampreys respond to hormonal signaling by the host or synchronize migration with host movements? Alternatively, might lampreys initiate spawning migration on reaching a necessary size or growth rate? The general plasticity in lamprey life histories argues against genetic programming. For example, sea lamprey spend approximately two years feeding in the Atlantic Ocean and only one year feeding in the Great Lakes (Applegate 1950; F. W. H. Beamish 1980a). In the lake environment, stream finding in sea lamprey is active and extensive (Vrieze et al. 2011), but thus far there is no documentation of lamprey behavior at sea or the mechanisms of physiological control. The following section outlines the little that is known about the physiological changes that precede freshwater entry in anadromous lampreys.

Relatively more is known about the physiology of upstream migrants in fresh water. This is in large part due to recent research aimed at improving passage success and fitness of native lampreys at man-made barriers (or developing barriers or traps in the case of sea lamprey in the Great Lakes). Hence, there have been large advances in our understanding of the mobilization of energy reserves, swimming performance, and energetics. An extensive literature on the lamprey sensory system has also emerged in recent decades, and this topic is covered in Sect. 5.4.

5.3.1 Preparation for Freshwater Migration

While the marine phase of the spawning migration in anadromous lampreys is poorly understood, there is circumstantial evidence that some species undertake extensive free-swimming migrations in the ocean and estuary prior to freshwater entry (see Sect. 5.2.1). Data collected during open-ocean trawl surveys reveal that lampreys can undergo seasonal changes in condition that could be related to preparation for spawning migration (Orlov et al. 2008). What triggers lampreys to transition from feeding to free-swimming oceanic or estuarine migration is unknown. Larsen (1980) suggested that intestinal atrophy likely begins when lampreys are still at sea. Gonadal hormones have been implicated in intestinal atrophy and the cessation of feeding but, in some species at least, freshwater entry precedes sexual maturation and the accompanying increase in sex steroids (see Sect. 5.3.2). The existence of praecox forms of European river lamprey, Arctic lamprey, and Pacific lamprey suggests that the length of the marine phase may be truncated in some cases (Abou-Seedo and Potter 1979; Kucheryavyi et al. 2007; Clemens 2011; Hess et al. 2013; Docker and Potter in press; Renaud and Cochran in press), but what regulates length of the feeding phase is an enigma.

Lamprey physiology in the estuarine environment, as migrants transition from sea water to fresh water, is equally mysterious. The duration of estuarine residence and the course of changes in osmoregulatory function have not been adequately described. Riverine entry requires excretion of large volumes of urine, cessation of drinking, intestinal atrophy, and a reversal in ion transport across the gills to allow survival in fresh water (Bartels and Potter 2007). While these processes and their controls have been reviewed in the past (Morris 1972; F. W. H. Beamish 1980b; Larsen 1980), recent work has provided additional insight regarding the mechanisms controlling hyper-osmoregulation by migrating adult anadromous lampreys (Rankin 1997; Brown and Rankin 1999; Rankin et al. 2001). These studies have identified a renin-angiotensin system in European river lamprey which is activated by exposure to decreasing environmental salinity. Interestingly, the circulating angiotensin appears to be controlled by both volume/pressor receptors and osmoreceptors (Brown et al. 2005). There is evidence that the transport-related proteins involved in osmoregulation in elasmobranch and teleost fishes (e.g., Na^+/K^+-ATPase) are present in the gills of upstream migrating pouched lamprey captured in the Derwent River in Tasmania (Choe et al. 2004). This study, however, does not specify how far from the estuary these individuals were captured. Very little is known about the role these enzymes play in upstream migrating lampreys, while in salmonids and other anadromous fishes it is comparatively well-studied (see Shrimpton 2013).

5.3.2 Physiology of Freshwater Migrants

Upstream migrants do not feed and can experience dramatic and well-documented decreases in length and weight following entry into fresh water. As a consequence of

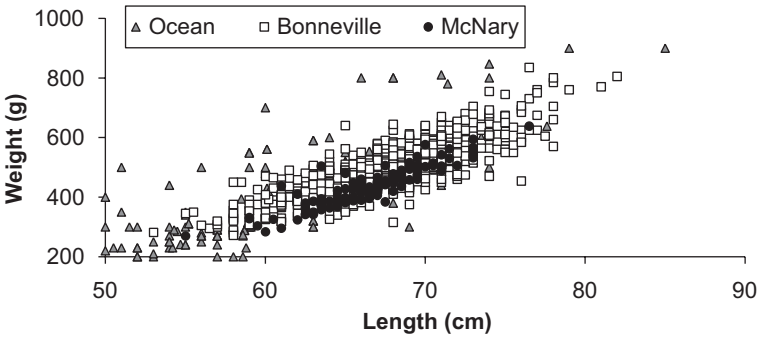

Fig. 5.1 Length-weight relationship of Pacific lamprey collected in the ocean (*gray triangles*, Orlov et al. 2008), at Bonneville Dam (*white squares*, Columbia rkm 235) and at McNary Dam (*black dots*, Columbia rkm 470, Cummings 2007)

this natural starvation, or synchony (Larsen 1980), lampreys mobilize lipid and protein reserves accumulated during the parasitic phase (reviewed in Hardisty 2006). Anadromous European river lamprey from the River Severn in England decreased in body weight by more than 30 % during the course of the upstream migration (between December and March; see Larsen 1980). Anadromous sea lamprey in North America decreased in body length by an estimated 19–24 % between the initial migration and post-spawning (F. W. H. Beamish 1980a). Anadromous sea lamprey on the Iberian Peninsula showed little or no change in body length between river entry and capture near the spawning grounds 65 km upstream, but weight decreased by an average of 20.4 % in males and 22.7 % in females (Araújo et al. 2013). In Caspian lamprey, the length difference between pre-spawning and spawning adults was found to average 22 % (see Holčík 1986b). Shrinkage in Arctic lamprey length during migration may be as high as 25 %, with females experiencing a greater reduction in length than males (see Holčík 1986a), and Pacific lamprey males and females decreased in body length by an estimated 15 % and 23 %, respectively (R. J. Beamish 1980). Furthermore, in Pacific lamprey, weight at a given length decreased noticeably between capture in the open ocean (Orlov et al. 2008; Fig. 5.1) and after traveling at least 235 km through the Columbia River estuary. Shrinkage was even more pronounced further upstream (470 km) (Cummings 2007; Fig. 5.1). Clemens et al. (2009) observed an increase in the rate at which energy stores were mobilized (i.e., a greater proportional decrease in body weight) when Pacific lamprey were held in the laboratory at warm summer temperatures (20–24 °C) relative to cooler temperatures (13.6 °C).

Decreases in lamprey body size with synchony is not confined to large-bodied anadromous forms, but parasitic species that migrate entirely within fresh water appear to experience less shrinkage. Potamodromous sea lamprey in the Great Lakes decreased an estimated 8–10 % in body length (O'Connor 2001) and length differences in mature and immature Vancouver lamprey suggested that shrinkage in this freshwater species is on the order of 6 % (R. J. Beamish 1982).

It has been hypothesized that body size and/or lipid content of adult lampreys at the onset of freshwater migration is correlated with migration distance among river systems (Hardisty and Potter 1971; Larsen 1980; Bird and Potter 1983). Lampreys with larger body size might be more able to complete lengthy migrations, either because their greater fat reserves can better sustain them during this non-trophic period or because only large lamprey are able to overcome obstacles such as dams and waterfalls to reach the most upstream regions (Keefer et al. 2009a, b). Recent studies of Pacific lamprey in the Columbia and Snake river basins lend further support to the idea that migratory distance is controlled by body size and/or condition (Keefer et al. 2009a). Using an extensive tagging program with passive integrated transponders, Keefer et al. (2009a) revealed that lamprey traveling furthest had the largest body size and earliest onset of migration. Moreover, successful passage to the uppermost extent of these drainages was highly dependent on body size, with small lamprey exhibiting the greatest attrition (Keefer et al. 2009b).

Reductions in body size are accompanied by a reduction in flesh quality and deterioration of organs to fuel sexual maturation (e.g., Larsen 1980; Clemens et al. 2009). Lipids and proteins are mobilized primarily from the body wall, whilst relatively small changes in liver weight in European river lamprey indicated that the liver plays little role in fueling upstream migration and sexual maturation (Larsen 1980). The intestine atrophies, liver and blood often become green, skin may turn yellow, and teeth degenerate (see Larsen 1980). Changes in the relative weight of various other organs during upstream migration and spawning, as well as their lipid, protein, and carbohydrate composition, are described for both anadromous sea lamprey (Beamish et al. 1979; Araújo et al. 2013) and European river lamprey (see Larsen 1980).

More recently, biochemical parameters have been measured during upstream migration of Pacific lamprey. Mesa et al. (2010a) examined the concentration of nutritional indicators (plasma protein, triglycerides, and glucose) in Pacific lamprey that were captured in September after traveling over 200 km upstream. After initial testing, these animals were held in the laboratory and sampled periodically over winter and during sexual maturation. There was a gradual decline in levels of plasma protein and triglycerides (e.g., plasma protein declined from about 45 mg/ml in mid-September to about 35 mg/ml in early April), although mobilization of protein and lipids would presumably be higher in actively migrating lamprey. Protein levels declined rapidly at sexual maturation (more dramatically in females), while triglycerides and glucose increased in some individuals.

In some lamprey species, upstream migration and sexual maturation occur at the same time, particularly those that undergo short migrations. Lampreys that undergo lengthy migrations (e.g., Pacific lamprey) will likely be at an advanced stage of synchony at the time of gonad development compared to those migrating over a shorter distance. Mesa et al. (2010a) observed the onset of synchony (e.g., shrinkage in length by about 20% and a gradual decline in plasma protein levels) months before there were measurable increases in circulating sex steroids (estradiol 17ß, progesterone, 15α-hydroxytestosterone) and thyroid hormones (plasma thyroxine and triiodothyronine). In contrast, synchony in European river lamprey is apparently

controlled by gonadal steroids. Gonadectomy performed on early migrant river lamprey prevented intestinal atrophy, hormone replacement therapy normalized the rate of atrophy, and treatment of intact lamprey with sex hormones increased the rate of atrophy (Pickering 1976a, b).

The results of Mesa et al. (2010a) suggest that lampreys entering fresh water with relatively undeveloped gonads may not yet be experiencing an increase in gonadal hormones (see Docker et al. in press). Follow-up research by Mesa and colleagues, however, suggests that sex steroids may play some role in mediating spawning migration behavior. Pacific lamprey trapped soon after freshwater entry were analyzed for sex steroids, implanted with a radio transmitter, and released 3 km downstream from the collection site. Both males and females that resumed upstream migration exhibited higher levels of estradiol than those that did not (Matthew G. Mesa, U.S. Geological Survey, Columbia River Research Laboratory, Cook, WA, personal communication, 2012).

5.3.3 Swimming Performance and Energetics

The natural progression of synchony is undoubtedly related to the rate and duration of exercise and oxygen consumption during the spawning migration. Beamish (1979) measured the caloric content and weight of anadromous sea lamprey collected at known distances up the St. John River in New Brunswick, Canada. He estimated the caloric cost of migration based on the relationship between lamprey weight and the energetic cost of swimming. Comparison of measured versus predicted caloric costs indicated that sea lamprey were expending more energy than predicted based on the linear travel distance. Thus, Beamish (1979) hypothesized that sea lamprey do not travel in a straight line in streams. The advent of miniaturized radio transmitters, passive integrated transponder (PIT) tags, and their respective detection systems has allowed direct assessment of spawning migration routes and rates in a number of different lamprey species and river systems (Table 5.1). For example, recent telemetry results have shown that sea lamprey in Lake Huron indeed travel in a straight line while in the open lake searching for the odor of spawning river plumes, but likely expend energy migrating vertically while doing so (Vrieze et al. 2011; see Sect. 5.4.1.2). The latter strategy presumably increases their chances of encountering river plumes which can be either submersed or superficial depending on water temperatures and densities.

Perhaps the most intensively studied freshwater spawning migrations are those of Pacific lamprey in the Columbia and Snake River basins (Moser et al. 2002, 2005, 2013a, b; Robinson and Bayer 2005; Keefer et al. 2009a, Keefer et al. b, 2013a, b; Mesa et al. 2010b). A number of studies in this system indicate that mean adult Pacific lamprey migration rates are similar over large spatial scales. That is, Pacific lamprey move at similar rates both while ascending large river systems and in tributaries (Table 5.1). However, early migrants do not move as rapidly as those migrating later in the year, when water temperatures are higher (Moser et al. 2005;

Table 5.1 Migration rates of adult lampreys during spawning migrations as documented using half-duplex passive integrated transponders (PIT) and radio transmitters (Radio); rkm = river kilometer

Species	Study area	Mean ground speed (km/day)	Method	Reference
Pacific lamprey *Entosphenus tridentatus*	Columbia River (rkm 235–470)	12.4–13.7	PIT	Keefer et al. (2009a)
		10.1	Radio	Keefer et al. (2009c)
	Columbia River (rkm 235–308)	20.8, 5.6	Radio	Moser and Close (2003); Keefer et al. (2009c), respectively
	Columbia River (rkm 308–347)	13.9, 4.2	Radio	Moser and Close (2003); Keefer et al. (2009c), respectively
	Columbia River basin tributaries:			
	Willamette River	5.3–6.8	Radio	Mesa et al. (2010b)
	John Day River	11.1[a]	Radio	Robinson and Bayer (2005)
	Snake River	18.2–19.1[a]	Radio	McIlraith (2011)
	Clearwater River	12.0–14.2	Radio	McIlraith (2011)
Sea lamprey *Petromyzon marinus* (anadromous)	River Mondego	36.0	Radio	Almeida et al. (2002a)
	River Vouga	15.6–33.4	Radio	Andrade et al. (2007)
	Connecticut River	24.0	Radio	Stier and Kynard (1986)
Sea lamprey *Petromyzon marinus* (landlocked)	Lake Huron	28.8–36.0	Radio	Vrieze et al. (2011)
	Lake Superior tributaries	1.3	Radio	Kelso and Gardner (2000)
European river lamprey *Lampetra fluviatilis*	River Derwent, England	c. 18.0	Radio	Lucas et al. (2009)
Pouched lamprey *Geotria australis*	Pigeon Bay Stream, NZ	<0.1	Radio	Kelso and Glova (1993)
	Okuti Stream, NZ	<0.1	Radio	Jellyman et al. (2002)
	Mataura River, NZ	0.5	Radio	Jellyman et al. (2002)

[a] Includes recovery time

Keefer et al. 2009a). Claridge and Potter (1975) noted that standard metabolic rates for both male and female European river lamprey increased by up to a factor of two during the final months prior to spawning. Thus, the energetic cost of the spawning migration increases as lamprey rates of movement increase, temperatures rise, and the standard metabolic rates increase as a function of gonadal maturation.

Continuous ground speed estimates (distance traveled upstream divided by time) for anadromous lampreys migrating in Northern Hemisphere rivers are remarkably similar among species. Adult Pacific lamprey traveled at rates of up to 20 km/day (Table 5.1), which translates to approximately 0.4 body lengths/s. These data are based on point-to-point estimates of movement against the current and include rest periods. Therefore, actual swim speeds were much greater. Stier and Kynard (1986) found that overall mean movement rates for anadromous sea lamprey were also approximately 0.4 body lengths/s; however, when they computed ground speed only during periods of continuous movement, the mean rate of migration increased to 0.6 body lengths/s. Andrade et al. (2007) also found that sea lamprey migrating in the Vouga River basin, Portugal, exhibited overall mean ground speeds of 0.2–0.4 body lengths/s. The effect of current velocity on swimming speed was clearly illustrated in radiotelemetry results from sea lamprey tracked in the River Mondego (Almeida et al. 2002a). In that study, lamprey ground speed was negatively correlated with river discharge, and the median rate of movement during periods of low river discharge was 0.55 body lengths/s.

For some species, a protracted period of freshwater residence may be necessary for sexual maturation, resulting in slow and extended freshwater movements (Bird and Potter 1983). Radiotelemetry has revealed that pouched lamprey in New Zealand travel at rates of less than 100 m each day (Kelso and Glova 1993; Jellyman et al. 2002; Table 5.1). Moreover, this species is among the largest of lampreys, with mean lengths of 655 mm (range = 503–788 mm) at the start of the freshwater spawning migration (Potter et al. 1983). Thus, slow rates of movement cannot be attributed to small body size, particularly when considering the European river lamprey, which is considerably smaller (e.g., mean length 356–412 mm) but exhibits rapid rates of movement (e.g., median 18 km/day in unobstructed portions of the River Derwent in England; Lucas et al. 2009). Migration rate may simply be related to distance, with species that face the longest migrations traveling more rapidly (see Sect. 5.2). This is clearly seen in comparisons of anadromous sea lamprey, which exhibit much faster riverine rates of movement in their native range than do landlocked forms that do not have far to go (Clemens et al. 2010). Upon entering spawning tributaries, landlocked sea lamprey ground speeds are slow (0.1–1.3 km/day), even though they are capable of very rapid ground speeds (up to 10 km/day) in the open lake environment prior to entering spawning tributaries (Vrieze et al. 2011; Table 5.1).

Laboratory investigations of swimming ability have indicated that for both sea lamprey and Pacific lamprey, swimming speed and duration are closely correlated with temperature (reviewed in Moser and Mesa 2009). That is, as temperature increases, lampreys are able to swim both more rapidly and for a longer time. Lampreys are not considered capable of high burst swimming speeds (e.g., in river lam-

prey, the only species for which this has been measured, maximum burst velocities range from approximately 1.3 to 2.12 m/s; Laine et al. 1998; Kemp et al. 2011; Russon et al. 2011), and velocity barriers have been reported for several species (McAuley 1996; Keefer et al. 2010). Critical swimming speed (i.e., the speed at which a fish becomes fatigued after incremental increases in swimming speed; Beamish 1978) for adult Pacific lamprey is 86.2 cm/s at 15 °C (Mesa et al. 2003). Thus, Mesa et al. (2003) predicted that at swimming speeds above this level (approximately 1.3 body lengths/s), there would be an increased contribution of anaerobic metabolism. Indeed, in this and other laboratory experiments where adult lampreys swam to exhaustion, significant increases in blood lactate levels and decreases in blood pH were recorded (Tufts 1991; Boutilier et al. 1993; Wilkie et al. 1997). In all of these studies, lampreys were able to recover from a single bout of exhaustive exercise in a short time (1–4 h). However, field observations of anadromous sea lamprey monitored with electromyogram transmitters indicated that these animals exhibit multiple bursts of high-intensity exercise (Fig. 5.2), that the recovery time between bursts was short (1.5–2.5 min), and that the lamprey seemed to experience fatigue as a result of successive high-energy bursts (Quintella et al. 2004, 2009).

Lampreys exhibit extreme bouts of exercise when confronted with obstacles to upstream migration, and some species are even capable of climbing vertical surfaces (see Sect. 5.5.3). Large-bodied forms (pouched and Pacific lampreys) use this unique mode of movement to scale waterfalls and other natural barriers (James 2008; Moser and Mesa 2009). The kinematics of climbing in Pacific lamprey have been described in detail for both movement on angled surfaces (Reinhardt et al. 2008) and on perfectly vertical walls (Kemp et al. 2009). In both cases, lamprey used the oral disc to attach to the surface and then compressed the body upward, released, and re-attached to the surface in a highly synchronized series of movements. Using a genetic algorithm simulation, Zhu et al. (2011) identified the combination of kinematic parameters which would correspond to optimally efficient climbing in lamprey (i.e., the gravitational potential energy gained in each climbing step divided by the energy spent to activate the motion). They found that the optimized parameters were similar to laboratory observations of lamprey motion, suggesting that this unique style of lamprey locomotion has been optimized for near maximum efficiency via evolution.

Use of the oral disc to attach to substrate is a key component of lamprey climbing, swimming, and holding during the spawning migration. Electromyogram studies of free-swimming sea lamprey indicated that they appear to move through arduous passage routes with a saltatory movement pattern, alternately bursting forward, attaching, and resting (Quintella et al. 2004, 2009). In both laboratory and field experiments, Keefer et al. (2010) demonstrated that Pacific lamprey movements were hindered when no suitable attachment surfaces were available. The morphology of the oral disc allows attachment to a variety of surface types with apparently minimal energetic cost (Adams and Reinhardt 2008). However, if the surface is too porous or rough, the oral fimbriae are incapable of conforming to the surface and lamprey are not able to attain an adequate seal. In these situations, the cost of attachment is likely increased, as lamprey must expend more energy in the creation

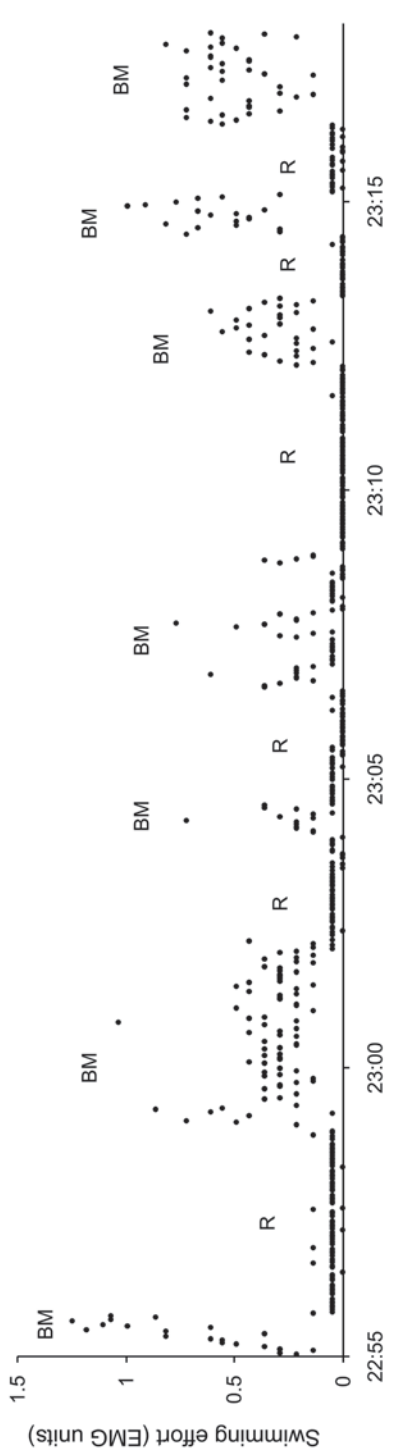

Fig. 5.2 Typical sea lamprey (length 95 cm, weight 1.5 kg) swimming effort pattern obtained from electromyogram (EMG) transmitter output as this individual moved through a 100 m rapid flow reach. Periods of burst movement (BM) and rest (R) are indicated. (This figure was originally published in Quintella et al. (2009) and reproduced with permission of John Wiley & Sons, Inc.)

of buccal pressure (Adams and Reinhardt 2008). Clearly, the oral disc is a wonderfully adapted structure that is key to lamprey success during the parasitic phase (for attachment to the host; see Renaud and Cochran in press), through spawning migration (for attachment to substrate), and throughout nest building and mating (for attachment to nest material and mates; see Chap. 6).

5.4 Sensory Systems

As lampreys pass from the marine or lake environment and into rivers and small streams, their sensory systems are challenged with a new range of stimuli and functional requirements. In lakes and the ocean, lampreys probably rely on a combination of vision, electrosensitivity, and olfaction to find hosts and avoid predators (e.g., Farmer and Beamish 1973; Farmer 1980). In addition, lampreys at sea probably rely on these senses to direct shoreward movements. For Great Lakes sea lamprey, olfaction is critical to detection and localization of river plumes; they do not find the rivers if their olfactory systems have been blocked (Vrieze et al. 2010). Stream odor is now known to be a mixture of pheromonal and other stream odors detected by the olfactory sense (Sorensen et al. 2003; see Sect. 5.6.1). Olfaction likely plays an important role in marine forms as well. The adult lamprey pineal organ is highly sensitive to changes in light (Tamotsu and Morita 1986) and may be implicated during orientation in open water, as described for tunas and other vertebrates (e.g., Deutschlander et al. 1999; Willis et al. 2009). Southern bluefin tuna *Thunnus maccoyii* potentially use spike dives, or rapid vertical movements, at dawn and dusk to aid navigation. The only telemetry study of adult lamprey movement in open water also described extensive vertical movement, which is undoubtedly important for lamprey orientation (Vrieze et al. 2010, 2011).

The role of vision during passage through estuaries and rivers is poorly understood, as lampreys are primarily nocturnal and can encounter extreme reductions in visibility due to increased turbidity (Keefer et al. 2013b). Binder and McDonald (2007) demonstrated that spawning migration and diel behavior patterns do not rely on vision, as experimentally blinded sea lamprey migrated upstream at the same rate as lamprey that were not blinded. Rather, although adult lampreys possess fully functional eyes, it appears that they continue to use dermal photophores (which mediate light avoidance in the blind larvae) to orient and find daytime refuge during the spawning migration (Binder and McDonald 2007, 2008a; see Sect. 5.5.1). As lamprey neared the spawning areas, dermal receptor sensitivity decreased, and lamprey exhibited less profound patterns in diel activity (Binder and McDonald 2007, 2008a).

The eyes of adult lampreys are used to convey images for vision (Binder and McDonald 2008b), and it appears that physiological changes occur as lampreys transition from the ocean to fresh water. Visual pigments in other fishes exhibit change as they adapt to light spectra more commonly encountered in fresh water than in the ocean (Britt et al. 2001). Wald (1957) described in detail the ontogenetic changes

in retinal pigment that occurred as anadromous sea lamprey metamorphosed and migrated to sea and again in upstream migrants. He reported that downstream migrants appear to "anticipate" the transition biochemically by assuming, while still in fresh water, the marine type of visual system. Conversely, the freshwater form of visual system was once again seen in sexually mature adults. However, visual pigments expressed at different phases of the spawning migration have not been examined. More recently, retinal anatomy and neurochemistry have been examined in the Southern Hemisphere lampreys (e.g., Collin et al. 1999, 2003, 2004; Collin and Potter 2000; Nivison-Smith et al. 2013). Unlike the Northern Hemisphere lampreys, which have a dichromatic visual system (i.e., with two structurally and spectrally distinct photoreceptor types), the pouched lamprey potentially has a pentachromatic system (see Nivison-Smith et al. 2013). Anatomical changes have been reported in the retinas of upstream versus downstream migrating and feeding-phase pouched lamprey. Whereas the eyes of feeding-phase lamprey appear to be adapted to avoid avian predators in the well-lit surface waters of the ocean, upstream migrants show changes in the retina (e.g., increase in size of the retina and its photoreceptors, changes in retinal pigments and amino acid neurochemistry; Nivison-Smith et al. 2013) that appear to correspond with the changing light environment as they move into fresh water and exhibit more nocturnal migratory activity (see Collin and Potter in press).

In recent years, a large body of research has been directed towards the description of olfaction in lampreys and its function in orientation during the spawning migration. Therefore, the following sections of this chapter provide a review of work on this important sensory system in lampreys.

5.4.1 Olfaction and its Role in Orientation

5.4.1.1 Olfaction

The olfactory system of lampreys is highly developed and specialized. Relative to brain size, it might be the most developed of all the vertebrates (Kleerekoper 1972). Although well studied only in sea lamprey, olfactory form and function appear similar among those lamprey species that have been examined (Imamura 1928; Kleerekoper 1972). Olfactory development is especially pronounced in adult lampreys, and the olfactory organ of the sea lamprey has been shown to increase many-fold at the time of metamorphosis, growing to about a quarter of the size of the brain (vanDenBossche et al. 1995).

The olfactory organ is located in a deep, two-chambered nasal pit and is irrigated by a single nasopore which has an associated pumping organ that permits sniffing (Kleerekoper and van Erkel 1960). The sensory sheet is comprised of approximately two dozen lamellae that contain three types of olfactory sensory neurons, including a ciliated type resembling that of teleost fishes (Laframboise et al. 2006). The lamprey olfactory bulb is also well-developed and receives projections from

the olfactory nerve. As in other craniates, the lamprey olfactory bulb is divided into functional units, which presumably allow for discrimination of complex odor mixtures (Fronti et al. 2003).

A number of studies have examined the olfactory sensitivity of adult sea lamprey using electro-olfactogram recordings. These studies have demonstrated that sea lamprey adults are acutely and specifically sensitive to only two classes of odorants: bile acids and their derivatives, some of which function as pheromones (Siefkes and Li 2004; Fine and Sorensen 2008), and a single amino acid, L-arginine, which presumably functions as a feeding cue (Li and Sorensen 1992). Notably, the sea lamprey detects petromyzonamine disulfate, at concentrations below 10^{-13} Molar, a possible record for vertebrates (Sorensen et al. 2005). This substance is the principal component of a pheromone released by larval sea lamprey and used by migratory adults to locate rearing and spawning grounds (see Sect. 5.4.1). The specificity with which the olfactory organ detects such bile steroids is also extraordinary in that a single change to odorant structure is detected by sea lamprey (Li and Sorensen 1997; Siefkes and Li 2004; Fine and Sorensen 2008).

Interestingly, the olfactory sensitivity of adult sea lamprey peaks just prior to upstream migration and then declines during spawning (Sorensen et al. 1995). There is evidence for maturation-related change in olfactory sensitivity in the Pacific lamprey as well, although this species appears to be slightly less sensitive than sea lamprey (Robinson et al. 2009). Olfactory sensitivity in the silver lamprey appears similar to that of the sea lamprey, though perhaps slightly less sensitive to bile acids (Fine et al. 2004). Olfactory occlusion studies in sea lamprey have shown that the olfactory sense is absolutely essential to localization of both spawning streams (Vrieze et al. 2010; see Sect. 5.4.1.2) and mates (Johnson et al. 2006; see Chap. 6). Olfaction is certainly one of the most important senses to lampreys. However, much remains to be learned, especially regarding the role of olfaction in other species of lampreys.

5.4.1.2 Orientation

Diadromous fishes have evolved at least two evolutionary pathways with respect to stream-finding during migration. One group (e.g., salmonids, sturgeons) returns or "homes" to natal streams for spawning (i.e., exhibits philopatry). Fishes that use the second strategy simply locate the best possible nursery or spawning habitat independent of where they came from; Waldman et al. (2008) referred to this as the "suitable river strategy." This strategy imparts considerable flexibility and seems well suited to species that are small or otherwise unable to control their movement patterns with high precision. Prominent examples of the second strategy include catadromous freshwater eels (*Anguilla* spp.), whose larvae locate rearing streams using odors that seem to be innately recognized (Sorensen 1986). Amphidromous galaxiids are another example; these juveniles locate streams from the ocean using pheromones produced by adults (Baker and Hicks 2003). Evidence strongly suggests that the sea lamprey (and probably other lamprey species) also use this strategy (see below). Both strategies are known to be mediated by odors. Olfactory cues

learned as juveniles contribute to homing in salmon (Scholze et al. 1976), while innate recognition of optimal habitat serves as the basis of this second strategy. These mechanisms are fundamentally different. A strategy based on innate recognition of optimal spawning/nursery habitat makes particular evolutionary sense for lampreys, since they are not strong swimmers and the adults are unable to control their whereabouts owing to the unpredictable movements of the host (Sorensen et al. 2005; Waldman et al. 2008; Spice et al. 2012).

Evidence for the lack of homing in lampreys comes from field studies, laboratory experiments, and genetic work. Perhaps the strongest evidence comes from trapping records in the Great Lakes, which showed that adult sea lamprey are highly selective in the choice of spawning streams (Morman et al. 1980), choosing only those streams that had high densities of larval lamprey (Moore and Schleen 1980). Thus, tagging of out-migrant juvenile sea lamprey and recapture of them as adults on spawning grounds has shown no evidence of homing to natal streams (Bergstedt and Seelye 1995). Especially compelling is evidence that streams traditionally favored by adult lamprey failed to attract them in the year following eradication of larval lamprey with 3-trifluoromethyl-4-nitrophenol (Moore and Schleen 1980). Adults instead entered proximate, untreated streams that still contained larvae and their pheromonal odors (Sorensen and Vrieze 2003). A large number of laboratory studies have since shown that adult sea lamprey are specifically attracted to the odor of larval lamprey and the pheromonal compounds they release (Vrieze et al. 2010, 2011). Finally, genetic studies of sea lamprey in the Great Lakes and along the Atlantic seaboard have failed to provide evidence of significant stock structure (Bryan et al. 2005; Waldman et al. 2008).

That adult landlocked sea lamprey recognize and choose streams based on odors released by larval lamprey (i.e., pheromones) has been further demonstrated in a series of related laboratory and field behavioral studies described in detail by Sorensen and Hoye (2007) and summarized here. Although this work has shown that pheromones are the most important set of olfactory cues used by adult sea lamprey in the Great Lakes, other stream odors and likely temperature play synergizing roles. The first step in this work was testing whether adult sea lamprey would consistently choose waters collected from streams with larval lamprey over water from streams without larvae (Vrieze and Sorensen 2001; Fine et al. 2004). This work has shown that when adult lamprey are offered the choice of lake water and water from streams with larval lamprey, they consistently prefer the latter, even when it is diluted over a thousand-fold (a dilution factor that might be expected at a stream mouth; Vrieze and Sorensen 2001). Experiments using larval odor showed that a single 1-g larva activated at least 300 L of stream water each hour (i.e., gave it attractive properties), more than enough to explain the potency of natural stream waters. Notably, stream water lacking larvae (which was mildly attractive) became highly attractive when larval water was added; indeed stream and larval odor appear to synergize each other's activity (Derby and Sorensen 2008). Further, larval odor is not fully attractive unless found in natural stream waters, suggesting a natural synergism between the pheromone and its normal odor context (Vrieze and Sorensen 2001). These laboratory findings have been further reinforced by field studies that have

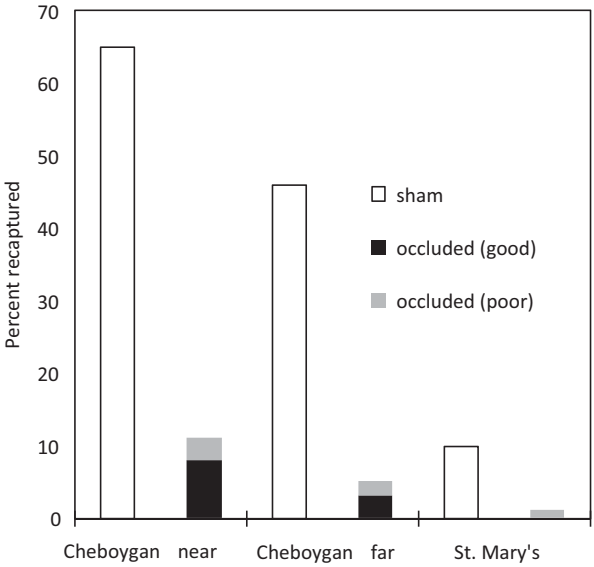

Fig. 5.3 Riverine recapture rates of landlocked sea lamprey released into Lake Huron at two sites outside the Cheboygan River and at a site 65 km downstream from traps in the St. Mary's River. The nasopore in these animals was either sham treated with gelatin or blocked with inert dental impression material. Upon recapture, occluded animals were divided into groups of good or poor occlusion based on visual inspection of the nasal plug. (Data from Vrieze et al. 2010)

found that anosmic sea lamprey do not find rivers (Vrieze et al. 2010; Fig. 5.3) and, even after they enter rivers, their upstream movement rates are suppressed (Vrieze et al. 2010; Fig. 5.4). Together, these behavioral studies confirm the key role larval odors play in orientation during sea lamprey migration.

Bioassay guided fractionation combined with chemical synthesis has shown that the larval pheromone used by adult sea lamprey is comprised of at least three novel

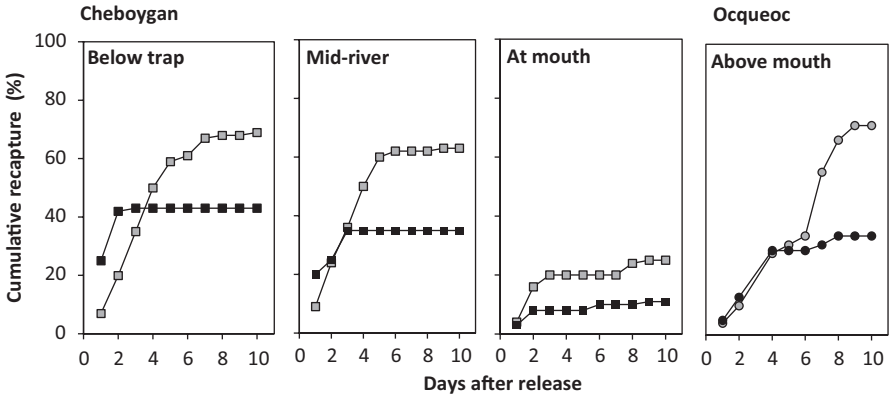

Fig. 5.4 Recapture rates of sea lamprey captured and re-released in two Great Lakes streams after occluding their nasopores (*black dots*) or sham treating them with gelatin (*gray*). Note that while the olfactory sense was especially important in the river mouth (after initial entry), it became relatively less important with time and distance upstream. (This figure was originally published in Vrieze et al. (2010) and reproduced with permission of John Wiley & Sons, Inc)

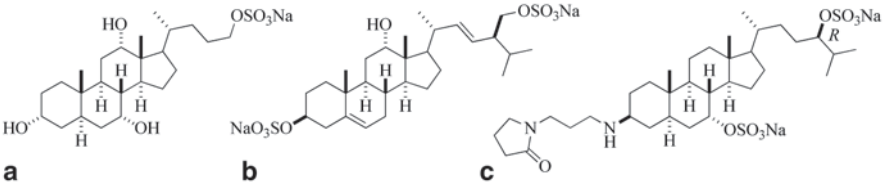

Fig. 5.5 The three sulfated sterols that serve as the primary components of the sea lamprey migratory (larval) pheromone. These components are complemented by other still unidentified odors. This figure was originally published in Sorensen and Hoye (2007) and reproduced with permission of John Wiley & Sons, Inc

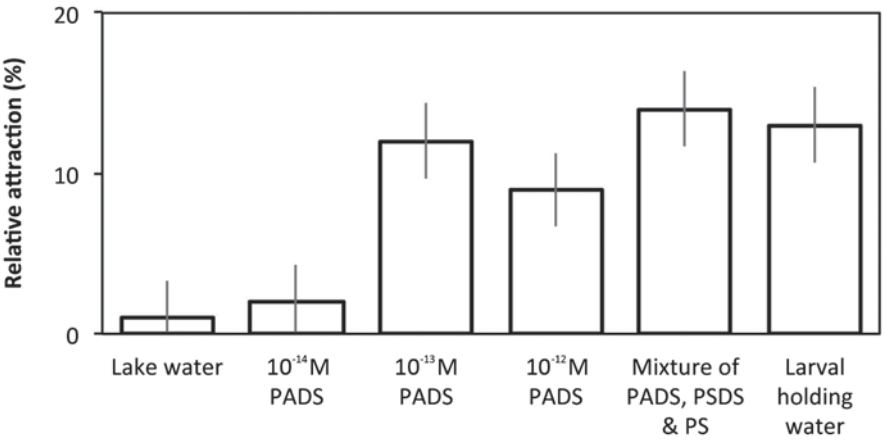

Fig. 5.6 Behavioral responses of landlocked sea lamprey to the migratory pheromone and its components in a two-choice maze. (Data from Sorensen et al. (2005); Fine and Sorensen (2008))

sulfated steroids: petromyzonamine disulfate (PADS), petromyzosterol disulfate (PSDS), and petromyzonol sulfate (PS) (Sorensen and Hoye 2007; Fig. 5.5). These three steroids are released at rates of 5–25 ng/larva/h. Electro-olfactogram recordings by Fine and Sorensen (2008) demonstrate that the adult sea lamprey olfactory system detects these steroids with both great sensitivity (at concentrations between 10^{-12} and 10^{-13} Molar) and specificity, and that these compounds drive behavioral activity (Sorensen et al. 2005; Fine and Sorensen 2008; Fig. 5.6). Thus, the rates at which steroids are released by larvae are more than adequate to account for the behavioral potency of both the larval holding water and whole stream water (Vrieze and Sorensen 2001). They also explain the potency of river plumes, in which larval pheromones have been measured (Polkinghorne et al. 2001; Fine and Sorensen 2010). To put this into perspective, approximately 500 g each of these compounds will, if added to stream water by larvae or managers, activate a cubic kilometer of water for one month. Recent field experiments suggest that other, as yet unidentified components are also found in this migratory pheromone (Meckley et al. 2012). Use of larval pheromones for sea lamprey control in the Great Lakes is now being

tested; however, synthesis is costly, and development of synthetic analogs has been advised (Sorensen and Hoye 2007; Burns et al. 2011; see Sect. 5.6.1). Further, behavioral studies suggest most if not all pheromonal components need to be present to effect strong behavioral activity (Fine and Sorensen 2008; Meckley et al. 2012), although—unlike insect pheromones—the specific ratio may not be of critical importance (Derby and Sorensen 2008). Full activity is, of course, essential for control in natural settings where competing whole odors are present.

There is evidence that other lamprey species use migratory pheromones that resemble those of the sea lamprey. Because lampreys have similar nursery habitat requirements regardless of species (see Chap. 3), there is apparently no evolutionary pressure for larvae to produce species-specific pheromones (Fine et al. 2004). Attraction to larval pheromones has been demonstrated in adult silver lamprey which, like sea lamprey, is attracted to the odor of its own larvae, as well as that of American (*Lethenteron appendix*) and northern (*Ichthyomyzon fossor*) brook lampreys (Fine et al. 2004). Tests of caged sea lamprey in stream environments corroborate that this species is attracted to larval odor (Bjerselius et al. 2000; Wagner et al. 2006, 2009). Interestingly, just as physiological sensitivity to odorants peaks in migratory sea lamprey, so does behavioral responsiveness to larval odor (Bjerselius et al. 2000). Gaudron and Lucas (2006) found that migratory-phase European river lamprey adults were highly attracted to the odor of conspecific larvae. Pacific lamprey has exhibited olfactory activity and behavioral attraction to water activated by larval conspecifics (Yun et al. 2011). However, this species reportedly exhibits relatively low olfactory sensitivity to larval pheromone isolates (Robinson et al. 2009). This suggests that other more important components exist or that the role of this cue differs in species that have a much longer migratory phase (see Sect. 5.2.1). The continuing collapse of lamprey fisheries across the globe is consistent with their reliance upon larval pheromones: once larvae are extirpated from these systems, a natural and self-sustaining cycle is broken (see Chap. 8).

The olfactory-mediated orientation mechanisms used by landlocked sea lamprey to find streams have also now been elucidated. In telemetry studies, tagged sea lamprey released outside of river plumes pursued remarkably straight bearings while swimming actively and performing extensive and frequent forays from the lake bottom to the surface (Vrieze et al. 2011). This likely represents a form of searching behavior in the absence of odors. Lamprey with occluded nasopores exhibited the same straight-line swimming behaviors, except that they swam directly offshore. In contrast, adult untreated sea lamprey released into or near a river plume exhibited dramatic turning behaviors, and one-third of these lamprey eventually encountered and entered the river. This type of behavior can be classified as an odor-driven kinesis that allows migrating lampreys to "sample" entire shorelines effectively and find suitable spawning streams no matter where they left their last host. Homing Atlantic salmon *Salmo salar* exhibit some of these orienting behaviors although, instead of circling after encountering stream odor, they explore and then track (via "zig-zagging") home stream waters at depth (Døving et al. 1985). This strategy seems reasonable for a strong swimmer that is obliged to track a specific home river odor plume. In contrast, sea lamprey may benefit from a more flexible system that ultimately allows them to locate a nearby stream that is suitable for spawning.

Very little work has been conducted on stream-finding in other lamprey species. A single field study, which displaced Pacific lamprey in the Columbia River basin, failed to show evidence of homing (Hatch and Whiteaker 2009). However, the fact that Pacific lamprey exhibits size differences among river systems and in migration timing suggests that there is some local adaptation in this species (R. J. Beamish 1980; Kostow 2002; Keefer et al. 2009a; Hess et al. 2013). Moreover, Beamish and Withler (1986) demonstrated significant differences in Pacific lamprey allozyme allele frequencies between two rivers in British Columbia, and a study using amplified fragment length polymorphism (AFLP) analysis also found significant differences among Pacific lamprey adults from eight sites in the Pacific Northwest, Alaska, and Japan (Lin et al. 2008). Other genetic studies using mitochondrial DNA (Goodman et al. 2008), microsatellite markers (Spice et al. 2012), and single nucleotide polymorphism (SNP) loci (Hess et al. 2013), however, suggest high gene flow among locations. This supports the hypothesis that Pacific lamprey does not home, since natal homing tends to minimize gene flow among locations (see Chap. 8). While the degree of homing in this and other anadromous lamprey species has not been completely resolved, future research on lamprey dispersal and orientation at sea are critically important to understanding stream selection in these lampreys.

5.5 Behavior of Upstream Migrants

Natural and anthropogenic physical and chemical features can impede migrating animals and fragment habitat, which in fluvial ecosystems results in lost longitudinal and lateral connectivity. In an effort to understand how environmental barriers impact fish migrations, quantitative measures of swimming (and leaping or climbing) performance have been developed. For lampreys, this work has largely stemmed from the need to mitigate the negative effects of barriers on native species or to control the range expansion and/or abundance of invasive species (e.g., sea lamprey in the Great Lakes). As is the case for other diadromous species, lampreys are likely influenced by multiple environmental variables over a range of scales in both the initiation of movement and the control of migration efficiency. The following section explores what is currently known of lamprey migrational behavior, their responses to physical structures, and their ability to negotiate barriers.

5.5.1 Nocturnal Migratory Behavior

Lampreys are negatively phototaxic, moving upstream in fresh water primarily during dusk and darkness (Almeida et al. 2000, 2002a; Moser and Mesa 2009; Nazari and Abdoli 2010) and seeking refuge before dawn (Kelso and Glova 1993; Andrade et al. 2007; Fig. 5.7). Similar nocturnal behavior was observed by Almeida et al. (2000) during the estuarine phase of sea lamprey migration in the Mondego River basin in Portugal, and telemetry studies in Lake Huron likewise indicated that the

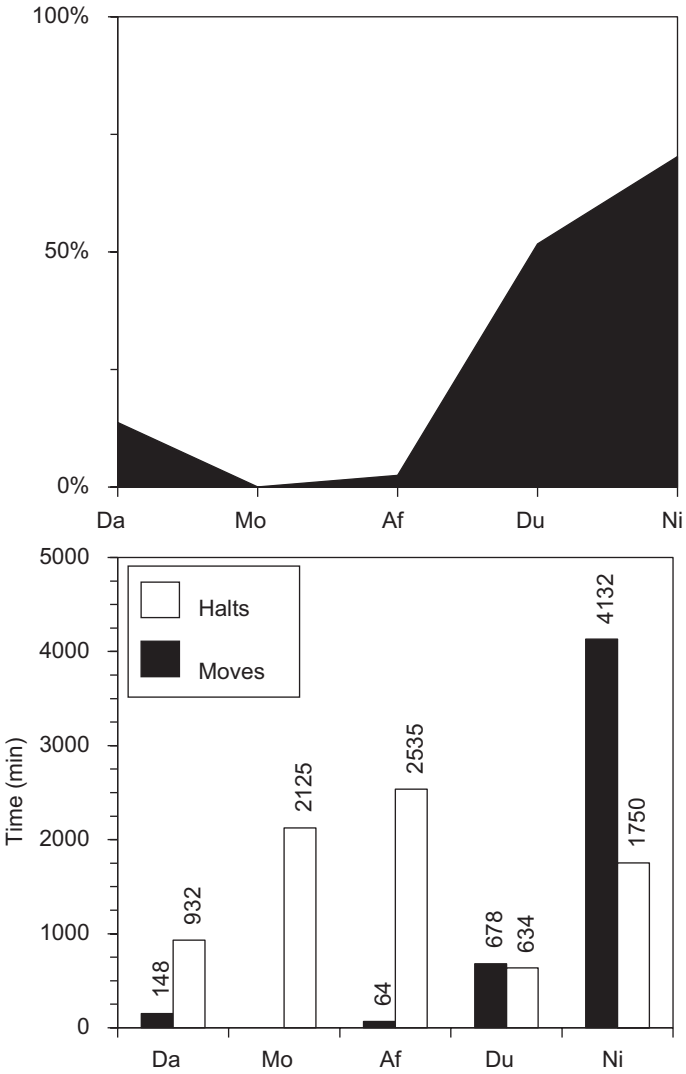

Fig. 5.7 Proportion of movement (*dark bars*) and resting times (*white bars*) throughout the day observed during the sea lamprey migration in the River Mondego, Portugal. Dawn (*Da*) = 1 h before to 1 h after sunrise, Morning (*Mo*) = 1 h after sunrise to 11:59 GMT, Afternoon (*Af*) = 12:00 GMT to 1 h before sunset, Dusk (*Du*) = 1 h before to 1 h after sunset, and Night (*Ni*) = 1 h after sunset to 1 h before sunrise. (This figure was originally published in Almeida et al. (2002a) and reproduced with kind permission from Springer Science+Business Media)

landlocked sea lamprey is strongly nocturnal prior to entering tributaries (Vrieze et al. 2011). The adaptive value of nocturnal behavior might be related to the greater protection from predation afforded by darkness. Pacific lamprey exhibited almost purely nocturnal behavior in environments with the greatest complexity and evolutionary novelty (e.g., fishway entrances with high turbulent flows and high

abundance of predators), but moved more frequently during daylight in less challenging reservoir areas (Keefer et al. 2013b). According to Andrade et al. (2007), sea lamprey are more likely to search for shelter in rivers when the cumulative distance of their migration exceeds 6.4 km. A few weeks into their upstream migration, landlocked sea lamprey become diurnal (Manion and Hanson 1980). Binder and McDonald (2008a) demonstrated that these changes in activity pattern were associated with increasing temperature; at 20 °C, the nocturnal peak in activity was reduced, and lamprey became active during the day (presumably in preparation for spawning; see Chap. 6). European brook lamprey likewise exhibit nocturnal behavior early in the migration period, but become more active during the day as the spawning time approaches (Malmqvist 1980).

5.5.2 Environmental Triggers Initiating Migratory Behavior

In many lamprey species, run timing varies greatly both within and between years (see Sect. 5.2.1), suggesting that photoperiod is not the main trigger initiating upstream migration. Instead, temperature and flow appear to be the key triggers influencing timing of upstream migration.

While temperature has profound effects on fish physiology, its influence on behavior is not well understood. Temperature dictates fish distribution (e.g., Buisson et al. 2008) and controls initiation and efficiency of movement (e.g., Jonsson 1991; Rodriguez-Ruiz and Granado-Lorencio 1992). In conjunction with flow, the spawning migration of Pacific lamprey in the Columbia River is strongly influenced by temperature, being earliest in warm years when flows are low and latest during cold years when flows are high (Keefer et al. 2009b). Similarly, the spawning migration of sea lamprey after entering tributaries of the Great Lakes has been best predicted by mean stream temperature (Binder et al. 2010), which peaks at approximately 15 °C. This temperature threshold apparently triggers odor-driven rheotaxis and the start of upstream movement (Vrieze 2008). Change in temperature also drives migratory behavior; in Lake Ontario tributary streams, sea lamprey migration was stimulated when mean stream temperature increased between consecutive days and inhibited when it decreased (Binder et al. 2010). Change in temperature also appears to signal cessation of movement in preparation for overwintering by Pacific lamprey in the Columbia River basin (Robinson and Bayer 2005; Mesa et al. 2010b; Clemens et al. 2012).

European river lamprey in northern England (River Ouse catchment) migrate when river temperatures range between 2 °C and 15 °C (Masters et al. 2006; Lucas et al. 2009), with no relationship evident between catch per unit effort and temperature over this range (Masters et al. 2006). River lamprey begin moving into Baltic Sea rivers (e.g., Perhonjoki and Kalajoki rivers in Finland) in early autumn at temperatures in excess of 10 °C, although this declines to 5–10 °C during the main migratory period (Jukka Tuohino, North Ostrobothnian Centre for Economic Development, Transport and the Environment, Finland, personal communication, 2011). Although Kemp et al. (2011) found that upstream movement of river lamprey was

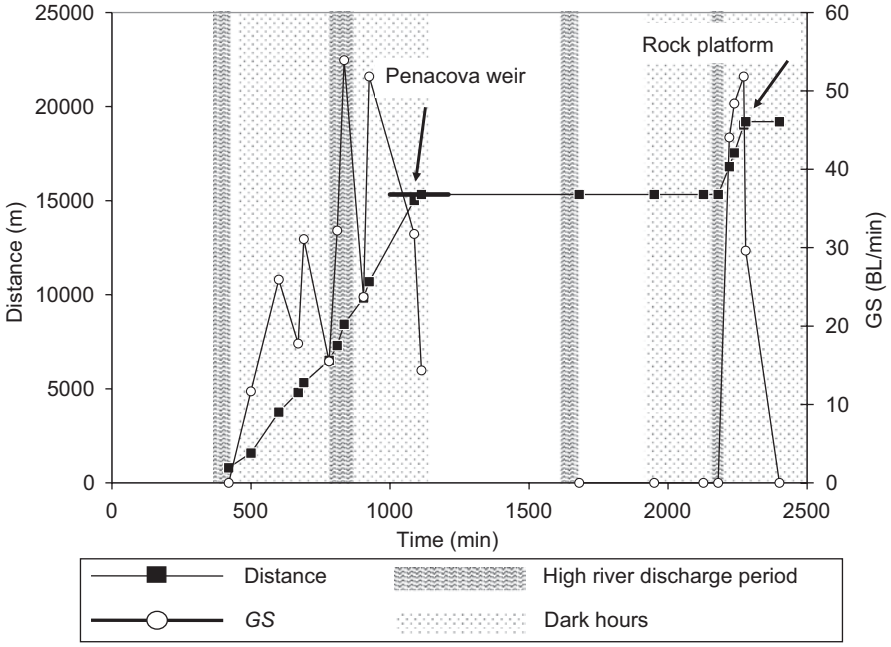

Fig. 5.8 Effect of river discharge on ground speed (*GS,* body length/min) and distance (m) travelled by sea lamprey observed in the River Mondego, Portugal. Note the unchanged behavior during the day (*unshaded area*). (This figure was originally published in Almeida et al. (2002a) and reproduced with kind permission from Springer Science+Business Media)

positively related to temperature under experimental conditions (11.5–19.2 °C), there was little other obvious effect on behavior, with the possible exception that a weir was more frequently approached from along the channel walls under warmer conditions. This may have reflected some strategy to utilize low-velocity routes and minimize energy expenditure at higher temperatures. Temperature-dependent migratory activity is presumably an adaptation to ensure arrival at the spawning grounds near the time that temperature is optimal for reproduction (Binder et al. 2010).

Like temperature, river discharge is known to influence movements of migratory fishes (e.g., Jensen et al. 1986; Jonsson and Jonsson 2002), and in many anadromous lampreys, migratory activity seems to be stimulated by changes in flow. In the U.K., for example, European river lamprey spawning runs tend to occur during the high winter flows (Masters et al. 2006; Lucas et al. 2009), although the degree to which high flows initiate upstream movement is not known. Studies on the anadromous sea lamprey (Almeida et al. 2002a; Andrade et al. 2007) have similarly demonstrated that migratory activity increases with increased stream discharge (Fig. 5.8). Interestingly, in landlocked sea lamprey, water level was a reliable predictor of migratory activity in only the two smallest of six Lake Ontario tributaries studied (Binder et al. 2010); landlocked sea lamprey thus seem to rely more heavily on a thermal trigger than on flow variation. Another interesting contrast is seen in

Pacific lamprey in the Columbia River. Here, the peak of upstream migration occurs in summer, after the period of maximum discharge (Keefer et al. 2009a). It may be that lampreys in the Columbia River and other large river systems take advantage of periods when water velocity is relatively low to avoid velocity barriers and reduce costs of upstream movement.

Very high flows also appear to prevent migration in other lamprey species. Masters et al. (2006) observed decreases in catch per unit effort in the European river lamprey commercial fishery in the tidal River Ouse when flows were particularly high (in excess of approximately 40 m^3/s). Although this decrease in catch could also be the result of poor trap efficiency under high-flow conditions or movement of lampreys outside the main channel (as suggested by Kelly and King 2001), side-trap catches in the River Ouse were generally lower than midstream catches, even when flows were very high (Masters et al. 2006). Jellyman et al. (2002) also reported that upstream movement in pouched lamprey in New Zealand was stimulated by increased flow only up to a point; after that, very high flows (approximately 10–25 m^3/s) decreased migratory activity.

Depending on the conditions, therefore, both low and high river discharge can result in barriers to upstream movement. For European river lamprey, high flows can facilitate movement past physical impediments that would be partially or fully exposed under lower flow conditions (Lucas et al. 2009). If the spawning migration coincides with periods of low flow, physical barriers, including relatively low-head structures, can impede or delay upstream movement [e.g., Nunn et al. (2008) for river lamprey; Jackson and Moser (2012) for Pacific lamprey]. Significant reductions in river discharge due to dams and weirs can thus diminish the attractive potential of a river and hence the numbers of spawners entering it (see Chap. 8). On the other hand, performance barriers can form under high flows when water is funneled through a constricted space (e.g., narrowed channel or culvert) at velocities greater than lamprey swimming capability (see Sect. 5.3.3).

5.5.3 Behavioral Responses to Barriers

Many non-anguilliform species assume a “burst-and-glide” gait, rather than continuous swimming, to enhance their locomotory performance under high velocities (e.g., Tudorache et al. 2007). Similarly, lampreys use their oral disc to attach to substrate and rest in between bouts of energetic swimming, a strategy referred to as “burst-and-attach” [Quintella et al. (2009) for sea lamprey; Keefer et al. (2010) for Pacific lamprey; Kemp et al. (2011) for European river lamprey]. European river lamprey apparently use this behavior to reduce energetic costs; this species typically attaches to structures during conditions of high flow and when encountering high velocities when approaching an undershot weir (Kemp et al. 2011). Experimental estimates of swimming capability derived using traditional swim-chamber tests can therefore be misleading, because lampreys prevented from attaching to substrate are unlikely to exhibit performance-enhancing behaviors (Mesa et al. 2003).

As flows change, other hydraulic parameters such as water velocity and turbulence do likewise. Lampreys may have particular difficulty in negotiating chaotic flow patterns as their elongated body morphology and lack of paired fins likely reduce stability (Liao 2007). Time-to-event, or "hazard analysis," was applied to radiotelemetry records for adult Pacific lamprey as they approached and entered fishways at hydropower dams during highly turbulent periods of spill (i.e., when water was released over the dam spillway) and less turbulent conditions when no spill occurred (Moser et al. 2005). This analysis indicated that there was no significant effect of the spill treatment, either during the period when lamprey occupied the tailrace at the base of the dam or after they had entered the fishways. However, behavioral observations at a much higher spatial resolution indicated that adult Pacific lamprey had more difficulty negotiating high-velocity areas with bottom structures that created turbulence than areas of similar velocity but without the structures (Keefer et al. 2010, 2013a). Haro and Kynard (1997) also speculated that turbulent and confusing flows were impediments to sea lamprey passage through a Connecticut River fishway.

To overcome natural barriers, some lamprey species—especially the large anadromous species—have developed remarkable abilities (see Sect. 5.3.3). Pacific lamprey, for example, are able to ascend the 12-m-high Willamette Falls in Oregon (Clemens et al. 2010), and pouched lamprey have been reported successfully negotiating a 14-m-high dam on the Arnold River in New Zealand (McDowall 1990). Both species exhibit strong vertical climbing on wetted surfaces out of water (Tweed 1987; James 2008; Reinhardt et al. 2008; Kemp et al. 2009). Sea lamprey can reportedly "creep" over lower obstacles (1.5–1.8 m; Scott and Crossman 1973), but laboratory experiments have shown that landlocked sea lamprey cannot climb vertically (Reinhardt et al. 2009). In laboratory studies and in the field, Pacific lamprey demonstrated high motivation and success after repeated ascents of a 1.5-m-high vertical structure (Kemp et al. 2009; Moser et al. 2013a). In addition, several different species of lampreys use the oral disc to maintain station in high flows (Pacific lamprey: Moser et al. 2002; Keefer et al. 2010; sea lamprey: Quintella et al. 2009; European river lamprey: Kemp et al. 2011). This strategy helps the animals to move through areas of difficult passage (Quintella et al. 2004), but can only be utilized if adequate attachment surface on the substrate is available (Keefer et al. 2009b). Optimal attachment sites feature regular surfaces made of a slightly rugous material that allows the fimbriae and oral disc to form a tight seal (Adams and Reinhardt 2008).

Fine-scale observations of adult Pacific lamprey have been made at physical barriers to elucidate individual behaviors as the lamprey encounter obstacles. Keefer et al. (2010) used both an experimental flume and field observations to document adult Pacific lamprey behaviors in fishways at a large hydropower dam. For pool and weir type fishways, they found that lamprey were obstructed by vertical steps and floor grating near weir orifices, which prevented attachment with the oral disc. In these tests, the adult lamprey also required more time to pass through orifices when there was an adjacent step up or floor grating immediately downstream from

the orifice (Keefer et al. 2010). For vertical slot fishways, Keefer et al. (2010) demonstrated that more attachment surfaces were provided, and that lamprey exhibited higher and more rapid passage, when bulkheads were rounded rather than square. In similar sets of experiments, Keefer et al. (2011) found that adult lamprey were able to find and use relatively low-velocity routes when given a choice between high (2.9–3 m/s) and lower velocity (0.1–1.2 m/s) treatments. In these experiments, lamprey took advantage of low-velocity boundary layers along the floor and flume walls.

Generally, anguilliform locomotion is considered to be highly efficient when compared with the carangiform mode (van Ginneken and Maes 2005), but it is not as powerful. Hence maximum burst swimming velocities tend to be lower (Dauble et al. 2006). Compared to salmonids, for which screening and fish passages have traditionally been designed, the lower burst swimming capacities of anguilliform fishes, such as lampreys, may limit the effectiveness of these facilities for multiple species. This is important because the impacts of reduced habitat connectivity are considered to be major contributory factors in declines of European river lamprey populations over the past approximately 20–50 years (Masters et al. 2006; see Chap. 8).

Kemp et al. (2011) evaluated the ability of groups of European river lamprey to pass small overshot and undershot sluices or weirs under experimental conditions. Lamprey tended to approach the weirs along the substrate, a finding that agreed with field observations (Lucas et al. 2009). Lamprey approached the weirs less frequently, and attached to structures using their oral disc more frequently, when flows were high. Overall, relatively low-elevation overshot weirs posed an impediment and delayed upstream movement. Similar findings have been observed for other weir types, such as those commonly used for gauging river flows (e.g., Crump and Flat-v weirs, Russon et al. 2011) and at irrigation diversions (Jackson and Moser 2012).

5.5.4 Effect of Chemosterilization on Migration Behavior

To establish whether chemical sterilization could be used as a control technique (i.e., whether sterile males could compete successfully with fertile males), spawning migration behavior was examined in male landlocked sea lamprey injected with bisazir (P,P-bis(1-aziridinyl)-n-methylphosphinothoic amide; see Marsden and Siefkes in press). In this method of sterilization, hormone cycles are not disrupted and adult males can successfully fertilize eggs, but the embryos do not survive to the larval stage (Hanson and Manion 1980). Radiotelemetry revealed that chemosterilized and unsterilized male sea lamprey traveled similar distances upstream, exhibited similar daily movement rates, selected identical habitats, and showed no detectable differences in nest-building or spawning behavior (Kelso and Gardner 2000; Kelso et al. 2001).

5.6 Management Implications

Recent advances in understanding the basic biology of the lamprey spawning migration have been funded largely to address specific management goals. For the invasive sea lamprey in the Great Lakes, this work stems from a need to understand and control the process of lamprey colonization and proliferation in spawning streams (see Marsden and Siefkes in press). For native sea and river lampreys in Europe and for Pacific lamprey along the west coast of the United States, research has been directed towards identification of and mitigation for impediments to adult passage (see Chap. 8).

5.6.1 Pheromones

Identification of the primary olfactory cues used by sea lamprey to find and colonize tributaries in the Great Lakes (see Sect. 5.4.1) has obvious management implications. By knowing the sensitivity of sea lamprey to these compounds, it may be possible to predict streams that lamprey are likely to find and to manipulate/disrupt normal patterns of colonization (Sorensen and Vrieze 2003). Additionally, the sea lamprey migratory pheromone and several inexpensive analogs have now been synthesized (Burns et al. 2011), making it possible to examine use of these substances and related ones to increase trapping efficiency (Sorensen and Hoye 2007) or to divert adult sea lamprey to locations where spawning might fail. Clearly, however, given the complexity of adult orientation behavior and the role of other odorants (Fine and Sorensen 2008; Meckley et al. 2012), as well as the cost of producing these components and gaining regulatory approval for their use (Sorensen and Hoye 2007), this pest control strategy would need to be carefully targeted. Another much more practical application would be to use the amount of naturally-produced pheromone in the water to gauge larval abundance (Fine and Sorensen 2005; Fine et al. 2006; Stewart et al. 2012). This method could aid in directing lampricide treatments as part of an integrated pest management plan. A similar idea has recently been proposed for measurement of adult male abundance using sex pheromone (Xi et al. 2011; see Chap. 6). Finally, another option would be to selectively nurture (manage) populations of native Great Lakes lampreys (e.g., northern and American brook lampreys) already located above barriers and traps so that that their pheromone plumes (which appear identical to those of the sea lamprey) would aid in attracting adult sea lamprey to block their spawning or perhaps remove them (Sorensen and Vrieze 2003). This idea is promising because the upper reaches of many Great Lakes tributaries already support substantial but presently unmanaged native brook lampreys (see Chap. 3), but have barriers with traps in the lower stretches. These areas could be simultaneously managed to promote native lampreys (whose numbers are threatened in some locations) while serving to improve trapping of sea lamprey. Thus, the identification of potent pheromones has opened the door to more efficient

upstream trapping and removal of invasive sea lamprey while also supporting native lamprey conservation.

Detailed information on the cues native lampreys use to find spawning streams has equally important management applications for lamprey conservation (Fine et al. 2004; Yun et al. 2011). If native lampreys primarily use pheromones to find spawning areas, it may be possible to predict the minimum levels of recruitment needed to sustain a detectable pheromone plume (Nunn et al. 2008). Moreover, positive feedback is likely when lamprey numbers start to rebound (Sorensen and Vrieze 2003). Information on pheromones could lead to direct management actions that employ pheromones either in directing lamprey to benign passage routes or as a method to improve trapping and translocation activities (Ward et al. 2012).

The role of chemical signaling also has important implications for lamprey restoration via artificial propagation or translocation. Use of lamprey hatcheries for research or supplementation of wild stocks is underway in Europe (e.g., Finland, Estonia, and Latvia; Sjöberg 2011) and is a management tool being considered in North America (Ward et al. 2012). Hatchery operations have a high potential to alter the natural distribution and/or concentration of pheromone signals. This could have unforeseen consequences for wild stocks attracted to hatchery effluents. In the northwestern United States, translocation of Pacific lamprey from lower parts of the Columbia River to upper portions of the drainage has been gaining traction in recent years (Close et al. 2009; see Chap. 8). Such efforts to "re-seed" lamprey in areas where populations are diminished or extirpated need to account for the potential effects of pheromone production by the offspring of successful spawners (Ward et al. 2012).

5.6.2 Passage Performance

Over the past century, native populations of anadromous lampreys have declined in the U.K. (Masters et al. 2006), the Baltic countries (Tuunainen et al. 1980; Thiel et al. 2009), Portugal (Cabral et al. 2005), France, Switzerland, the Czech and Slovak republics (Kelly and King 2001), and in the U.S.A. (Beamish and Northcote 1989; Close et al. 2002). In extreme cases, populations have been extirpated (e.g., the European river lamprey from Switzerland and the Rhine-Meuse hydro-system; Renaud 1997). Of current concern in several European countries is the status of river lamprey (Masters et al. 2006), which as a species listed under the European Commission Habitats Directive 92/43/EEC (EC 1992) must be afforded Special Areas of Conservation by member states (Bell and McGillivray 2006; see Chap. 8).

The decline of lamprey populations has been attributed to multiple factors, including commercial fishing (Masters et al. 2006), pollution (Renaud 1997), adverse oceanic conditions (Close et al. 2004), and loss of or reduced access to key habitat due to river engineering (Tuunainen et al. 1980; Renaud 1997; Close et al. 2002; Oliveira et al. 2004; Nunn et al. 2008; Lucas et al. 2009; see Chap. 8). Lucas et al. (2009) noted that 98 % of lamprey spawning habitat in the River Derwent, England,

occurred more than 51 km upstream. However, only 1.8 % of radio-tagged European river lamprey were recorded there due to the presence of multiple small-scale barriers to migration. However, even though it is acknowledged that adult anadromous lampreys must have access to upstream spawning areas, these species are rarely considered during the design or modification of traditional fish passage structures (Kemp et al. 2011; Moser et al. 2011). Consequently, most fish passage facilities are not efficient for lampreys (Bochechas 1995; Haro and Kynard 1997; Laine et al. 1998; Lucas and Baras 2001; Moser et al. 2002). This is due to both the relatively poor swimming performance of lampreys (Dauble and Moursund 2000; Mesa et al. 2003) and their unique use of the oral disc to attach and rest in high velocity situations (Haro and Kynard 1997; Moser et al. 2002; Keefer et al. 2010; see Sect. 5.3.3).

Furthermore, to be effective, fishway design must match the capability and behavior of each lamprey species (Moser et al. 2011), which can vary considerably, especially depending on body size (see Sect. 5.5.3). According to Quintella et al. (2009), vertical slot and pool-and-weir type fishways are favorable for successful adult sea lamprey passage. Small, near-vertical barriers, rock-ramp fishways, and nature-like bypass structures are also likely to be highly successful for passing adult sea lamprey (Martyn C. Lucas, Durham University, Durham, U.K., personal communication, 2011). Pacific lamprey are able to rapidly negotiate pool-and-weir fishways that have orifices flush with the bottom, but are obstructed by serpentine weirs that present them with confusing and turbulent flows (Moser et al. 2002; Keefer et al. 2010). Sea lamprey also appear to be obstructed by turbulence in fishways (Haro and Kynard 1997). Recent work showed that the rate of passage for upstream-migrating adult European river lamprey is higher for undershot than overshot weirs, and negatively related to the maximum velocity at the weir (Kemp et al. 2011). On the other hand, Denil fishways are almost impassable for this species (Laine et al. 1998), although there are some records of them being used by Pacific and sea lampreys (Slatick and Basham 1985; Martyn C. Lucas, personal communication, 2011). In a study performed on the Garonne and Dordogne rivers in France, it was documented that pool-type fish passage facilities with vertical slots and fish elevators were successfully used by sea lamprey during their upstream migration, although the efficiency of each type of installation was never estimated (Travade et al. 1998). According to Bochechas (1995), the Borland-type fish pass installed in 1986 in the Berver Dam (Tagus River, Portugal) frequently fails to work, and has proved inefficient for anadromous lamprey species. In contrast, the Holyoke Dam fish lift on the Connecticut River (at river kilometer 140) regularly passes sea lamprey upstream (Stier and Kynard 1986). In some cases, however, dam removal may be the only recourse to restore access to upstream spawning habitats (e.g., Gardner et al. 2012).

Fishway entrance areas are usually designed to accommodate strong-swimming salmonids, and often present water velocities that exceed the critical adult lamprey swim speed (Sect. 5.3.3). This is the case for Pacific lamprey in the Columbia River basin (Keefer et al. 2009b); however, recent efforts to reduce fishway velocities at night show promise for reducing velocity barriers during periods when lamprey are most active (Johnson et al. 2012). Besides the hydraulic conditions at fishway entrances, other structural challenges reduce passage efficiency and lengthen passage

times (Keefer et al. 2010). These include lack of suitable attachment surfaces, sharp-edged corners, turbulent flows, grates embedded in the fishway floors and walls, and unscreened channels that allow lampreys access to dead-end locations (Moser et al. 2002, 2008; Daigle et al. 2005; Keefer et al. 2010).

Despite lamprey-friendly modifications to aid Pacific lamprey passage at large hydropower dams in the Columbia River basin (i.e., elimination of vertical steps at orifices, rounding of entrance bulkheads, and installation of plates over floor grates), only modest passage improvements were realized (Keefer et al. 2010). In some cases, it is necessary to provide lamprey-specific routes at impassable obstacles (Moser et al. 2011). Structures designed specifically for adult Pacific lamprey take advantage of the climbing ability demonstrated by this species (Reinhardt et al. 2008; Kemp et al. 2009). Laboratory testing of lamprey fishways, in conjunction with field deployments, has resulted in development of passage structures that allow lamprey to surmount obstacles and have achieved very high rates of passage efficiency (Keefer et al. 2011; Moser et al. 2011). Thus, by providing Pacific lamprey with climbing routes at dams, they may be afforded passage at a relatively low metabolic cost (Zhu et al. 2011).

Although spawning migration is the focus of this chapter, impediments to downstream migration and direct mortality of juvenile lampreys during their seaward migration are also important management concerns (see Chaps. 3 and 8). A study performed in the Columbia River by Dauble et al. (2006) showed that the low burst speeds of juvenile Pacific lamprey (i.e., mean of 71 ± 5 SD cm/s) are not sufficient to avoid impingement at intake screens or other in-water structures designed either to collect debris or to bypass fishes at dams. Downstream migration of juvenile lampreys is seldom considered during mitigation planning and/or population recovery strategies (Moser and Mesa 2009). However, as recovery strategies that encompass translocation and propagation of lampreys are considered, it will be important to ensure the safe passage of both downstream migrating juveniles and upstream migrating adults.

5.7 Conclusions

Recent research using advanced telemetry methods, large-scale marking programs, and elegant laboratory studies have provided a wealth of information on the spawning behavior and movements of anadromous lampreys. However, as is often the case, a review of the state of our knowledge highlights the large gaps in information that remain. Of note is the lack of information on initiation, timing, extent, and rate of the marine phase of the spawning migration in anadromous forms. What cues do anadromous lampreys use to orient and enter coastal streams and rivers? How long do they reside in estuaries, and what are survival rates during this phase of the lamprey life history? Capturing and monitoring lamprey movements at sea will be challenging, but new research on the European eel *Anguilla anguilla* demonstrates that technologies are available to tackle these questions (Aarestrup et al. 2009).

Research is needed to determine the effects of dams and other water control structures on adfluvial parasitic lampreys and stream-resident non-parasitic forms. The movements of potamodromous lampreys have largely been ignored, even though they are probably as susceptible or more susceptible to anthropogenic obstacles to passage. Adfluvial parasitic and brook lampreys, with their smaller body sizes relative to anadromous species, probably have reduced swimming performance and no ability to climb. Movement of small potamodromous lampreys are likely impeded by seemingly inconsequential structures such as poorly designed culverts, irrigation diversions, or low-elevation weirs (see Chap. 8).

Innovations in pheromone research have moved from conceptualized models to identification of specific compounds and their synthesis. Questions remain regarding the degree to which various lamprey species use this orientation mechanism, the complete identity of the entire suite of cues used, ontogeny of olfactory sensitivity, and how sensitivity to pheromones translates to lamprey orientation and movement in oceans, rivers, and streams. How precisely does the bile acid pheromone model identified in landlocked sea lamprey apply to its anadromous form as well as to other species? There are clear synergies of both chemical signals and the environmental conditions where they occur. Further research is needed to elucidate these mechanisms.

With the increasingly urgent need for conservation of native lampreys and for new methods to control invasive sea lamprey in the Great Lakes, management applications for research on lamprey spawning migration abound. A better understanding is needed of the role pheromones play in controlling lamprey distribution. Such information is critical to decisions regarding propagation and translocation of native lampreys for conservation. Elaboration of the existing research is needed to develop more effective (and less expensive) methods of sea lamprey control. Fine-scale measurements of the effects of turbulence, light, and chemical signals on lampreys as they approach dams and other passage impediments are needed to understand the environmental factors that control lamprey behavior. Armed with this information, more rapid advances could be made in the development of aids to native lamprey passage and recovery of imperiled native stocks (Nunn et al. 2008). Hopefully, in the course of addressing these management concerns, new insights into the basic biology of both parasitic and non-parasitic lampreys will also emerge.

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References

Aarestrup K, Oklund F, Hansen MM et al (2009) Oceanic spawning migration of the European eel (*Anguilla anguilla*). Science 325:1660

Abou-Seedo FS, Potter IC (1979) The estuarine phase in the spawning run of the river lamprey *Lampetra fluviatilis*. J Zool 188:5–25

Adams RD, Reinhardt UG (2008). Effects of texture on surface attachment of spawning-run sea lampreys *Petromyzon marinus*: a quantitative analysis. J Fish Biol 73:1464–1472

Ahmadi M, Amiri BM, Abdoli A, Fakharzade SME, Hoseinifar SH (2011) Sex steroids, gonadal histology and biological indices of fall and spring Caspian lamprey (*Caspiomyzon wagneri*) spawning migrants in the Shirud River, Southern Caspian Sea. Environ Biol Fish 92:229–235

Alexandrino PJB (1990) Dispositivos de transposição de barragens para peixes migradores, em deslocação para montante. MSc thesis, Universidade do Porto, Oporto, Portugal

Almeida PR, Silva HT, Quintella BR (2000) The migratory behaviour of the sea lamprey *Petromyzon marinus* L., observed by acoustic telemetry in the River Mondego (Portugal). In: Moore A, Russel I (eds) Advances in fish telemetry. CEFAS, Lowestoft, pp 99–108

Almeida PR, Quintella BR, Dias NM (2002a) Movement of radio-tagged anadromous sea lamprey during the spawning migration in the River Mondego (Portugal). Hydrobiologia 483:1–8

Almeida PR, Quintella BR, Dias NM, Andrade N (2002b) The anadromous sea lamprey in Portugal: biology and conservation perspectives. In: Moser M, Bayer J, MacKinlay D (eds) The biology of lampreys, symposium proceedings. International Congress on the Biology of Fish. American Fisheries Society, 21–25 July, Vancouver, pp 49–58

Andrade NO, Quintella BR, Ferreira J et al (2007) Sea lamprey (*Petromyzon marinus* L.) spawning migration in the Vouga river basin (Portugal): poaching impact, preferential resting sites and spawning grounds. Hydrobiologia 582:121–132

Applegate VC (1950) Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. US Fish and Wildlife Service Special Scientific Report 55, Washington, DC

Araújo MJ, Ozório ROA, Bessa RJB et al (2013) Nutritional status of adult sea lamprey (*Petromyzon marinus* Linnaeus, 1758) during spawning migration in the Minho river, NW Iberian Peninsula. J Appl Ichthyol 29:808–814

Baker CF, Hicks BJ (2003) Attraction of migratory inanga (*Galaxias maculatus*) and koaro (*Galaxias brevipinnis*) juveniles to adult galaxiid odours. N Z J Freshw Mar Res 37:291–299

Bartels H, Potter IC (2007) Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys: implications for osmoregulation in fresh and seawater. J Exp Biol 207:3447–3462

Beamish FWH (1978) Swimming capacity. In: Hoar WS, Randall DJ (eds) Fish physiology, vol VII. Academic Press, New York, pp 101–187

Beamish FWH (1979) Migration and spawning energetics of the anadromous sea lamprey, *Petromyzon marinus*. Environ Biol Fish 4:3–7

Beamish FWH (1980a) Biology of the North American anadromous sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 37:1924–1943

Beamish FWH (1980b) Osmoregulation in juvenile and adult lampreys. Can J Fish Aquat Sci 37:1739–1750

Beamish FWH (1982) Biology of the southern brook lamprey, *Ichthyomyzon gagei.* Environ Biol Fish 7:305–320

Beamish FWH, Potter IC (1975) The biology of the anadromous sea lamprey (*Petromyzon marinus*) in New Brunswick. Can J Zool 177:57–72

Beamish FWH, Potter IC, Thomas E (1979) Proximate composition of the adult anadromous sea lamprey, *Petromyzon marinus,* in relation to feeding, migration and reproduction. J Anim Ecol 48:1–19

Beamish RJ (1980) Adult biology of the river lamprey (*Lampetra ayresi*) and the Pacific lamprey (*Lampetra tridentata*) from the Pacific coast of Canada. Can J Fish Aquat Sci 37:1906–1923

Beamish RJ (1982) *Lampetra macrostoma*, a new species of freshwater parasitic lamprey from the west coast of Canada. Can J Fish Aquat Sci 39:736–747

Beamish RJ (1987) Status of the lake lamprey, *Lampetra macrostoma*, in Canada. Can Field-Nat 101:186–189

Beamish RJ, Northcote TG (1989) Extinction of a population of anadromous parasitic lamprey, *Lampetra tridentata*, upstream of an impassable dam. Can J Fish Aquat Sci 46:420–425

Beamish RJ, Withler RE (1986) A polymorphic population of lamprey that may produce parasitic and a nonparasitic varieties. In: Uyen et al (eds) Info-Pacific fish biology. Proceedings of the

Second International Conference on Indo-Pacific Fishes, Ichthyology Society of Japan, Tokyo, pp 31–49
Beamish RJ, Youson JH (1987) Life history and abundance of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the Strait of Georgia. Can J Fish Aquat Sci 44:525–537
Bell S, McGillivray D (2006) Environmental law, 6th edn. Oxford University Press, Oxford
Bergstedt RA, Seelye J (1995) Evidence for lack of homing by sea lamprey. Trans Am Fish Soc 124:235–239
Bigelow HB, Schroeder WC (1948) Fishes of the western North Atlantic, part 1, cyclostomes. Mem Sears Found Mar Res 1:29–58
Binder TR, McDonald DG (2007) Is there a role for vision in the behaviour of sea lampreys (*Petromyzon marinus*) during their upstream spawning migration? Can J Fish Aquat Sci 64:1403–1412
Binder TR, McDonald DG (2008a) The role of temperature in controlling diel activity in upstream migrant sea lampreys (*Petromyzon marinus*). Can J Fish Aquat Sci 65:113–1121
Binder TR, McDonald DG (2008b) The role of dermal photoreceptors during the sea lamprey (*Petromyzon marinus*) spawning migration. J Comp Physiol A 194:921–928
Binder TR, McLaughlin RL, McDonald DG (2010) Relative importance of water temperature, water level, and lunar cycle to migratory activity in spawning-phase sea lampreys in Lake Ontario. Trans Am Fish Soc 139:700–712
Bird DJ, Potter IC (1983) Proximate composition of various stages of adult life in the Southern Hemisphere lamprey, *Geotria australis*. Comp Biochem Physiol 74:623–633
Bjerselius R, Li W, Teeter JH et al (2000) Direct behavioral evidence that unique bile acids released by larval sea lamprey function as a migratory pheromone. Can J Fish Aquat Sci 57:557–569
Bochechas JHR (1995) Condições de funcionamento e de eficácia de eclusas para peixes: casos das barragens de Crestuma-Lever e de Belver. MSc thesis, Universidade Técnica de Lisboa, Lisbon
Boutilier RG, Ferguson RA, Henry RP, Tufts BL (1993) Exhaustive exercise in the sea lamprey (*Petromyzon marinus*): relationship between anaerobic metabolism and intracellular acid–base balance. J Exp Biol 178:71–88
Britt L, Loew ER, McFarland WN (2001) Visual pigments in the early life stages of Pacific Northwest marine fishes. J Exp Biol 204:2581–2587
Brown JA, Rankin JC (1999) Lack of glomerular intermittency in the river lamprey *Lampetra fluviatilis* acclimated to sea water and following acute transfer to iso-osmotic brackish water. J Exp Biol 202:939–946
Brown JA, Cobb CS, Frankling SC, Rankin JC (2005) Activation of the newly discovered cyclostome renin-angiotensin system in the river lamprey *Lampetra fluviatilis*. J Exp Biol 208:223–232
Bryan MB, Zalinski D, Filcek KB et al (2005) Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. Mol Ecol 14:3757–3773
Buisson L, Blanc L, Grenouillet G (2008) Modeling stream fish species distribution in a river network: the relative effects of temperature versus physical factors. Ecol Freshw Fish 17:244–257
Burns AC, Sorensen PW, Hoye TR (2011) Synthesis and olfactory activity of unnatural, sulfated 5β-bile acid derivatives in the sea lamprey (*Petromyzon marinus*). Steroids 76:291–300
Cabral MJ, Almeida J, Almeida PR et al (eds) (2005) Livro vermelho dos vertebrados de Portugal. Instituto de Conservação da Natureza, Lisbon
Choe KP, O'Brien S, Evans D, Toop T, Edwards S (2004) Immunolocalization of Na^+/K^+-ATPase, carbonic anhydrase II, and vacuolar H^+-ATPase in the gills of freshwater adult lampreys, *Geotria australis*. J Exp Zool 301A:654–665
Claridge PN, Potter IC (1975) Oxygen consumption, ventilatory frequency and heart rate of lampreys (*Lampetra fluviatilis*) during their spawning run. J Exp Biol 63:193–206
Clemens BJ (2011) The physiological ecology and run diversity of adult Pacific lamprey, *Entosphenus tridentatus*, during the freshwater spawning migration. PhD thesis, Oregon State University, Corvallis, OR

Clemens BJ, van de Wetering S, Kaufman J, Holt RA, Schreck CB (2009) Do summer temperatures trigger spring maturation in Pacific lamprey, *Entosphenus tridentatus*? Ecol Freshw Fish 18:418–426

Clemens BJ, Binder TR, Docker MF, Moser ML, Sower SA (2010) Similarities, differences, and unknowns in biology and management of three parasitic lampreys of North America. Fisheries 35:580–596

Clemens BJ, Mesa MG, Magie RJ, Young DA, Schreck CB (2012) Pre-spawning migration of adult Pacific lamprey, *Entosphenus tridentatus*, in the Willamette River, Oregon, USA. Environ Biol Fish 93:245–254

Close DA, Fitzpatrick MS, Li HW (2002) The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fish 27:19–25

Close DA, Jackson AD, Conner BP, Li HW (2004) Traditional ecological knowledge of Pacific lamprey (*Entosphenus tridentatus*) in northeastern Oregon and southeastern Washington from indigenous peoples of the Confederated Tribes of the Umatilla Indian Reservation. J Northwest Anthropol 38:141–161

Close DA, Currens KP, Jackson A et al (2009) Lessons from the reintroduction of a noncharismatic, migratory fish: Pacific lamprey in the upper Umatilla River, Oregon. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 233–253

Cochran PA, Lyons J (2004) Field and laboratory observations on the ecology and behavior of the silver lamprey (*Ichthyomyzon unicuspis*) in Wisconsin. J Freshw Ecol 19:245–253

Cochran PA, Marks JE (1995) Biology of the silver lamprey, *Ichthyomyzon unicuspis*, in Green Bay and the lower Fox River, with a comparison to the sea lamprey, *Petromyzon marinus*. Copeia 1995:409–421

Cochran PA, Lyons J, Gehl MR (2003) Parasitic attachments by overwintering silver lampreys, *Ichthyomyzon unicuspis*, and chestnut lampreys *Ichthyomyzon castaneus*. Environ Biol Fish 68:65–71

Collin SP, Potter IC (2000) The ocular morphology of the southern hemisphere lamprey *Mordacia mordax* Richardson with special reference to a single class of photoreceptor and a retinal tapetum. Brain Behav Evol 55:120–138

Collin SP, Potter IC (in press) Sensory ecology and behavior in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Collin SP, Potter IC, Braekevelt CR (1999) The ocular morphology of the southern hemisphere lamprey *Geotria australis* Gray, with special reference to optical specialisations and the characterisation and phylogeny of photoreceptor types. Brain Behav Evol 54:96–118

Collin SP, Hart NS, Shand J, Potter IC (2003) Morphology and spectral absorption characteristics of retinal photoreceptors in the southern hemisphere lamprey (*Geotria australis*). Vis Neurosc 20:119–130

Collin SP, Hart NS, Wallace KM, Shand J, Potter IC (2004) Vision in the southern hemisphere lamprey *Mordacia mordax*: spatial distribution, spectral absorption characteristics, and optical sensitivity of a single class of retinal photoreceptor. Vis Neurosci 21:765–773

COSEWIC (2010) COSEWIC assessment and status report on the Chestnut Lamprey *Ichythyomyzon castaneus* (Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson River populations) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

COSEWIC (2011) COSEWIC assessment and status report on the Silver Lamprey, Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson Rivers populations *Ichthyomyzon unicuspis* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

Cummings D (2007) Direct and indirect effects of barriers to migration—Pacific lamprey at McNary and Ice Harbor dams in the Columbia River Basin. MS thesis, University of Idaho, Moscow, ID

Daigle WR, Peery CA, Lee SR, Moser ML (2005) Evaluation of adult Pacific lamprey passage and behavior in an experimental fishway at Bonneville Dam. Idaho Cooperative Fish and Wildlife Research Unit, Technical Report 2005–1 to the U.S. Army Corps of Engineers, Portland, OR

Daniels RA (2001) Untested assumptions: the role of canals in the dispersal of sea lamprey, alewife, and other fishes in the eastern United States. Environ Biol Fish 60:309–329
Dauble DD, Moursund RA (2000) Effects of extended length bypass screens on juvenile Pacific lamprey. HydroVision 2000 technical paper. HCL Publications, Kansas City
Dauble DD, Moursund RA, Bleich MD (2006) Swimming behaviour of juvenile Pacific lamprey, *Lampetra tridentata*. Environ Biol Fish 75:167–171
Derby CD, Sorensen PW (2008) Neural processing, perception and behavioral responses to natural chemical stimuli by fish and crustaceans. J Chem Ecol 34:898–914
Deutschlander ME, Borland SC, Phillips JB (1999) Extraocular magnetic compass in newts. Nature 400:324–325
Docker MF (2009) A review of the evolution of non-parasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 71–114
Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Docker MF, Beamish FWH, Bryan MB, Spice EK (in press) The lamprey gonad. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Døving KB, Westerberg H, Johnsen PB (1985) Role of olfaction in the behavioral and neuronal responses of Atlantic salmon, *Salmo salar*, to hydrographic stratification. Can J Fish Aquat Sci 42: 1658–1667
Farmer GJ (1980) Biology and physiology of feeding in adult lampreys. Can J Fish Aquat Sci 37:1751–1761
Farmer GJ, Beamish FWH (1973) Sea lamprey (*Petromyzon marinus*) predation on freshwater teleosts. J Fish Res Board Can 30:601–605
Fernholm B (1990) Petromyzontidae. In: Gon O, Heemstra PC (eds) Fishes of the Southern Ocean. JLB Smith Institute of Ichthyology, Grahamstown, pp 79–80
Fine JM, Sorensen PW (2005) Biologically-relevant concentrations of petromyzonol sulfate, a component of the sea lamprey migratory pheromone, measured in stream waters. J Chem Ecol 31:2205–2210
Fine JM, Sorensen PW (2008) Isolation and biological activity of the multi-component sea lamprey migratory pheromone and new information in its potency. J Chem Ecol 34:1259–1267
Fine JM, Sorensen PW (2010) Production and fate of the sea lamprey migratory pheromone. Fish Physiol Biochem 36:1013–1020
Fine JM, Vrieze LA, Sorensen PW (2004) Evidence that petromyzontid lampreys employ a common migratory pheromone that is partially comprised of bile acids. J Chem Ecol 30:2091–2110
Fine JM, Sisler SP, Vrieze LA, Swink WD, Sorensen PW (2006) A practical method for obtaining useful quantities of pheromones from sea lamprey and other fishes for identification and control. J Great Lakes Res 23:832–838
Fronti A, Zaidi A, Hua H (2003) Glomerular territories in the olfactory bulb from the larval stage of the sea lamprey *Petromyzon marinus*. J Comp Neurol 465:27–37
Gardner C, Coghlan SM Jr, Zydlewski J (2012) Distribution and abundance of anadromous sea lamprey spawners in a fragmented stream: current status and potential range expansion following barrier removal. Northeast Nat 19:99–110
Gaudron SM, Lucas MC (2006) First evidence of attraction of adult river lamprey in the migratory phase to larval odour. J Fish Biol 68:640–648
Goodman DH, Reid SB, Docker MF, Haas GR, Kinziger AP (2008) Mitochondrial DNA evidence for high levels of gene flow among populations of a widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae). J Fish Biol 72:400–417
Hanson LH, Manion PJ (1980) Sterility method of pest control and its potential role in an integrated sea lamprey (*Petromyzon marinus*) control program. Can J Fish Aquat Sci 37:2108–2117
Hardisty MW (1986a) *Petromyzon marinus* Linnaeus, 1758. In: Holčík J (ed) The freshwater fishes of Europe, part 1, Petromyzontiformes, vol 1. AULA, Wiesbaden, pp 96–116

Hardisty MW (1986b) *Lampetra fluviatilis* (Linnaeus, 1758). In: Holčík J (ed) The freshwater fishes of Europe, part 1, Petromyzontiformes, vol 1. AULA, Wiesbaden, pp 249–278
Hardisty MW (1986c) General introduction to lampreys. In: Holčík J (ed) The freshwater fishes of Europe, part 1, Petromyzontiformes, vol 1. AULA, Wiesbaden, pp 19–83
Hardisty MW (1986d) *Lampetra planeri* (Bloch, 1784). In: Holčík J (ed) The freshwater fishes of Europe, part 1, Petromyzontiformes, vol 1. AULA, Wiesbaden, pp 279–304
Hardisty MW (2006) Lampreys: life without jaws. Forrest Text, Tresaith
Hardisty MW, Potter IC (1971) The general biology of adult lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys. Academic Press, London, pp 127–205
Haro A, Kynard B (1997) Video evaluation of passage efficiency of American shad and sea lamprey in a modified Ice Harbor fishway. N Am J Fish Manag 17:981–987
Hatch DR, Whiteaker JM (2009) A field study to investigate repeat homing in Pacific lampreys. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 191–201
Hess JE, Campbell NR, Close DA, Docker MF, Narum SR (2013) Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. Mol Ecol 22:2898–2916
Holčík J (1986a) *Lethenteron japonicus* (Martens, 1868). In: Holčík J (ed) The freshwater fishes of Europe, part 1, Petromyzontiformes, vol 1. AULA, Wiesbaden, pp 198–219
Holčík J (1986b) *Caspiomyzon wagneri* (Kessler, 1870). In: Holčík J (ed) The freshwater fishes of Europe, part 1, Petromyzontiformes, vol 1. AULA, Wiesbaden, pp 119–142
Imamura Y (1928) Beitrage zur metamorphose des geruchsorganes von *Petromyzon planeri*. Acta Med Keijo 11:1–22
IUCN (2013) IUCN Red List of Threatened Species. Version 2013.1. http://www.iucnredlist.org. Accessed 12 Dec 2013
Jackson AD, Moser ML (2012) Low-elevation dams are impediments to adult Pacific lamprey spawning migration in the Umatilla River, Oregon. N Am J Fish Manag 32:548–556
James A (2008) Ecology of the New Zealand lamprey (*Geotria australis*): a literature review. Department of Conservation, Wanganui Conservancy, Wanganui, NZ
Jellyman DJ, Glova GJ, Sykes JRE (2002) Movements and habitats of adult lamprey (*Geotria australis*) in two New Zealand waterways. N Z J Freshw Mar Res 36:53–65
Jensen AJ, Heggberget TG, Johnsen BO (1986) Upstream migration of adult Atlantic salmon, *Salmo salar* L., in the River Vefsna, northern Norway. J Fish Biol 29:459–465
Johnson NS, Luehring MA, Siefkes MJ, Li W (2006) Mating pheromone reception and induced behavior in ovulating female sea lampreys. N Am J Fish Manag 26:88–96
Johnson EL, Caudill CC, Keefer ML et al (2012) Movement of radio-tagged adult Pacific lampreys during a large-scale fishway velocity experiment. Trans Am Fish Soc 141:571–579
Jonsson, N (1991) Influence of water flow, water temperature and light on fish migration in rivers. Nord J Freshw Res 66:20–35
Jonsson N, Jonsson B (2002) Migration in anadromous brown trout *Salmo trutta* in a Norwegian river. Freshw Biol 47:1391–1401
Kan TT, Bond CE (1981) Notes on the biology of the Miller Lake lamprey *Lampetra (Entosphenus) minima*. Northwest Sci 55:70–74
Keefer ML, Moser ML, Boggs CT, Daigle WR, Peery CA (2009a) Variability in migration timing of adult Pacific lamprey (*Lampetra tridentata*) in the Columbia River, U.S.A. Environ Biol Fish 85:253–264
Keefer ML, Moser ML, Boggs CT, Daigle WR, Peery CA (2009b) Effects of body size and river environment on the upstream migration of adult Pacific lampreys. N Am J Fish Manag 29:1214–1224
Keefer ML, Peery CA, Caudill CC et al (2009c) Adult Pacific lamprey migration in the lower Columbia River: 2008 radiotelemetry and half-duplex PIT tag studies. Final Report to the U.S. Army Corps of Engineers, Portland District, Portland, OR
Keefer ML, Daigle WR, Peery CA et al (2010) Testing adult Pacific lamprey performance at structural challenges in fishways. N Am J Fish Manag 30:376–385

Keefer ML, Peery CA, Lee SR et al (2011) Behavior of adult Pacific lampreys in near-field flow and fishway design experiments. Fish Manag Ecol 18:177–189
Keefer ML, Caudill CC, Clabough TS et al (2013a) Fishway passage bottleneck identification and prioritization: a case study of Pacific lamprey at Bonneville Dam. Can J Fish Aquat Sci 70:1551–1565
Keefer ML, Caudill CC, Peery CA, Moser ML (2013b) Context-dependent diel behavior of upstream-migrating anadromous fishes. Environ Biol Fish 96:691–700
Kelly FL, King JJ (2001) A review of the ecology and distribution of three lamprey species *Lampetra fluviatilis*, *Lampetra planeri*, and *Petromyzon marinus*: a context for conservation and biodiversity considerations in Ireland. Proc R Irish Acad B 101:165–185
Kelso JRM, Gardner WM (2000) Emigration, upstream movement, and habitat use by sterile and fertile sea lampreys in three Lake Superior tributaries. N Am J Fish Manag 20:144–153
Kelso JRM, Glova GJ (1993) Distribution, upstream migration and habitat selection of maturing lampreys, *Geotria australis*, in Pigeon Bay Stream, New Zealand. Aust J Freshw Mar Res 44: 749–759
Kelso JRM, Gardner WM, McDonald RB (2001) Interactions among fertile male, female, and sterile male sea lampreys during spawning in the Carp River, Lake Superior. N Am J Fish Manag 21:904–910
Kemp PS, Tsuzaki T, Moser ML (2009) Linking behaviour and performance: intermittent locomotion in a climbing fish. J Zool 277:171–178
Kemp PS, Russon IJ, Vowles AS, Lucas MC (2011) The influence of discharge and temperature on the ability of upstream migrant adult river lamprey (*Lampetra fluviatilis*) to pass experimental overshot and undershot weirs. River Res Appl 27:488–498
Kleerekoper H (1972) The sense organs. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 2. Academic Press, New York, pp 373–404
Kleerekoper H, van Erkel GA (1960) The olfactory apparatus of *Petromyzon marinu*s. Can J Zool 38:209–223
Kostow K (2002) Oregon lampreys: natural history, status, and analysis of management issues. Oregon Department of Fish and Wildlife, Portland
Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes. Kottelat, Cornol
Kucheryavyi AV, Savvaitova KA, Pavlov DS et al (2007) Variations of life history strategy of the Arctic lamprey *Lethenteron camtschaticum* from the Utkholok River (Western Kamchatka). J Ichthyol 47:37–52
Laframboise AJ, Ren X, Chang S, Dubuc R, Zielinski BS (2006) Olfactory sensory neurons in the sea lamprey display polymorphisms. Neurosci Lett 414:277–281
Laine A, Kamula R, Hooli J (1998) Fish and lamprey passage in a combined Denil and vertical slot fishway. Fish Manag Ecol 5:31–44
Larsen LO (1980) Physiology of adult lampreys, with special regard to natural starvation, reproduction, and death after spawning. Can J Fish Aquat Sci 37:1762–1779
Lasne E, Sabatie MR, Evanno G (2010) Communal spawning of brook and river lampreys (*Lampetra planeri* and *L. fluviatilis*) is common in the Oir River (France). Ecol Freshw Fish 19:323–325
Li W, Sorensen PW (1992) The olfactory sensitivity of sea lamprey to amino acids is specifically restricted to arginine. Chem Senses 17:658
Li W, Sorensen PW (1997) Highly independent olfactory receptor sites for conspecific bile acids in the sea lamprey, *Petromyzon marinus*. J Comp Physiol A 180:429–438
Liao JC (2007) A review of fish swimming mechanics and behaviour in altered flows. Phil Trans R Soc Biol Sci 362:1973–1993
Lin B, Zhang Z, Wang Y, Currens KP, Spidle A, Yamazaki Y, Close D (2008) Amplified fragment length polymorphism assessment of genetic diversity in Pacific lampreys. N Am J Fish Manag 28:1182–1193
Lorion CM, Markle DF, Reid SB, Docker MF (2000) Redescription of the presumed-extinct Miller Lake lamprey, *Lampetra minima*. Copeia 2000:1019–1028

Lucas MC, Baras E (2001) Migration of freshwater fishes. Blackwell, Oxford
Lucas MC, Bubb DH, Jang MH, Ha K, Masters JEG (2009) Availability of and access to critical habitats in regulated rivers: effects of low-head barriers on threatened lampreys. Freshw Biol 54:621–634
Machado-Cruz JM, Valente ACN, Alexandrino PJB (1990) Contribuição para a caracterização ecológica e económica da pesca de migradores a jusante da barragem de Belver, Rio Tejo. Actas 1º Congresso do Tejo. Que Tejo, que futuro? pp 189–200
Malmqvist B (1980) The spawning migration of the brook lamprey *Lampetra planeri* Bloch in a south Swedish stream. J Fish Biol 16:105–114
Manion PJ, Hanson LH (1980) Spawning behavior and fecundity of lampreys from the upper three Great Lakes. Can J Fish Aquat Sci 37:1635–1640
Marsden JE, Siefkes MJ (in press) Sea lamprey control in the Great Lakes, Finger Lakes, and Lake Champlain. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Masters JEG, Jang MH, Ha K et al (2006) The commercial exploitation of a protected anadromous species, the river lamprey (*Lampetra fluviatilis* (L.)), in the tidal River Ouse, north-east England. Aquat Conserv Mar Freshw Ecosyst 16:77–92
McAuley TC (1996) Development of an instream velocity barrier to stop sea lamprey migrations in the Great Lakes. MSc thesis, University of Manitoba, Winnipeg, MB
McDowall RM (1990) The Reed field guide to New Zealand freshwater fishes. Reed, Auckland
McIlraith BJ (2011) The adult migration, spatial distribution, and spawning behaviors of anadromous Pacific lamprey (Lampetra tridentata) in the lower Snake River. MS thesis, University of Idaho, Moscow, ID
Meckley TD, Wagner CM, Luehring MR (2012) Field evaluation of larval odor and mixtures of synthetic pheromone components for attracting migrating sea lamprey in rivers. J Chem Ecol 38:1062–1069
Mesa MG, Bayer JM, Seelye JG (2003) Swimming performance and physiological responses to exhaustive exercise in radio-tagged and untagged Pacific lampreys. Trans Am Fish Soc 132:483–492
Mesa MG, Bayer JM, Bryan MB, Sower SA (2010a) Annual sex steroid and other physiological profiles of Pacific lampreys (*Entosphenus tridentatus*). Comp Biochem Physiol A 155:56–63
Mesa MG, Magie RJ, Copeland ES (2010b) Passage and behavior of radio-tagged adult Pacific lampreys (*Entosphenus tridentatus*) at the Willamette Falls project, Oregon. Northwest Sci 84:233–242
Moore HH, Schleen LP (1980) Changes in spawning runs of sea lamprey (*Petromyzon marinus*) in selected streams of Lake Superior after chemical control. Can J Fish Aquat Sci 37:1851–1860
Morman RH, Cuddy, DW, Rugen PC (1980) Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:1811–1826
Morris R (1972) Osmoregulation. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 2. Academic Press, London, pp 193–239
Moser ML, Close DA (2003) Assessing Pacific lamprey status in the Columbia River basin. Northwest Sci 77:116–125
Moser ML, Mesa MG (2009) Passage considerations for lamprey. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 115–124
Moser ML, Ocker PA, Stuehrenberg LC, Bjornn TC (2002) Passage efficiency of adult Pacific lampreys at hydropower dams on the Lower Columbia River, USA. Trans Am Fish Soc 131:956–965
Moser ML, Zabel RW, Burke BJ, Stuehrenberg LC, Bjornn TC (2005) Factors affecting adult Pacific lamprey passage rates at hydropower dams: using “time to event” analysis of radiotelemetry data. In: Spedicato MT, Marmulla G, Lembo G (eds) Aquatic telemetry: advances and applications. FAO-COISPA, Rome, pp 61–70

Moser ML, Pennington HT, Roos JM (2008) Grating size needed to protect adult Pacific lamprey in the Columbia River Basin. N Am J Fish Manag 28:557–562

Moser ML, Keefer ML, Pennington HT, Ogden DA, Simonson JE (2011) Development of Pacific lamprey fishways at a hydropower dam. Fish Manag Ecol 18:190–200

Moser ML, Jackson AD, Tsuzaki T, Kemp PS (2013a) Do surgically-implanted radio transmitters alter the climbing ability of adult Pacific lamprey (*Lampetra tridentata*)? Fish Manag Ecol 20:374–376

Moser, ML, Keefer ML, Caudill CC, Burke BJ (2013b) Migratory behavior of adult Pacific lamprey and evidence for effects of individual temperament on migration rate. In: Ueda H, Tsukamoto K (eds) Physiology and ecology of fish migration. CRC, Boca Raton, pp 130–149

Nazari H, Abdoli A (2010) Some reproductive characteristics of endangered Caspian Lamprey (*Caspiomyzon wagneri* Kessler, 1870) in the Shirud River southern Caspian Sea, Iran. Environ Biol Fish 88:87–96

Nivison-Smith L, Collin SP, Zhu Y et al (2013) Retinal amino acid neurochemistry of the southern hemisphere lamprey, *Geotria australis*. PLoS ONE 8:e58406

Nunn AD, Harvey JP, Noble RAA, Cowx IG (2008) Condition assessment of lamprey populations in the Yorkshire Ouse catchment north-east England, and the potential influence of physical migration barriers. Aquat Conserv Mar Freshw Ecosyt 18:175–189

O'Connor LM (2001) Spawning success of introduced sea lampreys (*Petromyzon marinus*) in two streams tributary to Lake Ontario. MSc thesis, University of Guelph, Guelph, ON

Oliveira JM, Ferreira MT, Pinheiro AN, Bochechas JH (2004) A simple method for assessing minimum flows in regulated rivers: the case of sea lamprey reproduction. Aquat Conserv 14: 481–489

Orlov AM, Savinyh VF, Pelenev DV (2008) Features of the spatial distribution and size structure of Pacific lamprey *Lampetra tridentata* in the North Pacific. Rus J Mar Biol 34:276–287

Pickering AD (1976a) Effects of gonadectomy, oestradiol and testosterone on the migrating river lamprey, *Lampetra fluviatilis* (L). Gen Comp Endocrinol 28:473–480

Pickering AD (1976b) Stimulation of intestinal degeneration by oestradiol and testosterone implantation in the migrating river lamprey, *Lampetra fluviatilis* L. Gen Comp Endocrinol 30:340–346

Polkinghorne CN, Olson JM, Gallaher DG, Sorensen PW (2001) Larval sea lamprey release two unique bile acids to the water at a rate sufficient to produce detectable riverine pheromone plumes. Fish Physiol Biochem 24:15–30

Potter IC, Prince PA, Croxall JP (1979) Data on the adult marine and migratory phases in the life cycle of the southern hemisphere lamprey, *Geotria australis* Gray. Environ Biol Fish 4:65–69

Potter IC, Hilliard RW, Bird DJ, Macey DJ (1983) Quantitative data on morphology and organ weights during the protracted spawning-run period of the Southern Hemisphere lamprey *Geotria australis* J Zool 200:1–20

Quintella BR, Andrade NO, Koed A, Almeida PR (2004) Behavioural patterns of sea lampreys' spawning migration through difficult passage areas, studied by electromyogram telemetry J Fish Biol 65:961–972

Quintella BR, Povoa I, Almeida PR (2009) Swimming behaviour of upriver migrating sea lamprey assessed by electromyogram telemetry. J Appl Ichthyol 25:46–54

Rankin JC (1997) Osmotic and ionic regulation in cyclostomes. In: Hazon N, Eddy FB, Filk G (eds) Ionic regulation in animals. Cambridge University Press, London, pp 50–69

Rankin JC, Cobb CS, Frankling SC, Brown JA (2001) Circulating angiotensins in the river lamprey, *Lampetra fluviatilis*, acclimated to freshwater and seawater: possible involvement in the regulation of drinking. Comp Biochem Physiol B 129:311–318

Reinhardt UG, Eidietis L, Friedl SE, Moser ML (2008) Pacific lamprey climbing behavior. Can J Zool 86:1264–1272

Reinhardt, UG, Binder TR, McDonald DG (2009) Ability of adult sea lampreys to climb inclined surfaces. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, manage-

ment, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 125–138
Renaud CB (1997) Conservation status of Northern Hemisphere lampreys (Petromyzontidae). J Appl Ichthyol 13:143–148
Renaud CB (2011) Lampreys of the world. An annotated and illustrated catalogue of lamprey species known to date. FAO Species Catalogue Fishery Purposes 5, FAO, Rome
Renaud CB, Cochran PA (in press) Post-metamorphic feeding in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Renaud CB, Holčík J (1986) *Eudontomyzon danfordi* Regan, 1911. In: J Holčík (ed) The freshwater fishes of Europe, part 1, Petromyzontiformes, vol 1. AULA, Wiesbaden, pp 146–164
Robinson TC, Bayer JM (2005) Upstream migration of Pacific lampreys in the John Day River, Oregon: behavior, timing, and habitat use. Northwest Sci 79:106–119
Robinson TC, Sorensen PW, Bayer JM, Seelye JG (2009) Olfactory sensitivity of Pacific lampreys to a lamprey bile acid. Trans Am Fish Soc 138:144–152
Rodriguez-Ruiz A, Granado-Lorencio C (1992) Spawning period and migration of three species of cyprinids in a stream with Mediterranean regiment (SW Spain). J Fish Biol 41:545–556
Russon IJ, Kemp PS, Lucas MC (2011) Gauging weirs impede the upstream migration of adult river lamprey *Lampetra fluviatilis*. Fish Manag Ecol 18:201–210
Scholz AT, Horrall RM, Cooper JC, Hasler AD (1976) Imprinting to chemical cues: the basis for homestream selection in salmon. Science 192:1247–1249
Schuldt RJ, Goold R (1980) Changes in the distribution of native lampreys in Lake Superior tributaries in response to sea lamprey (*Petromyzon marinus*) control, 1953–77. Can J Fish Aquat Sci 37:1872–1885
Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Bulletin Fisheries Research Board of Canada 184, Ottawa
Shrimpton JM (2013) Seawater to freshwater transitions in diadromous fish. In: McCormick SD, Farrell AP, Brauner CJ (eds) Euryhaline fishes, Fish physiology, vol 32. Academic Press, San Diego, pp 327–392
Siefkes MJ, Li W (2004) Electrophysiological evidence for detection and discrimination of pheromonal bile acids by the olfactory epithelium of female sea lampreys (*Petromyzon marinus*). J Comp Physiol A 190:193–196
Sjöberg K (2011) River lamprey *Lampetra fluviatilis* (L.) fishing in the area around the Baltic Sea. J North Stud 5:51–86
Slatick E, Basham, LR (1985) The effect of Denil fishway length on passage of some nonsalmonid fishes. Mar Fish Rev 47: 83–85
Sorensen PW (1986) Origins of the freshwater attractants of the elvers of the American eel (*Anguilla rostrata*). Environ Biol Fish 17:185–200
Sorensen PW, Hoye TE (2007) A critical review of the discovery and application of a migratory pheromone in an invasive fish, the sea lamprey, *Petromyzon marinus* L. J Fish Biol 71(Suppl D):100–114
Sorensen PW, Vrieze LA (2003) Chemical ecology and application of the sea lamprey migratory pheromone. J Great Lakes Res 29(Suppl 1):66–84
Sorensen PW, Li W, Bjerselius R, Zielinski B, Bowdin L (1995) Peripheral olfactory sensitivity of sea lamprey is greatest just prior to spawning migration and then rapidly deteriorates. Chem Senses 20:782
Sorensen PW, Vrieze LA, Fine J (2003) A multicomponent migratory pheromone in the sea lamprey. Fish Physiol Biochem 28:253–257
Sorensen PW, Fine JM, Dvornikovs V et al (2005) Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. Nat Chem Biol 1:324–328
Spice EK, Goodman DH, Reid SB, Docker MF (2012) Neither philopatric nor panmictic: microsatellite and mtDNA evidence suggests lack of natal homing but limits to dispersal in Pacific lamprey. Mol Ecol 21:2916–2930

Stewart M, Baker C, Cooney T (2012) A rapid, sensitive and selective method for quantification of the sea lamprey migratory pheromones in river water. Chem Ecol 37:1201–1209
Stier K, Kynard B (1986) Movement of sea-run sea lampreys, *Petromyzon marinus*, during the spawning migration in the Connecticut River. Fish Bull 84:749–753
Tamotsu S, Morita Y (1986) Photoreception in pineal organs of larval and adult lampreys, *Lampetra japonica*. J Comp Physiol A 159:1–5
Thiel R, Winkler HM, Riel P et al (2009) Endangered anadromous lampreys in the southern Baltic Sea: spatial distribution, long-term trend, population status. Endanger Species Res 8:233–247
Travade F, Larinier M, Boyer-Bernard S, Dartiguelongue J (1998) Performance of four fish pass installations recently built on two rivers in south-west France. In: Jungwirth M, Schmutz S, Weiss S (eds) Fish migration and fish bypasses. Fishing News Books, Oxford, pp 146–170
Tudorache C, Viaenen P, Blust R, De Boeck G (2007) Longer flumes increase critical swimming speeds by increasing burst-glide swimming duration in carp *Cyprinus carpio*, L. J Fish Biol 71:1630–1638
Tufts BL (1991) Acid-base regulation and blood gas transport following exhaustive exercise in an agnathan, the sea lamprey *Petromyzon marinus*. J Exp Biol 159:371–385
Tuunainen P, Ikonen E, Auvinen H (1980) Lampreys and lamprey fisheries in Finland. Can J Fish Aquat Sci 37:1953–1959
Tweed T (1987) And the rains came down, and the lamprey went up. Freshw Catch 33:10–11
VanDenBossche J, Seelye JG, Zielinski BS (1995) The morphology of the olfactory epithelium in larval, juvenile and upstream migrant stages of the sea lamprey, *Petromyzon marinus*. Brain Behav Evol 45:19–24
van Ginneken VJT, Maes GE (2005) The European eel (*Anguilla anguilla*, Linnaeus), its lifecycle, evolution and reproduction: a literature review. Rev Fish Biol Fish 15:367–398
Vrieze LA (2008) The migratory behavior of adult sea lampreys (*Petromyzon marinus*) in response to olfactory and temperature cues. PhD thesis, University of Minnesota, Minneapolis
Vrieze LA, Sorensen PW (2001) Laboratory assessment of the role of a larval pheromone and natural stream odor in spawning stream localization by migratory sea lamprey. Can J Fish Aquat Sci 58:2374–2385
Vrieze LA, Bjerselius R, Sorensen PW (2010) Importance of the olfactory sense to migratory sea lampreys *Petromyzon marinus* seeking riverine spawning habitat. J Fish Biol 76: 949–964
Vrieze LA, Bergstedt RA, Sorensen PW (2011) Olfactory-mediated stream finding behavior of migratory adult sea lamprey (*Petromyzon marinus*). Can J Fish Aquat Sci 68: 523–533
Wagner CM, Jones ML, Twohey MB, Sorensen PW (2006) A field test verifies pheromones can be useful for sea lamprey (*Petromyzon marinus*) control in the Great Lakes. Can J Fish Aquat Sci 63:475–479
Wagner CM, Twohey MB, Fine JM (2009) Conspecific cueing in the sea lamprey: do reproductive migrations consistently follow the most intense larval odour? Anim Behav 78:593–599
Wald G (1957) The metamorphosis of visual systems in the sea lamprey. J Gen Physiol 40:901–914
Waldman J, Grunwald C, Wirgin I (2008) Sea lamprey *Petromyzon marinus*: an exception to the rule of homing in anadromous fishes. Biol Lett 4:659–662
Waldman J, Daniels R, Hickerson M, Wirgin I (2009) Mitochondrial DNA analysis indicates sea lampreys are indigenous to Lake Ontario: response to comment. Trans Am Fish Soc 138:1190–1197
Ward DL, Clemens BJ, Clugston D et al (2012) Translocating adult Pacific lamprey within the Columbia River Basin: state of the science. Fisheries 37:351–361
Wilkie MP, Brobbel MA, Davidson K, Forsyth L, Tufts BL (1997) Influences of temperature upon the post-exercise physiology of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 54:503–511
Willis J, Phillips J, Muheim R, Diego-Rasilla FJ, Hobday AJ (2009) Spike dives of juvenile southern bluefin tuna (*Thunnus maccoyii*): a navigational role? Behav Ecol Sociobiol 64:57–68
Winter HV, Van Densen WLT (2001) Assessing opportunities for upstream migration of non-salmonid fishes in the weir-regulated River Vecht. Fish Manag Ecol 8:513–532

Xi X, Johnson NS, Brant CO, Yun SS, Chambers KL, Jones AD, Li W (2011) Quantification of a male sea lamprey pheromone in tributaries of Laurentian Great Lakes by liquid chromatography tandem mass spectrometry. Environ Health Tech 45:6437–6443

Yun S, Wildbill AJ, Siefkes MJ et al (2011) Identification of putative migratory pheromones from Pacific lamprey. Can J Fish Aquat Sci 68:2194–2203

Zhu, Q, Moser ML, Kemp P (2011) Analysis of vertical climbing in Pacific lamprey. Bioinspir Biomim 6:016005

Chapter 6
Reproductive Ecology of Lampreys

Nicholas S. Johnson, Tyler J. Buchinger and Weiming Li

Abstract Lampreys typically spawn in riffle habitats during the spring. Spawning activity and diel (i.e., during daylight and at night) behavioral patterns are initiated when spring water temperatures increase to levels that coincide with optimal embryologic development. Nests are constructed in gravel substrate using the oral disc to move stones and the tail to fan sediment out of the nest. Spawning habitat used by individual species is generally a function of adult size, where small-bodied species construct nests in shallower water with slower flow and smaller gravel than large-bodied species. The mating system of lampreys is primarily polygynandrous (i.e., where multiple males mate with multiple females). Lamprey species with adult total length less than 30 cm generally spawn communally, where a nest may contain 20 or more individuals of both sexes. Lamprey species with adult sizes greater than 35 cm generally spawn in groups of two to four. Operational sex ratios of lampreys are highly variable across species, populations, and time, but are generally male biased. The act of spawning typically starts with the male attaching with his oral disc to the back of the female's head; the male and female then entwine and simultaneously release gametes. However, alternative mating behaviors (e.g., release of gametes without paired courtship and sneaker males) have been observed. Future research should determine how multiple modalities of communication among lampreys (including mating pheromones) are integrated to inform species recognition and mate choice. Such research could inform both sea lamprey control strategies and provide insight into possible evolution of reproductive isolation mechanisms between paired lamprey species in sympatry.

Keywords Agnatha · Behavior · Heterospecific matings · Mate choice · Mating system · Pheromones · Sex ratio · Spawning habitat · Sympatric speciation

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6.1 Introduction

Biologists have long been fascinated by the reproductive behavior of lampreys and it is from their unique and signature nesting behavior (where the oral disc is used to move rocks) that lampreys (order Petromyzontiformes, "stone sucker") derive their name. In the late nineteenth century, naturalists reported unexpectedly discovering lampreys spawning in shallow riffle areas of clear headwater streams (Young and Cole 1900 and references therein). They watched with intrigue for hours and sometimes days as groups of lampreys remained vigorously entwined in what was taken as a romantic effort to contribute to the next generation during their terminal life stage. The allure of observing and characterizing lamprey reproduction has not diminished in the twenty-first century and is further motivated by the ecological, cultural, and economic importance of lamprey species around the world (see Chap. 1). An enhanced understanding of the reproductive ecology of lampreys is needed, both for the more than 20 lamprey species that are threatened or endangered in at least part of their range (Renaud 1997; see Chap. 8) and for control of the invasive sea lamprey in the Laurentian and other Great Lakes (see Marsden and Siefkes in press). Furthermore, as representatives of an ancient vertebrate lineage (Janvier 2010), lampreys provide a unique insight into vertebrate mating systems and sensory modalities.

The lampreys, one of the two surviving groups of agnathan (jawless) vertebrates, currently consist of at least 41 recognized species (see Chaps. 2 and 8). They exhibit an antitropical distribution (Renaud 2011); the four Southern Hemisphere species are placed in families Geotriidae (one species) and Mordaciidae (three species), and the remaining 37–40 Northern Hemisphere species are placed in Petromyzontidae (see Chap. 2). All lampreys pass through a prolonged filter-feeding larval stage (see Chap. 3). Following a dramatic metamorphosis (see Chap. 4), 18 species are parasitic, feeding on the blood or tissue of actinopterygian fishes or other vertebrates in marine or freshwater systems (see Renaud and Cochran in press). Some of the anadromous species (e.g., sea lamprey *Petromyzon marinus*, Pacific lamprey *Entosphenus tridentatus*, and pouched lamprey *Geotria australis*) can reach total lengths (TL) in excess of 60–80 cm (see Docker and Potter in press) and can migrate several 100 km to headwater streams to spawn (see Chap. 5); lampreys that are parasitic in fresh water are smaller at maturity (20–30 cm). The remaining 23–26 species are non-parasitic "brook" lampreys; they bypass the adult feeding phase (thus maturing at lengths of approximately 11–14 cm) and remain within their natal streams (see Docker 2009). Most parasitic lamprey species are "paired" with one or more non-parasitic species; these paired species are morphologically and genetically similar and generally overlap in their distribution (Docker 2009; Docker and Potter in press). Paired lamprey species are examples of possible sympatric speciation through assortative mating (Beamish and Neville 1992; Salewski 2003).

The lamprey spawning stage, although the shortest life stage, is most commonly documented due to its relatively high accessibility for observation compared to the larval and parasitic stage. Lampreys spawn during daylight and at night, typically

in large groups, and are not easily disturbed. However, accounts of lamprey reproductive ecology, although extensive and informative, are scattered in the literature and commonalities or differences among species are infrequently summarized. Furthermore, many studies may be inaccessible to English-speaking readers (e.g., are in Russian and German), are often in the older literature, and generally provide descriptions of the spawning behavior of a single species. Two insightful reviews by Hardisty and Potter (1971) and Malmqvist (1986) amalgamated studies describing lamprey reproduction, but there have been few recent efforts to synthesize new discoveries concerning lamprey mating systems, spawning site selection, reproductive behavior, and communication modalities (Jang and Lucas 2005).

This chapter therefore provides an updated synthesis of the reproductive ecology of lampreys. In many cases, information from the older papers referred to above has been taken from the species accounts provided in *The Freshwater Fishes of Europe*, Volume 1, Part 1, Petromyzontiformes (e.g., Holčík 1986a, b, c; Hardisty 1986a, b, c); interested readers are referred to the references therein. Topics will be presented chronologically, beginning with the migration to the spawning grounds and concluding with senescence. Discussions will be focused on those species that have been most intensively studied; hypotheses will be proposed to explain commonalities and differences among species and existing knowledge gaps will be highlighted.

6.2 Migration and Environmental Control of Spawning Behavior

6.2.1 Migration to Spawning Habitat

Lamprey upstream migration has been reviewed in this volume (Chap. 5), but highlights will be briefly reiterated here to provide background for discussions of reproductive ecology. In nearly all cases, lampreys have been observed spawning in streams (but see Sect. 6.3.2). Parasitic lamprey species, which may be displaced over hundreds of kilometers by host fishes, must first locate streams that are suitable for reproduction. Migration has been studied extensively in sea lamprey which—unlike salmonids—do not home to their natal streams (Bergstedt and Seelye 1995; Waldman et al. 2008). Instead, adult sea lamprey use their olfactory system to locate streams containing migratory pheromones excreted by conspecific larvae (Sorensen et al. 2005; Meckley et al. 2012). In the Great Lakes ecosystem, although some sea lamprey migrate into streams without significant larval populations (as evidenced by the rapid re-infestation of streams recently treated with lampricide), adults are more likely to enter streams that contain large numbers of larvae (Moore and Schleen 1980), presumably because larvae emit bile acids that are highly attractive to sexually immature migratory-phase adults (Sorensen et al. 2005). Once a suitable stream is located, migration into specific tributaries continues to be directed by larval odor (Wagner et al. 2006). Recent genetic evidence suggests that Pacific

lamprey likewise do not home to their natal streams (Goodman et al. 2008; Spice et al. 2012), and it appears that migratory pheromones emitted by larvae are conserved among lamprey species (Jellyman et al. 2002; Fine et al. 2004; Gaudron and Lucas 2006; Robinson et al. 2009; Yun et al. 2011).

The duration of the pre-spawning migration is highly variable among and within lamprey species (see Chap. 5). For example, sea lamprey migration occurs over a period of 2–3 months (Applegate 1950), while pouched lamprey migration occurs over 15–16 months (Potter et al. 1983). The reasons for such differences in the duration of migration remain unknown; they are not explained solely by differential migration distance, as sea lamprey and pouched lamprey migrate over similar distances. European river lamprey *Lampetra fluviatilis* have distinct autumn and spring spawning runs (Maitland et al. 1994) and have been found in estuaries (i.e., at the beginning of the upstream migration) between July and April (Abou-Seedo and Potter 1979). Pre-spawning migrations of non-parasitic species have also been documented in populations of the Far Eastern brook lamprey *Lethenteron reissneri* (Takayama 2002) and European brook lamprey *Lampetra planeri* (Hardisty 1961a; McIntyre 1969), but such migrations—which only need to be sufficient to correct for downstream larval drift—are typically less than 20 km and confined to their natal watershed (Hardisty 1944; Malmqvist 1980; Takayama 2002).

Natural barriers such as waterfalls and man-made barriers such as dams often limit access to spawning areas and are serious impediments to lamprey restoration efforts (Renaud 1997; Close et al. 2002; see Chap. 8), but are advantageous for the control of invasive sea lamprey in the Great Lakes (Lavis et al. 2003; Marsden and Siefkes in press).

6.2.2 Environmental Control of Adult Lamprey Behavior

The most critical environmental factor influencing the timing of the spawning migration, nest construction, and spawning itself is water temperature (Hardisty and Potter 1971), although pheromones may also play a significant role (see Sect. 6.6.1). Upstream migration is most intense during periods when water temperature and stream flow increase in the spring (Hardisty and Potter 1971; Robinson and Bayer 2005; Binder and McDonald 2010; see Chap. 5). Sexually immature adults begin to migrate at night when water temperatures are generally 2–6 °C below the temperatures at which spawning occurs (see below). Arrival at the spawning grounds coincides with the occurrence of water temperatures appropriate for spawning, the onset of diel behavioral patterns (i.e., showing activity during the day and night; Binder and McDonald 2008a), and the final stages of sexual maturation (Docker et al. in press). Temperature regulation of migration and spawning behavior probably developed in response to the strict thermal requirements for embryonic development (Clemens et al. 2010). Sea lamprey development, for example, occurs at temperatures between 15 and 25 °C (Piavis 1961; McCauley 1963); Pacific lamprey and western brook lamprey *Lampetra richardsoni* development is optimal between 10 and 18 °C (Meeuwig et al. 2005).

Northern Hemisphere lampreys generally spawn during the spring at temperatures ranging from 6 to 26 °C (Table 6.1). Given the importance of elevated water temperature in initiating spawning activity, spawning generally occurs later at higher (i.e., more northerly) latitudes than at lower latitudes. Lampreys occupying higher latitudes generally spawn between April and June: for example, Heard (1966) found ripe and spent Arctic lamprey *Lethenteron camtschaticum* in the Naknek River, Alaska (at approximately 58°40′N) in June; Pletcher (1963) observed western brook and Pacific lampreys spawning in the Salmon River, British Columbia (50°29′N), from April to June; and spawning chestnut lamprey *Ichthyomyzon castaneus* were observed in the Rat River, Manitoba (49°35′N) in mid-June. Sea lamprey in the upper Great Lakes generally spawn in June (although the spawning season may extend from May until September; Manion and Hanson 1980); in the anadromous sea lamprey in Connecticut and Maine, spawning occurs in late May to late June (Gardner et al. 2012). American brook lamprey *Lethenteron appendix* in the upper Great Lakes and Quebec spawn in late April to mid-May (Morman 1979; Mundahl and Sagan 2005), but have been observed spawning as early as March (Cochran et al. 2012). Lampreys occurring at southern latitudes generally spawn at similar temperatures but earlier in the spring or, in some cases, in the winter. Southern brook lamprey *Ichthyomyzon gagei* in Alabama (at approximately 32°30′N) were observed spawning in mid-April to early May (Beamish 1982), and populations of American brook lamprey at the southern edge of this species' range (L'Eau Frais Creek in Arkansas; 34°06′N) spawn in early March (Tumlison and Tumlison 1999). Even more dramatically, two lamprey species found at approximately 20° N (the Mexican lamprey and Mexican brook lamprey, *Tetrapleurodon spadiceus* and *T. geminis*, respectively) reproduce from November to January (Hardisty and Potter 1971).

Spawning seasons may also not be as coordinated and condensed at lower latitudes, presumably because water temperatures are suitable for embryonic development for several months, whereas temperatures at higher latitudes may only be suitable for a few weeks during the late spring. Cochran et al. (1996) postulated that the spawning period of the two Mexican species may exceed 6 months. Similarly, Renaud (1982) documented Macedonia brook lamprey *Eudontomyzon hellenicus* with developed secondary sex characteristics (see Sect. 6.5) in both January and May in Kefalárion Brook (at approximately 37°36′N), and suggested that this represents two distinct spawning periods. Ahmadi et al. (2011) reported Caspian lamprey *Caspiomyzon wagneri* in the final stages of maturity in both the fall and spring (at 34°44–50′N).

Spawning has not been described in any of the four Southern Hemisphere species (see Sect. 6.4.3.1), although the spawning period has been inferred from museum collections of advanced spawning-run adults and the appearance of the young-of-the-year larvae. In this manner, Potter (1970) inferred that short-headed lamprey *Mordacia mordax* spawn in the Moruya River in New South Wales (35°55′S) in the late austral winter or early spring (i.e., August to November) and Maskell (1929), Potter et al. (1983), and Potter and Hilliard (1986) likewise estimated that pouched lamprey spawn over a period of several months during the austral spring and winter.

Table 6.1 List of 22 Northern Hemisphere lamprey species, their adult feeding type (P = parasitic, NP = non-parasitic), migratory type (A = anadromous, F = freshwater), most prevalent mating system, other species that have been observed in the same nest (Heterospecific with), temperature at which spawning occurs, and number of adults observed on a nest (mean and/or range). In the reference column, "inferred" means that mating system was categorized by the authors based on references listed for that species

Species	Feeding type	Migratory type	Mating system	Heterospecific with	Temperature (°C)	Number on nest	Reference
Ichthyomyzon							
Silver lamprey *I. unicuspis*	P	F	Polygynandry				Inferred
				Sea, northern brook, American brook	13–23	2.2 (1–10)	Morman (1979)
					18.2	1–15	Cochran and Lyons (2004)
Northern brook lamprey *I. fossor*	NP	F	Polygynandry				Inferred
				Silver, sea	13–23	6.7 (3–13)	Morman (1979)
Chestnut lamprey *I. castaneus*	P	F	Polygynandry				Inferred
				Southern brook			Cochran et al. (2008)
				Sea, American brook	16–22	1.3 (1–4)	Morman (1979)
					17	c.50	Case (1970)
Southern brook lamprey *I. gagei*	NP	F	Polygynandry				Inferred
				Chestnut			Cochran et al. (2008)
					15–22		Cochran and Gripentrog (1992)
					14–24		Beamish (1982)
						Up to 20	Dendy and Scott (1953)
Mountain brook lamprey *I. greeleyi*	NP	F	Polygynandry	Ohio			Cooper (1983)
					18.8	5–9	Raney (1939)

Table 6.1 (continued)

Species	Feeding type	Migratory type	Mating system	Heterospecific with	Temperature (°C)	Number on nest	Reference
Petromyzon							
Sea lamprey *P. marinus*	P	A, F	Monogamy, polygyny				Inferred
			Lentic spawning (still-water enclosure in river)				Scott (1957)
				Chestnut, silver, American brook, northern brook	11–26	2.4 (1–10)	Morman (1979); Cochran et al. (2008)
					10–26	2–6	Applegate (1950); Beamish and Potter (1975); Manion and Hanson (1980); Gardner et al. (2012)
Caspiomyzon							
Caspian lamprey *C. wagneri*	P	A	–	–	15–23	–	Nazari and Abdoli (2010); Holčík (1986a)
Eudontomyzon							
Ukrainian brook lamprey *E. mariae*	NP	F	–	–	8–13.5	–	Holčík and Delić (2000)
					11–12		Abakumov (1960)
Tetrapleurodon							
Mexican lamprey *T. spadiceus*	P	F	–	–	16.2–22.5	–	Alvarez del Villar (1966)

Table 6.1 (continued)

Species	Feeding type	Migratory type	Mating system	Heterospecific with	Temperature (°C)	Number on nest	Reference
Mexican brook lamprey *T. geminis*	NP	F	–	–	16.2–22.5	–	Alvarez del Villar (1966)
Entosphenus							
Pacific lamprey *E. tridentatus*	P	A, F	Monogamy, polygyny				Inferred
				Western brook			Brumo (2006)
					10.1–17.3	1–3	Stone (2006)
					10–15		Robinson and Bayer (2005)
			Lentic spawning (c. 1%)			2	Russell et al. (1987)
Vancouver lamprey *E. macrostomus*	P	F	Lentic spawning	–	–	–	Beamish (1987)
Miller Lake lamprey *E. minimus*	P	F	Polygyny				Inferred
				–	12	5	Lorion et al. (2000)
			Lentic spawning				Kan and Bond (1981)
Lethenteron							
Arctic lamprey *L. camtschaticum*	P	A, F	Polygynandry				Inferred
				Anadromous, "praecox," and non-parasitic morphs (possibly Far Eastern brook lamprey)		6–44	Savvaitova and Maksimov (1979); Kucheryavyi et al. (2007a)
					12–15	5–8	Heard (1966)

Table 6.1 (continued)

Species	Feeding type	Migratory type	Mating system	Heterospecific with	Temperature (°C)	Number on nest	Reference
Siberian brook lamprey *L. kessleri*	NP	F	Polygynandry				Inferred
					8.7–13	Up to 6–8	Holčík (1986b)
Far Eastern brook lamprey *L. reissneri*	NP	F	Polygynandry	Possibly Arctic lamprey			Savvaitova and Maksimov (1979); Kucheryavyi et al. (2007a)
					6.0–12.2	13	Takayama (2002)
Alaskan brook lamprey *L. alaskense*	NP	F	Polygynandry	–	–	–	Vladykov and Kott (1978)
American brook lamprey *L. appendix*	NP	F	Polygynandry	Sea, chestnut, silver			Renaud (2011)
			Lentic spawning		7–21	2.6 (1–9)	Morman (1979)
					9–16	4.2 (2–14)	Mundahl and Sagan (2005)
						Up to 20	Gage (1928)
Lampetra							
European river lamprey *L. fluviatilis*	P	A, F	Polygynandry				Inferred
			Lentic spawning	European brook			Huggins and Thompson (1970); Hardisty (1986a, c); Lasne et al. (2010)
					11		Hagelin and Steffner (1958)
					8	12–16	Jang and Lucas (2005)

Table 6.1 (continued)

Species	Feeding type	Migratory type	Mating system	Heterospecific with	Temperature (°C)	Number on nest	Reference
European brook lamprey *L. planeri*	NP	F	Polygynandry				Malmqvist (1983); Hardisty (1986b)
				European river			Huggins and Thompson (1970); Hardisty (1986a, c); Lasne et al. (2010)
					10		Hardisty (1961a)
					12.3	3	McIntyre (1969)
					8.6–17.7	2 (1–13)	Rooney et al. (2013)
Western brook lamprey *L. richardsoni*	NP	F	Polygynandry				Inferred
				Pacific			Brumo (2006)
					9.4–16.0	2–12	Stone (2006)
					10.6–11.1		Schultz (1930)
			Lentic spawning (still-water tanks)				Russell et al. (1987)
Least brook lamprey *L. aepyptera*	NP	F	–	–	10		Trautman (1957)
						10–12	Brigham (1973)

Water temperature trends have large impacts on nesting and spawning activity. For a particular population of lamprey at higher latitudes, spawning seasons are shortest when water temperatures are warm and stable and longest when water temperatures are low and unstable, resulting in sporadic spawning activity (Hardisty and Potter 1971). Spawning is most vigorous at high and stable water temperatures (Case 1970), but spawning activity can decrease or completely cease with sudden drops in water temperatures of even 1 or 2 °C. Reductions in mating behavior are especially prevalent early in the spawning season and when water temperatures drop during the day (Applegate 1950; Hardisty 1961a).

6.3 Spawning Habitat and Nest Construction

6.3.1 Size-Assorted Spawning Habitat

Spawning accounts in either Geotriidae or Mordaciidae have yet to be published (Renaud 2011). Therefore, descriptions of spawning habitat and behavior herein are limited to the family Petromyzontidae. In nearly all accounts, lampreys spawn in riffle habitats (i.e., shallow areas with fast, turbulent water running over rocks) located in streams. Streams with gradients between 2 and 6 m/km often contain productive spawning riffles and larval beds (Baxter 1954). Lamprey nests are most commonly observed at the head of shallow riffles at transition areas between run (i.e., a deep area with fast water and little or no turbulence) and riffle. Lamprey species have been reported to spawn at a variety of depths and water velocities, as long as there is unidirectional flow and gravel substrate (Schleen et al. 2003; but see Sect. 6.3.2). Although larval lampreys are found in fine sediment (see Chap. 3), spawning lampreys either avoid a substrate of fine particles (< 2 mm diameter; Gardner et al. 2012) or the nest-building activities themselves reduce the amount of silt (see Sect. 6.3.3.1). Smaller lampreys are known to use vacant nests constructed by larger-bodied species (Morman 1979), and European river and brook lampreys have been reported using nests constructed several months earlier by sympatric salmonids (Nika and Virbickas 2010).

Lamprey species of different adult size often spawn in specific microhabitats within riffles, where small-bodied species spawn on smaller gravel substrate, in shallower water, with slower velocity (Table 6.2). Small lampreys are likely restricted to spawning on smaller substrate because they are unable to move larger stones (Cochran and Lyons 2004) or navigate in high water velocities. Therefore, size-assorted spawning habitat preferences may function as an ecological barrier to hybridization of paired species (Beamish and Neville 1992; but see Sect. 6.4.3.5). In the Great Lakes basin, landlocked sea lamprey (mean adult size 35 cm TL, range 11–60 cm) construct nests in gravel substrate 0.9–5.1 cm in diameter, in water 10–170 cm deep, with velocities of 50–150 cm/s (Applegate 1950). Silver lamprey *Ichthyomyzon unicuspis* (mean adult size 28 cm, range 9–39 cm), a parasitic freshwater species that is sympatric with sea lamprey in the Great Lakes region, construct

Table 6.2 Adult length, spawning habitat, and nest size of 16 Northern Hemisphere lamprey species

Species	Adult size (cm)	Habitat			Nest size		Reference
		Water depth (cm)	Substrate diameter (cm)	Velocity (cm/s)	Width (cm)	Depth below substrate (cm)	
Ichthyomyzon							
Silver lamprey *I. unicuspis*	9–39	60	0.4–3	–	30	11	Morman (1979); Manion and Hanson (1980); Cochran and Lyons (2004)
Chestnut lamprey *I. castaneus*	9–36	38	1	–	60	5	Case (1970)
Southern brook lamprey *I. gagei*	9–13	15	–	35	–	–	Beamish (1982)
Mountain brook lamprey *I. greeleyi*	11–17	10–20	–	–	20–25	5	Raney (1939); Beamish and Medland (1988)
Petromyzon							
Sea lamprey (landlocked) *P. marinus*	11–60	13–170	0.9–5.1	50–150	45	20	Applegate (1950); Manion and Hanson (1980)
Sea lamprey (anadromous) *P. marinus*	60–90	40–60	1–5	100–200	40–225	20–40	Hardisty (1986b); Gardner et al. (2012); Sousa et al. (2012)
Entosphenus							
Miller Lake lamprey *E. minimus*	12–14	30	–	–	10	3	Lorion et al. (2000)
Pacific lamprey *E. tridentatus*	10–80	28	2.7	20	–	7	Beamish (1980); Stone (2006)
Lethenteron							
Arctic lamprey *L. camtschaticum*	11–63	10–30	3–5	60–80	20–50	5–10	Holčík (1986b); Kucheryavyi et al. (2007a)
Far Eastern brook lamprey *L. reissneri*	11–20	22	–	20	–	–	Takayama (2002)
American brook lamprey *L. appendix*	10–22	31	1–2	14	16	4	Manion and Hanson (1980); Mundahl and Sagan (2005)

Table 6.2 (continued)

Species	Adult size (cm)	Habitat			Nest size		Reference
		Water depth (cm)	Substrate diameter (cm)	Velocity (cm/s)	Width (cm)	Depth below substrate (cm)	
Lampetra							
European river lamprey *L. fluviatilis*	9–49	20–150	–	–	105	7	Jang and Lucas (2005); Nika and Virbickas (2010)
European brook lamprey *L. planeri*	9–17	10	0.2–1.9	70	28	4	Hardisty (1961a); McIntyre (1969); Nika and Virbickas (2010)
Western brook lamprey *L. richardsoni*	8–15	20	1.5	12	–	3	Stone (2006)
Least brook lamprey *L. aepyptera*	8–18	–	–	100	20	–	Brigham (1973)
Eudontomyzon							
Ukrainian brook lamprey *E. mariae*	12–22	20–30	–	10–15	5–10	–	Holčík and Renaud (1986)

nests in gravel substrate 0.4–3.0 cm in diameter (Manion and Hanson 1980), in water 47–68 cm deep (Cochran and Lyons 2004). American brook lamprey (mean adult size 16 cm, range 10–22 cm), which is broadly sympatric with sea and silver lampreys in the Great Lakes region, but is non-parasitic, constructs nests in gravel substrate 1.0–2.0 cm in diameter (Manion and Hanson 1980), in water 15–55 cm deep, at velocities 5–21 cm/s (Mundahl and Sagan 2005).

A similar relationship between lamprey body size and spawning habitat is observed in Europe and western North America. European river lamprey (mean adult size 30 cm, range 9–49 cm) construct nests in gravel 1.6–6.4 cm in diameter, in water 11–40 cm deep, at velocities 20–90 cm/s, whereas European brook lamprey (mean adult size 13 cm, range 9–17 cm) construct nests in gravel 0.8–3.2 cm in diameter, in water 8–33 cm deep, at velocities around 15–75 cm/s (Nika and Virbickas 2010; Rooney et al. 2013). Pacific lamprey (mean adult size 40 cm, range 10–80 cm) construct nests in gravel 2.7 cm in diameter, in water 28 cm deep, at velocities around 20 cm/s, whereas western brook lamprey (mean adult size 12 cm, range 8–15 cm) construct nests in gravel 1.5 cm in diameter, in water 20 cm deep, at velocities around 12 cm/s (Stone 2006).

Note, however, that heterospecific spawning associations involving both large- and small-bodied lampreys are not uncommon (Table 6.1, Sect. 6.4.3.3).

6.3.2 Alternative Spawning Habitats

Although spawning in riffle habitats is most common, alternative spawning habitats have been reported. Cochran and Gripentrog (1992) provided detailed accounts of chestnut lamprey, northern brook lamprey *Ichthyomyzon fossor*, and southern brook lamprey spawning beneath cover (e.g., boulders, woody debris and, at one site, vegetation). In these species, nesting beneath cover was most likely to occur in larger rivers, where swift currents may reduce accessibility to riffle habitats or where cover may have allowed spawning despite the faster-flowing conditions. Lampreys nesting beneath cover may also experience reduced predation, although this hypothesis has not been directly tested (Cochran and Gripentrog 1992). Gunckel et al. (2009), defining cover as any structure under which an adult lamprey could hide, found that 86 % of western brook lamprey nests were associated with a variety of cover types (predominantly large cobble substrates, but to a lesser extent, wood and vegetation), but found that only 43 % of Pacific lamprey nests—presumably because of their larger size—were associated with cover. Rooney et al. (2013) also reported a small number of European brook lamprey spawning among woody debris in the River Liffey, Ireland, but it appears that spawning beneath vegetation or woody debris is rare in lampreys. Holčík (1986a) reported that Caspian lamprey only rarely makes nests in areas with submerged vegetation, and some species (e.g., European brook lamprey, sea lamprey) show a preference for sunlit areas when spawning is in progress (Hardisty 1986a, b).

Lampreys have been observed spawning in depths greater than 5 m. Silver lamprey and sea lamprey spawn at depths greater than 5 m in the connecting channels of

the Great Lakes (Lamsa et al. 1980; Morse et al. 2003). Cochran and Lyons (2004) postulated that silver lamprey may spawn in swifter deeper water than other *Ichthyomyzon* species do, and observed silver lamprey spawning at deeper depths (>1 m) when present with sea lamprey. Caspian lamprey eggs have been found at depths ranging from 3.5 to 19 m (see Holčík 1986a), suggesting that the adults may spawn at depths greater than 5 m. Use of deepwater sampling equipment such as suction dredges (e.g., Beamish and Youson 1987; Taverny et al. 2012) and deepwater electrofishers (e.g., Jolley et al. 2012) are increasingly detecting larval lampreys of various species (anadromous sea lamprey, Pacific lamprey, and North American river lamprey *Lampetra ayresii*) in deep water in large river systems (see Chap. 3). It is not known, however, to what extent the presence of larvae in these habitats is the result of deepwater spawning or downstream movement of larvae from smaller tributaries.

Spawning in lakes (lentic spawning)—or at least spawning in the absence of a unidirectional current—has been reported in Vancouver lamprey *Entosphenus macrostomus*, Miller Lake lamprey *Entosphenus minimus*, and Pacific, western brook, American brook, and landlocked sea lampreys (Table 6.1). This type of spawning in Pacific, sea, and western brook lampreys is a rare deviation from their typical stream riffle spawning habitat. For example, although Russell et al. (1987) observed anadromous Pacific lamprey spawning in shallow water in two regions of the Babine Lake system in British Columbia, the frequency of lentic to lotic spawning (i.e., spawning in flowing water) in this system was low. Likewise, a sea lamprey pair was reported to have successfully spawned in a no-flow enclosure of a small river (Scott 1957), but this is considered rare, and most occurrences of sea lamprey larvae in lentic habitats are thought to be the result of downstream movement (e.g., during periodic floods) from the lower portions of rivers (Fodale et al. 2003). In contrast, Vancouver lamprey primarily spawn on shallow gravel bars in nearshore lake habitat, although some spawning also likely occurs in streams (Beamish 1987). Lentic spawning in Vancouver and Miller Lake lampreys may have developed as a reproductive adaptation after becoming landlocked (Russell et al. 1987). This observation is relevant to management of landlocked sea lamprey in the Great Lakes, where chemical control effectively kills larvae in streams, but lentic treatments of larvae are much less effective and more costly (Schleen et al. 2003). If lentic spawning is a genetically-linked trait, highly effective chemical control of sea lamprey larvae in streams with less effective treatments in lentic environments may favor selection of lentic spawning in landlocked sea lamprey (Russell et al. 1987).

6.3.3 Nest Construction, Size, and Function

6.3.3.1 Nest Construction

Lampreys construct nests using their oral disc to move stones and their tail to fan small gravel and silt out of the nest site. Nest construction of landlocked sea lamprey has been described in detail and will be used to highlight typical behaviors. Male sea lamprey have been observed initiating nest construction up to 8 weeks

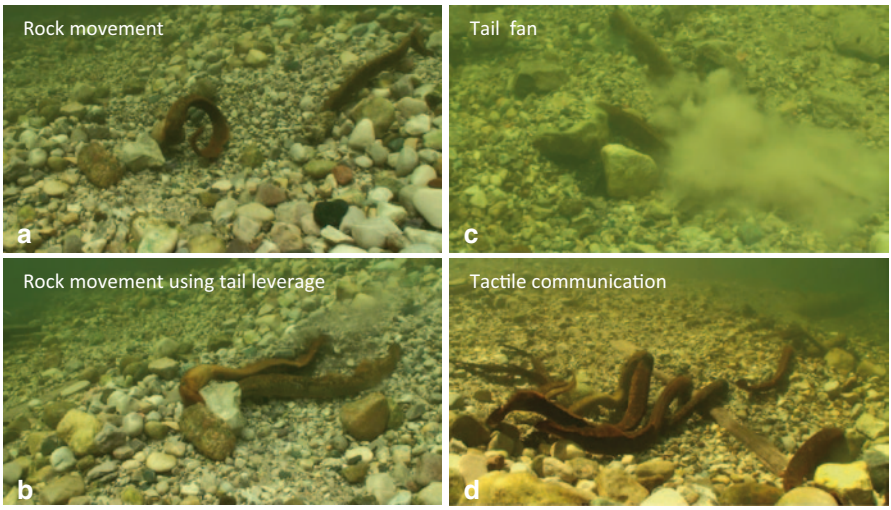

Fig. 6.1 Typical lamprey nesting behaviors. Photos are of sea lamprey spawning in the Cheboygan River, MI. **a** Rock movement by male. **b** Movement of large rock by leveraging tail against substrate. **c** Female cleaning sediment from the nest using rapid tail movements. **d** Tactile communication between male and female with oral discs. (Photos: Cory O Brant)

prior to spawning (Applegate 1950). Nest construction occurs day and night when water temperatures are suitable for mating (Table 6.1). Early in the spawning season, individual male sea lamprey construct several small nests and reside in one until joined by a female (Applegate 1950; Manion and Hanson 1980). Males vigorously defend nests from other males by attaching to intruding males and violently twisting and shaking as the current pushes them downstream of the nest (Applegate 1950; Manion and Hanson 1980). The victor, whether it was the male that established the nest or the intruder, quickly returns to the nest and awaits the arrival of a female. Male to male aggression has also been reported in European brook lamprey (Malmqvist 1983; Hardisty 1986a), European river lamprey (Hagelin and Steffner 1958), American brook lamprey (Young and Cole 1900), and in Siberian brook lamprey *Lethenteron kessleri* (Holčík 1986b), but not in Arctic lamprey (Heard 1966), Pacific lamprey (Brumo 2006; Stone 2006), or silver, chestnut, and northern brook lampreys (Manion and Hanson 1980).

Males and females participate in collaborative nest construction and rearrangement prior to and during spawning (e.g., Savvaitova and Maksimov 1979; Manion and Hanson 1980; Holčík 1986a, b, c; Sousa et al. 2012). Using the oral disc, lampreys latch onto stones and move them downstream using the assistance of the current, although sometimes stones are moved upstream and side stream (Fig. 6.1a). Large rocks are dragged from the nest by arching the back and leveraging the tail against the bottom to dislodge them (Fig. 6.1b). A few large stones typically remain at the upstream rim of the nest and are used as oral disc attachment points during nest cleaning and mating. Lampreys clear nests of silt by latching to a large rock in the upstream portion of the nest and vigorously fanning their tail laterally

(Fig. 6.1c). Rapid tail fanning stirs up sediment that is carried out of the nest by the current.

Deviations from typical lamprey nesting behavior have been reported. In European river lamprey, males arrive at the spawning riffles first, but females initiate nest construction (Jang and Lucas 2005). Female nest construction in European river lamprey is likely related to their generally promiscuous mating system (Jang and Lucas 2005), and potentially when there is a preponderance of females (see Sect. 6.4.2). Northern brook lamprey orient their body vertically, rather than horizontally as do most lampreys (Scott and Crossman 1973).

6.3.3.2 Nest Size

Nest size is generally a function of the body size of the species involved and the number of spawning adults present. For example, Great Lakes lamprey species excavate smaller nests with decreasing species size (Table 6.2). Landlocked sea lamprey nests are constructed to an average of 45 cm wide, 40 cm long, and 20 cm deep (Applegate 1950); silver lamprey nests are constructed to an average of 30 cm wide and 11 cm deep (Manion and Hanson 1980); and American brook lamprey nests are constructed to an average of 16 cm wide, 16 cm long, and 4 cm deep (Mundahl and Sagan 2005). The trend is also consistent in Europe where river lamprey nests average 105 cm wide, 129 cm long, and 7 cm deep; and average nest size for the much smaller European brook lamprey is 28 cm wide, 29 cm long, and 4 cm deep (Nika and Virbickas 2010). On both sides of the Atlantic, nests of the large anadromous sea lamprey may exceed 100–200 cm along their longest diameter (Hardisty 1986b; Gardner et al. 2012; Sousa et al. 2012). In the Coura River in Portugal, for example, where spawning sea lamprey averaged 882 mm total length, maximum nest length ranged from 80 to 225 cm (average 149 cm) and nest depth varied between 20 and 40 cm (average 28 cm; Sousa et al. 2012). Within a species, nest diameter and depth increases with the number of lamprey occupying the nest (Mundahl and Sagan 2005), and Rooney et al. (2013) reported the occurrence of "super redds," where smaller adjacent nests had merged into a single larger structure more than 40 cm wide.

6.3.3.3 Utility of Nest Physical Characteristics

The physical characteristics of the nest facilitate spawning behavior and embryo survival. A large rock located at the upstream rim of the nest (an "anchor") is central to nest cleaning and egg deposition in the nest. Without an anchor, the vigorous act of spawning will dislodge the pair, causing eggs to be scattered outside of the nest. The physical design of the nest produces upwelling of the water current, which increases oxygenation and reduces siltation (White 1990). Embryos retained in the nest have high survival rates (90% in sea lamprey, Manion 1968; Manion and Hanson 1980), likely due to reduced predation by fishes and crustaceans, increased oxygenation, and minimal silt accumulation. However, Smith and Marsden (2009) estimated that

85 % of sea lamprey eggs are not deposited within the nest. Eggs dislodge from nests stochastically and embryo survival outside the nest is nearly zero percent under most conditions, primarily due to predation and increased siltation (Smith and Marsden 2009). However, when stream flow is low, eggs that do not remain in the nest settle into gravel outside the nests and are thus better protected, resulting in higher production of embryos than during high stream flows (Smith and Marsden 2009).

6.4 Mating Systems, Sex Ratios, and the Spawning Act

6.4.1 Mating Systems

The reproductive behaviors of 16 lamprey species have been described in the literature sufficiently to allow a mating system to be assigned (Table 6.1 and references therein). However, mating system plasticity has been documented in most species. Polygynandry, defined here as multiple males mating with multiple females, is the most prevalent mating system in lampreys.

In species where mean adult size is less than 30 cm TL, communal spawning of multiple males and females is common. Spawning aggregations of over 20 individuals have been observed in American brook lamprey (Young and Cole 1900), European brook lamprey (Lasne et al. 2010), southern brook lamprey (Cochran et al. 2008), and Far Eastern brook lamprey (Takayama 2002). Small parasitic species such as silver, chestnut, Arctic, and European river lampreys are also polygynandrous communal spawners (Case 1970; Morman 1979; Savvaitova and Maksimov 1979; Jang and Lucas 2005; Kucheryavyi et al. 2007a), with up to 50 chestnut lamprey (Case 1970) and 44 Arctic lamprey (Savvaitova and Maksimov 1979) having been observed spawning in a single nest. Kucheryavyi et al. (2007a), however, also observed spawning in pairs in Arctic lamprey. In the European river lamprey, males are promiscuous and are found in multiple nests across a large area, while females remain in single nests (Jang and Lucas 2005).

Pacific and sea lampreys, which are greater than 35 cm in length as adults, have been described as monogamous tending toward polygyny, with generally fewer than five individuals per nest (Applegate 1950; Brumo 2006; Stone 2006; Gardner et al. 2012). However, large-bodied parasitic lamprey species show greater variation in mating systems than non-parasitic and small parasitic species. For example, the sea lamprey mating system can change through the spawning season from monogamy to polygyny (Applegate 1950). A genetic analysis of embryos produced by a known population of spawning sea lamprey showed that female sea lamprey may visit multiple nests and that males and females mate with several different partners (Gilmore 2004). If indeed this occurs widely in sea lamprey, the mating system of this species could also be described as polygynandrous. However, it appears that individual male sea lamprey most commonly defend nests from other males and females visit multiple nests, and polygynandry is common only in small-bodied species that spawn communally.

Communal spawning in lamprey species with adult sizes less than 30 cm TL may be an adaptation to increase embryo survival through increased egg retention in nests. In non-parasitic and small parasitic species, a communal group of males and females can construct larger nests than an individual male of the same species. Several Siberian brook lamprey together have been observed pushing away heavier stones during nest construction (see Holčík 1986b). In contrast, individual male sea and Pacific lampreys can construct sufficiently large nests on their own. Presumably larger nests would retain more eggs than smaller nests and embryo survival is positively correlated to egg retention in a nest (Smith and Marsden 2009). Conversely, communal spawning may also increase the probability of eggs being displaced from the nest by repeated spawning events and nest construction. However, McIntyre (1969) observed male European brook lamprey covering spawned eggs with gravel after each copulation event; such nest rearrangement might aid in egg retention. The hypothesis that communal spawning is an adaptation to increase egg retention in nests has not been experimentally evaluated.

6.4.2 Operational Sex Ratio

Operational sex ratios of spawning lampreys (i.e., the number of sexually competing males that are ready to mate relative to the number of sexually competing females that are ready to mate) vary across species, across populations of the same species, and through time within a population. A generally small, but variable, excess of males is present among most spawning adult lampreys (Hardisty 1954, 1961b; Hardisty and Potter 1971; Beamish 1982; Takayama 2002; Nazari and Abdoli 2010), particularly in non-parasitic species (Purvis 1970; Mundahl and Sagan 2005). However, exceptions do occur. Mundahl and Sagan (2005) report an overall sex ratio of 1:1 (male to female) in spawning American brook lamprey, but noted variability among streams, where one stream had more males and another more females. Other studies documented American brook lamprey sex ratios as high as 1:5 (Seagle and Nagel 1982). The sex ratio of adult Caspian lamprey was recently reported as 1:1 (Nazari and Abdoli 2010; Ahmadi et al. 2011), but has been reported as high as 3:1 (Ghasempouri 1993). A nearly equal number of European river lamprey males and females were observed over an entire mating season (Jang and Lucas 2005).

In several species, males are generally first to arrive at the spawning grounds (e.g., in American brook lamprey, Young and Cole 1900; sea lamprey, Applegate 1950; and southern brook lamprey, Beamish 1982), and the proportion of females thus tends to increase over the course of the spawning season (Applegate 1950). A similar shift toward a greater proportion of females later in the spawning season was reported in a population of European brook lamprey (Hardisty 1961a), although Pletcher (1963) reported the opposite for western brook lamprey in British Columbia. Jang and Lucas (2005) provide a systematic account of changes in operational sex ratio in a population of European river lamprey. During nest construction, females outnumbered males 1:3.5. Sex ratio then shifted to a preponderance of males (1:0.4) in spawning aggregations, and back again to a preponderance of females

in post-spawning aggregations (1:3.7). Shifts in operational sex ratio through the season could be attributed to sex differences in timing of maturation, longevity, or chemical signaling (Pletcher 1963; Jang and Lucas 2005), but this has yet to be investigated in detail.

At the population level, annual changes in adult lamprey sex ratios have been correlated with relative abundance. Hardisty (1961b) found a significant correlation between annual variability in adult sex ratios (1:1.2–1:3.4) and adult relative abundance in an isolated population of European brook lamprey. Similar changes in sex ratios have been observed in adult landlocked sea lamprey after chemical control of larval populations; an excess of males was observed when sea lamprey abundance was high but shifted to female-biased sex ratios following initiation of control measures (Applegate 1950; Wigley 1959; Torblaa and Westman 1980). Evidence for density-dependent sex determination in lampreys is discussed in Chap. 3 and Docker et al. (in press).

Operational sex ratios can influence nesting behavior and mating systems in lampreys. For example, although males often arrive first at the spawning grounds and initiate nest construction, female sea lamprey are also known to initiate nest construction when they are numerically dominant late in the season (Applegate 1950). Furthermore, mating system plasticity has been documented in most species and is often correlated to operational sex ratio. For example, the sea lamprey mating system can change from monogamy early in the mating season to polygyny late in the season (see Sect. 6.4.1), and mating system differences observed in European river lamprey may be related to differences in sex ratio or size of mating groups (Hagelin and Steffner 1958; Hagelin 1959; Jang and Lucas 2005).

6.4.3 The Spawning Act

6.4.3.1 General Spawning Description

Spawning has not been reported for any of the four Southern Hemisphere lamprey species. Glova (1995) and Jellyman et al. (2002) attempted to observe spawning in pouched lamprey by keeping adults in tanks for a year or more and either radio-tagging and releasing them just before sexual maturation for observation in a natural setting (Jellyman et al. 2002) or providing them in the laboratory with conditions thought suitable for spawning (Glova 1995); in neither case was spawning observed. Anecdotal reports that pouched lamprey are capable of moving tennis ball-sized stones with their oral discs (Renaud 2011) implies that spawning has been observed, presumably by Māori fishermen, for whom lampreys have historically had great value as a food source. Although speculative, it is conceivable that recent biologists have not observed spawning in Geotriidae or Mordaciidae because the spawning habitat or mating behavior of these lampreys is considerably different than what has been characterized for the family Petromyzontidae.

Within Petromyzontidae, described spawning behaviors are generally analogous among species. Hardisty and Potter (1971) reviewed mating behaviors in lampreys

(see Table 6.1 for additional references). A thorough description of European river lamprey spawning behavior has been provided by Hagelin and Steffner (1958) and Hagelin (1959). Applegate (1950) provided a useful description of sea lamprey spawning behavior. Pletcher (1963) described spawning behavior in western brook and Pacific lampreys; Brumo (2006) and Stone (2006) provide brief accounts of Pacific lamprey spawning. Species not listed in Table 6.1 have little or no information in the literature concerning spawning behavior. Common spawning behaviors will be highlighted here with a brief discussion and illustrations (Fig. 6.2). As far as the present authors can ascertain, all descriptions of lamprey spawning have had their origin from daytime observations.

Lampreys aggregated on a nest actively engage in courtship and nest maintenance behaviors when not copulating. Little is known about when spermiation and ovulation occur relative to the time active spawning begins. Copulations occur every few minutes during active spawning, but respites of over an hour can occur. Studies of European river lamprey indicate that copulation in this species is initiated by the female. In what has been described as courting behavior to signal readiness to males, female European river lamprey swim in circles over males occupied with nest building (Hagelin 1959 and references therein). Then, immediately prior to mating, the female will attach to a large rock at the upstream rim of the nest. A receptive male responds by gliding his head along the female's body from tail to head and, sometimes only briefly (for < 1 s), attaches to the female's head. Mating occurs, however, when male gliding is followed by firm attachment to the female's head. As the male attaches, he wraps his tail around the female, sliding and squeezing his tail in a posterior direction stopping at a few centimeters anterior of the female's urogenital region. The female responds to the male's tail wrapping and sliding by violently vibrating. The male vibrates together with the female, and with backs arched, gametes are simultaneously released. Fertilization occurs externally, with the male's genital papilla directing sperm toward the eggs. About 5 s pass from the male glide to gamete release. The number of eggs released per mating event has been reported to range from 10 to 50 in European brook lamprey (McIntyre 1969; Malmqvist 1983) and from 20 to 40 in landlocked sea lamprey (Applegate 1950). Eggs are highly adhesive and readily attach to stones and sand on the downstream rim of the nest. After gamete release, the pair unwinds and continues nest construction and maintenance. Occasionally after mating, the male and female will lay still side-by-side in the nest for several minutes. In the laboratory, females are typically spent after 1–3 days of active mating (Hagelin and Steffner 1958), but males may spawn for up to a week. Little empirical evidence is available to determine how long individuals actively spawn in the wild. Observations of European river lamprey suggest that individual lamprey only remain on spawning riffles for a few days (Jang and Lucas 2005).

6.4.3.2 Alternative Spawning Behaviors

Multiple lampreys can be involved in the act of spawning at the same time. Five chestnut lamprey have been observed attached to each other while spawning (Case

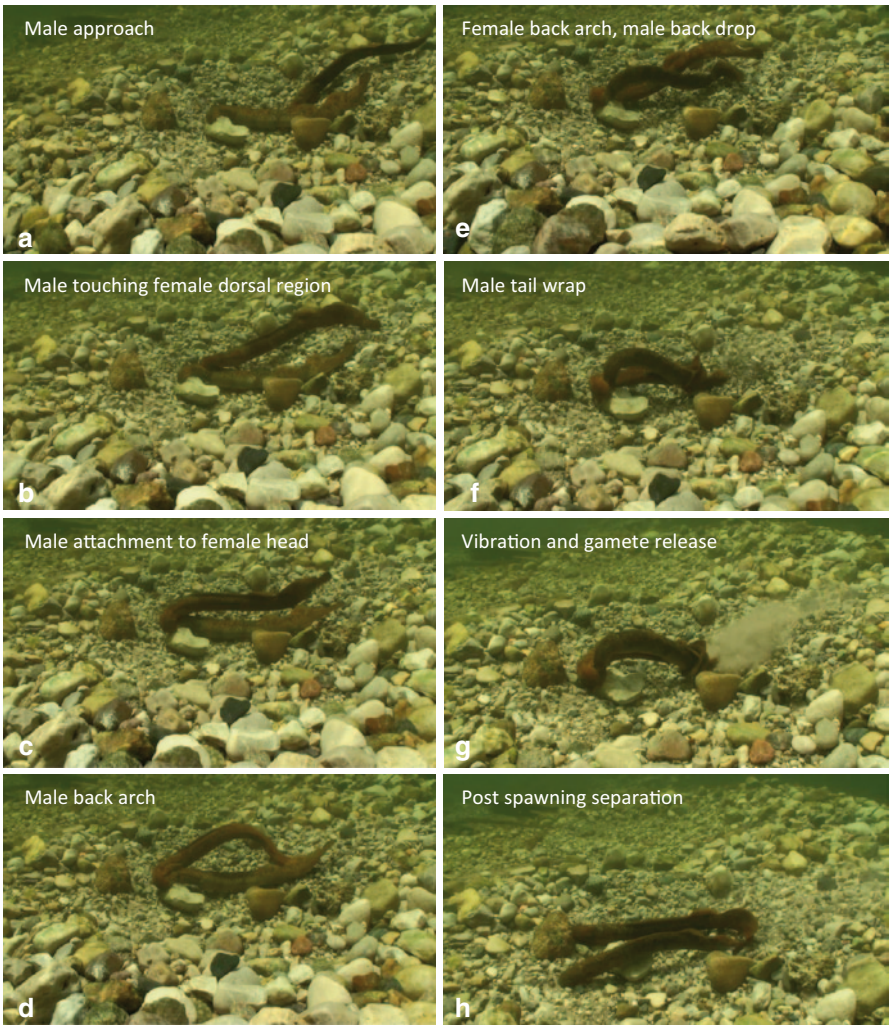

Fig. 6.2 Typical succession of behaviors during lamprey reproduction. Photos are of sea lamprey spawning in the Cheboygan River, MI. **a** Female attached to large rock at upstream end of the nest; male approaches the female from downstream. **b** Swimming along the female's back from posterior to anterior, the male touches the female dorsal region with its oral disc. **c** The male firmly attaches to the female's head with oral disc. **d** Immediately after the male attaches to the female's head, the male arches its back. **e** The female then arches its back allowing the male to slide its tail underneath the female's tail. **f** The male's tail tightly coils around the female between the dorsal fins, aligning their urogenital papillae. **g** Male and female vibrate violently stirring up sediment while releasing gametes. **h** Male and female release from each other after spawning. (Photos: Cory O Brant)

1970). Malmqvist (1983) reported several European brook lamprey coiled around a single female. Huggins and Thompson (1970) report two male European river lamprey mating with one female. Stone (2006) reported four male western brook lamprey coiled around two females.

An alternative reproductive behavior has been observed in European and American brook lampreys, where "satellite" males attempt to gain fertilizations by approaching the urogenital area of a mating pair and presumably attempting to fertilize the eggs as they are extruded by another male (Malmqvist 1983; Cochran et al. 2008). Recently, Hume et al. (2013a) demonstrated satellite (or "sneak male") mating tactics in anadromous and freshwater-resident European river lamprey and in European brook lamprey, observing this behavior both within and between species or morphs. In no case, however, has fertilization success of satellite males been determined.

Scott and Crossman (1973) noted an exception to the tail coil spawning behavior in northern brook lamprey, where males did not wrap around the female, but vibrated vigorously next to each other during spawning.

6.4.3.3 Heterospecific Mating Associations

Heterospecific mating associations are commonly observed between paired species and among two or more "unpaired" species that occur in sympatry (Table 6.1). Huggins and Thompson (1970) described heterospecific spawning of European brook and river lampreys, but did not report interspecific copulation attempts. Lasne et al. (2010) similarly documented that 54% of the nests they observed contained both European brook and river lampreys (the remaining nests contained only European river lamprey), but—contrary to Huggins and Thompson (1970)—did note interspecific copulation attempts in the mixed nests. They observed a male brook lamprey attempting to mate with a female river lamprey and another brook and two river lamprey males trying to mate with a single river lamprey female, but were unable to determine whether any successful fertilization occurred (see Sect. 6.4.3.5). Heterospecific spawning associations have also been documented in lamprey species in the Great Lakes, where the paired silver and northern brook lampreys, and chestnut, American brook, and sea lampreys have been observed spawning in various combinations on the same nest (Morman 1979; Manion and Hanson 1980). Cochran et al. (2008) observed the paired chestnut and southern brook lampreys spawning in the same nests in Wisconsin, and documented a southern brook lamprey male attempting to mate with a female chestnut lamprey (although no quivering or release of gametes was observed). "Unpaired" Pacific and western brook lampreys have also been documented in the same nests (Brumo 2006).

Two hypotheses, with potentially different fitness consequences, have been developed to explain the origins of heterospecific spawning associations. The first suggests that lampreys may find the increased nest size of larger heterospecifics attractive (Morman 1979), and their association with larger nests may thus provide fitness benefits through increased embryo survival and reduced predation risk to themselves (Cochran et al. 2008). This hypothesis seems reasonable considering the earlier postulations about the origin of communal mating in small-bodied lampreys (see Sect. 6.4.1). An alternative hypothesis is that lampreys are attracted to hetrospecifics, not the physical nest structure, through conserved pheromones (Cochran et al. 2008; Buchinger et al. 2013). Pheromones of anadromous species may be

released in larger amounts compared to non-anadromous species because their body size is larger and they signal (migrate) over longer distances. However, pheromonal attraction of lampreys to heterospecific nests may reduce fitness through wasted mating effort, interference with mating behavior, and increased predation risk due to enhanced conspicuousness.

6.4.3.4 Fertilization of Eggs

Lamprey gametes are generally viable for much longer than those of other fishes. In the laboratory, sea lamprey eggs could be fertilized for up to 3 h, although fertilization success rate declined significantly after 40 min (from 85–95% at 0–40 min to 32% after 3 h; Ciereszko et al. 2000). Arctic and sea lamprey sperm are viable in fresh water for several minutes (Kobayashi 1993; Ciereszko et al. 2000). Assuming all lamprey gametes have similar viabilities, the ecological consequence is that during communal and heterospecific spawning associations, males could fertilize eggs up to a few hours after deposition. Satellite males (Sect. 6.4.3.2) may also have an increased opportunity to fertilize eggs in the rear of the nest. Once eggs are fertilized, a fast block occurs at the plasma membrane level and then the perivitelline space acts as a permanent block to polyspermy (Arctic lamprey, Kobayashi and Yamamoto 1994).

6.4.3.5 Potential for Hybridization of Paired Species

Despite the occurrence of mixed-species spawning associations and even mating attempts between heterospecifics (see Sect. 6.4.3.3), natural hybrids of paired species have not been documented in nature (Beamish and Neville 1992; Yamazaki and Goto 1998). It is generally thought that size-assortative homogamy reduces the probability of paired species cross-fertilizing (Hagelin 1959; Malmqvist 1983). In North American river lamprey and western brook lamprey, for example, fertilization success is low when males and females differ in length by more than 20% due to misalignment of the urogenital regions (Beamish and Neville 1992). However, some fertilizations can occur even when size differences exceed 30% (Beamish and Neville 1992) and many lamprey species pairs differ in length by less than 20–30%, particularly paired species with freshwater-resident or "praecox" (i.e., small anadromous) parasitic forms (Docker 2009). Kucheryavyi et al. (2007b), for example, found considerable overlap in size and no evidence of assortative mating among three life history types of Arctic lamprey in the Utkholok River, Russia. Furthermore, "satellite" male behavior (Sect. 6.4.3.2), external fertilization, and extended gamete viability (Sect. 6.4.3.4) also increase the probability of genetic mixing between paired species, especially those that have viable hybrid offspring, such as northern brook and silver lampreys (Piavis et al. 1970), European river and brook lampreys (Enequist 1937; Hume et al. 2013b), and North American river and western brook lampreys (Beamish and Neville 1992). Therefore, although

size differences may have been an important factor allowing the sympatric speciation of non-parasitic species from parasitic species (Salewski 2003), additional barriers to hybridization such as spawning habitat differences (Sect. 6.3.1) and sensory cues (Sect. 6.6) may also have been needed to maintain reproductive isolation of paired species (Beamish and Neville 1992; Docker 2009).

Although hybrids have not been demonstrated to date, it is important to note that hybrids may be difficult to detect (certainly as larvae, when the species themselves are often indistinguishable; see Docker 2009). The taxonomic status of paired species could be challenged if successful hybridization is documented in the wild. Reclassification of paired species—where, instead of being considered separate species, they are considered different ecotypes of the same species (Enequist 1937)—could have profound impacts on conservation of rare or endangered lampreys (Docker 2009; Docker et al. 2012).

6.5 Secondary Sexual Characteristics

6.5.1 Male

The general secondary sexual characteristics for male and female lampreys have been previously reviewed (Vladykov 1949; Smith et al. 1968) and will be briefly highlighted with special emphasis on newly described structures and functions of previously described structures. During sexual maturation, male lampreys develop an elongated urogenital papilla (Hardisty and Potter 1971; Kott et al. 1988). The papilla is not used to internally fertilize eggs, but to direct milt towards eggs released by females (Kucheryavyi et al. 2007a). Mature males develop swollen cloacal lips, a straight or downwardly bent tail to aid in nest construction, and a heightened, serrated and vascularized second dorsal fin generally about 1 week before spawning. Mature male European river, European brook, and sea lampreys develop glandular cells in gill epithelial tissue when spermiated (Pickering and Morris 1977; Siefkes et al. 2003). Glandular cells of mature males likely function as transporters of mating pheromone from the blood into the riverine environment during respiration (Siefkes et al. 2003; see Sect. 6.6.1).

During sexual maturation of pouched lamprey and Chilean lamprey *Mordacia lapicida*, a highly pronounced gular pouch develops (Hardisty and Potter 1971). The gular pouch of these two Southern Hemisphere parasitic lamprey species has been described as a fibrous muscle with extensive vascularization resembling mammalian erectile tissue (Hardisty and Potter 1971; Potter and Welsch 1997). In the other parasitic Southern Hemisphere species, the short-headed lamprey, mature males may have some loose skin in the gular region (Renaud 2011). The gular pouch is also present in sexually immature and mature male parasitic lampreys in the Northern Hemisphere (including sea and Caspian lampreys; chestnut lamprey, silver lamprey, and Ohio lamprey *Ichthyomyzon bdellium*; Pacific and Miller Lake lampreys and Klamath lamprey *Entosphenus similis*), but in a much reduced state (Monette and Renaud

2005). The gular pouch of pre-spawning Pacific and sea lampreys contains lipids, which Monette and Renaud (2005) postulated could serve as an energy resource to support the protracted spawning migration of these anadromous species. The gular pouch is unlikely to aid in stone movement because male sea lamprey can move larger rocks than similarly-sized male pouched lamprey and yet have a smaller gular pouch. Where described, the gular pouch of mature males is larger than that of mature females and thus may also be involved in courtship (Monette and Renaud 2005).

A characteristic unique to mature male sea lamprey is a rope-like tissue that extends along the dorsal surface from approximately the posterior gill slit to the beginning of the anterior dorsal fin (Hardisty and Potter 1971). Unexpectedly, the rope was discovered to consist of a thermogenic adipose tissue that produces heat when in the presence of an ovulated female, but not when in the presence of other males (Chung-Davidson et al. 2013a). This is the first discovered thermogenic secondary sexual characteristic and the only example of a thermogenic fat outside the mammalian clade. Sea lamprey mate in generally the same manner as other Northern Hemisphere lampreys, so perhaps the rope tissue helps maintain reproductive isolation. Additional experiments are needed to determine if females can detect the heat produced in the rope and how heat production influences mate selection and mating behavior.

6.5.2 Female

Upon sexual maturation, female lampreys also develop an elongated urogenital papilla, but it is much reduced compared to that of a mature male conspecific. The female urogenital papilla helps direct eggs into the nest (Kucheryavyi et al. 2007a). Mature females also develop a keel behind the cloaca and upward bent tail (Applegate 1950; McIntyre 1969; Kott et al. 1988; Kucheryavyi et al. 2007a). The upward bent tail likely aids in nest cleaning or may be involved with expressing the eggs. In Arctic lamprey, the keel behind the cloaca is thought to be important for nest cleaning, mixing of eggs and sperm, and as a male tail "brake" helping to align male and female urogenital papillae (Kucheryavyi et al. 2007a). Similarly, a swelling at the anterior base of the posterior dorsal fin in mature female European river lamprey also appears to help align urogenital papillae during mating (Hagelin 1959). Females of the above mentioned Northern Hemisphere parasitic lamprey species (Sect. 6.5.1) have a gular pouch of reduced size relative to males of their own species (Monette and Renaud 2005).

6.6 Sensory Modalities that Facilitate Mating

Sensory modalities known to play roles in lamprey reproduction include olfaction, tactile sensation, and electroreception. Chemical communication is important for reproduction as adult lampreys have well-developed olfactory organs and

exceptionally large olfactory bulbs relative to the brain (Kleerekoper 1972). In sea lamprey, this anatomically dominant system is highly sensitive to compounds that direct spawning migrations into streams with established larval populations (Teeter 1980; Bjerselius et al. 2000; Sorensen et al. 2005; see Chap. 5) and to spawning grounds populated with spermiated males (Li et al. 2002; Siefkes et al. 2005; Johnson et al. 2009). The function of mating pheromones in sea lamprey reproduction has been reviewed (Li et al. 2007; Johnson and Li 2010), and will be briefly highlighted here. Additionally, other sensory modalities suspected to play a part in lamprey reproduction will be discussed.

6.6.1 Pheromones

6.6.1.1 Sea Lamprey Mating Pheromones

Upon spermiation, male sea lamprey release a mating pheromone that is highly attractive to ovulated females, luring them to nests. In natural populations, it is not known how far ovulated females need to travel to locate spermiated males or how long it takes for a female to locate a nest. A component of the mating pheromone released by males has been identified as 7α,12α,24-trihydroxy-3-one-5α-cholan-24-sulfate (3kPZS; Li et al. 2002; Yun et al. 2003) and shown to be highly attractive to ovulated female sea lamprey at in-stream concentrations ranging from 10^{-11} M to 10^{-13} M (Siefkes et al. 2005; Johnson et al. 2009). Only spermiated male sea lamprey release 3kPZS and only ovulated female sea lamprey are attracted to 3kPZS (Siefkes et al. 2005). Males release 3kPZS through their gills, likely through the profuse glandular cells with secretory papillae (Siefkes et al. 2003). Female olfactory sense is critical for locating spermiated males (Johnson 2005; Johnson et al. 2006). Ovulated females likely locate sources of 3kPZS by integrating tactile information concerning flow direction and olfactory information concerning pheromone concentration through a mechanism termed odor-conditioned rheotaxis (Johnson et al. 2012a). The male mating pheromone consists of multiple components that induce attraction to the nest, retention on the nest, rock movements, and tail fanning (Johnson et al. 2012b). 3,12-diketo-4,6-petromyzonene-24-sulfate (DKPES) is a minor component of the mating pheromone that, when mixed with 3kPZS at specific ratios, attracts more ovulated females than 3kPZS alone (Li et al. 2013). Additional components, however, remain unidentified; experiments directly comparing mixtures of 3kPZS and DKPES and water conditioned by spermiating males showed the latter to still be more attractive to females (Li et al. 2013).

The sea lamprey male mating pheromone also contains components that function as stimulatory and inhibitory priming pheromones. Sexually immature males and females exposed to washings of mature males or to synthesized 3kPZS mature faster than those exposed to water containing no pheromone (Chung-Davidson et al. 2013b). Further investigation revealed that immature sea lamprey exposed to 3kPZS exhibit increases in circulatory 15α-hydroxyprogresterone concentrations and forebrain gene expressions (Chung-Davidson et al. 2013b). However, exposure

of immature males to 7α,12α-dihydroxy-5α-cholan-3-one-24-oic acid (3kACA), which is released by mature males (Yun et al. 2003), inhibits steroidogenesis (Chung-Davidson et al. 2013c). Because 3kACA is released by mature males at a rate about 10 times less than 3kPZS, the inhibitory impacts of 3kACA likely only occur when males are at close proximity; for example, when competing for nest sites. Taken together, increases in spring water temperature and the presence of male mating pheromones are likely important triggers that synchronize maturation of early and late arriving migrants to enable spawning during the 2–3 week period.

The sea lamprey has become a model for how pheromones may be used in the control of invasive vertebrates (Li et al. 2007) because it is an ecologically and economically damaging pest in the Laurentian Great Lakes and Lake Champlain (Smith and Tibbles 1980; Marsden and Siefkes in press). Pheromone-baited traps show promise for increasing the capture of female sea lamprey (Johnson et al. 2009; Luehring et al. 2011), and disruption of pheromone communication through antagonists or application of high concentrations of 3kPZS may also be effective in reducing mating success (Johnson et al. 2009). When 3kPZS alone was applied to existing traps in the Great Lakes basin, trap efficiencies significantly increased (Johnson et al. 2013).

6.6.1.2 Mating Pheromones in Other Lamprey Species

Mating pheromones have been hypothesized to be used by European river, pouched, silver, and Pacific lampreys. Jang and Lucas (2005) reported that the majority of a population of European river lamprey in an 80 km segment of river spawned at a single site, indicating possible coordination of reproduction through mating pheromones released by mature adults. In this case, migratory pheromones released by larvae (Fine et al. 2004; Sorensen et al. 2005; see Chap. 5) would not likely coordinate reproduction, as larvae would be present downstream of spawning areas. The presence of glandular cells in mature male European river lamprey suggests that bile acid pheromones may be released across the gills to coordinate reproduction (Pickering and Morris 1977). Radio-tagged pouched lamprey all entered the same tributary immediately prior to the putative mating season, supporting the hypothesis that chemical cues may coordinate spawning site selection (Jellyman et al. 2002). Pacific lamprey have high olfactory sensitivity to the sea lamprey mating pheromone 3kPZS (Robinson et al. 2009), but no behavioral tests have been conducted to evaluate if females are attracted to synthesized 3kPZS. Given the protracted spawning migration of Pacific and pouched lamprey (see Sect. 6.2.1), it is possible that the newly arrived migratory cohort may be exposed to mating pheromones from the spawning adult cohort. Therefore, upon arrival to fresh water, Pacific and pouched lampreys may use mating pheromones released by the upstream spawning cohort while still in the migratory stage as an honest indicator of tributaries containing spawning habitat (Robinson et al. 2009). Silver lamprey appear to use 3kPZS as a migratory pheromone, but not as a mating pheromone as observed in sea lamprey (see Sect. 6.6.1.1). Female preference for 3kPZS in a migratory context may be a bias leading to male

signaling with 3kPZS, where male sea lamprey may have evolved to take advantage of an existing preference of females (Buchinger et al. 2013).

Questions remain concerning species specificity, production, and release of lamprey mating pheromones. As discussed above, spermiated male sea lamprey actively release mating pheromones at high rates (Sect. 6.6.1.1). Sea lamprey defend nests from other males, and therefore the male obtains direct benefit from attracting mates. A different ecology is true of communal spawners, where multiple males compete for mating opportunities in a nest. Perhaps communally spawning males, if they release mating pheromones, share fitness benefits with other males in the nest. Investigations of pheromone communication in satellite males (see Sect. 6.4.3.2) would be interesting because pheromone production may be suppressed to help them remain cryptic. Mating pheromones in general are expected to be species specific because reproductive isolation often provides significant fitness benefits (Wyatt 2003). Yamazaki and Goto (2000), for example, suggested that pheromones may prevent interbreeding in two undescribed *Lethenteron* species (*L.* sp. N and *L.* sp. S) where they occur sympatrically. Although spawning seasons and sizes at maturity overlap, heterospecific nesting assemblages have not been observed. Species specific mating pheromones may be another factor facilitating reproductive isolation within heterospecific spawning associations (Sect. 6.4.3.3), but this has not yet been investigated.

Pheromones have the potential to benefit restoration efforts in lamprey species (Robinson et al. 2009) whose populations are in decline (Renaud 1997; see Chap. 8). Migratory pheromones could direct migratory-phase adults into streams from which they had been extirpated or into specific tributaries with high quality spawning and larval rearing habitat. Mating pheromones could be used to direct spawning-phase adults to specific high quality spawning riffles. Both pheromones could be effective at low concentrations (i.e., 10^{-12} M), meaning that once identified, implementation of pheromone-based restoration techniques could be cost-effective. Additionally, advances in pheromone quantification in stream water could allow for non-invasive, rapid population assessment (Li et al. 2011; Stewart et al. 2011; Xi et al. 2011).

6.6.2 Additional Sensory Modalities used During Reproduction

6.6.2.1 Tactile

Tactile communication is important once lampreys are aggregated on nests and engaged in mating behavior (Fig. 6.1d, 6.2). Lampreys probe with their oral disc during nest construction to locate rocks to be moved. Lampreys likely use tactile cues to determine if the physical characteristics of the nest are suitable for spawning. Hagelin and Steffner (1958) reported that when mating, European river lamprey males glide their oral discs along the side of females immediately prior to attachment to the head. Reighard (1903) hypothesized that such tactile cues in American brook lamprey may determine sex when multiple lamprey are in the nest. For ex-

ample, when a male attaches to a receptive female, the female will remain attached to a rock, triggering the male to wrap his tail around her. In contrast, if the male attaches to another male, the second male will typically detach from the rock and drift around the nest. In sea lamprey, the increased heat generation in female presence and frequent contact with the female urogenital pore indicates that the male rope tissue may be involved in tactile communication on the spawning nest (Chung-Davidson et al. 2013a).

6.6.2.2 Electroreception

Adult lampreys respond to weak, low frequency electrical fields (Bodznick and Northcutt 1981), but limited knowledge is available concerning the function of electroreception during mating. Chung-Davidson et al. (2008) provided evidence that sea lamprey use electroreception to locate conspecifics or that electroreception may be used to regulate sexual behavior along with tactile cues. However, additional research is needed to determine the extent to which electroreception influences mate choice and reproductive behavior in lampreys.

6.6.2.3 Vision

There is no evidence that vision is used by adult lampreys to direct spawning migrations, locate mates, or to facilitate spawning behavior. Experimentally blinded sea lamprey migrated upstream at the same rate as lamprey that were not blinded, and both groups showed the same nocturnal activity patterns (Binder and McDonald 2007). The switch from migration only at night to becoming active during both day and night (i.e., showing diel behavior) has been shown in part to be mediated by the dermal photophores rather than the eyes (Binder and McDonald 2008a, b). Although all light-associated behaviors are mediated by the eyes in mammals, such "extraocular" photoreceptors are not uncommon among lower vertebrates (Foster and Hankins 2002). In sea lamprey, the reduction in light avoidance behavior in spawning-phase lamprey is the result of reduced dermal photosensitivity in response to elevated stream temperatures (Binder and McDonald 2008a, b).

There is anecdotal evidence to suggest that vision is also not used to locate mates or during reproduction. Applegate (1950) noted the degeneration of the eyes of spawning sea lamprey and suggested that vision was not important to them for mating. Further, lampreys can be readily approached and observed during the day without sign of being disturbed.

6.6.3 Acoustic

Acoustic signals produced by fishes have been widely implicated to facilitate species recognition and influence mate choice (Verzijden et al. 2010). Mating decisions

are often based on the collective information from multiple modalities (Johnstone 1996). Although lampreys have been shown to use chemical and tactile communication, acoustic signals could also serve an important role. To date, no studies have evaluated the role of acoustic cues in lamprey reproduction. Future research should investigate whether lampreys produce sound during mating and whether those sounds influence mate choice and mating behaviors.

6.7 Senescence

All lampreys die after spawning (i.e., they are semelparous). A few exceptions have been reported in the literature, that is, of possible repeat spawning in Pacific lamprey (Michael 1980, 1984) and survival of European river lamprey until the following year (at which time they may participate in the spawning migration but be unable to breed; see Hardisty 1986c), but it is questionable whether this is possible (Hardisty 1986c). Although survival can be extended if spawning is delayed or prevented (e.g., at low temperatures; Larsen 1980), survival after spawning seems highly unlikely given the severe atrophy of the intestine, degeneration of the liver and eyes, the inability of anadromous species to osmoregulate in salt water after spawning, and the depletion in lipids (Hardisty and Potter 1971; Larsen 1980). Caspian lamprey females have been reported to die immediately after releasing their eggs, while males were documented to survive until spermiation ceased (see Holčík 1986a). Pletcher (1963) observed that female western brook lamprey usually died within a week of spawning (with males living for 1–2 months). Female sea and European river lamprey that deposit all their eggs early in the season may survive up to a week and continue to participate in spawning behaviors (Applegate 1950; Hagelin and Steffner 1958). Post-spawn lampreys are believed to move downstream (Jang and Lucas 2005) and seek refuge under cover until death occurs (Hagelin 1959). The odor of dead sea lamprey is repulsive to migratory-phase conspecifics (Wagner et al. 2011), and may cue to newly arrived migrants that the spawning season has ended or there is considerable risk of mortality upstream.

6.8 Conclusion

As the terminal life stage of an unusual primitive fish, reproduction of lampreys has fascinated biologists for centuries. Much has been revealed concerning the reproductive ecology of lampreys such as spawning preferences, mating systems, and behavior. Critical knowledge gaps still exist, however. Spawning has never been reported for any of the four Southern Hemisphere lamprey species, perhaps because their reproductive ecology differs substantially from that of the well-studied Northern Hemisphere species. Furthermore, how multiple modalities of communication among lampreys (including mating pheromones) are integrated to inform species

recognition and mate choice remains poorly understood. This is especially interesting for ecologists in light of heterospecific mating associations and the apparent sympatric speciation of paired species. For conservationists and fisheries managers, an enhanced understanding of the reproductive ecology of lampreys is needed, both for the more than 20 lamprey species that are threatened or endangered in at least part of their range and for control of invasive sea lamprey in the Laurentian and other Great Lakes.

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References

Abakumov VA (1960) Systematics and ecology of the Ukrainian lamprey (*Lampetra mariae* Berg). Vop Ikhtiol 6:609–618 [in Russian]

Abou-Seedo FS, Potter IC (1979) The estuarine phase in the spawning run of the river lamprey *Lampetra fluviatilis*. J Zool Lond 188: 5–25

Ahmadi M, Amiri BM, Abdoli A, Fakharzade SME, Hoseinifar SH (2011) Sex steroids, gonadal histology and biological índices of fall and spring Caspian lamprey (*Caspiomyzon wagneri*) spawning migrants in the Shirud River, Southern Caspian Sea. Environ Biol Fish 92:229–235

Alvarez del Villar J (1966) Ictiologia michoacana. IV. Contribucion al conocimiento biológico y sistemático de las lampreas de Jacona, Mich., México. Annu Esc Nac Cienc Biol Mex 13:107–144

Applegate VC (1950) Natural history of the sea lamprey (*Petromyzon marinus*) in Michigan. US Fish and Wildlife Service Special Scientific Report 55, Washington, DC

Baxter EW (1954) Studies on the biology of lampreys. PhD thesis, London University, London

Beamish FWH (1982) Biology of the southern brook lamprey, *Ichthyomyzon gagei*. Environ Biol Fish 7:305–320

Beamish FWH, Medland TE (1988) Metamorphosis of the mountain brook lamprey *Ichthyomyzon greeleyi*. Environ Biol Fish 23:45–54

Beamish FWH, Potter IC (1975) The biology of the anadromous sea lamprey *Petromyzon marinus* in New Brunswick. J Zool Lond 177:52–72

Beamish RJ (1980) Adult biology of the river lamprey (*Lampetra ayresi*) and the Pacific lamprey (*Lampetra tridentata*) from the Pacific coast of Canada. Can J Fish Aquat Sci 37:1906–1923

Beamish RJ (1987) Status of the lake lamprey, *Lampetra macrostoma*, in Canada. Can Field Nat 101:186–189

Beamish R, Neville C (1992) The importance of size as an isolating mechanism in lampreys. Copeia 1992:191–196

Beamish RJ, Youson JH (1987) Life history and abundance of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the Strait of Georgia. Can J Fish Aquat Sci 44:525–537

Bergstedt R, Seelye J (1995) Evidence for lack of homing by sea lampreys. Trans Am Fish Soc 124:235–239

Binder TR, McDonald DR (2007) Is there a role for vision in the behaviour of sea lampreys (*Petromyzon marinus*) during their upstream spawning migration? Can J Fish Aquat Sci 64:1403–1412

Binder TR, McDonald DG (2008a) The role of temperature in controlling diel activity in upstream migrant sea lampreys (*Petromyzon marinus*). Can J Fish Aquat Sci 65:1113–1121
Binder TR, McDonald DG (2008b) The role of dermal photoreceptors during the sea lamprey (*Petromyzon marinus*) spawning migration. J Comp Physiol A 194:921–928
Binder TR, McDonald DG (2010) Relative importance of water temperature, water level, and lunar cycle to migratory activity in spawning-phase sea lampreys in Lake Ontario. Trans Am Fish Soc 139:700–712
Bjerselius R, Li W, Teeter JH et al (2000) Direct behavioral evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinu*s) function as a migratory pheromone. Can J Fish Aquat Sci 57:557–569
Bodznick D, Northcutt RC (1981) Electroreception in lampreys: evidence that the earliest vertebrates were electroreceptive. Science 212:465–467
Brigham WU (1973) Nest construction of the lamprey, *Lampetra aepyptera*. Copeia 1973:135–136
Brumo AF (2006) Spawning, larval recruitment, and early life survival of Pacific lamprey in the South Fork Coquille River, Oregon. MS thesis, Oregon State University, Corvallis, OR
Buchinger TJ, Wang H, Li W, Johnson NS (2013) Evidence for receiver bias underlying female preference for a male mating pheromone in sea lamprey. Proc R Soc B 280:1966–1973
Case B (1970) Spawning behaviour of chestnut lamprey (*Ichthyomyzon castaneus*). J Fish Res Board Can 27:1872–1874
Chung-Davidson Y-W, Bryan MB, Teeter J, Bedore CN, Li W (2008) Neuroendocrine and behavioral responses to weak electrical fields in adult sea lampreys (*Petromyzon marinus*). Horm Behav 54:34–40
Chung-Davidson Y-W, Priess MC, Yeh C-Y et al (2013a) A thermogenic secondary sexual character in male sea lamprey. J Exp Biol 216:2702–2712
Chung-Davidson Y-W, Wang H, Siefkes MJ et al (2013b) Pheromonal bile acid 3-ketopetromyzonol sulfate primes the neuroendocrine system in sea lamprey. BMC Neurosci 14:11–24
Chung-Davidson Y-W, Wang H, Bryan MB et al (2013c) An anti-steroidogenic inhibitor primer pheromone in male sea lamprey (*Petromyzon marinus*). Gen Comp Endocrinol 189:24–31
Ciereszko A, Glogowski J, Dabrowski K (2000) Fertilization in landlocked sea lamprey: storage of gametes, optimal sperm: egg ratio, and methods of assessing fertilization success. J Fish Biol 56:496–505
Clemens BJ, Binder TR, Docker MF, Moser ML, Sower SA (2010) Similarities, differences, and unknowns in biology and management of three parasitic lampreys of North America. Fisheries 35:580–594
Close DA, Fitzpatrick MS, Li W (2002) The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries 27:19–25
Cochran PA, Gripentrog AP (1992) Aggregation and spawning by lampreys (genus *Ichthyomyzon*) beneath cover. Environ Biol Fish 33:381–387
Cochran PA, Lyons J (2004) Field and laboratory observations on the ecology and behavior of the silver lamprey (*Ichthyomyzon unicuspis*) in Wisconsin. J Freshw Ecol 19:245–253
Cochran, PA, Lyons J, Merino-Nambo E (1996) Notes on the biology of the Mexican lampreys *Lampetra spadicea* and *L. geminis* (Agnatha: Petromyzontidae). Ichthyol Explor Freshw 7:173–180
Cochran PA, Bloom DD, Wagner RJ (2008) Alternative reproductive behaviors in lampreys and their significance. J Freshw Ecol 23:437–444
Cochran PA, Ross MA, Walker TS, Biederman T (2012) Early spawning by the American Brook lamprey (*Lethenteron appendix*) in southeastern Minnesota. Can Field Nat 126:204–209
Cooper EL (1983) Fishes of Pennsylvania and the northeastern United States. Pennsylvania State University Press, University Park
Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 71–114

Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Docker MF, Beamish FWH, Bryan MB, Spice EK (in press) The lamprey gonad. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Docker MF, Mandrak NE, Heath DD (2012) Contemporary gene flow between "paired" silver (*Ichthyomyzon unicuspis*) and northern brook (*I. fossor*) lampreys: implications for conservation. Conserv Genet 13:823–835

Dendy JS, Scott DC (1953) Distribution, life history, and morphological variations of the southern brook lamprey, *Ichthyomyzon gagei*. Copeia 1953:152–162

Enequist P (1937) *Lampetra planeri* as an ecological modification of *Lampetra fluviatilis* on the river and brook lampreys in Sweden. Ark Zool 29:1–22 [in German]

Fine JM, Vrieze LA, Sorensen PW (2004) Evidence that Petromyzontid lampreys employ a common migratory pheromone that is partially comprised of bile acids. J Chem Ecol 30:2091–2110

Fodale MF, Bronte CR, Bergstedt RA, Cuddy DW, Adams JV (2003) Classification of lentic habitat for sea lamprey (*Petromyzon marinus*) larvae using a remote seabed classification device. J Great Lakes Res 29(Suppl 1):190–203

Foster RG, Hankins MW (2002) Non-rod, non-cone photoreception in the vertebrates. Prog Retin Eye Res 21:507–527

Gage SH (1928) The lampreys of New York state-life history and economics. Biol Surv Oswego River System, New York Conservation Department Annual Report 17(Suppl):139–191

Gardner C, Coghlan SM Jr, Zydlewski J (2012) Distribution and abundance of anadromous sea lamprey spawners in a fragmented stream: current status and potential range expansion following barrier removal. Northeast Nat 19:99–110

Gaudron SM, Lucas MC (2006) First evidence of attraction of adult river lamprey in the migratory phase to larval odour. J Fish Biol 68:640–644

Ghasempouri SM (1993) The Caspian lamprey. Abzeeyan 4:18–21 [in Farsi]

Gilmore SA (2004) Genetic assessment of adult mating success and accuracy of statolith aging in the sea lamprey *Petromyzon marinus*. MS thesis, Michigan State University, Lansing, MI

Glova G (1995) The secret life of the lamprey. NIWA Water Atmos 3:20–21

Goodman DH, Reid SB, Docker MF, Haas GR, Kinziger AP (2008) Mitochondrial DNA evidence for high levels of gene flow among populations of a widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae). J Fish Biol 72:400–417

Gunckel SL, Jones KK, Jacobs SE (2009) Spawning distribution and habitat use of adult Pacific and western brook lampreys in Smith River, Oregon. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society Symposium 72, Bethesda, pp 173–189

Hagelin L-O (1959) Further aquarium observations on the spawning habits of the river lamprey (*Petromyzon fluviatilis*). Oikos 10:50–64

Hagelin L-O, Steffner N (1958) Notes on the spawning habits of the river lamprey (*Petromyzon fluviatilis*). Oikos 9:221–238

Hardisty MW (1944) The life history and growth of the brook lamprey (*Lampetra planeri)*. J Anim Ecol 13:110–122

Hardisty, MW (1954) Sex ratio in spawning populations of *Lampetra planeri*. Nature 173:874–875

Hardisty MW (1961a) Studies on an isolated spawning population of the brook lamprey (*Lampetra planeri*). J Anim Ecol 30:339–355

Hardisty MW (1961b) Sex composition of lamprey populations. Nature 191:1116–1117

Hardisty MW (1986a) *Lampetra planeri* (Bloch, 1784). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 279–304

Hardisty MW (1986b) *Petromyzon marinus* (Linnaeus, 1758). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 94–116

Hardisty MW (1986c) *Lampetra fluviatilis* (Linnaeus, 1758). In: Holčík J (ed), The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 249–278

Hardisty MW, Potter IC (1971) The general biology of adult lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys. Academic Press, London, pp 127–206
Heard WR (1966) Observations on lampreys in the Naknek River system of southwest Alaska. Copeia 1966:332–339
Holčík J (1986a) *Caspiomyzon wagneri* (Kessler, 1870). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 119–142
Holčík J (1986b) *Lethenteron kessleri* (Anikin, 1905). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 220–236
Holčík J (1986c) *Lethenteron japonicum* (Martens, 1868). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 198–219
Holčík J, Delić A (2000) New discovery of the Ukrainian brook lamprey in Croatia. J Fish Biol 56:73–86
Holčík J, Renaud CB (1986) *Eudontomyzon mariae* (Berg, 1931). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part 1, Petromyzontiformes. AULA, Wiesbaden, pp 165–185
Huggins RJ, Thompson A (1970) Communal spawning of brook and river lampreys, *Lampetra planeri* Bloch and *Lampetra fluviatilis* L. J Fish Biol 2:53–54
Hume JB, Adams CE, Mable B, Bean CW (2013a) Sneak male mating tactics between lampreys (Petromyzontiformes) exhibiting alternative life-history strategies. J Fish Biol 82:1093–1100
Hume JB, Adams CE, Mable B, Bean C (2013b) Post-zygotic hybrid viability in sympatric species pairs: a case study from European lampreys. Biol J Linn Soc 108:378–383
Jang M-H, Lucas MC (2005) Reproductive ecology of the river lamprey. J Fish Biol 66:499–512
Janvier P (2010) microRNAs revive old views about jawless vertebrate divergence and evolution. Proc Natl Acad Sci U S A 107:19137–19138
Jellyman DJ, Glova GJ, Sykes JRE (2002) Movements and habitats of adult lamprey (*Geotria australis*) in two New Zealand waterways. N Z J Mar Freshw Res 36:53–65
Johnson NS (2005) Investigating potential applications of the male mating pheromone in sea lamprey management. MS thesis, Michigan State University, East Lansing, MI
Johnson, NS, Li W (2010) Understanding behavioral responses of fish to pheromones in natural freshwater environments. J Comp Physiol A 196:701–711
Johnson NS, Luehring MA, Siefkes MJ, Li WM (2006) Mating pheromone reception and induced behavior in ovulating female sea lampreys. N Am J Fish Manag 26:88–96
Johnson NS, Yun S-S, Thompson HT, Brant CB, Li W (2009) A synthesized pheromone induces upstream movement in female sea lampreys and summons them into traps. Proc Natl Acad Sci U S A 106:1021–1026
Johnson NS, Mohammed A, Thompson HT, Chio J, Li W (2012a) Sea lampreys orient to a source of synthesized 3kPZS using odour-conditioned rheotaxis. Behav Ecol Sociobiol 66:1557–1567
Johnson NS, Yun S-S, Li W (2012b) Multiple functions of a multi-component mating pheromone in sea lamprey *Petromyzon marinus*. J Fish Biol 80:538–554
Johnson NS, Siefkes MJ, Wagner CM et al (2013) A synthesized mating pheromone component increases adult sea lamprey (*Petromyzon marinus*) trap capture in management scenarios. Can J Fish Aquat Sci 70:1101–1108
Johnstone RA (1996) Multiple displays in animal communication: 'backup signals' and 'multiple messages.' Philos Trans R Soc Lond B Biol Sci 351:329–338
Jolley JC, Silver GS, Whitesel TA (2012) Occupancy and detection of larval Pacific lampreys and *Lampetra* spp. in a large river: the Lower Willamette River. Trans Am Fish Soc 141:305–312
Kan TT, Bond CE (1981) Notes on the biology of the Miller lake lamprey *Lampetra* (*Entosphenus*) *minima*. Northwest Sci 55:70–74
Kleerekoper H (1972) The sense organs. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 2. Academic Press, London, pp 373–404
Kobayashi W (1993) Effect of osmolality on the motility of sperm from lamprey *Lampetra japonica*. Zool Sci 10:281–285
Kobayashi W, Yamamoto TS (1994) Fertilization of the lamprey (*Lampetra japonica*) eggs: implication of the presence of fast and permanent blocks against polyspermy. J Exp Zool 269:166–176

Kott E, Renaud CB, Vladykov VD (1988) The urogenital papilla in the Holarctic lamprey (Petromyzontidae). Environ Biol Fish 23:37–44

Kucheryavyi AV, Savvaitova KA, Gruzdeva MA, Pavlov DS (2007a) Sexual dimorphism and some special traits of spawning behavior of the Arctic lamprey *Lethenteron camtschaticum*. J Ichthyol 47:481–485

Kucheryavyi AV, Savvaitova KA, Pavlov DS et al (2007b) Variations of life history strategy of the Arctic lamprey *Lethenteron camtschaticum* from the Utkholok River (Western Kamchatka). J Ichthyol 47:37–52

Lamsa AK, Rovainen CM, Kolenosky DP, Hanson LH (1980) Sea lamprey (*Petromyzon marinus*) control-where to from here? Report of the SLIS control theory task force. Can J Fish Aquat Sci 37:2175–2192

Larsen LO (1980) Physiology of adult lampreys, with special regard to natural starvation, reproduction, and death after spawning. Can J Fish Aquat Sci 37:1762–1779

Lasne E, Sabatie M-R, Evanno G (2010) Communal spawning of brook and river lampreys (*Lampetra planeri* and *L. fluviatilis*) is common in the Oir River (France). Ecol Freshw Fish 19:323–325

Lavis DS, Hallett A, Koon EM, McAuley TC (2003) History of and advances in barriers as an alternative method to suppress sea lampreys in the Great Lakes. J Great Lakes Res 29(Suppl 1):362–372

Li W, Scott AP, Siefkes MJ et al (2002) Bile acid secreted by male sea lamprey that acts as a sex pheromone. Science 296:138–141

Li W, Twohey M, Jones M, Wagner M (2007) Research to guide use of pheromones to control sea lamprey. J Great Lakes Res 33:70–86

Li K, Wang H, Brant CO, Ahn S, Li W (2011) Multiplex quantification of lamprey specific bile acid derivatives in environmental water using UHPLC-MS/MS. J Chromatogr B 879:3879–3886

Li K, Brant CO, Siefkes MJ, Kruckman HG, Li W (2013) Characterization of a novel bile alcohol sulfate released by sexually mature male sea lamprey (*Petromyzon marinus*). PLoS ONE 8:e68157

Lorion CM, Markle DF, Reid SB, Docker MF (2000) Redescription of the presumed-extinct Miller Lake lamprey, *Lampetra minima*. Copeia 2000:1019–1028

Luehring MA, Wagner CM, Li W (2011) The efficacy of two synthesized sea lamprey sex pheromone components as a trap lure when place in direct competition with natural male odors. Biol Invasions 13:1589–1597

Maitland PS, Morris KH, East K (1994) The ecology of lampreys (Petromyzonidae) in the Loch Lomond area. Hydrobiologica 290:105–120

Malmqvist B (1980) The spawning migration of the brook lamprey, *Lampetra planeri* Bloch, in a south Swedish stream. J Fish Biol 16:105–114

Malmqvist B (1983) Breeding behavior of brook lampreys *Lampetra planeri*: experiments on mate choice. Oikos 41:43–48

Malmqvist B (1986) Reproductive ecology of lampreys. In: Uyeno T, Arai R, Taniuchi T, Marsuura K (eds) Proceedings of the 2nd international conference on Indo-Pacific Fishes. Ichthyological Society of Japan, Tokyo, pp 20–30

Manion PJ (1968) Production of sea lamprey larvae from nests in two Lake Superior streams. Trans Am Fish Soc 97:484–486

Manion PJ, Hanson LH (1980) Spawning behavior and fecundity of lampreys from the upper three Great Lakes. Can J Fish Aquat Sci 37:1635–1640

Marsden JE, Siefkes MJ (in press) Sea lamprey control in the Great Lakes, Finger Lakes, and Lake Champlain. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Maskell FG (1929) On the New Zealand lamprey, *Geotria australis* Gray. Trans N Z Inst 60:167–210

McCauley RW (1963) Lethal temperatures of the developmental stages of the sea lamprey, *Petromyzon marinus* L. J Fish Res Board Can 20:483–490

McIntyre JD (1969) Spawning behavior of the brook lamprey, *Lampetra planeri*. J Fish Res Board Can 26:3252–3254
Meckley TD, Wagner CM, Luehring MA (2012) Field evaluation of larval odor and mixtures of synthetic pheromone components for attracting migrating sea lampreys in rivers. J Chem Ecol 38:1062–1069
Meeuwig MH, Bayer JM, Seelye JG (2005) Effects of temperature on survival and development of early life stage Pacific and western brook lamprey. Trans Am Fish Soc 134:19–27
Michael JH (1980) Repeat spawning of Pacific lamprey. Calif Fish Game 66:186–187
Michael JH (1984) Additional notes on the repeat spawning by Pacific lamprey. Calif Fish Game 70:278–285
Monette SN, Renaud CB (2005) The gular pouch in northern hemisphere parasitic lampreys (Petromyzontidae). Can J Fish Aquat Sci 83: 527–535
Moore HH, Schleen LP (1980) Changes in spawning runs of sea lamprey (*Petromyzon marinus*) in select streams in Lake Superior after chemical control. Can J Fish Aquat Sci 37:1851–1860
Morman RH (1979) Distribution and ecology of lampreys in the Lower Peninsula of Michigan, 1957–75. Great Lakes Fishery Commission, Technical Report 33, Ann Arbor
Morse TJ, Ebener MP, Koon EM et al (2003) A case history of sea lamprey control in Lake Huron: 1979 to 1999. J Great Lakes Res 29(Suppl 1):559–614
Mundahl ND, Sagan RA (2005) Spawning ecology of the American brook lamprey. Environ Biol Fish 73:283–292
Nazari H, Abdoli A (2010) Some reproductive characteristics of endangered Caspian lamprey (*Caspiomyzon wagneri* Kessler, 1870) in the Shirud river southern Caspian Sea, Iran. Environ Biol Fish 88:87–96
Nika N, Virbickas T (2010) Brown trout *Salmo trutta* redd superimposition by spawning *Lampetra* species in a lowland stream. J Fish Biol 77:2358–2372
Piavis GW (1961) Embryological stages in the sea lamprey and effects of temperature on development. US Fish Wildl Serv Fish Bull 61:111–143
Piavis GW, Howell JH, Smith AJ (1970) Experimental hybridization among five species of lampreys from the Great Lakes. Copeia 1970:29–37
Pickering AD, Morris R (1977) Sexual dimorphism in the gills of the spawning river lamprey, *Lampetra fluviatilis* L. Cell Tissue Res 180:1–10
Pletcher FT (1963) The life history and distribution of lampreys in the Salmon and certain other rivers in British Columbia, Canada. PhD thesis, University of British Columbia, Vancouver
Potter IC (1970) The life cycles and ecology of Australian lampreys of the genus *Mordacia*. J Zool Lond 161:487–511
Potter IC, Hilliard RW (1986) Growth and the average duration of larval life in the Southern Hemisphere lamprey, *Geotria australis* Gray. Experientia 42:1170–1173
Potter IC, Welsch U (1997) The structure of the gular pouch of mature males of the lamprey *Geotria australis*. Acta Zool 78:97–106
Potter IC, Hilliard RW, Bird DJ, Macey DJ (1983) Quantitative data on the morphology and organ weights during the protracted spawning-run period of the Southern Hemisphere lamprey *Geotria australis*. J Zool Lond 200:1–20
Purvis A (1970) Growth, age at metamorphosis, and sex ratio of northern brook lamprey in a tributary of southern Lake Superior. Copeia 1970:326–332
Raney EC (1939) The breeding habits of *Ichthyomyzon greeleyi* Hubbs and Trautman. Copeia 1939:111–112
Reighard J (1903) An experimental study of the spawning behavior of *Lampetra wilderi*. Science 17:529
Renaud CB (1982) Revision of the lamprey genus *Eudontomyzon* Regan (1911). MSc thesis, University of Ottawa, Ottawa
Renaud CB (1997) Conservation status of Northern Hemisphere lampreys (Petromyzontidae). J Appl Ichthyol 13:143–148
Renaud CB (2011) Lampreys of the world. An annotated and illustrated catalogue of lamprey species known to date. FAO Species Catalogue for Fishery Purposes 5, FAO, Rome

Renaud CB, Cochran PA (in press) Post-metamorphic feeding in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Robinson TC, Bayer JM (2005) Upstream migration of Pacific lampreys in the John Day River, Oregon: behavior, timing, and habitat use. Northwest Sci 79:106–119
Robinson TC, Sorensen PW, Bayer JM, Seelye JG (2009) Olfactory sensitivity of Pacific lampreys to lamprey bile acids. Trans Am Fish Soc 138:144–152
Rooney SM, O'Gorman NM, Greene F, King JJ (2013) Aspects of brook lamprey (*Lampetra planeri* Bloch) spawning in Irish waters. Proc R Irish Acad B 113:13–26
Russell JE, Beamish FWH, Beamish RJ (1987) Lentic spawning by the Pacific lamprey, *Lampetra tridentata*. Can J Fish Aquat Sci 44:476–478
Salewski V (2003) Satellite species in lampreys: a worldwide trend for ecological speciation in sympatry. J Fish Biol 63:267–279
Savvaitova KA, Maksimov VA (1979) Spawning of the Arctic lamprey of the genus *Lampetra* in connection with the problem of the taxonomic status of small forms. J Ichthyol 18:555–560
Schleen LP, Christie GC, Heinrich JW et al (2003) Development and implementation of an integrated program for control of sea lampreys in the St Marys River. J Great Lakes Res 29(Suppl 1):677–694
Schultz LP (1930) The life history of *Lampetra planeri*, Bloch, with a statistical analysis of the rate of growth of the larvae from Western Washington. Occas Pap Mus Zool Univ Mich 221:1–35
Scott DP (1957) Spawning requirements of sea lamprey. Annual Report of the Biological Station and of the Technological Unit, London, ON
Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Bulletin Fisheries Research Board of Canada 184, Ottawa
Seagle HH, Nagel JW (1982) Life cycle and fecundity of the American brook lamprey, *lampetra appendix*, in Tennessee. Copeia 1982:362–366
Siefkes MJ, Scott AP, Zielinski B, Yun S-S, Li W (2003) Male sea lampreys, *Petromyzon marinus* L., excrete a sex pheromone from gill epithelia. Biol Reprod 69:125–132
Siefkes MJ, Winterstein SR, Li WM (2005) Evidence that 3-keto petromyzonol sulphate specifically attracts ovulating female sea lamprey, *Petromyzon marinus*. Anim Behav 70:1037–1045
Smith SJ, Marsden JE (2009) Factors affecting sea lamprey egg survival. N Am J Fish Manag 29:859–868
Smith BR, Tibbles JJ (1980) Sea lamprey *Petromyzon marinus* in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936–78. Can J Fish Aquat Sci 37:1780–1801
Smith AJ, Howell JH, Piavis GW (1968) Comparative embryology of five species of lampreys of the Upper Great Lakes. Copeia 3:461–469
Sorensen PW, Fine JM, Dvornikovs V et al (2005) Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. Nat Chem Biol 1:324–328
Sousa R, Araújo MJ, Antunes C (2012) Habitat modifications by sea lampreys (*Petromyzon marinus*) during the spawning season: effects on sediments. J Appl Ichthyol 28:766–771
Spice EK, Goodman DH, Reid SB, Docker MF (2012) Neither philopatric nor panmictic: microsatellite and mtDNA evidence suggests lack of natal homing but limits to dispersal in Pacific lamprey. Mol Ecol 21:2916–2930
Stewart M, Baker CF, Cooney T (2011) A rapid, sensitive, and selective method for quantification of lamprey migratory pheromones in river water. J Chem Ecol 37:1203–1207
Stone J (2006) Observations on nest characteristics, spawning habitat, and spawning behavior of Pacific and western brook lamprey in a Washington stream. Northwest Nat 87:225–232
Takayama M (2002) Spawning activities and physical characteristics of the spawning ground of *Lethenteron reissneri* at the headstream of the Himekawa River, central Japan. Ichthyol Res 49:165–170
Taverny C, Lassalle G, Ortusi I et al (2012) From shallow to deep waters: habitats used by larval lampreys (genus *Petromyzon* and *Lampetra*) over a western European basin. Ecol Freshw Fish 21:87–99
Teeter J (1980) Pheromone communication in sea lampreys (*Petromyzon marinus*): implications for population management. Can J Fish Aquat Sci 37:2123–2132

Torblaa RL, Westman RW (1980) Ecological impacts of lampricide treatments on sea lamprey (*Petromyzon marinas*) ammocoetes and metamorphosed individuals. Can J Fish Aquat Sci 37:1835–1850

Trautman MB (1957) The fishes of Ohio. Ohio State University Press, Columbus

Tumlison R, Tumlison C (1999) An extralimital population of *Lampetra appendix* (Petromyzontidae) in southwestern Arkansas. Southwest Nat 44:106–108

Verzijden MN, Heusden JV, Bouton N et al (2010) Sounds of male Lake Victoria cichlids vary within and between species and affect mate preferences. Behav Ecol 21:548–555

Vladykov VD (1949) Quebec lampreys (Petromyzonidae). List of species and their economical importance. Department of Fisheries, Province of Quebec, Contribution 26

Vladykov VD, Kott E (1978) A new nonparasitic species of the Holarctic lamprey genus *Lethenteron* (Petromyzontidae) from northwestern North America with notes on other species of the same genus. Biol Pap Univ Alaska 19:1–74

Wagner CM, Jones ML, Twohey MB, Sorensen PW (2006) A field test verifies that pheromones can be useful for sea lamprey (*Petromyzon marinus*) control in the Great Lakes. Can J Fish Aquat Sci 63:475–479

Wagner CM, Stroud EM, Meckley TD (2011) A deathly odor suggests a new sustainable tool for controlling a costly invasive species. Can J Fish Aquat Sci 68:1157–1160

Waldman J, Grunwald C, Wirgin I (2008) Sea lamprey *Petromyzon marinus*: an exception to the rule of homing in anadromous fishes. Biol Lett 4:659–662

White DS (1990) Biological relationships to convective flow patterns within stream beds. Hydrobiologia 196:49–158

Wigley RL (1959) Life history of the sea lamprey of Cayuga Lake, New York. US Fish Wildl Serv Fish Bull 59:559–617

Wyatt TD (2003) Pheromones and animal behavior: communication by smell and taste. Cambridge University Press, Cambridge

Xi X, Johnson NS, Brant CO et al (2011) Quantification of a male sea lamprey pheromone in tributaries of Laurentian Great Lakes by liquid chromatography-tandem mass spectrometry. Environ Sci Tech 45:6437–6443

Yamazaki Y, Goto A (1998) Genetic structure and differentiation of four *Lethenteron* taxa from the Far East, deduced from allozyme analysis. Environ Biol Fishes 52:149–161

Yamazaki Y, Goto A (2000) Breeding season and nesting assemblages in two forms of *Lethenteron reissneri*, with reference to reproductive isolating mechanisms. Ichthyol Res 47:271–276

Young RT, Cole LJ (1900) On the nesting habits of the brook lamprey (*Lampetra wilderi*). Am Nat 34:617–620

Yun S-S, Scott AP, Li W (2003) Pheromones of the male sea lamprey, *Petromyzon marinus* L: structural studies on a new compound, 3-keto allocholic acid, and 3-keto petromyzonol sulfate. Steroids 68:297–304

Yun S-S, Wildbill AJ, Siefkes MJ et al (2011) Identification of putative migratory pheromones from Pacific lamprey (*Lampetra tridentata*). Can J Fish Aquat Sci 68:2194–2203

Chapter 7
The Reproductive Hypothalamic-Pituitary Axis in Lampreys

Stacia A. Sower

Abstract This chapter reviews the knowledge of the hypothalamic-pituitary axis in the control of reproduction in lampreys. In gnathostomes, the hypothalamus and pituitary have well-defined roles in the control of reproduction. Up until the late 1970s, it was thought that the agnathan lampreys did not have the same neuroendocrine control of reproduction seen in the jawed vertebrates, in part due to their lack of the typical anatomical hypothalamic-pituitary connection. Since then and during the past three decades, there have been rapid advances in our knowledge of the structure and function of the hypothalamic and pituitary hormones and respective receptors in lampreys. This chapter highlights the delineation of the neuroendocrine system that has come from 30 years of research on the biochemical, molecular, anatomical, immunohistochemical, and functional studies that have established that lampreys, similar to the jawed vertebrates, have a hypothalamic-pituitary-gonadal axis and that there is a high conservation of the mechanisms of gonadotropin-releasing hormone action. These findings also show that the neuroendocrine factors share common functional and developmental features compared to later evolved vertebrates.

Keywords Estradiol · GABA · Glycoprotein receptors · Gonadotropin · Gonadotropin inhibiting hormone · Gonadotropin-releasing hormone · GnIH · GnRH · GnRH receptors · Hypothalamus · Hypothalamic-pituitary-gonadal axis · Kisspeptin · Neuropeptide Y · NPY · Pituitary · Pituitary glycoprotein hormones · *Petromyzon marinus* · RFamide peptides · Thyrostimulin

7.1 Introduction

In the late 1970s, it was thought that lampreys did not have the same neuroendocrine control of reproduction as in the gnathostomes (i.e., the jawed vertebrates), in part due to their lack of the typical anatomical hypothalamic-pituitary connection. The question whether there is hypothalamic control over reproduction in lampreys

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has special significance since these fishes, along with the hagfishes, represent the oldest lineage of extant vertebrates and hold a basal position to all other vertebrates (see Chaps. 1, 2). However, considering that lampreys are seasonal animals and responsive to environmental cues such as temperature and photoperiod (e.g., see Chaps. 4, 6), it was postulated that there would be some sort of neuroendocrine control over various physiological processes such as metamorphosis and reproduction. Testing this concept, Drs. Joe Crim (Crim et al. 1979a) and Stacia Sower et al. (1983), as postdoctoral associates of Professor Aubrey Gorbman, performed the first immunohistochemical study and experimental study, respectively, suggesting that lampreys may have neuroendocrine control of reproduction. Since then and during the past three decades, there have been rapid advances in our knowledge of the structure and function of the hypothalamic and pituitary hormones and respective receptors in lampreys. This chapter highlights the delineation of this reproductive neuroendocrine system that has come from 30 years of research.

Modern vertebrates are classified into two major groups, the gnathostomes (jawed vertebrates) and the agnathans (jawless vertebrates). There are only two surviving agnathan lineages—the hagfishes (order Myxiniformes) and lampreys (Petromyzontiformes)—while the gnathostomes constitute all other living vertebrates including the bony and cartilaginous fishes and the tetrapods (see Chap. 1). In this chapter, lampreys and hagfishes are considered to be monophyletic. Although the phylogenetic relationship between hagfishes, lampreys, and the jawed vertebrates is still not completely resolved (e.g., Forey and Janvier 1994; Near 2009), recent studies have provided strong evidence for "cyclostome" (i.e., agnathan) monophyly (Ota et al. 2007; Heimberg et al. 2010; Janvier 2010; see Chap. 2).

It is generally believed that two large-scale genome duplications (2R) occurred during the evolution of early vertebrates, although there is controversy on whether the 2R duplications occurred in the lineage leading to all extant vertebrates (including the hagfishes and lampreys) or whether there was one round of duplication prior to and one round of duplication after divergence of the jawless vertebrates (Ohno 1970; Holland et al. 1994; Vandepoele et al. 2004). The sequencing, annotation and synteny analysis (i.e., examining similar blocks of genes in the same relative positions in genomes from different species) of the sea lamprey *Petromyzon marinus* whole genome—a monumental effort performed by an international group of researchers led by Professor Weiming Li (Michigan State University)—now provides support for the first hypothesis (i.e., two rounds of genome-wide duplication in the ancestor to both agnathans and gnathostomes; Smith et al. 2013). Genome duplications, which provide a source of genetic material for subsequent mutation, drift, and selection, are generally considered to make new evolutionary opportunities possible (Crow and Wagner 2006).

The hypothalamic-pituitary system is considered to be a vertebrate innovation and seminal event that emerged prior to or during the differentiation of the ancestral agnathans (Sower et al. 2009) likely due to the two whole rounds of genome duplication. Reproduction in vertebrates is controlled by a hierarchically organized endocrine system (Fig. 7.1). In spite of the very diverse patterns of life cycles, reproductive strategies, and behaviors, this endocrine system is remarkably

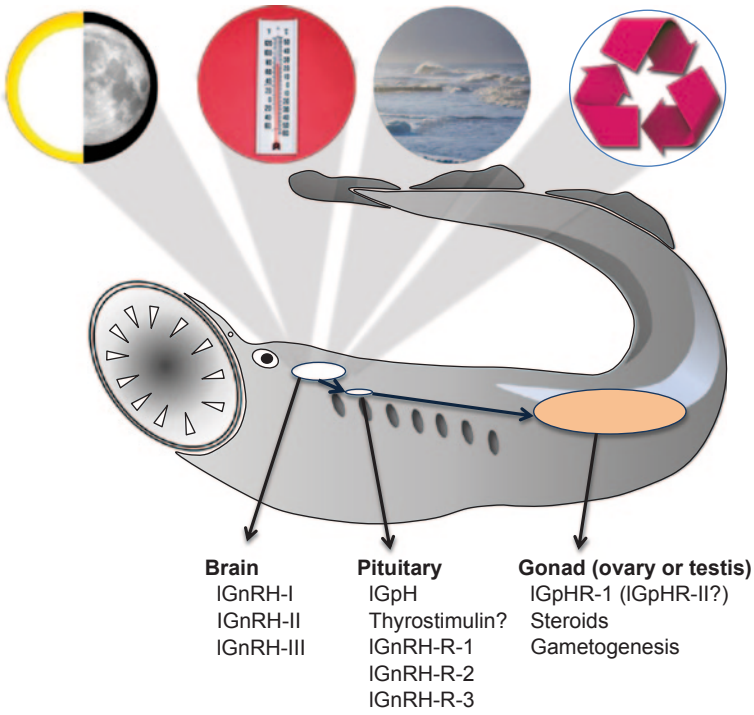

Fig. 7.1 Schematic diagram of the reproductive hypothalamic-pituitary-gonadal (HPG) system in lampreys highlighting the GnRH-GTH system. Lampreys have three gonadotropin-releasing hormones released from the hypothalamus in the brain (lGnRH-I, lGnRH-II, lGnRH-III), one pituitary glycoprotein hormone/gonadotropin (lGpH or GTH), and one glycoprotein receptor (lGpHR-1) in the gonad (Sower et al. 2009). In comparison, gnathostomes generally have one or two GnRHs that act as hypothalamic hormones, two pituitary gonadotropins (LH and FSH), and one gonadal FSH receptor and one LH receptor (see Table 7.3). The hypothalamus integrates photoperiod, temperature, seasonal changes, and feedback cues and, in response, releases one or more GnRHs that act at the pituitary controlling the pituitary-gonadal axis. (Lamprey figure courtesy of Dr. Marty Wong; environmental icons courtesy of Wayne A. Decatur)

conserved throughout the vertebrate lineages. As this chapter describes, it has now been clearly demonstrated that lampreys possess a hypothalamic-pituitary system. Evidence for neuroendocrine control of reproduction in hagfishes is more recent and far less extensive, but it likewise suggests that hagfishes possess a hypothalamic-pituitary system (Sower et al. 1995a; Sower and Kawauchi 2011; Uchida et al. 2010, 2013). The vertebrate neuroendocrine system consists of neurosecretory neurons located in specific nuclei within the hypothalamus of the brain and the pituitary gland or hypophysis. The vertebrate pituitary is composed of two structural components, the neurohypophysis (posterior pituitary) that is actually part of the brain floor and the adenohypophysis (or anterior pituitary) that is composed mostly of cords of secretory cells. The neurohypophysis in most vertebrates (except agnathans and most teleost fishes; see Sect. 7.2) is further divided into two

components: the pars nervosa and the median eminence that is the neurohemal structure that conveys the neurohormones to the adenohypophysis via portal blood vessels (Gorbman 1965).

Although the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) systems overlap (Sower et al. 2009), this chapter focuses on the hypothalamic-pituitary axis as it relates to reproduction. Gonadotropin-releasing hormone (GnRH, previously called luteinizing hormone releasing hormone LHRH) produced in the hypothalamus is a regulatory neurohormone controlling reproduction in all vertebrates (see Guilgur et al. 2006; Kah et al. 2007; Kavanaugh et al. 2008; Okubo and Nagahama 2008). Upon response to external and internal cues, GnRH is released and acts at specific G-protein coupled receptors (GPCR) at the pituitary gland to stimulate the synthesis and release of the gonadotropin(s), which in turn travel via the blood and act via specific GPCRs at the gonads to stimulate and/or regulate steroidogenesis and gametogenesis. Generally, gnathostomes have one or two GnRHs that act as hypothalamic hormones, two pituitary gonadotropins—luteinizing hormone (LH or lutropin) and follicle stimulating hormone (FSH or follitropin), and one gonadal FSH receptor and one LH receptor. In comparison, lampreys have three hypothalamic GnRHs, only one pituitary gonadotropin-type hormone, and one gonadal glycoprotein receptor (Fig. 7.1; Sower et al. 2009). This chapter summarizes the latest information on the hypothalamic-pituitary axis in lampreys, with a focus on its regulation of reproduction; coverage of the lamprey gonad itself is provided by Docker et al. (in press). Most of the information available on the lamprey neuroendocrine system is from the well-studied sea lamprey, with some information from some of the other 40 species of lampreys. Although our knowledge of the neuroendocrine axis in lampreys is far from complete, the following information—acquired from 30 years of research—will serve as a basis for future studies that will continue to extend our understanding of this system in lampreys and vertebrates in general.

7.2 Neuroanatomy of the Hypothalamus

Within all vertebrate brains, there is a region called the hypothalamus. The hypothalamus contains a number of small nuclei and is located below the thalamus, just above the brain stem, and forms the ventral part of the diencephalon. The telencephalon and the diencephalon constitute the forebrain.

In lampreys, the hypothalamus comprises the largest part of the diencephalon (Butler and Hodos 2005). Although there is a considerable body of reported information on the neuroendocrinology of the lamprey hypothalamus, there are relatively few modern reports on its cytoarchitecture or connections (Butler and Hodos 2005). The most ventral part of the hypothalamus surrounds the infundibular recess, a widening of the third ventricle (Nieuwenhuys 1977). The hypothalamus receives afferent axons from all parts of the telencephalon, including the olfactory bulb, and from both the rectum and tegmentum of the mid- and dorsal thalamus

(Nieuwenhuys 1977; Butler and Hodos 2005). Efferents from the lamprey hypothalamus are mainly to the tegmentum of the midbrain (the mesencephalon) and the hindbrain (rhombencephalon), as well as to the dorsal thalamus, ventral thalamus, and the olfactory bulb (Nieuwenhuys 1977; Butler and Hodos 2005).

In mammals, releasing hormones are secreted from the terminal boutons in the median eminence and enter the vascular portal network through capillary beds (Butler and Hodos 1996). The releasing hormones subsequently act on the glandular tissue of the adenohypophysis, inducing the synthesis and release of the anterior pituitary hormones (Butler and Hodos 1996). Of all vertebrates, only the agnathan and non-osteoglossomorph teleost fishes lack a portal vascular system (median eminence) for transferring neurohormones from the hypothalamus to the adenohypophysis (Gorbman 1965; Tsuneki and Nozaki 1989; Fig. 7.2). The adaptive importance of such a portal system is that it makes possible central nervous regulation of such vital processes as reproduction by external (and internal) cycling environmental conditions. The teleosts have solved this structural problem by direct innervation of the pars distalis in the anterior pituitary by appropriate neurosecretory neurons from the adjacent hypothalamus (Gorbman et al. 1983). The agnathans, however, have no nervous or vascular communication between the brain and neurohypophysis (Tsuneki and Gorbman 1975), leading to speculation that nervous regulation of the agnathan pars distalis is by diffusion of brain peptides from the adjacent neurohypophysis, across the thin connective tissue layer that separates the neural from the glandular tissues (Nozaki et al. 1975).

Proof that diffusion is an adequate basis for brain regulation of the pars distalis has rested on such experiments as those of Nozaki et al. (1975) and Tsukahara et al. (1986), who injected substances of varying molecular size (a colloidal particulate dye, a protein, and an ion) into the third ventricle of the inshore hagfish *Eptatretus burgeri*. Staining revealed that, within minutes of injection, significant amounts of molecular substances as large as the protein horseradish peroxidase (44 kDa) had diffused from the third ventricle, through the neurohypophysis, to the pars distalis. These authors also suggested that tanycytes, special ependymal cells located in the floor of the third ventricle and with processes extending deep into the hypothalamus, might also be responsible for transport. There were concerns that experiments with hagfishes might not represent the diffusion hypothesis fairly (Nozaki et al. 1994)—e.g., because of aberrant features of their adenohypophysis (Norris and Carr 2013) and because they may not have an environmentally regulated reproductive cycle (Gorbman and Dickhoff 1978)—but subsequent anatomical evidence also supported the concept of a "diffusional median eminence" in lampreys (King et al. 1988; Tsuneki 1988; Nozaki et al. 1994). In both sea lamprey and Pacific lamprey *Entosphenus tridentatus*, GnRH-like neurons identified by immunocytochemistry were shown to project their fibers primarily into the neurohypophysis from the preoptic region (PO; also abbreviated POA for preoptic area, or PON for preoptic nucleus) of the hypothalamus (Crim et al. 1979a, b; Nozaki and Kobayashi 1979; Nozaki and Gorbman 1984; King et al. 1988). Crim (1981) and King et al. (1988) also showed that GnRH neurons project into the third ventricle, and likewise suggested that there might be an additional route of GnRH movement via secretion into the

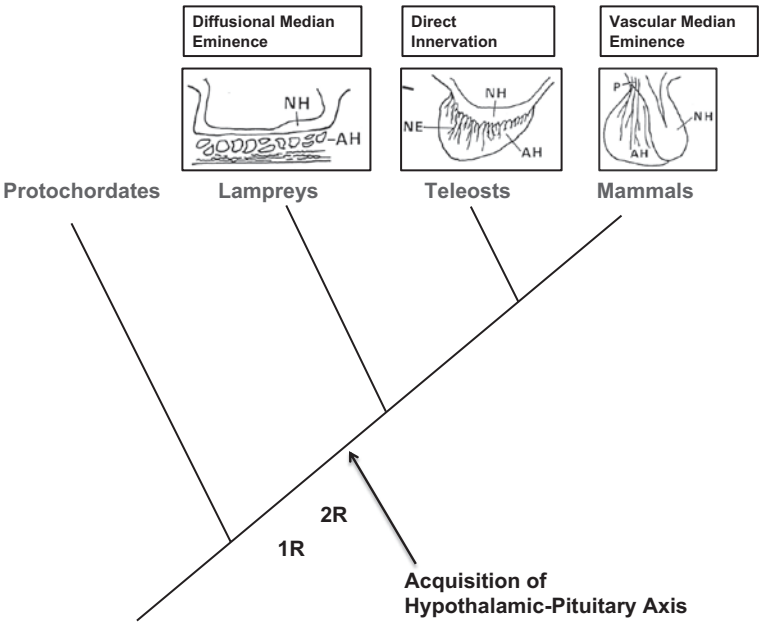

Fig. 7.2 Schematic of the three types of regulation of the adenohypophysis (anterior pituitary) developed in the vertebrates: the agnathan diffusional type (in hagfishes and lampreys), the teleostean direct innervational type (in non-osteoglossomorph teleosts), and the vascular type seen in all other vertebrates (e.g., cartilaginous fishes, most non-teleost bony fishes, and tetrapods). Adenohypophysis (*AH*), neurohypophysis (*NH*), neurohypophysial axonal extensions (*NE*), portal blood vessel (*P*) from vascular median eminence, and first (*1R*) and second round (*2R*) of whole genome duplication in vertebrates are shown. (Adapted from Nozaki et al. 1994)

third ventricle and transport by tanycytes to the adenohypophysis (King et al. 1988). As in hagfish, experimental studies in sea lamprey showed that horseradish peroxidase, injected into the third ventricle of the brain of adults, rapidly passed (within 5–15 min) through the neurohypophysis, which forms the floor of the third ventricle, and diffused throughout the connective tissue separating the adenohypophysial follicles from the neurohypophysis and into intracellular spaces in the adenohypophysis (Nozaki et al. 1994). These authors concluded that neurosecretory peptides like GnRH diffuse from the brain (neurohypophysis) to the adenohypophysis, and thus regulate its secretory activity in lampreys.

It thus appears that, in the evolutionary sense, there have been three types of brain regulation of the adenohypophysis developed in the vertebrates: the agnathan diffusional type, the teleostean direct innervational type, and the vascular type seen in all other vertebrates (Nozaki et al. 1994; Fig. 7.2). These authors suggested that the principal advantage of the vascular median eminence type of control of the pars distalis by the brain is that it permitted development of larger and thicker glands as vertebrates became larger and more complicated in form and the distance between the hypothalamus and pituitary increased significantly. Teleost fishes secondarily acquired a new system of integration with direct release of the neurohormones into

the adenohypophysis (Lagios 1970; Peter et al. 1990), whereas in extant agnathans, structural features of the pituitary and surrounding tissues appear to have evolved to make diffusion as efficient as possible for pituitary regulation by brain peptides (Nozaki et al. 1994).

7.3 Pituitary: Neurohypophysis and Adenohypophysis

As stated above, the pituitary gland of lampreys (as in all other vertebrates) consists of the neurohypophysis and adenohypophysis. The neurohypophysis, which is not as highly developed in lampreys as it is in hagfishes (Gorbman et al. 1983), consists of a thin anterior section that is the floor of the diencephalon. The posterior part is somewhat thickened and is the terminating neurohemal structure for neurons whose cell bodies are in the preoptic region of the hypothalamus (Fig. 7.3b, c); it is thus similar to the pars nervosa found in most other vertebrates (Gorbman et al. 1983). The lamprey adenohypophysis, in contrast to the neurohypophysis, is much better differentiated than in hagfishes (see Sower and Kawauchi 2011). As in the jawed fishes, it is differentiated into the rostral pars distalis (RPD), the proximal pars distalis (PPD), and the pars intermedia (PI). Neither the hagfishes nor lampreys have the anatomical equivalent of a median eminence to convey the neurohormones to the adenohypophysis; brain regulation of the pituitary is achieved instead via diffusion (see Sect. 7.2).

The adenohypophysis of the pituitary gland in turn secretes a number of protein hormones that regulate a variety of physiological processes in vertebrates. The adenohypophysial hormones can be classified, on the basis of structural and functional similarity, into three groups: (1) the proopiomelanocortin (POMC) family; (2) the growth hormone/prolactin/somatolactin (GH/PRL/SML) family; and (3) the glycoprotein hormone (GpH) family which includes gonadotropins (GTHs) and thyroid-stimulating hormone (TSH or thyrotropin) and that now also includes thyrostimulin (Kawauchi and Sower 2006; Sower et al. 2009); another member of the GpH family, chorionic gonadotropin (CG), is not expressed in the pituitary and is only found in placental mammals (see Sect. 7.10). The POMC family includes adrenocorticotropin (ACTH) and melanocyte stimulating hormone (MSH). The classical GpH family hormones each consist of two non-covalently bound subunits, α and β (Kawauchi et al. 1989); within a species, the α subunit is the same among all the GpH family hormones, while the β subunits are different and convey hormone specificity (Kawauchi et al. 1989; Swanson et al. 1991; Huhtaniemi 2005). Somatolactin is only found in bony fishes (including sturgeons, teleosts, and lungfishes; Amemiya et al. 1999; Fukamachi and Meyer 2007). Each hormone family is believed to have evolved from an ancestral gene by duplication and subsequent mutations (Kawauchi and Sower 2006; Fukamachi and Meyer 2007).

The pituitary hormones in agnathans had been an enigma until the laboratories of Professors Kawauchi and Sower with other collaborators characterized hormones from these three families in the sea lamprey (Kawauchi and Sower 2006). In general,

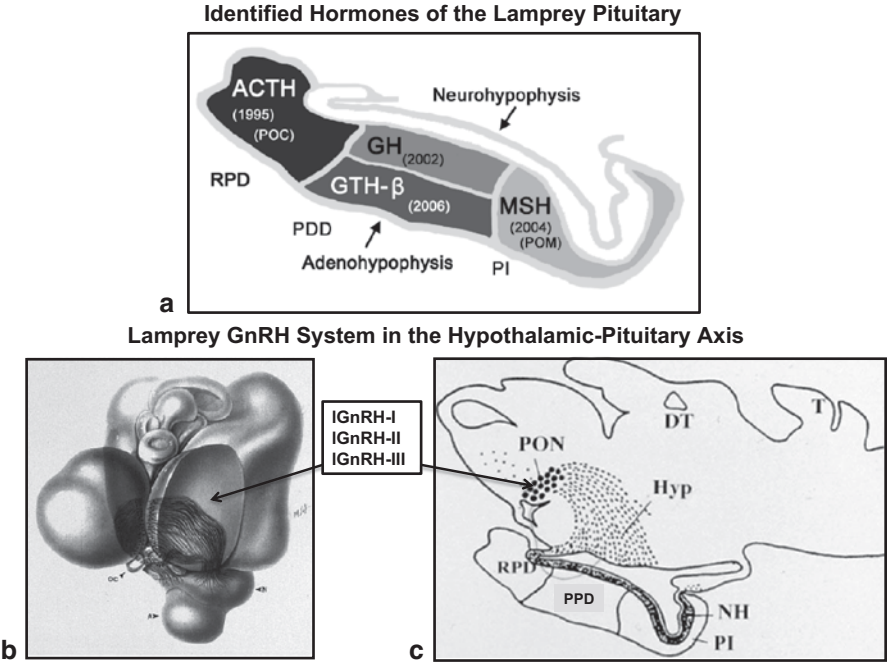

Fig. 7.3 **a** A schematic diagram of the lamprey pituitary consisting of the adenohypophysis (divided into the rostral pars distalis *RPD*, proximal pars distalis *PPD*, and pars intermedia *PI*) and neurohypophysis. Main sites of expression of the following adenohypophysial hormones are indicated (along with date of discovery in lampreys): adrenocorticotropin (*ACTH*), gonadotropin-β (*GTH-β*), growth hormone (*GH*), melanocyte stimulating hormone (*MSH*). ACTH (and one β-endorphin) are encoded by the proopiocortin (*POC*) gene; MSH (and a different β-endorphin) are encoded by the proopiomelanotropin (*POM*) gene (Drawing courtesy of Professor Hiroshi Kawauchi). **b** Diagrammatic representation of the system of GnRH cells and their projections in the brain of the lamprey. The brain is viewed from the front so that the telencephalon appears as large rounded masses, the optic chiasm (*oc*) is seen on the ventral surface rostral to the adenohypophysis (*A*) and neurohypophysis (*N*). Projections of ventral preoptic cells join those of the dorsal preoptic cells to form the preoptico-hypophyseal tract; both these cell groups project along the external surface of the brain to the neurohypophysis and their processes directly contact the midline third ventricle. **c** Distribution of irGnRH containing cell bodies (*circles*) and fibers (*broken lines*) in approximate mid-sagittal planes in the brain of the sea lamprey. Dorsal thalamus (*DT*), hypothalamus (*Hyp*), neurohypophysis (*NH*), pars intermedia (*PI*), proximal pars distalis (*PPD*), preoptic nucleus (*PON*), rostral pars distalis (*PD*), and optic tectum (*T*) are shown; T is found within the midbrain; Hyp (including PO) and DT are located within the forebrain. (Adapted from Nozaki and Gorbman (1984). **b** was originally published in King et al. (1988) and reproduced with kind permission from Springer Science+Business Media)

pituitary cells of a given type are located in specific regions of the adenohypophysis (Fig. 7.3a). ACTH and MSH, together with distinct endorphins in the sea lamprey, are encoded by two distinct genes, proopiocortin (POC) and proopiomelanotropin (POM), respectively, while they are encoded by the same POMC gene in the jawed vertebrates (Heinig et al. 1995; Takahashi et al. 1995a, b). The POC and POM are

expressed specifically in the RPD and PI, respectively. ACTH cells are distributed in most parts of the RPD, as well as some in the PPD, whereas MSH cells are found in almost all parts of the PI (Nozaki et al. 1995). Growth hormone (GH) appears to be the only member of the GH/prolactin/somatolactin family in the sea lamprey (Kawauchi et al. 2002). Its gene consists of five exons and four introns spanning 13.6 kilobases, which is the largest among known GH genes. GH is expressed in the cells of the dorsal half of the PPD (Fig. 7.3a).

Within the glycoprotein hormone (GpH) family, although two GTHs (LH and FSH) were known from all taxonomic groups of gnathostomes by the 1990s (Suzuki et al. 1988; Kawauchi et al. 1989; Quérat et al. 2000, 2004), only one GTH has been identified in the agnathans, in both lampreys (Sower et al. 2006) and hagfishes (Uchida et al. 2010, 2013; see Sect. 7.10). Further discussion of the potential origin and/or divergence of the GTHs among vertebrates is provided in Sect. 7.10. In lampreys, the GTHβ is expressed in the ventral portion of the PPD (Fig. 7.3b).

7.4 Gonadotropin-Releasing Hormone (GnRH) Isoforms

The primary structure of the first GnRH (GnRH-I) in lampreys was determined by Sherwood and Sower and colleagues in 1986 (Sherwood et al. 1986). The primary structure of a second form (GnRH-III) was determined in 1993 (Sower et al. 1993), and the complementary DNA (cDNA) encoding a third form (GnRH-II) was cloned and identified in 2008 (Kavanaugh et al. 2008). In the 1986 Sherwood et al. paper, the amino acids but not the sequence of a second putative form of GnRH were identified and it was called lamprey GnRH-II. Therefore, when lamprey GnRH-III was discovered in 1993, it was called -III instead of -II. This was fortunate since the lamprey GnRH-II (which was not the same as identified in 1986) turned out to be a GnRH-2 type form. Like GnRH in all other vertebrates (see below), lamprey GnRH is a decapeptide (Table 7.1). Similar to other neuropeptides, GnRH is derived from a larger precursor protein (or prohormone), prepro-GnRH, which is then processed to the final decapeptide (Suzuki et al. 2000). The precursor protein is a tripartite molecule, consisting of a signal peptide; the GnRH decapeptide and cleavage (or processing) site; and a GnRH-associated peptide, GAP (Suzuki et al. 2000). The GnRH-I, -II, and -III prohormone peptides consist of 87, 105, and 93 amino acid residues, respectively, in the sea lamprey. Each of the GnRH precursor proteins are expressed by separate genes (Suzuki et al. 2000; Silver et al. 2004); the cDNA encoding these precursors are 638, 694, and 718 nucleotides (or base pairs, bp) in length in sea lamprey for prepro-lamprey GnRH-I, -II, and -III (Suzuki et al. 2000; Silver et al. 2004; Kavanaugh et al. 2008). In seven other lamprey species (including representatives from all three families; see below), the cDNA encoding prepro-lamprey GnRH-II was 666–774 bp in length and encoded 92 or 94 amino acids (Silver et al. 2004).

Table 7.1 Amino acid sequences of the 15 vertebrate GnRH decapeptides. Non-identical residues are in bold. The GnRHs are divided into three types: GnRH-1; GnRH-2 and GnRH-2-like; and GnRH-3. GnRH-I and -III in lampreys share a common ancestry with both GnRH-2 and -3 (see Sect. 7.4). The original names such as mammal and guinea pig GnRH are based on the taxon in which the forms were discovered. Lamprey GnRH-I and -III are the only vertebrate GnRHs to have different amino acids, Glu and Asp, respectively, in the sixth position. As is standard for peptides, the sequences are given from amino terminus to carboxyl terminus; the NH_2 at the carboxyl terminus indicates that it terminates as a carboxamide

GnRH	1	2	3	4	5	6	7	8	9	10
GnRH-1										
Mammal	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	$GlyNH_2$
Guinea Pig	pGlu	**Tyr**	Trp	Ser	Tyr	Gly	**Val**	Arg	Pro	$GlyNH_2$
Chicken-I	pGlu	His	Trp	Ser	Tyr	Gly	Leu	**Gln**	Pro	$GlyNH_2$
Rana	pGlu	His	Trp	Ser	Tyr	Gly	Leu	**Trp**	Pro	$GlyNH_2$
Seabream	pGlu	His	Trp	Ser	Tyr	Gly	Leu	**Ser**	Pro	$GlyNH_2$
Whitefish	pGlu	His	Trp	Ser	Tyr	Gly	**Met**	**Asn**	Pro	$GlyNH_2$
Medaka	pGlu	His	Trp	Ser	**Phe**	Gly	Leu	**Ser**	Pro	$GlyNH_2$
Catfish	pGlu	His	Trp	Ser	**His**	Gly	Leu	**Asn**	Pro	$GlyNH_2$
Herring	pGlu	His	Trp	Ser	**His**	Gly	Leu	**Ser**	Pro	$GlyNH_2$
GnRH-2 and GnRH-2-like										
Chicken-II	pGlu	His	Trp	Ser	**His**	Gly	**Trp**	**Tyr**	Pro	$GlyNH_2$
Dogfish	pGlu	His	Trp	Ser	**His**	Gly	**Trp**	**Leu**	Pro	$GlyNH_2$
Lamprey-II	pGlu	His	Trp	Ser	**His**	Gly	**Trp**	**Phe**	Pro	$GlyNH_2$
GnRH-3										
Salmon	pGlu	His	Trp	Ser	Tyr	Gly	**Trp**	**Leu**	Pro	$GlyNH_2$
GnRH-2/3										
Lamprey-III	pGlu	His	Trp	Ser	**His**	**Asp**	**Trp**	**Lys**	Pro	$GlyNH_2$
Lamprey-I	pGlu	His	**Tyr**	Ser	**Leu**	**Glu**	**Trp**	**Lys**	Pro	$GlyNH_2$

The GnRH family currently includes at least 32 GnRHs, with 15 having been identified from representative vertebrate species (Kavanaugh et al. 2008), 10 from non-vertebrate chordates (all from the urochordates or tunicates; Adams et al. 2003), at least six from protostomian invertebrate species (molluscs and annelids; Tsai and Zhang 2008; Zhang et al. 2008; Sun et al. 2012), and one putative GnRH from the deuterstome echinoderms (Rowe and Elphick 2012). Whereas all chordate GnRHs are decapeptides, non-chordate GnRHs identified to date consist of 11 or 12 amino acids (Tsai and Zhang 2008; Sun et al. 2012). Each new GnRH was named according to the first species in which that particular peptide sequence was found (see Fernald and White 1999; Table 7.1), although these GnRHs are not restricted to these taxa; chicken-II, for example, is found in all gnathostomes (Fernald and White 1999; Kavanaugh et al. 2008). To date, two to three GnRHs have been identified in lampreys (see above) and representative species of all classes of gnathostomes (Gorbman and Sower 2003; Guilgur et al. 2006; Kah et al. 2007; Kavanaugh et al. 2008; Okubo and Nagahama 2008), and growing evidence suggests that at least two GnRH forms are expressed within the brain of any one species (Kah et al. 2007; Kavanaugh et al. 2008). Chromatographical and immunocytochemical studies have identified a GnRH molecule in the brain of both Pacific hagfish *Eptatretus stouti* (Braun et al. 1995) and Atlantic hagfish *Myxine glutinosa* (Sower et al. 1995a). This GnRH was characterized as being more structurally similar to lamprey GnRH-III than to any other vertebrate GnRH (Sower et al. 1995a), but its gene and primary structure has not yet been identified. Lampreys are thus the earliest evolved group for which multiple GnRH isoforms have been identified.

Early analyses by Grober et al. (1995) and Fernald and White (1999) suggested that there were three paralogous (i.e., related by duplication) groups of GnRH (GnRH-1, GnRH-2, and GnRH-3) in gnathostome brains. Most vertebrate species have forms from the GnRH-1 and GnRH-2 groups; GnRH-3 has been found only in teleosts (Fernald and White 1999; Chen and Fernald 2008), although not all teleost species possess three forms (Guilgur et al. 2006). The primary amino acid sequences of GnRH-2 and GnRH-3 forms are highly conserved in gnathostomes. The primary amino acid sequence of chicken GnRH-II (GnRH-2) is identical from sharks to mammals, and only one other GnRH-2 sequence, dogfish GnRH (Lovejoy et al. 1992), is known. Salmon GnRH found only in teleosts is the only known GnRH-3 in gnathostomes (Kah et al. 2007). GnRH-1 sequences, however, have diverged within the gnathostome lineage (Table 7.1). The GnRH-1 neurons are primarily located in the forebrain (i.e., the preoptic area of the hypothalamus or telencephalon); GnRH-2 is produced primarily in the midbrain or mesencephalon, and the teleost-specific GnRH-3 neurons are located in the terminal nerve ganglion near the olfactory bulb (Fernald and White 1999; Guilgur et al. 2006; Kah et al. 2007; Chen and Fernald 2008).

More recent reviews of GnRHs and their respective receptors have proposed various other scenarios on the phylogenetic relationships among gnathostome GnRHs and receptors (e.g., Guilgur et al. 2006; Kah et al. 2007; Okubo and Nagahama 2008), but agnathan GnRHs were not included. Inclusion of lamprey GnRHs, including prepro-lamprey GnRH-III cDNAs from all three lamprey families, had

previously suggested four paralogous lineages: GnRH1, -2, -3, and -4 (Silver et al. 2004). This study, using phylogenetic analysis, function, neural distribution, and developmental origin, confirmed the model of three gnathostome GnRH lineages and placed lamprey GnRH-I and -III into a fourth group. Identification of two mollusc GnRHs and one annelid GnRH suggested a fifth group consisting of invertebrate GnRHs, and upheld the fourth group of lamprey GnRH-I and -III (Tsai and Zhang 2008; Zhang et al. 2008).

The subsequent discovery of a third lamprey GnRH, GnRH-II, however, offered a new paradigm of the origin of the vertebrate GnRH family (Kavanaugh et al. 2008). Phylogenetic analyses suggested that, although lamprey GnRH-I and -III were included within a fourth vertebrate GnRH group, lamprey GnRH-II appeared sister to all the gnathostome GnRHs. These authors thus hypothesized that, likely due to a genome/gene duplication event, an ancestral gene gave rise to two lineages of GnRHs—the gnathostome GnRH and lamprey GnRH-II. The gene duplication events that generated the different fish and tetrapod paralogous groups likely took place within the gnathostome lineage, after its divergence from the ancestral agnathans (Kavanaugh et al. 2008).

However, recent synteny data provide evidence for an alternate view of the evolution of the GnRH family and suggest that all duplication events that generated the different fish and tetrapod GnRH groups likely took place before the split of the ancestral lamprey and gnathostome lineages (Decatur et al. 2013; Smith et al. 2013). GnRH-1 has been lost from lampreys, as it has in zebrafish *Danio rerio* (Kuo et al. 2005). In addition, the analysis corroborates recent views (Kim et al. 2011; Tostivint 2011) that GnRH-3 was lost in the tetrapod lineage and did not arise in the teleost lineage as a result of a third round of whole genome duplication (3R; Decatur et al. 2013). There is not any biochemical evidence in lampreys for an extant GnRH-4-like paralog which was proposed to have arisen from tetraploidizations in the early stages of vertebrate evolution (Tostivint 2011). Furthermore, there was not any obvious GnRH-4-like candidate identified from preliminary analysis of the genome, suggesting that the homolog of this gene was lost in lampreys, similar to its loss in the other vertebrate lineages. With respect to the agnathans and GnRH, the analysis of the synteny agrees with the previous proposal in that lamprey GnRH (lGnRH)-I and -III resulted from a duplication event within the lamprey lineage (Kavanaugh et al. 2008; Decatur et al. 2013). However, the data now suggest a substantially different view of the evolutionary history of the GnRH family in vertebrates. Significantly, the current evidence suggests that all of the genome duplication events that generated the different fish and tetrapod paralogous groups (Kuraku et al. 2009) likely took place before the divergence of the ancestral agnathan and gnathostome lineages and that the GnRHs in lampreys that were previously proposed (erroneously) as members of group IV (lGnRH-I and -III) share a more recent common ancestry with GnRH-2 and -3 (GnRH-2/3; Table 7.1). Given the single amino acid difference between mature lGnRH-II and GnRH-2, it is proposed that a GnRH-2-like gene existed before the lamprey/gnathostome split (Decatur et al. 2013).

7.5 Immunolocalization of GnRH in the Brain

As stated above (see Sect. 7.4), in gnathostomes, the GnRH-1 neurons are primarily located in the preoptic area of the hypothalamus, GnRH-2 predominates in extra-hypothalamic regions (i.e., the midbrain and numerous peripheral tissues, although there are species in which chicken GnRH-II is found in the hypothalamic region), and the teleost-specific GnRH-3 neurons are located in the terminal nerve ganglion near the olfactory bulb (Fernald and White 1999; Guilgur et al. 2006; Chen and Fernald 2008; Kavanaugh et al. 2008). As described in the following sections, it appears that lampreys may possess three hypothalamic GnRHs, and their respective predominance during different stages of the life cycle may help to infer their functions.

7.5.1 GnRH-I and GnRH-III Distribution and Localization

In lampreys, there is a general pattern of GnRH distribution in the anterior-preoptic-neurohypophysial tract to the neurohypophysis of adult lampreys as determined by immunocytochemical studies using mammalian GnRH antisera (e.g., Crim et al. 1979a, b; Nozaki and Kobayashi 1979; Nozaki et al. 1984; King et al. 1988) or, following determination of the primary amino acid structure of lamprey GnRH-I (Sherwood et al. 1986), with specific lamprey GnRH antisera (e.g., Tobet et al. 1995, 1996; Eisthen and Northcutt 1996). These findings were later verified with antisera to lamprey GnRH-III and -II (e.g., Tobet et al. 1996; Fig. 7.4). In the earlier studies using mammalian GnRH or lamprey GnRH-I antisera, GnRH immunoreactive cells were shown to project their fibers primarily into the neurohypophysis from the preoptic area in adult Pacific lamprey (Crim et al. 1979b), western brook lamprey *Lampetra richardsoni* (Crim et al. 1979a), sea lamprey (Nozaki and Kobayashi 1979; Nozaki et al. 1984; King et al. 1988), and silver lamprey *Ichthyomyzon unicuspis* (Eisthen and Northcutt 1996). As described by King et al. (1988), immunopositive neuronal perikarya (i.e., cell bodies) were present in an arc-shaped population extending from ventral to dorsal preoptic areas. Fibers from these cells projected to the neurohypophysis via the preoptico-hypophyseal tract, but in addition also protruded into the third ventricle. Additionally, some fibers coursed along the external surface of the brain, suggesting the release of GnRH into meningeal compartments.

In a later study designed to distinguish GnRH-I and -III, specific antisera were generated, followed by the use of two kinds of immunostaining: one was a single immunostaining by one of the two GnRH antisera using two successive sections; the other was double immunostaining of a single section (Nozaki et al. 2000). In adult sea lamprey brain, a dense accumulation of neuronal cells immunoreactive (ir) to antisera against either lamprey GnRH-I or -III was found in the arc-shaped preoptico-anterior hypothalamic area. Additional smaller numbers of irGnRH cells were found in the periventricular zone of the posterior hypothalamus. In the

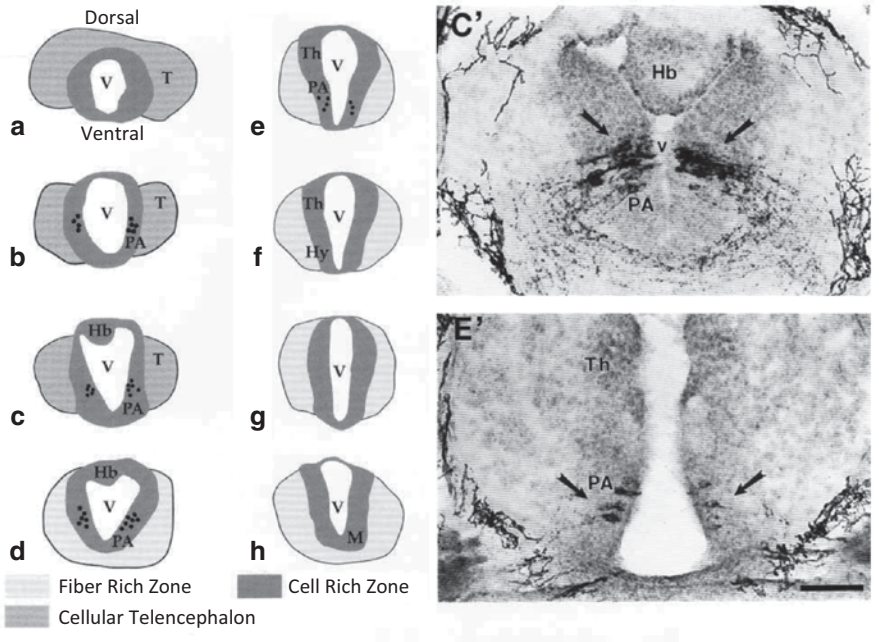

Fig. 7.4 Schematic diagrams of coronal sections illustrate the position of cells containing immunoreactive lamprey GnRH-III in a representative 47-day-old larval lamprey. Immunoreactive (ir) cells are represented by *solid black circles*. Different *shades of gray* indicate the cell-rich medial zone and the fiber-rich lateral zone of the diencephalon (sections A–G) and mesencephalon (section H), and the telencephalon (*T*) that was comprised of more loosely packed cells (sections A–C). The cell-rich medial zone of the diencephalon was only 10–20 cells deep at this point in development. The sections are arranged from rostral (A) to caudal (H) and are approximately 30 μm apart. Ventricle (*V*), habenula (*Hb*), thalamus (*Th*), preoptic area (*PA*), hypothalamus (*Hy*), and mesencephalon (*M*) are shown. Representative photomicrographs (C' and E') from a 66-day-old larval lamprey at schematic levels C and E are presented on the right. *Arrows* in the photomicrographs indicate the positions of cells containing ir lamprey GnRH-III. Dark but immunonegative melanophores are visible at the edges of the tissue. The scale bar in E' also applies to C' and = 50 μm. (This figure was originally published in Tobet et al. (1996) and reproduced with permission of John Wiley & Sons, Inc.)

above-mentioned locations, the distribution of both irGnRH-I and -III cells was intermixed and very similar, but the cells exhibiting GnRH-III immunoreactivity were distinctly different from those exhibiting GnRH-I immunoreactivity. The relative numbers of irGnRH-III cells were larger than those of irGnRH-I cells in the preoptico-anterior hypothalamic area, and more than 90 % of GnRH cells in the posterior hypothalamus were irGnRH-III cells (Nozaki et al. 2000). Both irGnRH-I and -III cells projected their fibers primarily into the neurohypophysis. The relative densities of the accumulated irGnRH-III fibers were similar to those of irGnRH-I fibers in the anterior neurohypophysis but higher than those of irGnRH-I fibers in the posterior neurohypophysis. In larval sea lamprey, the majority of irGnRH in the brain was lamprey GnRH-III (Tobet et al. 1995; see Sect. 7.5.2). However, in contrast to the findings above where irGnRH-III cells

in adults appeared to be distinct from irGnRH-I cells (Nozaki et al. 2000), these authors showed co-localization of GnRH in cells (i.e., when lamprey GnRH-I was seen, it was in cells that appeared to contain both forms of GnRH). A later study using in situ hybridization also supported co-localization of the mature GnRH-I and -III proteins in cells of the preoptic region of adult sea lamprey, as did Youson et al. (2006) throughout the life cycle of the non-parasitic American brook lamprey *Lethenteron appendix* (see Sect. 7.5.2). Further detailed studies using multiple immunostaining will be needed to distinguish whether the three different forms (i.e., including the more recently-discovered GnRH-II; Kavanaugh et al. 2008) are co-localized or found in distinct cells.

Immunohistochemical studies also provide evidence for the presence of lamprey GnRH-I and -III in the brain of representatives of the two Southern Hemisphere lamprey families, Geotriidae and Mordaciidae (Sower et al. 2000). Moreover, the distribution pattern of irGnRH cell bodies and fibers in adult specimens from these families was similar to that observed in the Holarctic lampreys (e.g., sea and Pacific lampreys). The situation in the larvae, however, was somewhat different. Wright et al. (1994) and Tobet et al. (1995) studied the distribution of lamprey GnRH in the brain of larval and metamorphosing sea lamprey, and found extensive irGnRH cell bodies and tracts. In the pouched lamprey *Geotria australis*, Sower et al. (2000) found some of these irGnRH cell bodies and tracts, but they were less widely distributed, being confined to the dorsal preoptic area (POA), with simple tracts projecting only to the rostral and caudal neurohypophysis. The more extensive tracts described for sea lamprey larvae were found only in the adult pouched lamprey. This reduced distribution of lamprey GnRH in the brain of the pouched lamprey larvae may be related to the later development of the gonads in this species as compared to sea lamprey. In large, premetamorphic pouched lamprey, the testis is still small and apparently undifferentiated with only a few germ cells present among numerous somatic cells (Hardisty et al. 1986; see Docker et al. in press). A more intriguing difference between pouched and sea lampreys is the presence of irGnRH cells in the adenohypophysis (anterior pituitary) of both larvae and adults of the former species. There is no mention of these cells in any of the sea or Pacific lamprey studies (Nozaki et al. 1984; King et al. 1988; Wright et al. 1994; Tobet et al. 1995), which suggests that they were not observed in these Northern Hemisphere species. The significance of these cells in the pituitary of pouched lamprey remains to be investigated, but they may indicate an additional regulatory pathway that is unknown. With the identity of a third lamprey GnRH, further detailed studies will be needed to examine GnRH in the anterior pituitary.

7.5.2 Distribution and Activity of GnRH at Different Stages of the Life Cycle

Several studies have examined GnRH distribution at different stages of the lamprey life cycle and show that GnRH activity is not restricted to the final reproductive phase. There have been many immunohistochemical studies showing GnRH

distribution in the brain throughout the life cycle of the sea lamprey (King et al. 1988; Wright et al. 1994; Tobet et al. 1995, 1996, 1997; Nozaki et al. 2000; Reed et al. 2002; Root et al. 2005), for example, and in the pouched lamprey from the Southern Hemisphere (Sower et al. 2000). Prominent changes have been demonstrated during embryogenesis (Tobet et al. 1996), larval development (e.g., Tobet et al. 1995), and metamorphosis (e.g., Youson and Sower 1991, 2001). In larval sea lamprey, both lamprey GnRH-I and -III are found in the cell bodies in the rostral hypothalamus and preoptic area (Wright et al. 1994; Tobet et al. 1995). However, the majority of irGnRH in developing larval lampreys was GnRH-III, which suggests that this is the more active form during early gonadal development (Tobet et al. 1995). A small number of cells found in the caudal hypothalamus contain only irGnRH-III, which may constitute a functional subgroup within the population of GnRH neurons (Tobet et al. 1995). In lampreys undergoing metamorphosis, there is a large increase in reaction product in all GnRH-containing cells and fibers in the rostral and preoptic hypothalamic areas (Crim et al. 1979a; Wright et al. 1994; Tobet et al. 1995). In addition, there is a noted increase of irGnRH cells in the ventral hypothalamic area in larger larvae and during metamorphosis, which suggests that these cells play a unique role during metamorphosis (Tobet et al. 1995).

In the most comprehensive study of its kind to date, Youson et al. (2006) investigated brain GnRH immunoreactivity throughout the entire lamprey life cycle (with the exception of embryogenesis) in the non-parasitic American brook lamprey. Whereas both the sea and pouched lamprey are parasitic as adults (i.e., delaying sexual maturity for one or more years after metamorphosis, during which time the sexually immature juveniles feed on actinopterygian fishes), non-parasitic lampreys begin sexual maturation before the completion of metamorphosis and bypass the adult feeding phase altogether (see Docker 2009; Docker and Potter in press). Youson et al. (2006) found neurosecretory cells and fibers that were immunoreactive (ir) with sea lamprey GnRH-I and -III antisera in the neurohypophysis and preoptic area of the brain of late larval, metamorphosing (stages 1–7; see Chap. 4), juvenile, and prespawning and spawning adults. Using the antisera and preabsorption testing first validated by Tobet et al. (1995) and then again by Nozaki et al. (2000), this study found that there were some cells and fibers that seem to contain both forms of the hormone (see Sect. 7.5.1). An earlier immunohistochemical study on larval and maturing adult western brook lamprey, also non-parasitic, used an antiserum to mammalian GnRH to show some life-cycle differences in immunoreactivity that were related to the events of metamorphosis and sexual maturation (Crim et al. 1979a).

Consistent with previous studies in larval sea lamprey (Tobet et al. 1995), Youson et al. (2006) also found that intensity of immunoreactive staining in the preoptic area of the American brook lamprey is higher (or at least equivalent) for the GnRH-III peptide than it is for GnRH-I prior to metamorphosis. Brain concentrations based on radioimmunoassay (RIA) likewise indicate that most of the immunoreactivity for GnRH in sea lamprey during the larval stage and early metamorphosis is GnRH-III (Sower 2003). The situation is reversed, however, following metamorphosis. In the American brook lamprey, staining intensity of GnRH-III is

superseded at mid-metamorphosis (stage 4) by GnRH-I (Youson et al. 2006). Likewise, the RIA data indicate that—although GnRH-III seems to predominate in sea and western brook lamprey up to stages 6 and 4 of metamorphosis, respectively—GnRH-I appeared to be the dominant adult form (Youson et al. 1995; Youson and Sower 2001). This provides further evidence that metamorphosis is a prominent phase of upregulated activity for GnRHs to act either locally or on other components of the reproductive axis. This activity is likely consistent for all lampreys, irrespective of their adult life history, although Youson et al. (1995) observed that levels of both forms were lower in the non-parasitic western brook lamprey than in the parasitic sea lamprey. The dominance of GnRH-III in larvae, however, seems to run counter to the view that it is the more active form during gonadal maturation (Sower 2003), since the histological observations indicate that the gonads of stage 4 American brook lamprey were in a maturation phase and GnRH-I intensity is the most prominent (Youson et al. 2006). However, the intensity of staining for GnRH-III indicates a continuous rising trend up until the last stage (7) of metamorphosis. Tobet et al. (1995) noticed a large increase in immunoreactive product (mostly GnRH-III) in GnRH-containing cells and fibers during sea lamprey metamorphosis and suggested that GnRH-III is important during maturation of GnRH cells and fibers. This role of maturation of GnRH cells, that attained their positions in the preoptic and hypothalamic areas before metamorphosis, is possibly a key event at this interval of ontogeny. If GnRH-III is to be accepted as the form that is most active during gonadal maturation, then what—given that GnRH-I is the dominant GnRH in both mid- to late metamorphic American brook and sea lampreys—is the function of GnRH-I at the same stage of metamorphic development of a non-parasitic and parasitic lamprey when the latter will not undergo sexual maturation for at least another year (see Sect. 7.7)?

The aforementioned results of the distribution of the immunoreactive cell bodies and fibers in the brain of American brook lamprey (Youson et al. 2006) corresponds to that described in earlier immunohistochemical studies of lampreys of different species (Crim et al. 1979a, b; King et al. 1988; Wright et al. 1994; Tobet et al. 1995; Nozaki et al. 2000; Sower et al. 2000; Reed et al. 2002; Root et al. 2005). In larval and metamorphic stage 1–3 animals, most ir fibers were confined to the dorsal region of the neurohypophysis, directly beneath the ventricular lining of ependymal cells. The lower irGnRH in larvae followed by apparently more peptide with which to immunoreact during metamorphosis is similar to that found in other studies (Crim et al. 1979a; Wright et al. 1994; Tobet et al. 1995; Root et al. 2005). However, the apparent increased synthesis of GnRH peptide is further suggested by the changing appearance of the ir neurons in the Youson et al. (2006) study. The ir neurons in the larval, metamorphic, and adult stages of this study were rounded, early elongated cuboidal, and pear-shaped, respectively. This pattern of changes in neuronal shape is similar to that found in sea lamprey (King et al. 1988; Tobet et al. 1995). The striking increase during metamorphosis in irGnRH in a ventral hypothalamic cell group caudal to the optic chiasm that was barely detectable in larvae, coupled with their location and the orientation of their processes, led the authors to speculate that the cell group might have a role during metamorphosis or in

subsequent development of the reproductive system. This cell group was also noted in sea lamprey at the same time in the life cycle (Tobet et al. 1995). Unfortunately, the RIA study of the non-parasitic western brook lamprey did not have data for the last stage (7) of metamorphosis or juveniles for comparison with this study (Youson et al. 1995). The intensity data of the preoptic area indicate that this is the peak time for GnRH-I, and perhaps GnRH-III, in American brook lamprey. Furthermore, the western brook lamprey whole brains showed a prominent increase of both forms during metamorphosis, with GnRH-I being the dominant form at maturity (Youson et al. 1995), perhaps indicating its role in reproductive behavior (Sower 2003; see Sect. 7.7). At the time of maturity, the preoptic area of the brain of American brook lamprey had declining intensity of immunoreactivity. Given that the neurohypophysis shows intense immunostaining during the immediate time preceding and during spawning in this species, it may not be feasible to compare whole brain concentrations of western brook lamprey with intensity of immunoreactivity in the preoptic area of American brook lamprey. Histological comparisons of mammalian LHRH immunoreactivity in non-reproductive and reproductive adults of western brook lamprey show intense staining of the neurohypophysis in both adult types but only intense staining of PO cells and fibers during reproduction (Crim et al. 1979a). The Youson et al. (2006) study provided monthly observations of immunoreactivity during the critical period of final sexual maturation from January to May to show, in the PO area, a relatively constant intensity of staining until the spawning period when this area of the brain was greatly depleted of immunoreactivity but the neurohypophysis was intensely stained. During their spawning migration, sea lamprey have equal numbers of GnRH-I and -III fibers in the anterior neurohypophysis but higher numbers of GnRH-III fibers in the posterior neurohypophysis (Nozaki et al. 2000). It appears that, in agnathans, hormones such as GnRH can diffuse from the neurohypophysis to the adenohypophysis (Nozaki et al. 1994; see Sect. 7.2); the existence of this diffusion pathway would explain the intense staining of the neurohypophysis in reproductive adults of non-parasitic species (Crim et al. 1979a; Youson et al. 2006).

In summary, these results collectively suggest that metamorphosis is an important phase of stimulation to the reproductive system of lampreys, irrespective of their adult life history type, and that GnRH-I and -III may have different roles in the development of sexual maturation in both adult types (Sower 2003; see Sect. 7.7). These studies were done before the identification of lamprey GnRH-II; therefore, it is likely that lamprey GnRH-II also has a distinct role in sexual maturation.

7.5.3 GnRH-II Distribution

With the identification of the third form of lamprey GnRH, lamprey GnRH-II, specific antisera were also generated (Kavanaugh et al. 2008). In this case, both in situ hybridization and immunohistochemical studies were done. In situ hybridization of the brain showed expression and localization of the transcript in the hypothalamus, medulla, fourth ventricle, and olfactory regions, whereas

immunohistochemistry using a specific antiserum showed mostly ir-lamprey GnRH-II nerve fibers originating from cells in the arc-shaped hypothalamic/preoptic areas ending at the neurohypophysis, and proposed to form the preoptico-hypophyseal GnRH tract. The distribution of irGnRH-II neurons was quite similar to distributions of lamprey GnRH-I and -III neurons, which were studied previously in the sea lamprey brain (Nozaki et al. 2000). In the Kavanaugh et al. (2008) study, there did not appear to be irGnRH processed to protein in the olfactory or midbrain regions of the adult brain. This is similar to previously reported immunohistochemical studies, both irGnRH-I and -III were found in the cell bodies of the rostral hypothalamus and preoptic area in larval and adult sea lamprey and not expressed in extra-hypothalamic regions of the brain (Tobet et al. 1995; Nozaki et al. 2000; see Sect. 7.5.1). Although dual-label in situ hybridization indicated that GnRH-I and -III messenger RNA (mRNA) are co-localized in the same cells in the preoptic nucleus/hypothalamic regions in adult lamprey (Root et al. 2005; see Sect. 7.5.1), in the Kavanaugh et al. (2008) study, it was difficult to know whether GnRH-II is co-localized in GnRH-I and/or -III cells or present in different GnRH cells because anti-GnRH-II used in the present study exhibited slight cross-reactivity to both GnRH-I and -III. These in situ and immunohistochemistry data show that lamprey GnRH-II is expressed and processed in the hypothalamus-preoptic region, but further detailed examination of all three GnRH ligands and receptors during the developmental and maturational stages will be critical in our full understanding of the neuroendocrine system in lampreys.

7.6 Developmental and Spatial Relationship Studies of GnRH and GABA

7.6.1 Origin of GnRH Neurons

The system of GnRH neurons is intimately connected to the olfactory system from the earliest points in development and functionally into adulthood in all vertebrates that have been studied (Tobet et al. 1996). Neurons that contain forms of GnRH that govern the hypothalamic-pituitary-gonadal axis are thought to be derived from progenitor cells in embryonic olfactory placodes (Tobet et al. 1996). The early data from the late 1980s and early 1990s from gnathostomes including mouse *Mus musculus* (Schwanzel-Fukuda and Pfaff 1989; Wray et al. 1989a, b), chicken *Gallus gallus* (Akutsu et al. 1992; Murakami and Arai 1994; Norgren and Gao 1994) and amphibians (Murakami et al. 1992; Northcutt and Muske 1994) showed that these critical neurons migrate from birth sites in epithelia of medial olfactory placodes, across the nasal compartment and cribriform plate to the forebrain. Although these results led to suggestions that some GnRH neurons have a nasal origin in all vertebrates, there is evidence (outlined below) to suggest that GnRH neurons in lampreys are not derived from the olfactory placode

(Muske 1993; Tobet et al. 1996). The characterization of GnRH neuronal system development and function has become much more complicated because there are many different forms of GnRH (see Sect. 7.4), and some GnRHs such as GnRH-2 may not regulate pituitary gonadotropin (Tobet and Schwarting 2006). It is likely that neurons producing different forms within the same species may have different developmental origins (Gorbman and Sower 2003; Amano et al. 2004).

As described above, data showing GnRH immunoreactive neurons in adult (Crim et al. 1979a, b; King et al. 1988; Wright et al. 1994; Tobet et al. 1995) and larval (Crim et al. 1979a; Wright et al. 1994; Tobet et al. 1995) lampreys showed cells restricted to a single bilateral dense arc along the third ventricle in the rostral hypothalamus and preoptic area. Neurons containing lamprey GnRH-III, which is a more prevalent form of GnRH in early development (Youson and Sower 1991), were found in the preoptic area/hypothalamus of larval lampreys, and the only fibers visible in olfactory regions originated from these more caudal cells (Tobet et al. 1995). The absence of GnRH cells and fibers in the olfactory system is consistent with the suggestion that lamprey GnRH neurons are not derived from the olfactory placode (Muske 1993). To experimentally address the question of the origin of GnRH in lampreys, experiments were conducted in the mid-1990s to characterize the earliest development of neurons containing lamprey GnRH using antisera directed against lamprey GnRH-I or -III in relation to the developing olfactory system by collaborators Stuart Tobet and Stacia Sower (Tobet et al. 1996). Eggs from fertile adult sea lamprey were fertilized in the laboratory, and larvae were maintained for up to 100 days. GnRH neurons were visualized within the lamprey preoptic area and hypothalamus as soon as GnRH was detectable (22 days after fertilization). The number of neurons increased with age through day 100. GnRH neurons were never seen within the olfactory system. As shown in a representative schematic diagram and data from immunohistochemistry, the position of cells containing irGnRH-III in a representative 47-day old larval lamprey were noted in the same location in the preoptic-area/anterior hypothalamus (Fig. 7.4). No immunoreactive cells were noted in the telencephalic lobes.

In addition, in these same studies, the cells and fibers of the olfactory system were identified using the lectin, Grifonia Simplicifolia-1 (GS-1) (Tobet et al. 1996). Overlap between the olfactory and GnRH systems were at the level of fiber projections. GS-1 reactive cells of apparent placodal origin did not enter the region of the preoptic area or hypothalamus that contained GnRH neurons. Recently-divided cells were labeled with the thymidine analog, bromodeoxyuridine (BrdU). The positions of BrdU-labeled cells after different survival times suggested a predominant medial-lateral radial neuron migration with a small number in positions suggestive of migration between the olfactory epithelium and the telencephalic lobes. Regardless of survival time, these cells were always found close to their entry point into the brain, suggesting minimal rostral-caudal migration. Based on these results, Tobet et al. (1996) hypothesized that GnRH neurons in developing lamprey originate within proliferative zones of the diencephalon and not in the olfactory system.

Based on the overlap of olfactory- and GnRH-containing fibers from prolarval stages to metamorphosis, olfactory stimuli may play a major role in the regulation of GnRH secretion in lamprey.

7.6.2 Distribution of GABA Neurotransmitter

The distribution of the amino acid neurotransmitter gamma-aminobutyric acid (GABA) in neurons in the brain and central nervous system has been examined in adult animals representing several classes of vertebrates. In the vertebrate species examined, GABA neurons were shown to be distributed throughout the brain and have been found consistently in the preoptic area of the hypothalamus and in the olfactory bulbs of the telencephalon (Domenici et al. 1988; Franzoni and Morino 1989; Martinoli et al. 1990; Bennis et al. 1991; Lauder 1993; Medina et al. 1994; Barale et al. 1996). Co-localization of dopamine and GABA neurons were also observed in the dopaminergic populations of adult sea lamprey (Barreiro-Iglesias et al. 2009). A few studies have examined the spatial relationship of GABA-containing neurons to regions of the brain containing gonadotropin-releasing hormone (GnRH or LHRH) neurons. GABA is considered the primary inhibitory neurotransmitter in the central nervous system across all vertebrates, although its action can be excitatory if chloride concentrations are higher inside target cells than outside (Cherubini and Conti 2001). GABA plays an important role in the regulation of GnRH and gonadotropin (GTH) release in vertebrates (Kah et al. 1992; Sloley et al. 1992; Trudeau et al. 1993a, b, c, 2000). In the rat *Rattus rattus*, GABAergic axons synapse on LHRH neurons found in the preoptic area (Leranth et al. 1985). In teleost fishes, GABA-containing cell bodies were found in the preoptic area and tuberal regions of the hypothalamus (Martinoli et al. 1990), and studies suggest that GABA is important in the early development of the teleost central nervous system (Ekstrom and Ohlin 1995; Doldan et al. 1999). Furthermore, direct innervation of the neurohypophysis and adenohypophysis by GABA neurons has been demonstrated in goldfish *Carassius auratus* (Kah et al. 1987; Kah and Dufour 2010). Only a limited number of studies examining the distribution and functions of GABA as related to GnRH have been completed in fish, and only one in developing lamprey (Reed et al. 2002).

In the Reed et al. (2002) study, the topographic distribution of GABA- and GnRH-containing cells was examined in the brains of developing and adult sea lamprey using immunocytochemistry and in situ hybridization. In the prolarval sea lamprey, distinct populations of GABA-containing cells were visible in the forebrain by 20 days after fertilization. These GABA-containing cells occurred throughout the olfactory bulb region of the telencephalon and in the diencephalon, particularly in the periventricular region of the rostral preoptic area. The GABAergic cells remained distributed in these separate populations throughout the lamprey prolarval developmental stages. In the adult sea lamprey, GABAergic elements appeared ubiquitous throughout the brain, making cell bodies of origin difficult to discern. Nonetheless,

cell bodies were discernible in the rostral hypothalamus. The distribution of cells containing GABA was then compared to that of GnRH-I cells using brain sections matched for coronal or horizontal planes within the diencephalon from both larval and adult lamprey. The GnRH-containing cells were found in the same distribution as described previously (Tobet et al. 1996), with GnRH-containing cells arising in the rostral diencephalon after 20 days of development. This study showed that in the lamprey, GABA-containing cells are discernable earlier in development than GnRH-containing cells. The GABA-containing cells were first visualized in the hypothalamus at 10–20 days after fertilization, whereas GnRH appeared in the same region as GABA between 20 and 30 days after fertilization. The matched section analysis in the Reed et al. (2002) study suggested that GABA and GnRH cell populations in the rostral hypothalamus and preoptic area are closely apposed, yet likely distinct (Fig. 7.5).

To further establish a proximate relationship between GABA and GnRH, glutamate decarboxylase (GAD, the GABA-synthesizing enzyme) and GnRH mRNA expression were examined by in situ hybridization in the brains of larval lamprey, thus providing the first GAD expression data for an agnathan (Reed et al. 2002). Similar to the results obtained by immunocytochemistry, GnRH and GAD mRNA were present in cell populations in and around the third ventricle of the hypothalamus. If the close proximity of these elements in the developing and adult hypothalamus provides for specific neural communication, then there is the potential for a regulatory role for GABA on GnRH neuronal development and reproductive function in the lamprey (Reed et al. 2002).

GABA is one of the earliest neurotransmitters to appear in the brain during development (Lauder et al. 1986; Roberts et al. 1987; Barale et al. 1996; Anadón et al. 1998), thus it is not surprising that GABA might affect GnRH neuronal development in the lamprey. Studies in the zebrafish demonstrated that GABA was distributed in discrete brain regions during early development (Doldan et al. 1999). Another study also described the developmentally dependent appearance of GABA-ir neurons in the early brain of another teleost, the threespine stickleback *Gasterosteus aculeatus*; GABA appeared to be expressed in the first differentiated neuronal populations of the brain (Ekstrom and Ohlin 1995). In the African clawed frog *Xenopus laevis* tadpole, GABA was found in the prosencephalon (which later forms the telencephalon and diencephalon) along the prosencephalic vesicle, and in the ventral thalamus and the hypothalamus early in embryonic development (Barale et al. 1996). In the rat, a population of GABAergic neurons was found in the diencephalon, including the hypothalamus, early in development. In teleosts, GABA neurons emerge early in development within the rostral prosencelphalon (Ekstrom and Ohlin 1995; Doldan et al. 1999). In amphioxus *Branchiostoma lanceolata*, GABA-ir cells were localized caudal to the infundibular organ, which is thought to correspond to the GABA-ir fibers observed in the ventral hindbrain of *Xenopus* embryos (Anadón et al. 1998). Taken together with the results obtained in the Reed et al. (2002) study, it appears that the early establishment and development of GABAergic systems is a phylogenetically old

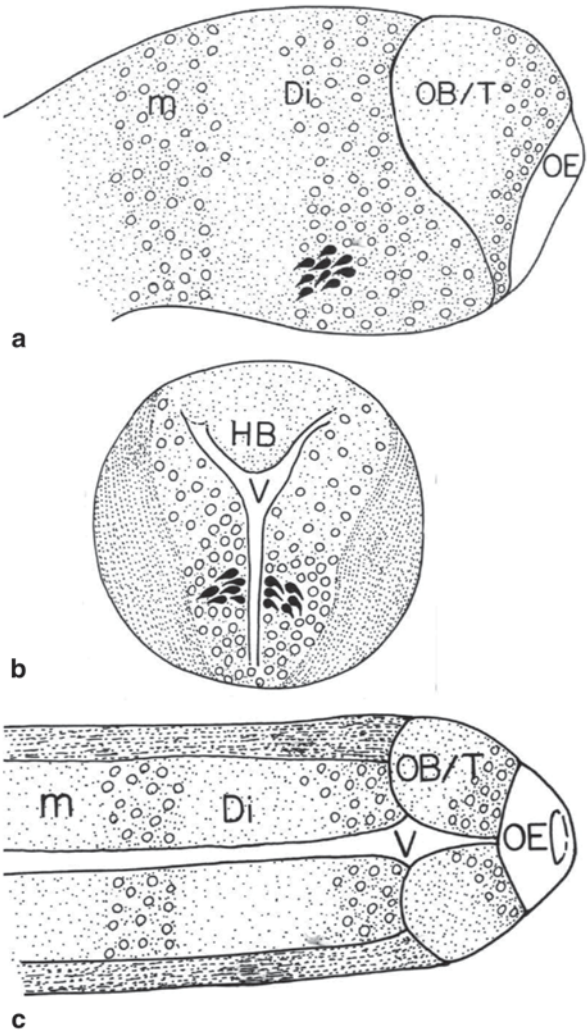

Fig. 7.5 Schematic diagrams of sagittal (**a**), coronal (**b**), and horizontal (**c**) sections illustrating the positions of GABA and lamprey GnRH immunoreactive (ir) cells in the heads and brains of larval sea lamprey (days 30 or 40). *Round circles* represent GABA ir cells and *filled teardrop shapes* represent lamprey GnRH ir cells within the preoptic area/rostral hypothalamus. The horizontal plane chosen (**c**) shows the different rostral-caudal populations for cells containing ir GABA and is located dorsal to the region containing GnRH neurons. Habenula (*HB*), diencephalon (*Di*), midbrain (*m*), olfactory bulb/telencephalon (*OB/T*), olfactory epithelium (*OE*), ventricle (*V*) are shown. (This figure was originally published in Reed et al. (2002) Copyright © 2002 Karger Publishers, Basel, Switzerland)

developmental pattern. The early appearance of GABA could be due to its suggested dual role as a trophic factor as well as a neurotransmitter (Lauder 1993).

If the GABA-containing cells defined in the Reed et al. (2002) study communicate with the GnRH-containing cells that were found in close proximity, then this could provide a mechanism for GABA in influencing the development and establishment of GnRH cell populations in the sea lamprey. Studies on the physiological role of GABA, in relation to GnRH, at any stage during the life cycle of the sea lamprey are limited (Root et al. 2004, 2005). In the mouse, it has been shown that GABA influences the development of the GnRH system (Fueshko et al. 1998; Bless et al. 2000). GABA is transiently expressed during development (von Bartheld and Rubel 1989; Barale et al. 1996; Tobet et al. 1996), and in explants from embryonic

mice, synaptic input from GABAergic cells caused spontaneous activity in GnRH neurons (Kusano et al. 1995). The published results in other species suggest that GnRH neurons possess GABA receptors and are responsive to GABA early in development. The data to date show that GABA is present early in the development of the prolarval sea lamprey as well as in the larval and adult stage lamprey brains (Reed et al. 2002; Root et al. 2005). The GABA cells clustered in several distinct populations within the forebrain. One of the populations of GABA cells, in the rostral hypothalamus/preoptic area, was closely apposed to GnRH cells in the same region. Based on the results of this study, the authors hypothesized that GABA influences the development and function of GnRH neurons in the sea lamprey (Reed et al. 2002). As stated above, this suggests that the early establishment and development of GABAergic systems within the lamprey brain, particularly the forebrain, is a phylogenetically ancient pattern.

The cDNA of lamprey GnRH-III had not yet been identified at the time of the Reed et al. (2002) study. With the cloning of lamprey GnRH-III (Silver et al. 2004) and lamprey GAD (Lariviere et al. 2002) cDNAs, the relationship between mRNA expression of these genes in the sea lamprey were compared using dual-label in situ hybridization in adult, juvenile, and larval sea lamprey (Root et al. 2005). In this study, GAD-expressing cells were distributed in several populations throughout the adult sea lamprey brain (Fig. 7.6). A population of GAD-expressing cells was localized in the olfactory bulb of the telencephalon, a second smaller cell population was seen in the ventral anterior hypothalamic region, and a third larger cell population was identified, stretching from the medial ventral hypothalamus and neurohypophysis along the dorsal and ventral divisions of the periventricular arcuate nucleus to the anterior region of the rhombencephalon (Fig. 7.6; Root et al. 2005). GAD-expressing cells were also detected in the dorsal thalamus, widely scattered between the habenular region and the optic tectum. A similar distribution of GAD populations was observed in larval and metamorphosing lampreys, although the reaction product did not appear to be as concentrated as in adults.

These data suggested a relationship of the GnRH- and GABA-expressing neurons in the ventral hypothalamus. Dark reaction product was detected in the hypothalamus near the preoptic region stretching along the ventral hypothalamus and neurohypophysis in the adult sea lamprey. In the brain of parasitic-phase lampreys, GAD-expressing neurons were detected in this same region of the hypothalamus along the neurohypophysis. In larvae, GAD mRNA was seen in the developing medial and ventral hypothalamic regions. What is significant is that these GAD-expressing cells were observed in similar regions of the hypothalamus as GnRH-expressing cell populations in all three lamprey life stages. Under the fluorescent microscope, GAD-expressing cells were seen remarkably close to those populations expressing GnRH in the preoptic area suggesting direct interaction between these neurons. These data are in agreement with previous immunocytochemistry data from Reed et al. (2002) where GABA-immunoreactive neurons were detected near GnRH-immunoreactive neurons. This possible interaction between GABAergic neurons and GnRH neurons is further supported by recent in vitro and in vivo studies. In vitro administration of muscimol (GABA receptor A agonist) in adult

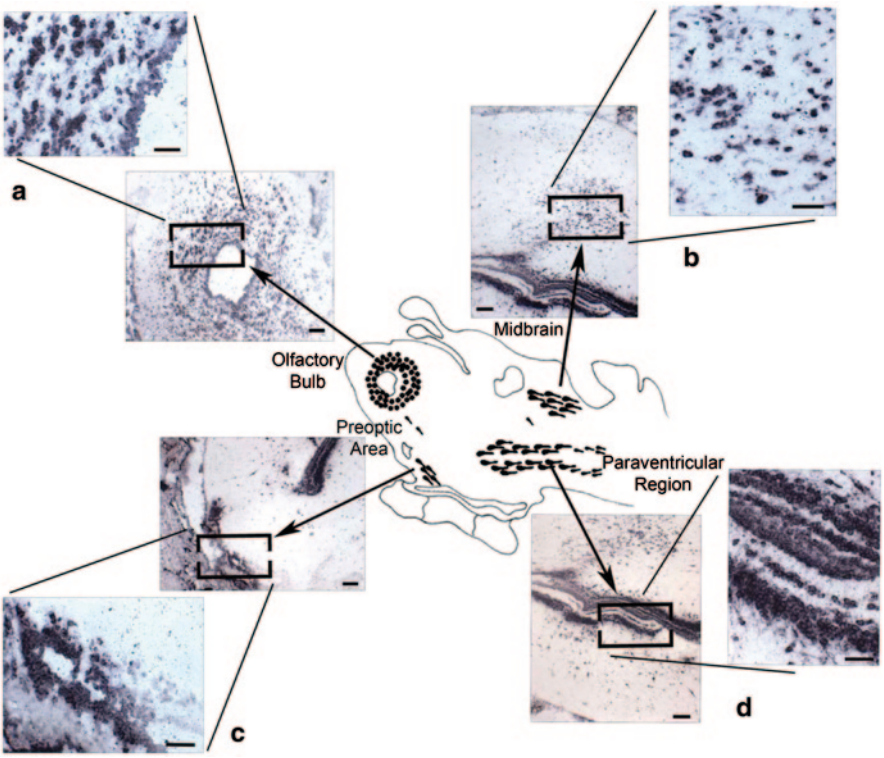

Fig. 7.6 Digoxigen (*DIG*)-labeled in situ hybridization for lamprey glutamate decarboxylase (*GAD*) in adult lamprey and schematic diagram illustrating GAD expression. Digital photographs of sagittal tissue sections from adult (**a**–**d**) sea lamprey brains showing distinct cell populations expressing lamprey GAD mRNA. Reaction product was detected in the olfactory bulb (**a**), preoptic nucleus and ventral hypothalamus (**b**), dorsal thalamus extending to the base of the habenular region (**c**), and ventral and dorsal periventricular arcuate nuclei (**d**). Scale bars = 25 μm. (Adapted from Root et al. 2005)

female sea lamprey showed an increase in GnRH-III release compared to controls, whereas in vivo administration of GABA and muscimol showed an increase of both forms of GnRH compared to controls in adult female sea lamprey brains (Root et al. 2004). These data suggested that GABA has a direct action on GnRH neurons as a neurotransmitter.

The occurrence of GAD mRNA expression in the forebrain of the sea lamprey is further supported by previous immunocytochemical studies for GABA in larval (Melendez-Ferro et al. 2001, 2002; Reed et al. 2002) and adult (Pombal et al. 1997; Pombal and Puelles 1999) lamprey, by a GAD microassay study (Wald et al. 1981), and by GAD *in situ* hybridization studies in other fishes (Anglade et al. 1999). These studies have shown collectively that both GABA and GAD are present and that GAD is functionally active in the sea lamprey. Melendez-Ferro et al. (2002) and Reed et al. (2002) separately demonstrated that GABA is present in the forebrain of

the embryonic and larval lamprey, appearing in the telencephalon and diencephalon 20 days after fertilization. In the olfactory bulb, Melendez-Ferro et al. (2001) reported that at least five types of cells containing GABA were present in all of the olfactory layers, with the majority present in the glomerular layer and the regions surrounding the olfactory nerve. In the Root et al. (2005) study, GAD-expressing cells were detected in the glomerular layer and mitral and granular cell layers, agreeing well with data presented by Melendez-Ferro et al. (2001) with regard to distribution and intensity. These data suggested that GABA is involved in processing in the olfactory bulb of lampreys. GAD-expressing cells located in the dorsal thalamus near the optic tectum suggest a possible role for GABA as a neurotransmitter affecting the optic nerve in the lamprey. Neurons projecting to the medial hypothalamus and eventually to the rhombencephalon support a role in the oculomotor system as suggested by Melendez-Ferro et al. (2001). Although these data offer support to the above hypotheses, they are not definitive evidence and, as such, further research is needed. The Root et al. (2005) study has shown that GAD mRNA is expressed in four distinct cell populations in the lamprey brain, ranging from the telencephalon and diencephalon of the forebrain to the mesencephalon and rhombencephalon of the midbrain and hindbrain. The close distribution of GAD and lamprey GnRH in the preoptic region also reported further supports the hypothesis that GABA might act on the reproductive axis through the feedback on GnRH neurons (Reed et al. 2002; Root et al. 2005).

7.7 Biological Activity of GnRHs

Prior to the 1980s, there was little evidence for a regulatory influence of the hypothalamus on the pituitary-gonadal axis in agnathans (Sower 1997, 2003). The first experimental evidence of the neuroendocrine control of reproduction in lampreys was obtained using a mammalian GnRH analog (Sower et al. 1983). This was followed by the subsequent identification of lamprey GnRH-I in 1986 (Sherwood et al. 1986), lamprey GnRH-III in 1993 (Sower et al. 1993), and lamprey GnRH-II in 2008 (Kavanaugh et al. 2008; see Sect. 7.4). The many functional studies that followed in testing these GnRHs and respective analogs along with the immunohistochemical and anatomical studies have clearly demonstrated that lampreys are the most basal vertebrates for which there are demonstrated functional roles for multiple GnRH neurohormones that are involved in pituitary-reproductive activity (Sower 2003; Sower et al. 2009) (Table 7.2). Investigations on the role of each of the GnRHs in lampreys had and have been impeded by the lack of a purified gonadotropin that can be used in assays to directly measure pituitary response/function. Even though gonadotropin(s) have not been fully identified from lamprey pituitaries, there is substantial direct and indirect evidence of pituitary responsiveness to lamprey GnRHs. As each of the GnRHs has been identified, biological activity has been assessed by indirect measures of determining steroidogenesis or gametogenesis in in vivo or in vitro studies (Sower et al. 1985b, 1987, 1995b; Sower

Table 7.2 Schematic representation of the actions of lamprey GnRH-I, -II, and -III at the pituitary-gonadal axis. Inositol phosphate (IP) activity was determined by stimulation with increasing doses of lamprey GnRH-I, -II, or -III using each of transiently transfected lamprey GnRHR-1, -2, or -3 receptors. Lamprey GnRH-I ligand did not activate the lamprey GnRHR-2 or -3. The pituitary or gonadal response represents an overall summary of data from various studies when the lampreys were treated either with *in vivo* or *in vitro* GnRH ligands at different doses when held at water temperatures above 15 °C or below 15 °C (see Sect. 7.7). OH-steroids = steroids with an additional hydroxyl group at the C15 position; *nd* not determined

Ligands		Lamprey GnRH-I		Lamprey GnRH-II		Lamprey GnRH-III	
Pituitary-IP response	lGnRH-R-1	+		+		+	
	lGnRH-R-2	−		+		+	
	lGnRH-R-3	−		+		+	
Pituitary cAMP response	lGnRH-R-1	+		+		+	
	lGnRH-R-2	−		−		−	
	lGnRH-R-3	−		−		−	
Pituitary response	GTH-β	+		nd		+	
Gonadal response	**Testes**	**<15 °C**	**>15 °C**	**<15 °C**	**>15 °C**	**<15 °C**	**>15 °C**
	Spermiation	No	**Yes**	nd	nd	No	**Yes**
	Estradiol	No	**Yes**	nd	**Yes**	No	**Yes**
	Testosterone	No	No	nd	nd	No	No
	Progesterone	No	**Yes**	nd	nd	No	**Yes**
	OH-Steroids	nd	**Yes**	nd	nd	nd	nd
	Ovary						
	Ovulation	No	**Yes**	nd	nd	No	**Yes**
	Estradiol	No	**Yes**	nd	**Yes**	No	**Yes**
	Progesterone	No	**Yes**	nd	nd	No	**Yes**
	OH-Steroids	nd	**Yes**	nd	nd	nd	**Yes**

1989; Deragon and Sower 1994; Barannikova et al. 1995; Gazourian et al. 1997, 2000; Kavanaugh et al. 2008). The first direct evidence of GnRH stimulating the pituitary was provided by Knox et al. (1994), in which the lamprey pituitary was shown to contain two high-affinity binding sites for GnRH. Other methods for directly measuring pituitary responsiveness were done using co-cultures of pituitaries and gonads with varying doses of GnRH (Sower 1990; Gazourian et al. 2000; Kavanaugh et al. 2008) and receptor binding studies (Materne et al. 1997; Knox et al. 1994). Most recently, the cDNAs of three GnRH pituitary receptors have been identified, and it has been shown that each receptor activated the inositol triphosphate (IP_3) or cAMP signaling system; stimulation with lamprey GnRH-I, -II or -III led to dose-dependent responses in COS7 cells transiently transfected with lamprey GnRH-R-1, -2, -3 (Silver et al. 2005; Silver and Sower 2006; Joseph et al. 2012). In addition, seasonal correlations between changes in brain GnRHs and gametogenic and steroidogenic activity of the gonads in adult male and female sea lamprey have been demonstrated (Fahien and Sower 1990; Bolduc and Sower 1992; Sower et al. 2011). While these studies strongly support the actions of GnRHs directly or indirectly at the pituitary, it will be unknown how these GnRHs differentially regulate the pituitary-gonadal axis until the gonadotropin/glycoprotein hormone(s) responses can be measured.

As stated previously, in 1986, the primary structure of GnRH-I was identified in the sea lamprey (Sherwood et al. 1986). Using synthetic lamprey GnRH-I and analogs, various studies provided the first evidence of neuroendocrine control of reproduction in lampreys (Sower et al. 1983, 1985b; Sower 1987, 1989, 1990; Fahien and Sower 1990; Sower and Larsen 1991; Youson and Sower 1991; Bolduc and Sower 1992). Once lamprey GnRH-III was identified in 1993, the first experimental studies were done and showed that lamprey GnRH-III was also a neurohormone involved in reproduction, based on its ability to produce a significant elevation of estradiol in adult female sea lamprey (Sower et al. 1993) and on the occurrence of this peptide in lampreys at different stages of metamorphosis coinciding with the acceleration of gonadal maturation (Youson and Sower 1991).

7.7.1 Plasma Sex Steroid Responses to GnRH

Concentrations of plasma estradiol (E2) and progesterone (P) have been used as measures of pituitary response to GnRH, reproductive development, and gonadal activity in sea lamprey (Sower 1987, 1989, 1990) and other lampreys (reviewed in Bryan et al. 2008). E2 is considered to be a major reproductive hormone in both male and female lampreys (Sower et al. 2011). The role of E2 in reproduction is further supported by the cloning of an estrogen-like receptor in sea lamprey (Thornton 2001). In sea lamprey and Arctic lamprey *Lethenteron camtschaticum*, E2 concentrations increased during spermiation (Fukayama and Takahashi 1985; Sower et al. 1985a; Fahien and Sower 1990) and decreased during ovulation (Sower et al. 1985a; Bolduc and Sower 1992). In the first reported study examining sex steroid profiles in the Pacific lamprey during overwintering and sexual maturation, E2

concentrations were usually higher in males than in females and increases coincided with the development of secondary sex characteristics (Mesa et al. 2010). In another study, there were higher plasma concentrations of E2 in females compared to males and, in both sexes, plasma E2 significantly increased as the season progressed, correlating with a temperature increase that is in general agreement with these earlier studies (Sower et al. 2011). In males, higher E2 concentrations corresponded to males that have mature sperm (Fukayama and Takahashi 1985; Sower et al. 1985a; Linville et al. 1987) and are consistent with the presence of an estrogen receptor in the testis (Ho et al. 1987). While E2 is considered to be a major steroid involved in reproductive processes, the precise function(s) of E2 in both male and female lampreys need to be elucidated. There are still many questions remaining as to the type of steroids that are synthesized and their respective functions (reviewed in Bryan et al. 2008; Docker et al. in press). For example, there is growing evidence that all lampreys produce gonadal steroids that are different from those of other vertebrates by possessing an additional hydroxyl group at the C15 position (Bryan et al. 2006, 2008). Plasma concentrations of 15α-hydroxylated steroids increased in both sea and Pacific lampreys when GnRH was administered (Bryan et al. 2004; Young et al. 2004). These studies suggested that GnRH-III was more potent than GnRH-I in Pacific lamprey (Young et al. 2004), but only in some instances for sea lamprey (Bryan et al. 2004; Young et al. 2004). In addition, there is evidence that 15α-hydroxyprogesterone is a hormone in lampreys and that androstenedione, a precursor to vertebrate androgens, is the main androgen (Bryan et al. 2008), but much more detailed research will be required on the steroids and respective receptors in lampreys.

Sower (1989) demonstrated that lamprey GnRH-I stimulated plasma P and E2 in adult male sea lamprey after single and two successive injections of lamprey GnRH-I. In this same study, lamprey GnRH-I was determined to induce spermiation in adult male sea lamprey compared to controls after four successive injections of lamprey GnRH-I. Lamprey GnRH-III was also shown to stimulate plasma concentrations of both P and E2 in the adult male lamprey after a single injection of lamprey GnRH-III, and induce spermiation after four successive injections of lamprey GnRH-III (Deragon and Sower 1994). In both studies, neither lamprey GnRH-III nor lamprey GnRH-I appeared to produce a dose-related response in plasma concentrations of E2 and P. The percent spermiation data demonstrated that the injection of adult male sea lamprey with lamprey GnRH-III induced a higher percent spermiation after days 16 and 21, indicating that lamprey GnRH-III may be more potent as a neurohormone than lamprey GnRH-I in the adult male sea lamprey. This is supported by the fact that lamprey GnRH-III brain content concentration was determined to be three times greater than that of lamprey GnRH-I (Sower et al. 1993) and about four times greater than that of lamprey GnRH-II (Sower et al. 2011). In another study, both lamprey GnRH-I and -III stimulated steroidogenesis and induced ovulation in adult female sea lamprey during their final reproductive stage (Gazourian et al. 1997). One injection of lamprey GnRH-III at 0.1 or 0.2 μg/g lamprey stimulated plasma E2 concentrations in lamprey held at each of three water temperatures, 13, 17, and 19 °C, corresponding to increasing stages of maturation.

Four successive injections, 3–4 days apart, of lamprey GnRH-III at 0.1 or 0.2 μg/g body weight induced ovulation in 100 or 88 % of lampreys, respectively, compared to 21 % in controls by day 31. In contrast to the Deragon and Sower (1994) study, in which lamprey GnRH-III was more potent than lamprey GnRH-I in inducing spermiation in adult male sea lamprey, the results from the Gazourian et al. (1997) study indicated that lamprey GnRH-I and -III are equally potent in inducing ovulation and stimulating steroidogenesis in female sea lamprey. The varying results from these studies are likely reflected in the timing of injections, water temperature, and slight differences in reproductive stages.

The hypothalamic role of lamprey GnRH-II is not well understood at this time. Lamprey GnRH-II was shown to be biologically active as determined by significantly increased concentrations of plasma E2 in the in vivo studies and significantly increased media E2 in the co-culture ovary/pituitary in vitro studies (Kavanaugh et al. 2008). Lamprey GnRH-III was slightly less effective, compared with lamprey GnRH-II. In these co-culture ovary/pituitary studies, media E2 was significantly potentiated, compared with media E2 from ovary culture treated with lamprey GnRH-II. In the ovary culture only, and similar to earlier studies on lamprey GnRH-I and -III (Gazourian et al. 2000), lamprey GnRH-II also had a slight direct effect on stimulating media E2, compared with controls. In the Kavanaugh et al. (2008) study, administration of GnRH-II in vivo and in vitro induced significant pituitary-gonadal responses. However, until the release rates of lamprey GnRH-I, -II and -III are known, and gonadotropins directly measured, the differences in potency between the GnRHs can only be inferred.

7.7.2 Effect of Temperature on GnRH Activity

Early experiments in sea lamprey suggested that injections of salmon gonadotropin or a mammalian GnRH analog (see below) were insufficient to evoke ovulation at lower temperatures (13 °C) despite being sufficient to elevate plasma E2 (Sower et al. 1983), but did induce ovulation when the temperature was increased to 21 °C (Table 7.2). Temperature has been considered an important environmental factor for the final maturational processes in adult sea lamprey (Fahien and Sower 1990; Sower 1990; Bolduc and Sower 1992). Upstream spawning sea lamprey kept at temperatures below 15.5 °C will not ovulate or spermiate unless the temperature is elevated close to their optimal spawning temperatures of 21 °C (Sower 1990), and decreased spawning activity has been associated with sudden drops in temperature (Applegate 1950; Manion and Hanson 1980; Linville et al. 1987). In the European river lamprey *Lampetra fluviatilis*, a rise in temperature (from 6 to 8 °C) was shown not to be a necessary factor for development of secondary sex characteristics and spermiation/ovulation, but there was a delay in sexual maturation of more than one month compared to controls held at increasing temperatures (6–11 °C; Larsen 1973). However, the normal spawning temperature is only 8–11 °C for the river lamprey (Hagelin and Steffner 1958; Jang and

Lucas 2005), so the low temperatures were not as extreme as those used in the sea lamprey studies. Temperature is considered to be an important environmental factor for initiating spawning activity in lampreys in general (see Chap. 6). Thus, it would be of great interest to know the dynamic and temporal patterns of the GnRH ligands and receptors held under varying photoperiod and temperature regimes during the reproductive cycles.

7.7.3 Structure-Function Activity of GnRH and Analogs

A series of studies, summarized in the following paragraphs, evaluated the biological effects of mammalian and lamprey GnRH and respective analogs (i.e., synthetic peptides designed to interact with the GnRH receptor and modify its effect). Because GnRH molecules are simple decapeptides (see Sect. 7.4), they can be easily synthesized and different amino acids can be inserted at any position to produce analogs that can be more or less potent than the natural form (Harvey and Carolsfeld 1993). Such analogs can either activate the receptor (i.e., be agonists) or block the receptor (i.e., be antagonists). In the above-mentioned Sower et al. (1983) study (Sect. 7.7.2), injections of the synthetic agonist of mammalian GnRH ([D-Ala^6, Pro^9] NEt mammalian GnRH) significantly elevated plasma E2 and advanced ovulation by at least several weeks in adult female lamprey. Note that in these analogs, D means that a D-amino acid, the mirror image form of the naturally occurring L-forms, has been inserted at that position (making the analog more resistant to degradation), and NEt means that the analog is missing the tenth amino acid and instead ends with an ethylamide (Harvey and Carolsfeld 1993). In the same 1983 study, a mammalian GnRH antagonist ([Ac-3 Pro^1, 4-FD-Phe^2, D-Trp^{3,6}] mammalian GnRH), which is a competitive inhibitor of GnRH in mammalian systems, had no apparent effect on plasma E2 concentrations or on timing of ovulation. These data confirmed that the receptors for GnRH in the sea lamprey are specific and can distinguish between variants in this molecule.

[D-Phe^{2,6}, Pro^3] lamprey GnRH was one of the first lamprey GnRH analogs tested and found to be a putative antagonist (Sower 1987). It inhibited ovulation in mature female lamprey, and inhibited spermiation and reduced plasma P concentrations in male sea lamprey (Sower 1987, 1989). Some GnRH-I analogs (but not GnRH-III) have been shown to influence spawning behavior in lampreys (Sower 2003). Such effects of GnRH-I and analogs on spawning behavior in adult male and female sea lamprey were investigated by Sower and Hanson (1992) during three successive spawning seasons. In each of these experiments, three or four groups of 12 sea lamprey each were injected two times with saline, lamprey GnRH-I, lamprey GnRH-I agonist [D-Ala^6, Pro^9 NEt lamprey GnRH], or a GnRH-I antagonist [D-Phe^{2,3}, Pro^3 lamprey GnRH]. After the second injection, the lamprey were introduced into an artificial stream channel and behaviors of spawning activity, resting, nest building, swimming, and fanning were monitored. In one of the experiments, spawning behavior was inhibited in females treated with

a lamprey GnRH-I agonist or antagonist compared to controls. However, in the males, lamprey GnRH-I agonist or antagonist stimulated earlier spawning activity compared to the controls. In another experiment of this study, lamprey GnRH antagonist induced earlier spawning activity in males, while lamprey GnRH agonist inhibited spawning activity, and lamprey GnRH delayed spawning activity compared to the controls. These data suggested that lamprey GnRH-I influences spawning behavior in sea lamprey. Furthermore, the responses to lamprey GnRH-I and analogs were different in males compared to females, suggesting that different neuroendocrine mechanisms may be involved (Sower 2003). In later studies examining the effects of lamprey GnRH-III on behavior, it was shown that this GnRH ligand did not influence the lamprey spawning behaviors (Sower and Hanson unpublished data).

The above studies evaluating the biological effects of lamprey GnRH-I and -III and analogs were done prior to the discovery of lamprey GnRH-II, a type 2-like GnRH (see Sect. 7.4). The expression pattern of GnRH-2 varies from species to species in jawed vertebrates, but is generally expressed in the brain and numerous peripheral tissues (Gorbman and Sower 2003; Kah et al. 2007). The function(s) of GnRH-2 in peripheral tissues and brain, however, have not been established. In the brain of gnathostomes, GnRH-2 predominates in extrahypothalamic regions and has been suggested to act as a neuromodulator/neurotransmitter, although there are species in which GnRH-2 is found in the hypothalamic regions and acts at the pituitary in stimulating gonadotropin function (King and Millar 1991). Several laboratories have been examining the role of gnathostome GnRH-2 in reproductive behavior. GnRH-2 appears to have one or more regulatory functions acting as a neurohormone or neuromodulator distinct from stimulation of LH secretion (Rissman et al. 1995). In several mammalian species, exogenous GnRH-2 treatments can regulate various behaviors such as promoting mating and reducing short-term food intake (Temple et al. 2003; Kauffman and Rissman 2004a, b). In birds, it was shown that the GnRH system in songbirds is modulated by social context (Stevenson et al. 2008). Confirming that lamprey GnRH-I (and its receptor) is involved in lamprey behavior and examining the potential roles of lamprey GnRH-II and -III (and their receptors) will require detailed studies and is ripe for investigation.

The effects of lamprey GnRH-I, -III, and analogs on plasma E2 in male landlocked sea lamprey were determined at different temperatures and different stages of reproduction (Gazourian et al. 2000). Both lamprey GnRH-I and lamprey GnRH-III significantly elevated plasma E2 levels for 24 h at 8 °C, but not at 16 °C. This is consistent with a previous study, where injections of lamprey GnRH-I significantly elevated plasma E2 levels in male sea lamprey for up to 48 h at a low temperature, 10 °C (Sower 1989). In female sea lamprey, it was found that plasma E2 remained significantly elevated for 24 h after injections of lamprey GnRH-I and -III at 13 °C, but not at 19 °C (Gazourian et al. 1997). These combined data suggest a greater metabolic turnover or degradation of lamprey GnRH, GTH, or their respective receptors at higher temperatures or later stages of reproductive maturity. In an in vitro study, lamprey GnRH-I and -III significantly stimulated the pituitary to release a

putative GTH capable of stimulating the ovaries to release E2 when incubated at 18 °C (Gazourian et al. 2000). [D-Glu^6] lamprey GnRH-I at all doses suppressed pituitary response on the testis at 14 °C, whereas cyclo-[Glu^6-Trp^7-Lys^8] lamprey GnRH-I only suppressed the pituitary at a dose of 100 and 1,000 ng/ml. It was proposed that the constrained analogs might interact with the pituitary GnRH receptor and in turn inhibit putative GTH release or cause the release of a substance capable of inhibiting steroidogenesis in the lamprey testis.

Lamprey GnRH-I and lamprey GnRH-III are the only vertebrate GnRHs that do not have glycine in the sixth position (see Sect. 7.4); instead they have amino acid substitutions glutamate and aspartate, respectively (Sower et al. 1993). Thus, in earlier studies, cyclized GnRH analogs were examined to test whether the close proximity of the N and C termini is important for binding of GnRH-I to its receptor in lampreys. Sower et al. (1995b) determined the in vivo effects of two lamprey GnRH-I analogs with substitutions of D-glutamate and glycine in the sixth position of the molecules, [D-Glu^6] lamprey GnRH-I and [Gly^6] lamprey GnRH-I, respectively. Two additional analogs, cyclo-[Glu^6-Trp^7-Lys^8] lamprey GnRH-I and cyclo-[D-Glu^6-Trp^7-Lys^8] lamprey GnRH-I, with their respective R groups linked by amide bonds at positions six and eight, were also studied to determine how restricting the flexibility of the molecule would influence its activity. In the Sower et al. (1995b) study, [Gly^6] lamprey GnRH-I acted antagonistically by delaying ovulation by 3 weeks as compared to controls, while [D-Glu^6] lamprey GnRH-I advanced ovulation. All GnRH-I analogs tested significantly elevated plasma E2 levels compared to controls, suggesting that the sixth position of the lamprey GnRH peptide is important for its function. The suggested active conformation of mammalian GnRH contains a type IIβ-bend at the level of Gly^6-Leu^7, which brings the putative binding sites on the amino and carboxy termini into proximity (Struthers et al. 1985). Considering that lamprey GnRH-I and -III have different amino acids in the sixth position, it is possible that lamprey GnRH-I or -III have a different conformation compared to the putative conformation of the other members of the vertebrate GnRH family.

In the Gazourian et al. (2000) study, GnRH analogs were tested which had modifications in the second and third positions of the native molecule. The putative binding domains of the mammalian GnRH molecule are considered the amino and carboxy termini (Struthers et al. 1985); therefore, substitutions of amino acids in these termini may affect receptor binding and/or activation. In this study, the activity of [Phe^2] lamprey GnRH-I, [Trp^3] lamprey GnRH-I, and others was examined. In the in vivo studies, [Phe^2] lamprey GnRH-I elevated plasma E2 levels after 4 hours, but had no effect after 24 h. In the in vitro studies, [Phe^2] lamprey GnRH-I only stimulated E2 production with 1,000 ng/ml at 14 and 18 °C. Since this analog initially had a stimulatory effect on plasma E2 levels and acted directly on the testis, it apparently was able to bind, and subsequently activate, the GnRH receptor. The inability of this analog to sustain elevated plasma E2 levels for 24 h suggests that this analog was susceptible to enzymatic degradation which shortened its plasma half-life. The presence of an endopeptidase capable of degrading mammalian GnRH analogs at the His^2-Trp^3 position has been suggested (Brudel et al. 1994); however, it is not

known whether this enzyme is active in the lamprey system. Lamprey GnRH-I is the only member of the vertebrate GnRH family to have an amino acid other than tryptophan in the third position. In the Gazourian et al. (2000) study, replacement of the native Tyr^3 of lamprey GnRH-I with tryptophan rendered the analog completely inactive, suggesting that the third position of lamprey GnRH-I is critical for binding and/or activation of the receptor.

The effects of [Gly^6] lamprey GnRH-I, [Gly^6] lamprey GnRH-III, [D-Glu^6] lamprey GnRH-I, and cyclo-[Glu^6-Trp^7-Lys^8] lamprey GnRH-I were examined in the male sea lamprey (Gazourian et al. 2000). Substitution of the native sixth position amino acid of lamprey GnRH-I or -III with glycine resulted in increased potency of the analogs for 24 h in vivo. Cyclo-[Glu^6-Trp^7-Lys^8] lamprey GnRH-I or [D-Glu^6] lamprey GnRH-I also stimulated plasma E2 in vivo. In the in vitro studies, [Gly^6] lamprey GnRH-I at all doses and [Gly^6] lamprey GnRH-III at 100 and 1,000 ng/ml directly stimulated E2 production in the testis of the male lamprey incubated at 14 °C. At 18 °C, only 1,000 ng/ml of [Gly^6] lamprey GnRH-I and -III directly elevated E2 production. These data support the Gazourian et al. (2000) in vivo data with both [Gly^6] lamprey GnRH-I and -III elevating E2 levels. In addition, cyclo-[D-Glu^6-Trp^7-Lys^8] lamprey GnRH-I at 10 and 1,000 ng/ml directly stimulated the testis at 14 °C, whereas [D-Glu^6] lamprey GnRH-I at 100 and 1,000 ng/ml significantly stimulated E2 production at 18 °C. Sower et al. (1995b) also showed that cyclo-[D-Glu^6-Trp^7-Lys^8] lamprey GnRH-I and [D-Glu^6] lamprey GnRH-I elevated E2 levels in vivo. The lower activity of [Gly^6] lamprey GnRH-I and -III at 18 °C, as compared to 14 °C, may be due to increased enzymatic degradation of the peptide or the inability of the peptide to interact with the receptor, which may be enhanced at lower temperatures. In the in vivo studies, it is possible that the noted increase in E2 was due to both the direct activation of the GnRH analogs on steroidogenesis in the testis and the action of the lamprey GnRH analog acting through the pituitary-gonadal axis.

It is proposed that the substitution of Gly^6 may have modified the structure of the molecule, possibly promoting the conformation required for receptor interaction, or that the substitution of Gly^6 augmented the resistance of the molecule to enzymatic degradation (Gazourian et al. 2000). Enzymatic degradation of both mammalian GnRH and salmon GnRH primarily results in cleavage of the Tyr^5-Gly^6 or Gly^6-Leu^7 bond (Goren et al. 1990). If these enzymes are present in the sea lamprey, a substitution of the less-bulky glycine in the sixth position should have resulted in increased degradation and decreased activity of the molecule. Since the Gly^6 substituted analogs consistently acted as the more potent analogs, this suggests that there may be different enzymes at work in the sea lamprey compared to other vertebrates.

In light of the newly identified lamprey GnRH receptors (see Sect. 7.8) and tools of molecular biology, testing the lamprey GnRH analogs in GnRH receptor assays can provide critical information on the functions of each of the amino acid residues of the GnRHs and analogs. New methods in molecular biology now allow one to screen many different GnRH analogs using transiently transfected cell lines all year long (e.g., Kavanaugh et al. 2008); the studies described above tested GnRH and analogs on sexually maturing lamprey, and were thus limited

to the final reproductive period of approximately six weeks every spring. There is now the opportunity of being able to fully evaluate the structure-function of each GnRH and respective analogs with each of the three GnRH receptors using transient cell lines.

7.7.4 Direct Gonadal Effects of GnRH

In a number of different species of vertebrates, GnRH has been detected in a wide range of tissues including the ovary and testis, although the functions in these tissues are not well understood and most often suggest that it acts in a paracrine function, that is, through release into adjacent cells or surrounding tissue rather than into the bloodstream (Gore 2002). In the goldfish, the expression of GnRH-2 and GnRH receptor were observed in the testis (Yu et al. 1998). In lampreys, it has been shown that lamprey GnRH-III has a direct steroidogenic effect on sea lamprey gonads, as evidenced by an increase in E2 levels (Gazourian and Sower 1994; Gazourian et al. 2000). Both lamprey GnRH-I and -III (1,000 ng/ml) had significant direct effects on sea lamprey testes. Based on the evidence that neither lamprey GnRH-I (Millar and King 1987; Fahien and Sower 1990) nor lamprey GnRH-III (Sower unpublished data) had been detected in the plasma of the sea lamprey, it was proposed that GnRH does not exert direct effects on the gonads via systemic circulation in the lamprey. In addition, GnRH binding sites have been demonstrated in both the testis and the ovary of the adult sea lamprey using an analog of mammalian GnRH ([D-Lys^6] mammalian GnRH) as a labeled ligand (Gazourian et al. 1997). Scatchard analysis suggested the presence of a high-affinity binding site in both the testis and the ovary (Gazourian et al. 1997). In this study, a single class of high affinity/high capacity binding sites was characterized in the testes with an equilibrium dissociation constant (Kd) of 0.187 nM and binding capacity of 1.55 pmol/mg protein. Ovarian data also demonstrated the presence of a single class of high affinity/high capacity binding sites with a Kd of 0.286 nM and binding capacity of 2.08 pmol/mg protein, respectively. The tissue expression data from the cloning of the first GnRH receptor (lamprey GnRHR-1) showed that the GnRH receptor was expressed in testis (Silver et al. 2005). The direct gonadal effects of GnRH and presence of high affinity binding sites suggest that there is a GnRH-like factor produced locally in sea lamprey gonads acting via GnRH receptors that may modulate gonadal function.

7.8 GnRH Receptors

7.8.1 Early Studies, 1990s

Many studies on the binding characteristics and kinetics of the GnRH receptor in vertebrates were performed throughout the 1970s and 1980s. In the early 1990s,

Tsutsumi et al. (1992) reported the first successful cloning of a GnRH receptor from the mouse using a homology-based PCR amplification scheme (Tsutsumi et al. 1992). Since this landmark study, more than 83 GnRH receptors have been cloned in invertebrates and vertebrates (see Millar et al. 2004; Sower et al. 2004; Silver et al. 2005). In lampreys, the first successful cloning of the receptor was reported in 2005 (Silver et al. 2005). Prior to this identification and during the 1990s, GnRH binding assays were performed using lamprey pituitaries. In 1994, it was reported that two high-affinity, specific classes of binding sites occurred in the lamprey pituitary (Knox et al. 1994). Quantitative in vitro autoradiography was used to characterize and localize these GnRH receptors in the anterior pituitary of the adult female sea lamprey. Scatchard analysis revealed two classes of high-affinity binding sites with Kds of 1.5×10^{-12} M and 5×10^{-9} M. Binding to the GnRH receptors was saturable, reversible, tissue specific, and time and temperature dependent. At the time, with the exception of the goldfish, only a single class of GnRH binding sites had been demonstrated in the following teleosts: stickleback (Andersson et al. 1989), African catfish *Clarias gariepinus* (De Leeuw et al. 1988), seabream *Sparus aurata* (Pagelson and Zohar 1992), and winter flounder *Pseudopleuronectes americanus* (Crim et al. 1988). Since the Sower laboratory has now cloned three GnRH receptors (GenBank accession numbers: lGnRHR-1, AF439802; lGnRH-R2, HM641828; lGnRH R3, HM641829; Silver et al. 2005; Joseph et al. 2012), it is likely that the two high affinity-binding sites in the lamprey pituitary from the Knox et al. (1994) study reflect two distinct receptors.

Displacement studies showed that a labeled mammalian GnRH analog could be displaced by chicken GnRH-I (GnRH-1), chicken GnRH-II (GnRH-2), synthetic mammal and salmon GnRHs, lamprey GnRH-I, lamprey GnRH-III, D-Ala^{6}-Pro^{9} NEt mammalian GnRH, and D-Phe^{2,6}-Pro^{3} lamprey GnRH (Knox et al. 1994). The proximal pars distalis region of the anterior pituitary contained most of the GnRH binding sites with slight binding in the rostral pars distalis. Subsequent studies on the identification of the lamprey gonadotropin-β (Sower et al. 2006) showed that the immunoreactive GTH cells are located in the proximal pars distalis of the pituitary, confirming the findings of the location of the lamprey GnRH receptors.

These described studies on the characterization of two high-affinity GnRH-binding sites were the first to show direct action of GnRH on the lamprey pituitary (Knox et al. 1994). In later studies, in vitro binding analysis was performed on pituitary sections in an effort to better understand the differential roles of the two GnRH binding sites throughout the development and sexual maturation (stage I, II, III, and ovulation) of the female sea lamprey, and to characterize the affinity for GnRH binding sites of four potential lamprey GnRH antagonists in the sexually mature male landlocked sea lamprey (Materne et al. 1997). Two high-affinity GnRH binding sites were observed throughout the development and final sexual maturation of female sea lamprey (Materne et al. 1997). Concentration of sites increased in correlation with increased gonadal maturation and brain GnRH concentration, peaking near and at ovulation. All four lamprey GnRH analogs demonstrated two specific binding compartments of high- and low-affinity with inhibition constants comparable to those of the native lamprey GnRH-I and -III.

7.8.2 Cloning, Identification and Functional Studies of Lamprey GnRH Receptors

GnRH action is mediated through high affinity binding with the GnRH receptor (GnRH-R), a class A or rhodopsin-like seven transmembrane G-protein coupled receptor (GPCR). GnRH receptors have been classified typically as type-1 (without a C-terminal tail) and type-II (with a C-terminal tail) (Silver et al. 2005; Guilgur et al. 2006). The type-1 GnRH receptor is unique among all GPCRs in that this receptor lacks the highly conserved intracellular carboxy-terminal (C-terminal) tail. A full-length transcript encoding a functional GnRH-R-1 was isolated and cloned from the pituitary of the sea lamprey (Silver et al. 2005; Guilgur et al. 2006). Recently, the full-length cDNA of lGnRHR-2 and lGnRHR-3 were cloned (Joseph et al. 2012). The cloned receptors retain the conserved structural features and amino acid motifs of other known GnRHRs and include C-terminal tails. lGnRHR-1 was shown to activate the inositol triphosphate (IP_3) signaling system; stimulation with lamprey GnRH-I, -II or -III led to dose-dependent responses in COS7 cells transiently transfected with lamprey GnRH-R-1 (Silver et al. 2005; Kavanaugh et al. 2008). The phylogenetic placement, structural, and functional features of lGnRHR-1 suggested that it is representative of an ancestral GnRH receptor.

More than 83 GnRH receptor cDNAs have been cloned since 1992 (Sect. 7.8.1). With the description of the catfish GnRH receptor 1, which was the first identified GnRH receptor to retain the evolutionarily conserved intracellular C-terminal tail, it has become evident that a major structural difference within the GnRH receptor family is the presence or absence of the intracellular C-terminal tail. This tail has been shown to affect not only effective GnRH binding and activation of signal transduction, but desensitization and internalization pathways as well (Heding et al. 1998; Pawson et al. 1998; Blomenrohr et al. 1999; McArdle et al. 1999; Willars et al. 1999; Vrecl et al. 2000; Hislop et al. 2005). Multiple GnRH receptors have been characterized in several species of vertebrates, suggesting that most organisms likely contain two or more functional GnRH receptors in the pituitary and brain (Illing et al. 1999; Millar et al. 2001; Neill et al. 2001; Okubo et al. 2001; Wang et al. 2001; Bogerd et al. 2002; Seong et al. 2003). Investigations in these organisms have demonstrated differential tissue distribution of GnRH receptor subtypes, as well as changes in receptor transcript expression based on reproductive stage (Illing et al. 1999; Wang et al. 2001).

A full-length transcript encoding a functional type-II GnRH receptor (lGnRHR-1) was isolated and cloned from the pituitary of the sea lamprey (Silver et al. 2005). This study was the first to identify a pituitary GnRH receptor transcript in an agnathan. The cloned receptor retains the conserved structural features and amino acid motifs of other known GnRH receptors (Fig. 7.7) and notably includes a C-terminal intracellular tail of about 120 amino acids, the longest C-terminal tail of any vertebrate GnRH receptor identified to date. The lamprey GnRH receptor-1 was shown to activate the inositol phosphate (IP) signaling system; stimulation with either lamprey GnRH-I or lamprey GnRH-III led to dose-dependent responses in

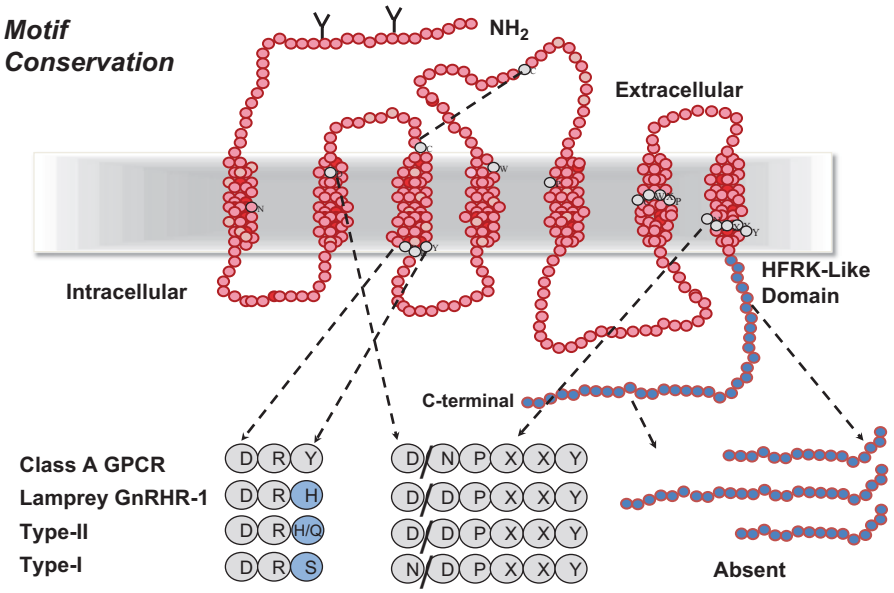

Fig. 7.7 Schematic representation of the gonadotropin-releasing hormone receptors (GnRHR) and conserved motifs. GnRH receptors have been classified typically as type-I (without a carboxy-terminal tail) and type-II (with a C-terminal tail). The type-I GnRH receptor is unique among all G-protein coupled receptors (GPCRs) in that it lacks the highly conserved intracellular C-terminal tail. Lamprey GnRHR-1 is a type-II receptor with the longest C-terminal tail of any vertebrate GnRH receptor identified to date, but it retains the conserved structural features and amino acid motifs of other known GnRH receptors. (Figure courtesy of Dr. Nathaniel Nucci)

transiently transfected COS7 cells. Furthermore, analyses of serially truncated lamprey GnRH receptor-1 mutants indicate that perturbations of the C-terminal tail disrupt IP accumulation; however, the tail-less lamprey GnRH receptor-1 was not only functional but was also capable of stimulating IP levels equal to wild type. Expression of the receptor transcript was demonstrated in the pituitary and testes using reverse transcriptase PCR (RT-PCR), whereas in situ hybridization showed expression and localization of the transcript in the proximal pars distalis of the pituitary. The phylogenetic placement and structural and functional features of this GnRH receptor suggested that it is representative of an ancestral GnRH receptor. In addition to having an important role in lamprey reproductive processes, the extensive C-terminal tail of this lamprey GnRH receptor may have great significance for understanding the evolutionary change of this vital structural feature within the GnRH receptor family.

Silver and Sower (2006) performed a series of experiments examining cAMP responses, binding kinetics, whole cell competitive binding assays, and internalization studies of the lamprey GnRH receptor-1 using a series of three C-terminal tail truncations (80, 40, and 0 amino acids) to better describe the functional significance of this unique vertebrate GnRH receptor. Activation of the lamprey GnRH receptor

was shown to stimulate cAMP production in a dose-dependant manner when treated with either lGnRH-I or lGnRH-III. Truncation analysis indicated that the membrane-proximal 40 amino acids (aa) of the lamprey GnRH receptor C-terminal tail contain a motif required for cAMP accumulation. Saturation binding assays using the wild type and truncated lamprey GnRH receptors revealed that all of the three truncated lamprey GnRH receptors were capable of binding lGnRH-I. Competitive, intact cell-binding assays suggested that the lamprey GnRH receptor is lamprey GnRH-III selective, based on the observed pharmacological profile. Finally, the lamprey GnRH receptor-1 was shown to undergo rapid ligand-dependent internalization, which was significantly diminished in the tail-less truncated form. These studies showed that this unique lamprey GnRH receptor-1 shares several characteristics of both type I and type II GnRH receptors which suggests that this receptor has retained ancestral characteristics that can provide insight into the function and evolution of the vertebrate GnRH receptor family (Silver et al. 2005; Silver and Sower 2006).

Pituitary GnRH receptors are thought to primarily signal through $G_{q/11}$, resulting in the stimulation of the IP_{3s} messenger system; however G_s activation and cAMP signaling has been reported as well (Arora et al. 1995; Stanislaus et al. 1998; Grosse et al. 2000; Liu et al. 2002; Oh et al. 2005). G-protein coupling to type I GnRH receptors clearly occurs within the intracellular loops (ILs), where several motifs have been identified that may be involved in G-protein coupling. For instance, the DRxxxI/VxxPL motif in IL2 and a conserved Ala residue in IL3 have been linked to $G_{q/11}$ coupling (Arora et al. 1995; Myburgh et al. 1998), while a BBxxB (where B is any basic amino acid) in IL1 was shown to be required for G_s coupling (Arora et al. 1998). Furthermore, the presence or absence of the C-terminal tail in the type II or type I GnRH receptors could possibly explain the signaling disparity between the two groups, whereas an HFRK motif in the membrane proximal region of the bullfrog type II GnRH receptor-1 was recently shown to be required for cAMP signaling, but not for IP signaling (Oh et al. 2005). In the Silver and Sower (2006) study, lamprey GnRH receptor-1 was shown to activate the cAMP signaling system, in a dose-dependent manner, in transiently transfected COS7 cells. Lamprey GnRH-III was a more potent activator of this system compared with lamprey GnRH-I, which supports the previous hypothesis, based on IP activation (Silver et al. 2005), that the lamprey GnRH receptor-1 is lamprey GnRH-III selective. These data have several interesting implications. The lamprey GnRH receptor-1 activates both the cAMP and IP signaling systems; however, the IP system is activated at an approximately 10-fold lower concentration of both lamprey GnRH-I and lamprey GnRH-III, and is also activated to a greater magnitude of approximately 4.5-fold, compared with c. 1.7-fold (lamprey GnRH-I) or c. 2.1-fold (lamprey GnRH-III) accumulation of cAMP. Not unexpectedly, truncation of the lamprey GnRH receptor C-terminal tail interfered with cAMP signaling; this is partially recovered by the 40 aa tail mutant, and lost again in the tail-less mutant form. The exact nature of GPCR/G-protein coupling is still in question since no conserved motifs that can be generally used to define G-protein specificity have been identified, nor has any particular domain been shown to be required. These current data indicate that a motif within the first

40 aa of the lamprey GnRH receptor-1 is involved in the G_s coupling, which is proposed by these authors to be the 'HFRK'-like motif (histidine–valine–arginine–arginine (HVRR) in lamprey) located within the membrane proximal region of the C-terminal tail (Silver and Sower 2006). Furthermore, this region contains the BBxxB shown to be involved in Gs coupling in type I GnRH receptors, in which case this motif is located in the first IL (Arora et al. 1998).

Two full-length cDNAs encoding novel GnRH receptors, lGnRH-R-2 and lGnRH-R-3, were identified and classified as type III receptors (Joseph et al. 2012). Analysis of the encoded amino acid sequences showed conservation of the characteristic motifs of GnRH receptors and high overall similarity to previously identified GnRH receptors. The ligand specificity and activation of intracellular signaling studies showed ligands lGnRH-II and -III induced an IP response in lGnRH-R-2 and lGnRH-R-3, whereas no response was detected at either receptor with lGnRH-I stimulation. lGnRH-II was a more potent activator of lGnRH-R-3 than lGnRH-III. lGnRH-R-2 has a higher binding affinity in response to lGnRH-III than lGnRH-II, whereas lGnRH-R-3 has a higher binding affinity in response to lGnRH-II than lGnRH-III. Stimulation of lGnRH-R-2 and lGnRH-R-3 with increasing doses of each of the three GnRH ligands did not elicit a cAMP response supporting evidence that a key motif (HVRR-like) in the C-terminal tail is required for cAMP activation. Lamprey GnRH-R-2 precursor transcript was detected in a wide variety of tissues including the pituitary in both male and female adult lampreys. Lamprey GnRH-R-3 precursor transcript was not as widely expressed and was primarily expressed in the brain and eye of male and female lamprey. A more recent study showed the presence of all three receptor transcripts in brain tissues for adult and parasitic-phase lamprey and all three receptor transcripts were expressed in the adult pituitaries, but not in the parasitic pituitaries (Hall et al. 2013). In this same study, in the larval phase, only lGnRH-R-1 was expressed in the larval brain and pituitary. From the phylogenetic analysis, lGnRH-R-1 is proposed to have evolved from a common ancestor of all vertebrate GnRH receptors, and lGnRH-R-2 and lGnRH-R-3 likely occurred due to a local gene duplication within the lamprey lineage. In summary, the findings of three receptor subtypes in the sea lamprey suggest that the plasticity in evolutionary recruitment of specific pituitary GnRH receptor subtypes for particular physiological functions seen in later evolved vertebrates was an ancestral character that first arose in a basal vertebrate.

The highly conserved DRY (Asp-Arg-Tyr) motif located at the end of the third transmembrane of G-protein-coupled receptors has been described as a key motif for several aspects of GPCR functions (Rovati et al. 2007). However, in the case of the vertebrate gonadotropin-releasing hormone receptor (GnRHR), the amino acid in the third position in the DRY motif is variable. The other notable characteristic of GnRHRs is the variation of a DRY motif of GPCRs that is a highly conserved amino acid triplet at the end or junction of the third transmembrane domain and the second intracellular loop. There are variable substitutions of the third amino acid in the DRY motif of GnRHRs from different classes of vertebrates. This region potentially contributes to GnRHR function. In many cases, type I receptor DRY motif is substituted with DR'S', while type II has DR'H/Q'. To date, there are few reports

about the functional significance of the Ser in DRS of type I receptors (Arora et al. 1995, 1997; Byrne et al. 1999). Thus, the functional significance of this variation of the DRY motif, particularly the type II GnRHR, has not been established. In the lamprey, the third amino acid of the DRY motif in lGnRHR-1 is His, while it is most often His/Gln in the type II GnRHR. To investigate the functional significance of the substitution of DRY to DRH in the GnRHR-1, second messenger signaling, ligand binding, and internalization of the wild-type and mutant lGnRH receptors were characterized with site-directed mutagenesis (Kosugi and Sower 2010). Treatment of the DRE^{151} and DRS^{151} mutant receptors with lamprey GnRH-I significantly reduced IP compared to wild-type (DRH^{151}) and DRY^{151} receptors. The $LogIC_{50}$ of wild-type receptor (−9.554+/−0.049) was similar to the $LogIC_{50}$ of DRE^{151}, DRS^{151}, and DRX^{151} mutants, yet these same mutants were shown to significantly reduce cell-surface expression. However, the DRY^{151} mutant compared to the wild-type receptor increased cell-surface expression, suggesting that the reduction of IP production was due to the level of the cell-surface expression of the mutant receptors. The rate of internalization of DRX^{151} (35.60 %) was reduced compared to wild-type and other mutant receptors. These results suggested that His^{151} of the lamprey GnRH receptor-1 may play a critical role in the retention of a certain level of cell-surface expression for subsequent cellular second messenger events.

7.9 Other Brain Neurohormones Potentially Involved in the Hypothalamic-Pituitary Axis: NPY, GnIH, TRH

There are several important brain neurohormones/factors that have been shown to stimulate/modulate gonadotropin releasing hormone (GnRH) and/or gonadotropin synthesis and/or release in vertebrates. In some teleost fishes, those neurohormones/factors include dopamine, neuropeptide Y (NPY), gamma-aminobutyric acid (GABA), and more recently gonadotropin-inhibitory hormone (GnIH) and kisspeptin (KiSS) (Kah and Dufour 2010). In lampreys, GABA (as described in Sect. 7.6.2) and NPY have been shown to be involved with brain GnRH and reproduction (Conlon et al. 1994; Root et al. 2004, 2005; Fig. 7.8).

Many factors have been identified in vertebrates that are able to modulate reproductive events through the influence on the hypothalamic-pituitary-gonadal axis. Two such modulators have been found to establish relationships with GnRH neurons from the earliest stages of their development. Thus, NPY, GABA, and dopamine (teleost fishes only) have been shown to influence reproductive processes in vertebrates, and have been found in and around GnRH neurons during their migration in several different species. In sea lamprey, both NPY (originally called peptide methionine-tyrosine, PMY, a neuropeptide Y-like hormone) and GABA were determined to interact with the reproductive neuroendocrine axis (MacIntyre et al. 1997; Reed et al. 2002). NPY is a 36 amino acid peptide that has been shown to act at the level of the hypothalamus and pituitary to alter GnRH and gonadotropin (GTH) release, respectively (reviewed in Larhammar 1996). Immunocytochemical

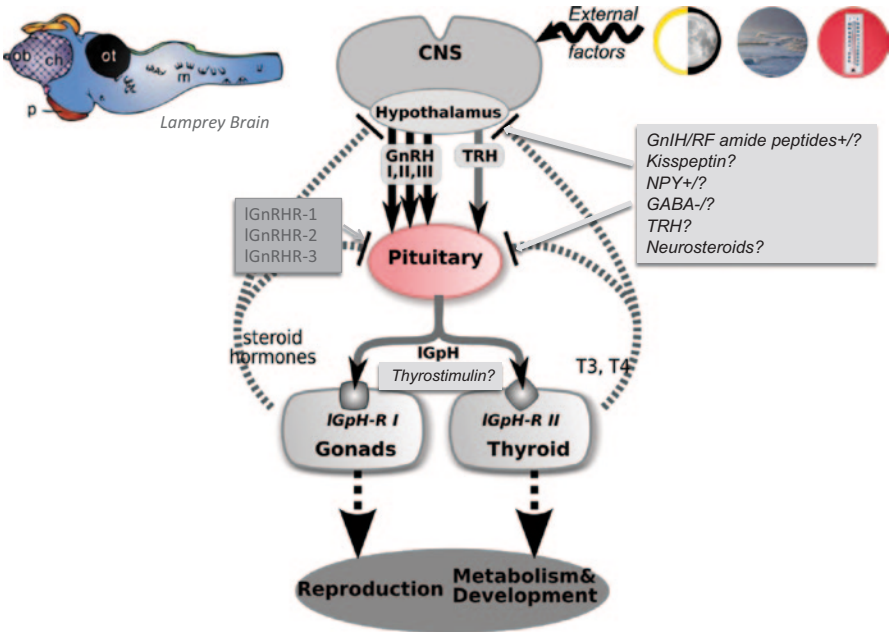

Fig. 7.8 Schematic diagram of the lamprey hypothalamic-pituitary-gonadal (HPG) axis. The following have been identified in lampreys: three GnRH ligands (lGnRH-I, -II, and -III); three GnRH receptors, all expressed in the pituitary (lGnRHR-1, -2, and -3); one gonadotropin β or lamprey glycoprotein hormone (lGpH) from the pituitary; and two glycoprotein hormone receptors (lGpHR-1 in the gonads and lGpH-R II in thyroid tissues). In addition, thyrostimulin has been identified in the lamprey but its function is unknown (see Sect. 7.10). There are several other important brain neurohormones/factors that have been shown to stimulate/modulate GnRH synthesis and/or release in vertebrates; their potential role in lampreys awaits further studies. Abbreviations: central nervous system (*CNS*), gonadotropin inhibiting hormone (*GnIH*), neuropeptide Y (*NPY*), gamma-aminobutyric acid (*GABA*), thyrotropin hormone releasing hormone (*TRH*), triiodothyronine (*T3*), thyroxine (*T4*). (Figure courtesy of Dr. Mihael Freamat)

studies have determined that, in both teleosts and mammals, NPY-containing cells can be identified in close proximity to GnRH-containing cells (Larhammar 1996). In teleosts, NPY is able to stimulate GnRH and GTH release from the hypothalamus and pituitary and potentiate GnRH-induced GTH release when conducive steroidal conditions exist (Larhammar 1996). Whether NPY exerts a stimulatory or inhibitory effect at either of these levels has proven to be highly dependent on the hormonal milieu. In some cyprinid fishes, dopamine has been demonstrated to inhibit GTH release (Peter et al. 1978; Peter and Paulencu 1980; Chang et al. 1983) while in many other teleost fishes, particularly marine species, dopamine has not been shown to inhibit GTH release (Copeland and Thomas 1989). The role of dopamine has not been examined in lamprey neuroendocrinology.

Lamprey NPY was isolated first from the intestine and then from the brain of the sea lamprey (Conlon et al. 1991, 1994). Lamprey NPY is structurally more similar to mammalian NPY than other NPY-family members as it has the same amino acid

residues at key positions identified in all other vertebrate forms of NPY (Conlon et al. 1991). Studies showed that lamprey NPY suppressed estradiol (E2) levels in female sea lamprey (MacIntyre et al. 1997). It was further demonstrated that lamprey NPY elevated brain lamprey GnRH-I and -III content, which is consistent with the function of NPY observed in other vertebrates (MacIntyre et al. 1997). At this time, it is undetermined whether NPY altered E2 concentration through direct action at the ovaries or if it affected pituitary function. Our understanding of NPY in lamprey reproduction is far from complete; further research would elucidate the interrelationships of lamprey NPY with the neuroendocrine system.

In 2000 and 2003, two new brain hormones were identified called GnIH and kisspeptin, respectively, which act on the hypothalamic-pituitary axis (Tsutsui et al. 2000; Seminara et al. 2003). GnIH is a dodecapeptide first identified in quail *Coturnix japonica* and shown to inhibit the synthesis and release of gonadotropins (Tsutsui et al. 2000). Subsequently, GnIH, which belongs to the LPXRF-amide family of peptides (see below), has been described in fish but its actions on the hypothalamic-pituitary axis have not been elucidated (Tsutsui et al. 2007). The Kiss1/GpR54 system was discovered and shown to be the central gatekeeper in the regulation of GnRH and puberty in mammals (Seminara et al. 2003; Seminara and Crowley 2008). In mammals, the kisspeptin system acts in regulating many aspects of reproductive functions including the mediation of steroid feedback (Roa et al. 2009). In adult sea lamprey brains, estrogen receptor expression was shown in the hypothalamic region using RT-PCR and in situ hybridization (Sower and Baron 2011). A key question remains as to whether E2 acts directly on GnRH-expressing neurons, or indirectly through a mechanism utilizing GABA or kisspeptin. Root et al. (2005) identified GABA-expressing neurons in close proximity to neurons expressing lamprey GnRH (see Sect. 7.6.2), supporting the possibility of an indirect action of E2 on GnRH. However, the critical evidence needed in order to establish the direct and/or indirect nature of E2–GnRH interaction in lampreys lies in the determination of cellular co-localization between GABA, GnRH, kisspeptin, and estrogen receptor. In teleosts, there are two KiSS genes, KiSS1 and KiSS2, expressed in hypothalamic and preoptic neurons; however, similar to GnIH, our knowledge of the functions of these new neurohormones on the hypothalamic-pituitary axis are limited (Felip et al. 2009; Kitahashi et al. 2009). While two kisspeptin genes (KiSS-1 and KiSS-2) were identified in the lamprey genome (Lee et al. 2009), the cloning and function of kisspeptin(s) and respective receptors in lampreys has not yet been elucidated.

RF (Arg-Phe) amide peptides, first discovered in invertebrates, have been identified in a few species of vertebrates (e.g., in birds: Tsutsui et al. 2000) and recently, in the lamprey also (Osugi et al. 2006). In fact, over the past decade (2000–2010), neuropeptides that have the RFamide motif at their C-termini have been identified in the brains of several vertebrates (Osugi et al. 2006). Based on the structures of vertebrate RFamide peptides, to date, at least five groups of the RFamide peptide family have been documented as follows: (a) PQRFamide peptide group (Yang et al. 1985; Yang and Martin 1995; Bonnard et al. 2001, 2003; Burlet-Schiltz et al. 2002); (b) LPXRFamide (X = L or Q) peptide group, including GnIH (Tsutsui et al. 2000; Fukusumi et al. 2001; Satake et al. 2001; Chartrel et al. 2002; Koda et al.

2002; Sawada et al. 2002; Ukena et al. 2002, 2003a, b; Ubuka et al. 2003; Yoshida et al. 2003; Osugi et al. 2004); (c) prolactin-releasing peptide (PrRP) group (Fujimoto et al. 1998; Hinuma et al. 1998; Moriyama et al. 2002; Seale et al. 2002); (d) metastin group, including metastin and kisspeptin (Kotani et al. 2001; Ohtaki et al. 2001); and (e) pyroglutamylated RFamide peptide (QRFP) group (Chartrel et al. 2003; Fukusumi et al. 2003).

Among the RFamide peptide groups, PQRFamide peptides, such as neuropeptide FF (NPFF) and neuropeptide AF (NPAF), share a common C-terminal Pro-Gln-Arg-Phe-NH_2 motif. LPXRFamide (X = L or Q) peptides, such as GnIH, frog growth hormone-releasing peptide (fGRP), goldfish LPXRFamide peptide, and mammalian RFamide-related peptides (RFRPs), also share a C-terminal Leu-Pro-Leu/Gln-Arg-Phe-NH_2 motif (Osugi et al. 2006). Such a similar C-terminal structure suggests that these two groups may have diverged from a common ancestral gene. Osugi et al. (2006) sought to clarify the evolutionary origin and divergence of these two groups, by identifying novel RFamide peptides from the brain of sea lamprey. A novel lamprey RFamide peptide was identified by immunoaffinity purification using the antiserum against LPXRFamide peptide. The lamprey RFamide peptide did not contain a C-terminal LPXRFamide motif, but had the sequence SWGAPAEKFWMRAMPQRFamide (lamprey PQRFa). A cDNA of the precursor encoded one lamprey PQRFa and two related peptides. These related peptides, which also had the C-terminal PQRFamide motif, were further identified as mature endogenous ligands. Phylogenetic analysis revealed that lamprey PQRFamide peptide precursor belongs to the PQRFamide peptide group. In situ hybridization demonstrated that lamprey PQRFamide peptide mRNA is expressed in the regions predicted to be involved in neuroendocrine and behavioral functions. This was the first demonstration of the presence of RFamide peptides in an agnathan brain. In subsequent studies, lamprey PQRFa at 100 μg/kg increased brain concentrations of lamprey GnRH-II in adult female lamprey compared to controls (Daukss et al. 2012). In these same studies, PQRFa, PQRFa-RP-1 and PQRFa-RP-2 did not significantly change brain protein concentrations of either lamprey GnRH-I, -III, or lamprey GTH-β mRNA expression in the pituitary. These data suggest that one of the PQRFamide peptides may act as a neuroregulator of at least the lamprey GnRH-II system in adult female lamprey. Lamprey PQRFamide peptides are considered to have retained the most ancestral features of PQRFamide peptides.

In another study, an LPXRFamide peptide gene was identified encoding three peptides (LPXRFa-1a, -1b and -2) from the brain of sea lamprey by synteny analysis and cDNA cloning, and the mature peptides by immunoaffinity purification and mass spectrometry (Osugi et al. 2012). The expression of lamprey LPXRFamide peptide precursor mRNA was localized in the brain and gonad by RT-PCR and in the hypothalamus by in situ hybridization. Immunohistochemistry showed appositions of lamprey LPXRFamide peptide immunoreactive fibers in close proximity to GnRH-III neurons, suggesting that lamprey LPXRFamide peptides act on GnRH-III neurons. In addition, lamprey LPXRFa-2 stimulated the expression of lamprey GnRH-III protein in the hypothalamus and gonadotropin β mRNA expression in the pituitary. Synteny and phylogenetic analyses provide evidence that the

LPXRFamide peptide gene diverged from a common ancestral gene likely through gene duplication in the basal vertebrates. These results suggest that one ancestral function of LPXRFamide peptides may be stimulatory compared to the inhibitory function seen in later-evolved vertebrates (i.e., birds and mammals). In time, the elucidation of the functions of these peptides will contribute to our understanding of the interrelationships of these peptides and the GnRH-GTH system.

In mammals, thyrotropin-releasing hormone (TRH) is considered a major hypothalamic hormone that acts on the pituitary to stimulate the synthesis and release of thyrotropin hormone (TSH, a member of the pituitary glycoprotein family; see Sect. 7.3); TSH in turn acts on the thyroid gland to stimulate the synthesis and/or release of the thyroid hormones, thyroxine and triiodothyronine. TRH has also been shown to release pituitary growth hormone (GH) (Guillemin 1978; Schally 1978) and prolactin (PRL) (Jackson and Reichlin 1977). The role of TRH in non-mammalian vertebrates is much less established (reviewed in De Andrés et al. 2002). To date, there are only two reports on TRH in lampreys. Youngs et al. (1985) showed that TRH was present in the pituitary, brain, and spinal cord of larval and adult sea lamprey and adult European river lamprey, as determined by radioimmunoassay. A more extensive immunocytochemistry study was done, in which the distribution of TRH mainly occurred in the preoptic region and the hypothalamus in large larvae and adult upstream migrating sea lamprey (De Andrés et al. 2002). Sower et al. (1985b) reported that treatment of adult lamprey with a partly purified salmon gonadotropin or a GnRH analog significantly elevated plasma thyroxine. It was hypothesized from these studies that one hypothalamic GnRH stimulated both the pituitary-thyroid and pituitary gonadal axes. In later studies, lamprey GnRH-I and -III were shown to be significantly correlated with the seven stages of lamprey metamorphosis (Youson and Sower 2001). Unlike the induction of frog metamorphosis and even amphioxus metamorphosis by thyroid hormones (Paris et al. 2008), thyroid hormones do not stimulate the process of metamorphosis in lamprey (see Chap. 4). Metamorphosis in sea lamprey is characterized by a significant decline in thyroid hormones, changes in lipid metabolism, and elevated GnRH (Youson and Sower 2001). Subsequently, it has been determined that lampreys likely have only one pituitary glycoprotein hormone (GTH-like hormone) and one gonadal glycoprotein receptor and one thyroid glycoprotein receptor (Freamat et al. 2006; Sower et al. 2006; Freamat and Sower 2008a; see Sect. 7.10). Therefore, the working hypothesis (as of 2009) for these authors is that the glycoprotein hormone/glycoprotein hormone receptor systems in lampreys are strongly interconnected (Sower et al. 2009).

The actions and interactions of these various neurohormones/neuromodulators are not known in lampreys and whether one or a combination of these hormones exert a stimulatory or inhibitory effect on the GnRH system is likely highly dependent on the hormonal milieu as well as the fish's reproductive and developmental stage and environmental factors including photoperiod and temperature. Further studies will be required to gain an understanding of the complexity of the hypothalamic-pituitary axis in controlling reproduction in lampreys.

Table 7.3 Number of identified pituitary glycoprotein hormones to date in the different groups of vertebrates. The duality of the gonadotropin hormones and thyroid hormone-stimulating hormone (*TSH*) has been established in all gnathostomes, as shown. As noted, there is only one glycoprotein hormone in hagfishes and one in lampreys that cannot be assigned to luteinizing hormone (*LH*), follicle stimulating hormone (*FSH*), or TSH; LH, FSH, and TSH are not found in invertebrates. The two subunits of thyrostimulin, a new member of the vertebrate glycoprotein pituitary hormone family, are the ancestral units of LH, FSH, and TSH (see Sect. 7.10). There is one putative thyrostimulin in lampreys. (Data obtained from NCBI Uni-ProtKB, Sower et al. unpublished data)

	LH	FSH	TSH	Thyrostimulin
Mammals	52	58	65	41
Birds	5	9	8	5
Reptiles	4	6	4	2
Amphibians	9	10	3	1
Bony fishes	82	38	25	8
Cartilaginous fishes	1	2	0	1
Agnathans		Hagfish: 1 GpH Lamprey: 1GpH		Lamprey: 1

7.10 Lamprey Gonadotropin-Glycoprotein Hormone Family

In gnathostomes, the classical pituitary glycoprotein hormone (GpH) family consists of two pituitary GTHs, LH and FSH, one thyroid-stimulating hormone (TSH), and a new member, thyrostimulin. In placental mammals, there is an additional member of the glycoprotein hormone family called chorionic gonadotropin (CG). Each of these GpHs consist of a heterodimer composed of an α- and β-subunit (see Sect. 7.3). Two GTHs have been identified in all taxonomic groups of gnathostomes (Suzuki et al. 1988; Kawauchi et al. 1989; Quérat et al. 2000, 2004; Huhtaniemi 2005; Table 7.3). After more than 20 years of intensive investigations, the laboratories of Sower and Kawauchi along with their students and collaborators identified the first and perhaps only GTH β-like protein by cDNA cloning in sea lamprey and it was shown to be localized in the proximal pars distalis (PPD) of the anterior pituitary (Sower et al. 2006).

Previous evidence from physiological and immunohistochemical studies had strongly supported the presence of a GTH-like molecule in lampreys (Sower 2003; Sower et al. 2006). Hypophysectomy and substitution therapy with pituitary extracts or mammalian GTHs indicated pituitary regulation of the gonads in European river lamprey (Larsen 1965). Injection of salmon GTH preparation into adult spawning sea lamprey advanced ovulation by several weeks and elevated plasma estradiol levels (Sower et al. 1983). Two high-affinity binding sites for lamprey GnRH-I and -III were found in the PPD of sea lamprey pituitary (Knox et al. 1994) and the cDNA of one pituitary GnRH receptor-1 was cloned and shown to be expressed in the PPD (Silver et al. 2005). Moreover, GTH-like immunoreactivity was identified in cells distributed in the ventral half of the PPD (Nozaki et al. 1999, 2001). These pituitary cells in the PPD were stained intensely by anti-ovine

LH including LHβ and moderately or weakly by several other antisera such as human LHβ. Therefore, it seemed that it would be easy to isolate GTH from pituitaries of adult sea lamprey that were close to spawning. One would expect a high content of GTH based on the reproductive stage and physiological data obtained from GnRH studies (Sower 2003). Sower et al. (1985b) speculated that perhaps the difficulty in identifying the lamprey GTH was due to its parasitic nature. Perhaps the lamprey had a "different" GTH and receptors in order for its system not to respond to other fish gonadotropins that could influence its own reproductive cycle when the lamprey was in its parasitic feeding phase, consuming the blood and other bodily fluids of its host. However, the fact that lampreys responded to salmon GTH did not support this speculation—although, as will be described below, the α subunit does not appear to be the typical vertebrate GTHα. For more than 20 years, the Sower and Kawauchi laboratories tried to isolate gonadotropin, assumed to be a heterodimer glycoprotein as in other vertebrates, from the pituitary extracts from landlocked adult female sea lamprey. However, despite these exhaustive efforts using molecular and biochemical techniques, no molecule related to LH or FSH was found. During these early years, a glycoprotein homodimer called nasohypophysial factor (NHF) was identified (Sower et al. 1995b) that corresponded to the N-terminal peptide of proopiocortin (Takahashi et al. 1995b). This NHF molecule was always found as the predominant glycoprotein (Sower et al. 1995b). Moreover, there were numerous unsuccessful attempts using molecular techniques to clone α and β subunits with a number of primers corresponding to conserved regions for the gonadotropin subunits. Finally, the success of determining the β subunit of the lamprey GTH-like protein was accomplished by expressed sequence tag analysis of the pituitary cDNA library (Sower et al. 2006) that allowed identification of three out of 2,208 clones showing sequence similarity to glycoprotein hormone β.

The mature lamprey gonadotropin β protein contains 12 cysteine residues at a homologous position to those of LH, FSH, and TSH and three N-glycosylation sites (Sower et al. 2006). Two of them are homologous to those of FSHβ, one to LHβ, and the other to TSHβ. In addition, the region of the molecule that has been proposed to control receptor binding specificity (i.e., the region between the 10th and 12th Cys residues) suggested that the proposed heterodimer would be more like a FSH than a LH (Cosowsky et al. 1997). The mature protein showed similar sequence identity to LHβ and FSHβ of shark *Scyliorhinus canicula* (Quérat et al. 2001), sturgeon *Acipenser baeri* (Quérat et al. 2000), and lungfish *Neoceratodus forsteri* (47%) (Quérat et al. 2004) compared to TSH-β of sturgeon and lungfish (41%). It was proposed that the β subunit would likely combine with the α subunit since it has a hydrophobic residue (Ile) that corresponds to hCGβ Val44, a residue that fits into a hydrophobic pocket in the α subunit (Cosowsky et al. 1997; Moyle et al. 2004). This is a highly conserved subunit interaction in most, if not all, gonadotropins, and slightly different than TSH. An unusual feature of the lamprey β-like protein is the tail that has a N-glycosylation signal, a phenomenon that is not common in vertebrate β subunits. Perhaps this is to prolong its half-life or to confer

receptor specificity, relative to other fish GTHs lampreys may encounter during their parasitic life phase (although one would assume that much of the ingested proteins would be digested by the intestinal system).

In accordance with the expression pattern of the isolated lamprey GTHβ-like protein, the antiserum against the synthetic peptide corresponding to the deduced amino acid sequence specifically stained most cells in the ventral half of the PPD, which had also been stained with anti-ovine LH (Nozaki et al. 1999). The results agree well with the GnRH-binding study showing GnRH-binding sites in the PPD (Knox et al. 1994). On the basis of sequence identity and histochemical characteristics, it is evident that this protein is a potential candidate for lamprey GTHβ. To obtain the definite proof, these authors examined whether GnRH could stimulate expression of the putative GTHβ gene in the pituitary of sea lamprey (Sower et al. 2006). After two intraperitoneal injections of 100 μg/g body weight at 24 h intervals in adult lamprey, both GnRH-I and GnRH-III stimulated the expression of mRNA of the putative GTHβ. In the same pituitary preparations, expression of other sea lamprey pituitary hormone genes such as GH, POC, and POM gene were investigated (see Sect. 7.3). The results demonstrated that lamprey GnRH also stimulated expression of GH, but not that of POC and POM in vivo. The stimulation of GH and GTH by GnRH is not novel in non-mammalian vertebrates. In previous studies, GnRH induced GH and GTH secretion from the goldfish pituitary (Marchant et al. 1989). These authors suggested that the secretion of GH and GTH in the goldfish are regulated, at least in part, through a common releasing factor, GnRH, whereas somatostatin and dopamine appear to act independently as GH and GTH release inhibitory factors, respectively (Marchant et al. 1989). Combining the biochemical characteristics, sequence identity, location of the GTH-like protein in the anterior pituitary and stimulation of GnRH, Sower et al. (2006) concluded that the identified glycoprotein hormone is gonadotropin-like in the lamprey pituitary.

The duality of gonadotropins (i.e., the presence of LH and FSH) has been established in all classes of gnathostomes (see Sect. 7.3). In the sea lamprey, however, Sower et al. (2006) found only a single GTHβ, which showed intermediate sequence similarity to LHβ and FSHβ of jawed fishes such as shark, sturgeon, and lungfish. In the molecular phylogenetic tree of β-subunits of glycoprotein hormones, sea lamprey is far removed from the β-subunits of LH, FSH, and TSH, and takes a position as an outgroup. In addition, immunohistochemical data suggested that there are no other cells that produce GTH. It has been shown that ACTH cells are in the RPD; GH and GTH cells are in the PPD; and MSH cells are in the PI (Nozaki et al. 1995; Fig. 7.3a). In later immunohistochemistry studies, GTH-like cells were not observed in the pituitary during the larval and metamorphic stages, but the numbers increased markedly during the parasitic period (Nozaki et al. 2008). These results strongly suggest that duality of GTH was established after the divergence of gnathostomes and agnathans.

The presence of a single GTH-like glycoprotein in agnathans was further supported by the first identification of gonadotropin in a hagfish. Recently, the presence and identity of a functional GpH was elucidated from the brown hagfish *Paramyxine atami* (Uchida et al. 2010). In contrast to the lamprey, phylogenetic analyses

suggest that the identified hagfish GpHα and β subunits are the typical yet ancestral GpHα and GpHβ subunits found in gnathostomes, but still support the hypothesis that the duality of the gonadotropins appeared with the gnathostomes.

To date, there has been no evidence to support the presence of TSH in lampreys (Kawauchi and Sower 2006). It now appears that lampreys have thyrostimulin and not TSH (Sower unpublished data; see paragraph below) In an earlier study from Sower et al. (1985b), a partly purified salmon gonadotropin and an analog of GnRH stimulated the elevation of thyroxine in adult female sea lamprey. These authors suggested the possibility that the thyroid and gonad may both be activated by one glycoprotein hormone. Uchida et al. (2010, 2013) have also suggested that the recently-identified hagfish glycoprotein has both gonadotropic and thyrotropic functions; they hypothesize that the ancestral GpH did not give rise to the multiplicity of GpHs (LH, FSH, TSH, and CG) seen in other vertebrates until the early evolution of gnathostomes. More recently, two kinds of glycoprotein hormone receptors (lGpH-R I and II) have been cloned in the sea lamprey: one (lGpH-R I) is predominantly expressed in the gonad and the other (lGpH-R II) is predominantly expressed in the thyroid tissue (Freamat et al. 2006; Freamat and Sower 2008a; see Sect. 7.11). A single GTH molecule may stimulate the gonads and thyroid glands through these receptors (Sower et al. 2009).

A fifth heterodimeric GpH in gnathostomes (after FSH, LH, TSH, and CG) was discovered in 2002 and termed thyrostimulin due to its thyroid-stimulating activity (Nakabayashi et al. 2002). The vertebrate thyrostimulin is expressed in the pituitary but, compared to GpH, has unique subunits called GpA2 (thyrostimulin α) and GpB5 (thyrostimulin β). GpA2 is homologous but not identical to the common α-subunit (GpA1 or α) in the other GpHs. With the discovery of GpA2 and GpB5 (thyrostimulin-β) homologs in invertebrates (including *Drosophila melanogaster*), Sudo et al. (2005) proposed that an ancestral heterodimeric GpH existed before the divergence of vertebrates and invertebrates, and that a later gene duplication event in vertebrates produced the thyrostimulin (GpA2 and GpB5) and GTH/TSH (GpA1 and LHβ/FSHβ/TSHβ) lineages. This ancestry of GpH is supported by recent studies in which GpB5 (Dos Santos et al. 2009; Tando and Kubokawa 2009) and GpA2 (Dos Santos et al. 2009, 2011) were identified from amphioxus, a basal chordate.

We now report the identification and characterization of a functional novel glycoprotein hormone, lamprey GpH (Sower et al. unpublished data). It consists of lGpA2 and lGTHβ (GpHβ), a combination of subunits that has not been reported in any other vertebrate. Our compelling new data (including cloning of the full-length cDNA of lGpA2) show that the single glycoprotein α subunit has higher similarity with mammalian GpA2 than GpA1 subunits (Sower et al. 2009, unpublished data). In situ hybridization revealed the GpA2 in lampreys is expressed in the rostral pars distalis, proximal pars distalis, and pars intermedia (Sower et al. unpublished data). Based on these studies, we propose that GpA2 is the ancestral α subunit and GpA1 was lost in the lamprey lineage; this is supported by syntenic and phylogenetic analyses. After the gnathostome-agnathan divergence, subsequent gene duplications produced the two α subunits (GpA1 and GpA2) and β subunits (FSHβ, LHβ, TSHβ, GpB5) in gnathostomes (Sower et al. 2009).

We thus propose that, during the course of lamprey evolution, GpA2 became adapted as a functional α subunit for the lamprey gonadotropin/thyroid hormone stimulating hormone. This raises many questions, however, on why lampreys have such a unique pituitary glycoprotein hormone—consisting of the typical β-like subunit of FSH, LH, and TSH, and the α-like subunit of thyrostimulin: is this because lampreys have a parasitic phase and need to "block" response to host fish gonadotropins? In lampreys, there are three hypothalamic GnRHs: how do three GnRHs regulate one pituitary glycoprotein hormone and perhaps the putative thyrostimulin? How does one pituitary glycoprotein hormone differentially regulate the gonad and thyroid? The overlapping of the pituitary-gonadal axis and pituitary-thyroid axis will be addressed in future studies. Furthermore, whether the lamprey thyrostimulin has a functional role in mediating the processes of the gonad or thyroid has yet to be determined. In gnathostomes, a cognant receptor has not been shown for thyrostimulin, nor has the function of thyrostimulin been established (Kleinau and Krause 2009); while thyrostimulin has been shown to activate the human TSH-receptor, it is not considered a major regulator of the thyroid gland.

In lampreys, therefore, the organization of the HPG axis is similar to that in gnathostomes in its most fundamental features, but with a simplified structure. The lamprey HPG axis overlaps with the pituitary-thyroid axis and involves a single glycoprotein hormone and perhaps thyrostimulin interacting with two receptors; this suggests an evolutionary plasticity of the gonadotropin/thyroid hormone stimulating hormone(s) in this basal group of vertebrates.

7.11 Glycoprotein Hormone Receptors

GTH and TSH hormone actions are mediated through a subfamily of GPCRs, namely the GpH receptors (Combarnous 1992). Known GpH-Rs share a number of unique features. They are composed of two functionally distinct modules of similar size: an extracellular N-terminal domain followed by a prototypical GPCR segment. The extracellular N-terminal domain is primarily responsible for high-affinity hormone binding and contains a central portion of nine Leu-rich repeat motifs, flanked by N- and C-terminal Cys-rich clusters. The C-terminal half of the receptor contains a transmembrane region with seven hydrophobic transmembrane α-helices, connected by intra- and extracellular loops and an intracellular C-terminal domain (Grossmann et al. 1997; Dufau 1998; Ascoli et al. 2002; Moyle et al. 2005). To date, approximately 79 GpH-Rs have been identified and described in 36 different species, mostly in mammals but also in three species of birds, two reptile species, one amphibian, and ten fish species (Hovergen Database, http://pbil.univ-lyon1.fr).

Until recently, there had been no GpH-Rs described in any agnathan species. One functional GpH receptor (lGpH-R I) (Freamat et al. 2006) was identified from lamprey testis and a second functional GpH-R receptor (lGpH-R II) was identified and shown to be expressed mainly in thyroid tissue (Freamat and Sower 2008a).

These authors hypothesized that lGpH-R I and lGpH-R II are the only members of the GpH receptor subfamily in lampreys (Freamat and Sower 2008a). They are descendants of the TSH receptor-like molecular ancestors of the GpH-Rs in gnathostomes and are likely the result of the genome duplication event hypothesized to have taken place before the divergence of agnathans (Sidow 1996; Kuratani et al. 2002).

The 719-amino acid full-length cDNA encoding lGpH-R I is highly similar and likely a homolog of the vertebrate GpH-Rs (including LH, FSH, and TSH receptors; Freamat et al. 2006). The key motifs, sequence comparisons, and characteristics of the identified GpH-R reveal a mosaic of features common to all other classes of GpH-Rs in vertebrates. The lGpH-R I was shown to activate the cAMP signaling system using human chorionic gonadotropin (hCG) in transiently transfected COS7 cells. The highest expression of the receptor transcript was demonstrated in the testes using RT-PCR. The high expression of lGpH-R I in the testis and the high similarity with gnathostome gonadotropin hormone receptors suggest that lGpH-R I functions as a receptor for lamprey GTHs.

The second GpH receptor (lGpH-R II) in the sea lamprey is a 781-residue protein and is approximately 43 % identical with mammalian TSH-R and FSH-R representative sequences (Freamat and Sower 2008a). Similar to these two classes of mammalian receptors, lGpH-R II is assembled from 10 exons. A synthetic ligand containing the lamprey GpHβ chain tethered upstream of a mammalian α chain activated the lGpH-R II expressed in COS7 cells but to a lesser extent than lGpH-R I. The most obvious feature of the coding sequence of lGpH-R II is the presence of a long linker fragment called signaling specificity domain (SSD) or "hinge" located between the Leu Rich Domain (LRD) of the extracellular segment and the transmembrane domain. This is one of the longest linker fragments described in all vertebrate GpH receptors. This is in contrast with the similar region of the lGpH-R I, which is the shortest SSD/hinge segment among all vertebrate GpH-Rs (Freamat et al. 2006). Molecular phylogenetic analysis of vertebrate GpH-R protein sequences provide evidence that the two lamprey GpH receptors form a sister group with gnathostome TSH receptors, suggesting that the ancestral receptors are more TSH receptor-like.

Therefore, at this point, a comparative perspective on this endocrine compartment in lampreys relative to the well established gnathostome paradigm suggests the involvement of one pituitary GpH, possibly thyrostimulin, and two GpH-Rs as opposed to three or four dimeric hormones and three receptors in gnathostomes. The role of this GpH/GpH-R system in lampreys has yet to be fully established. The recent identification and characterization of a GpA2 and GpB5 in lampreys (see Sect. 7.10) will now permit experimental studies to be performed. The existence of a thyrostimulin in lampreys with a distinct binding specificity to GpH-R I and II cannot be excluded. From the studies completed to date, Sower and colleagues (e.g., Freamat and Sower 2008a, b, 2010) hypothesized that there is lower specificity of lamprey GpH and its receptor in agnathans and that, during co-evolution of the ligand and its receptor in gnathostomes, there were increased specificities of interactions between each GpH (TSH, LH, and FSH) and its receptor.

7.12 Conclusions and Perspectives

Lamprey reproductive neuroendocrinology is still a young science, considering that the first paper identifying lamprey GnRH-I was published in 1986, anatomical studies of the brain-pituitary relations were determined in 1994, and the GTHβ hormone was identified in 2006. From the relatively short time from the late 1970s when virtually nothing was known about the neuroendocrine system in lampreys to the present, there is now an impressive body of knowledge. The molecular, biochemical, and functional studies of GnRHs, GTHs, and respective receptors show that these neuroendocrine factors share common functional and developmental features compared to later evolved vertebrates. Further comparative studies of the GnRH and GTH families will help provide clues on the evolution of reproductive mechanisms and insights into our understanding of gene duplication, structure-activity relations, and the molecular evolution and functional diversity of reproductive hypothalamic and pituitary hormones. With such increasing knowledge from genomics and proteomics, it will be necessary for us to understand the extent and intensity of changes in gene expression levels due to hormones, the interactions between signaling pathways due to hormones, and induced structural and functional changes to cells—all over a range of ontogenetic stages and under a wide variety of environmental conditions.

The hypothalamic-pituitary (HP) system is considered to be a seminal event that emerged prior to or during the differentiation of the ancestral agnathan vertebrates (Sower et al. 2009). Reproduction in vertebrates is controlled by a hierarchically organized endocrine system. In spite of the very diverse patterns of life cycles and reproductive strategies and behaviors, this endocrine system is remarkably conserved throughout the gnathostome lineages. A new paradigm was proposed by Sower et al. (2009) in that the neuroendocrine control of reproduction and thyroid functions in the agnathan sea lamprey exhibits an overlapping, simplified organization represented by one glycoprotein hormone putatively interacting with two receptors. Therefore, a working hypothesis is that the glycoprotein hormone/glycoprotein hormone receptor systems emerged as a link between the neuroendocrine and peripheral control levels during the early stages of gnathostome divergence (Fig. 7.9). This transforming paradigm serves as a model for analysis of the evolutionary mechanisms leading to emergence of the highly specialized gnathostome endocrine axes. Thus, the phylogenetic position of lampreys as a basal vertebrate allows them to be a basis for understanding genes that arose in the vertebrates. Techniques ranging from those of classical physiology to the sophisticated techniques of molecular biology and knowledge of the lamprey genome now available has greatly aided the expansion of our understanding of the neuroendocrine system. Yet, there are many challenges that lie ahead; future research can include the elucidation of the complexities of the GnRH–GnRH receptor system, the array of other neuroendocrine hormones, the GTH–GTH receptor system, and of course how these systems control reproduction during each of the reproductive stages and reproductive behavior. Future research will increase our understanding and insight into the molecular and functional evolution of brain and pituitary hormones and receptors in lamprey.

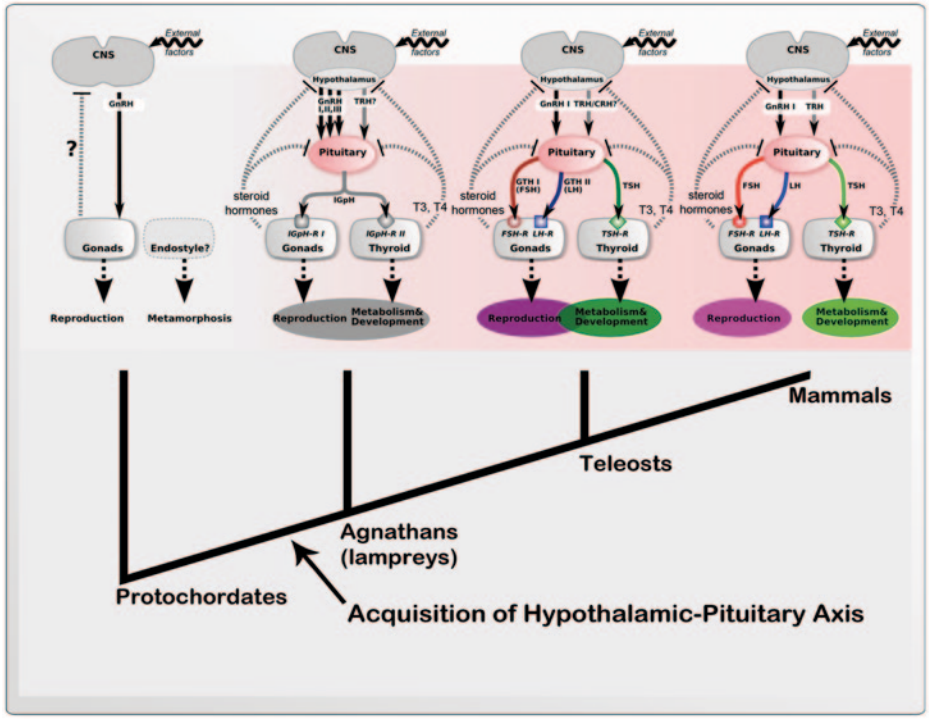

Fig. 7.9 Sower et al. (2009) hypothesized that the hypothalamic-pituitary-gonadal (*HPG*) and hypothalamic-pituitary-thyroid (*HPT*) endocrine systems evolved from an ancestral, pre-vertebrate, exclusively neuroendocrine mechanism by gradual emergence of the components of a new control level (GpHs/GpH-Rs) concomitantly with the development of the corresponding anatomical structure (pituitary). The endocrine control of reproductive and thyroid functions in lampreys may reflect an intermediary stage on the evolutionary pathway to the highly specialized gnathostome HPG and HPT axes. Abbreviations: corticotropin releasing hormone (*CRH*), gonadotropin I and II (GTH I and GTH II), follicle stimulating hormone (*FSH*), luteinizing hormone (*LH*), thyrotropin stimulating hormone (*TSH*), follicle stimulating hormone receptor (*FSH-R*), luteinizing hormone receptor (*LH-R*), thyroid stimulating hormone receptor (*TSH-R*); other abbreviations are as in Fig. 7.8. (This figure was originally published in Sower et al. (2009) and reproduced with permission of Elsevier)

Abbreviations and Acronyms

aa	Amino acid
ACTH	Adrenocorticotropic hormone
BrdU	Bromodeoxyuridine
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary DNA
CG	Chorionic gonadotropin
E2	Estradiol
fGRP	Frog growth hormone-releasing peptide
FSH	Follicle stimulating hormone or follitropin
GABA	Gamma-aminobutyric acid
GAD	Glutamate decarboxylase
GH	Growth hormone
GnIH	Gonadotropin-inhibitory hormone
GnRH	Gonadotropin-releasing hormone
GnRHR	Gonadotropin-releasing hormone receptor
GPA	Glycoprotein α (alpha) subunit
GpA2	Thyrostimulin α (alpha)
GPB	Glycoprotein hormone β (beta) subunit
GpB5	Thyrostimulin β (beta)
GPCR	G-protein coupled receptors
GpH	Glycoprotein hormone
GS-1	Grifonia Simplicifolia-1
GTH	Gonadotropin
hCG	Human chorionic gonadotropin
HP	Hypothalamic-pituitary
HPG	Hypothalamic-pituitary-gonadal
IL	Intracellular loop
IP	Inositol phosphate
IP_3	Inositol triphosphate
Ir	Immunoreactive
Kd	Equilibrium dissociation constant
KiSS	Kisspeptin
LH	Luteinizing hormone or lutropin
LHRH	Luteinizing hormone releasing hormone
LRD	Leu Rich Domain
mRNA	Messenger RNA
MSH	Melanocyte stimulating hormone, or melanotropin
NHF	Nasohypophysial factor
NPAF	Neuropeptide AF
NPFF	Neuropeptide FF
NPY	Neuropeptide Y
P	Progesterone
PD	Pars distalis

PPD Proximal pars distalis
PI Pars intermedia
PMY Peptide methionine-tyrosine
POA Preoptic area
POC Proopiocortin
POM Proopiomelanotropin
POMC Proopiomelanocortin
PON Preoptic nucleus
PO Preoptic region
PRL Prolactin
PrRP Prolactin-releasing peptide
QRFP Pyroglutamylated RFamide peptide
RFRP RF-amide-related peptide
RIA Radioimmunoassay
RPD Rostral pars distalis
RT-PCR Reverse transcriptase PCR
SML Somatolactin
SSD Signaling specificity domain
TRH Thyrotropin-releasing hormone
TSH Thyroid stimulating hormone, or thyrotropin

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References

Adams BA, Tello JA, Erchegyi J et al (2003) Six novel gonadotropin-releasing hormones are encoded as triplets on each of two genes in the protochordate, *Ciona intestinalis*. Endocrinology 144:1907–1919

Akutsu S, Takada M, Ohki-Hamazaki H, Murakami S, Arai Y (1992) Origin of luteinizing hormone-releasing hormone (LHRH) neurons in the chick embryo: effect of the olfactory placode ablation. Neurosci Lett 142:241–244

Amano M, Iigo M, Ikuta K et al (2004) Disturbance of plasma melatonin profile by high dose melatonin administration inhibits testicular maturation of precocious male masu salmon. Zool Sci 24:79–85

Anadón R, Melendez-Ferro M, Perez-Costas E, Pombal MA, Rodicio MC (1998) Centrifugal fibers are the only GABAergic structures of the retina of the larval sea lamprey: an immunocytochemical study. Brain Res 782(1–2):297–302

Andersson E, Borg B, de Leeuw R (1989) Characterization of gonadotropin-releasing hormone binding sites in the pituitary of the three-spined stickleback, *Gasterosteus aculeatus*. Gen Comp Endocrinol 76:41–45

Amemiya Y, Sogabe Y, Nozaki M, Takahashi A, Kawauchi H (1999) Somatolactin in the white sturgeon and African lungfish and its evolutionary significance. Gen Comp Endocrinol 114:181–190

Anglade I, Mazurais D, Douard V et al (1999) Distribution of glutamic acid decarboxylase mRNA in the forebrain of the rainbow trout as studied by *in situ* hybridization. J Comp Neurol 410:277–289

Applegate VC (1950) Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. US Fish and Wildlife Service Special Scientific Report 55, Washington, DC

Arora KK, Sakai A, Catt KJ (1995) Effects of second intracellular loop mutations on signal transduction and internalization of the gonadotropin-releasing hormone receptor. J Biol Chem 270:22820–22826

Arora KK, Cheng Z, Catt KJ (1997) Mutations of the conserved DRS motif in the second intracellular loop of the gonadotropin-releasing hormone receptor affect expression, activation, and internalization. Mol Endocrinol 11:1203–1212

Arora KK, Krsmanovic LZ, Mores N, O'Farrell H, Catt KJ (1998) Mediation of cyclic AMP signaling by the first intracellular loop of the gonadotropin-releasing hormone receptor. J Biol Chem 273:25581–25586

Ascoli M, Fanelli F, Segaloff DL (2002) The lutropin/choriogonadotropin receptor, a 2002 perspective. Endocr Rev 23:141–174

Barale E, Fasolo A, Girardi E, Artero C, Franzoni MF (1996) Immunohistochemical investigation of gamma-aminobutyric acid ontogeny and transient expression in the central nervous system of *Xenopus laevis* tadpoles. J Comp Neurol 368:285–294

Barannikova IA, Boev AA, Arshavskaya SV, Dyubin VP (1995) Feature of hormonal regulation of the reproduction of lamprey, *Lampetra fluviatilis*, during the final period of the sexual cycle. J Ichthyol 35:184–197

Barreiro-Iglesias A, Villar-Cervino V, AnadÏn R, Rodicio M (2009) Dopamine and γ-aminobutyric acid are colocalized in restricted groups of neurons in the sea lamprey brain: insights into the early evolution of neurotransmitter colocalization in vertebrates. J Anat 215:601–610

Bennis M, Calas A, Geffard M, Gamrani H (1991) Distribution of GABA immunoreactive systems in the forebrain and midbrain of the chameleon. Brain Res Bull 26:891–898

Bless EP, Westaway WA, Schwarting GA, Tobet SA (2000) Effects of gamma-aminobutyric acid(A) receptor manipulation on migrating gonadotropin-releasing hormone neurons through the entire migratory route *in vivo* and *in vitro*. Endocrinology 141:1254–1262

Blomenrohr M, Heding A, Sellar R et al (1999) Pivotal role for the cytoplasmic carboxyl-terminal tail of a nonmammalian gonadotropin-releasing hormone receptor in cell surface expression, ligand binding, and receptor phosphorylation and internalization. Mol Pharmacol 56:1229–1237

Bogerd J, Diepenbroek WB, Hund E et al (2002) Two gonadotropin-releasing hormone receptors in the African catfish: no differences in ligand selectivity, but differences in tissue distribution. Endocrinology 143:4673–4682

Bolduc TG, Sower SA (1992) Changes in brain gonadotropin-releasing hormone, plasma estradiol 17-β, and progesterone during the final reproductive cycle of the female sea lamprey, *Petromyzon marinus*. J Exp Zool 264:55–63

Bonnard E, Burlet-Schiltz O, Frances B et al (2001) Identification of neuropeptide FF-related peptides in rodent spinal cord. Peptides 22:1085–1092

Bonnard E, Burlet-Schiltz O, Monsarrat B, Girard JP, Zajac JM (2003) Identification of proNeuropeptide FF_A peptides processed in neuronal and non-neuronal cells and in nervous tissue. Eur J Biochem 270:4187–4199

Braun CB, Wicht H, Northcutt RGJ (1995) Distribution of gonadotropin-releasing hormone immunoreactivity in the brain of the Pacific hagfish, *Eptatretus stouti* (Craniata: Myxinoidea). Comp Neurol 353:464–476

Brudel M, Kertscher U, Berger H, Mehlis B (1994) Liquid chromatographic-mass spectrometric studies on the enzymatic degradation of gonadotropin-releasing hormone. J Chromatogr A 661:55–60

Bryan MB, Scott AP, Cerny I, Young BA, Li W (2004) 15α-Hydroxyprogesterone in male sea lampreys, *Petromyzon marinus* L. Steroids 69:473–481

Bryan MB, Young BA, Close DA et al (2006) Comparison of synthesis of 15α-hydroxylated steroids in males of four North American lamprey species. Gen Comp Endocrinol 146:149–156

Bryan MB, Scott AP, Li W (2008) Sex steroids and their receptors in lampreys. Steroids 73:1–12

Burlet-Schiltz O, Mazarguil H, Sol JC et al (2002) Identification of neuropeptide FF-related peptides in human cerebrospinal fluid by mass spectrometry. FEBS Lett 532:313–318

Butler AB, Hodos W (1996) Comparative vertebrate neuroanatomy: evolution and adaptation. Wiley-Liss, New York

Butler AB, Hodos W (2005) Comparative vertebrate neuroanatomy: evolution and adaptation, 2nd edn. Wiley, Hoboken

Byrne B, McGregor A, Taylor PL et al (1999) Isolation and characterisation of the marmoset gonadotrophin releasing hormone receptor: Ser^{140} of the DRS motif is substituted by Phe. J Endocrinol 163:447–456

Chang JP, Cook AF, Peter RE (1983) Influence of catecholamines on gonadotropin secretion in goldfish, *Carassius auratus*. Gen Comp Endocrinol 49:22–31

Chartrel N, Dujardin C, Leprince J et al (2002) Isolation, characterization, and distribution of a novel neuropeptide, Rana RFamide (R-RFa), in the brain of the European green frog *Rana esculenta*. J Comp Neurol 448:111–127

Chartrel N, Dujardin C, Anouar Y et al (2003) Identification of 26RFa, a hypothalamic neuropeptide of the RFamide peptide family with orexigenic activity. Proc Natl Acad Sci U S A 100:15247–15252

Chen C-C, Fernald RD (2008) GnRH and GnRH receptors: distribution, function and evolution. J Fish Biol 73:1099–1120

Cherubini E, Conti F (2001) Generating diversity at GABAergic synapses. Trends Neurosci 24:155–162

Combarnous Y (1992) Molecular basis of the specificity of binding of glycoprotein hormones to their receptors. Endocr Rev 13:670–691

Conlon JM, Bjornholm B, Jorgensen FS, Youson JH, Schwartz TW (1991) Primary structure and conformational analysis of peptide methionine-tyrosine, a peptide related to neuropeptide Y and peptide YY isolated from lamprey intestine. Eur J Biochem 199:293–298

Conlon JM, Balasubramaniam A, Sower SA (1994) Purification of a neuropeptide Y-related peptide from the brain of the sea lamprey and its effect on steroidogenesis. Regul Pept 50:167–175

Copeland P, Thomas P (1989) Control of gonadotropin release in the Atlantic croaker (*Micropogonias undulatus*): evidence for lack of dopaminergic inhibition. Gen Comp Endocrinol 74:474–483

Cosowsky L, Lin W, Han Y et al (1997) Influence of subunit interactions on lutropin specificity. Implications for studies of glycoprotein hormone function. J Biol Chem 272:3309–3314

Crim JW (1981) Immunoreactive luteinizing hormone releasing hormone and cerebral spinal fluid-contacting neurons in the preoptic nucleus of lamprey. In: Farner DS, Lederis K (eds) Neurosecretion: molecules, cells and systems. Plenum, London, pp 424–433

Crim J, Urano A, Gorbman A (1979a) lmmunocytochemical studies of luteinizing hormone-releasing hormone in brains of agnathan fishes. II. Patterns of immunoreactivity in larval and maturing Western brook lamprey (*Lampetra richardsoni*). Gen Comp Endocrinol 38:290–299

Crim JW, Urano A, Gorbman A (1979b) Immunocytochemical studies of luteinizing hormone-releasing hormone in brains of agnathan fishes. I. Comparisons of adult Pacific lamprey (*Entosphenus tridentata*) and the Pacific hagfish (*Eptatretus stouti*). Gen Comp Endocrinol 37(3):294–305

Crim LW, St Arnaud R, Lavoie M, Labrie F (1988) A study of LH-RH receptors in the pituitary gland of the winter flounder (*Pseudopleuronectes americanus* Walbaum). Gen Comp Endocrinol 69:372–377

Crow KD, Wagner GP (2006) What is the role of genome duplication in the evolution of complexity and diversity? Mol Biol Evol 23:887–892

Daukss D, Gazda K, Kosugi T et al (2012) Effects of lamprey PQRFamide peptides on brain gonadotropin-β mRNA expression. Gen Comp Endocrinol 177:215–219

De Andrés M, Anadón R, Manso MJ, González MJ (2002) Distribution of thyrotropin-releasing hormone immunoreactivity in the brain of larval and adult sea lampreys, *Petromyzon marinus* L. J Comp Neurol 453:323–335

Decatur WA, Hall JA, Smith JJ, Li W, Sower SA (2013) Insight from the lamprey genome: glimpsing early vertebrate development via neuroendocrine-associated genes and shared synteny of gonadotropin-releasing hormone (GnRH). Gen Comp Endocrinol 192:237–245

De Leeuw R, Conn P, Van't Veer C, Goos H, Van Ordt P (1988) Characterization of the receptor for gonadotropin-releasing hormone in the pituitary of the African catfish, *Clarias gariepinus*. Fish Physiol Biochem 5:99–107

Deragon KL, Sower SA (1994) Effects of lamprey gonadotropin-releasing hormone-III on steroidogenesis and spermiation in male sea lampreys. Gen Comp Endocrinol 95:363–367

Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 71–114

Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Docker MF, Beamish FWH, Bryan MB, Spice EK (in press) The lamprey gonad. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Doldan MJ, Prego B, Holmqvist BI, de Miguel E (1999) Distribution of GABA-immunolabeling in the early zebrafish (*Danio rerio*) brain. Eur J Morphol 37:126–129

Domenici L, Waldvogel HJ, Matute C, Streit P (1988) Distribution of GABA-like immunoreactivity in the pigeon brain. Neuroscience 25:931–950

Dos Santos S, Bardet C, Bertrand S et al (2009) Distinct expression patterns of glycoprotein hormone-α2 and -β5 in a basal chordate suggest independent developmental functions. Endocrinology 150:3815–3822

Dos Santos S, Mazan S, Venkatesh B, Cohen-Tannoudji J, Querat B (2011) Emergence and evolution of the glycoprotein hormone and neurotrophin gene families in vertebrates. BMC Evol Biol 11:332

Dufau ML (1998) The luteinizing hormone receptor. Annu Rev Physiol 60:461–496

Eisthen HL, Northcutt RG (1996) Silver lampreys (*Ichthyomyzon unicuspis*) lack a gonadotropin-releasing hormone- and FMRFamide-immunoreactive terminal nerve. J Comp Neurol 370:159–172

Ekstrom P, Ohlin LM (1995) Ontogeny of GABA-immunoreactive neurons in the central nervous system in a teleost, *Gasterosteus aculeatus* L. J Chem Neuroanat 9:271–288

Fahien CM, Sower SA (1990) Relationship between brain gonadotropin-releasing hormone and final reproductive period of the adult male sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 80:427–437

Felip A, Zanuy S, Pineda R et al (2009) Evidence for two distinct KiSS genes in non-placental vertebrates that encode kisspeptins with different gonadotropin-releasing activities in fish and mammals. Mol Cell Endocrinol 312:61–71

Fernald RD, White RB (1999) Gonadotropin-releasing hormone genes: phylogeny, structure, and functions. Front Neuroendocrinol 20:224–240

Forey P, Janvier P (1994) Evolution of the early vertebrates. Am Sci 82:554–566
Franzoni MF, Morino P (1989) The distribution of GABA-like-immunoreactive neurons in the brain of the newt, *Triturus cristatus carnifex*, and the green frog, *Rana esculenta*. Cell Tissue Res 255:155–166
Freamat M, Sower SA (2008a) A sea lamprey glycoprotein hormone receptor similar with gnathostome thyrotropin hormone receptor. J Mol Endocrinol 41:219–228
Freamat M, Sower SA (2008b) Glycoprotein hormone receptors in sea lamprey *Petromyzon marinus*. Zool Sci 25:1037–1044
Freamat M, Sower SA (2010) Functional divergence of glycoprotein hormone receptors. Integr Comp Biol 50:110–123
Freamat M, Kawauchi H, Nozaki M, Sower SA (2006) Identification and cloning of a glycoprotein hormone receptor from sea lamprey, *Petromyzon marinus*. J Mol Endocrinol 37:135–146
Fueshko SM, Key S, Wray S (1998) GABA inhibits migration of luteinizing hormone-releasing hormone neurons in embryonic olfactory explants. J Neurosci 18:2560–2569
Fujimoto M, Takeshita K, Wang X et al (1998) Isolation and characterization of a novel bioactive peptide, Carassius RFamide (C-RFa), from the brain of the Japanese crucian carp. Biochem Biophys Res Commun 242:436–440
Fukamachi S, Meyer A (2007) Evolution of receptors for growth hormone and somatolactin in fish and land vertebrates: lessons from the lungfish and sturgeon orthologues. J Mol Evol 65:359–372
Fukayama S, Takahashi H (1985) Changes in serum levels of estradiol-17b and testosterone in the Japanese river lamprey, *Lampetra japonica*, in the course of sexual maturation. Bull Fac Fish Hokkaido Univ 36:163–169
Fukusumi S, Habata Y, Yoshida H et al (2001) Characteristics and distribution of endogenous RFamide-related peptide-1. Biochim Biophys Acta 1540:221–232
Fukusumi S, Yoshida H, Fujii R et al (2003) A new peptidic ligand and its receptor regulating adrenal function in rats. J Biol Chem 278:46387–46395
Gazourian L, Sower SA (1994) Pituitary responsiveness to perifused lamprey GnRH-I or -III analogs in the adult sea lamprey, *Petromyzon marinus*. Am Zool 34:23A
Gazourian L, Deragon KL, Chase CF et al (1997) Characteristics of GnRH binding in the gonads and effects of lamprey GnRH-I and -III on reproduction in the adult sea lamprey. Gen Comp Endocrinol 108:327–339
Gazourian L, Evans EL, Hanson L, Chase CF, Sower SA (2000) The effects of lamprey GnRH-I, III and analogs on steroidogenesis in the sea lamprey (*Petromyzon marinus*). Aquaculture 188:147–165
Gorbman A (1965) Vascular relations between the neurohypophysis and adenohypophysis of cyclostomes and the problem of evolution of hypothalamic neuroendrocrine control. Arch Anat Microsc Morphol Exp 54:163–194
Gorbman A, Dickhoff WW (1978) Endocrine control of reproduction in hagfish. In: Gaillard PJ, Boer HH (eds) Comparative endocrinology. Elsevier, Amsterdam, pp 49–54
Gorbman A, Sower SA (2003) Evolution of the role of GnRH in animal (metazoan) biology. Gen Comp Endocrinol 134:207–213
Gorbman A, Dickoff WW, Vigna SR, Clark NB, Ralph CL (1983) Comparative endocrinology. Wiley, New York
Gore AC (2002) GnRH: the master molecule of reproduction. Kluwer, New York
Goren A, Zohar Y, Fridkin M, Elhanati E, Koch Y (1990) Degradation of gonadotropin-releasing hormones in the gilthead seabream, *Sparus aurata*. I. Cleavage of native salmon GnRH and mammalian LHRH in the pituitary. Gen Comp Endocrinol 79:291–305
Grober MS, Myers TR, Marchaterre MA, Bass AH, Myers DA (1995) Structure, localization, and molecular phylogeny of a GnRH cDNA from a paracanthopterygian fish, the plainfin midshipman (*Porichthys notatus*). Gen Comp Endocrinol 99:85–99
Grosse R, Roelle S, Herrlich A, Hohn J, Gudermann T (2000) Epidermal growth factor receptor tyrosine kinase mediates Ras activation by gonadotropin-releasing hormone. J Biol Chem 275:12251–12260

Grossmann M, Weintraub BD, Szkudlinski MW (1997) Novel insights into the molecular mechanisms of human thyrotropin action: structural, physiological, and therapeutic implications for the glycoprotein hormone family. Endocr Rev 18:476–501

Guilgur LG, Moncaut NP, Canario AV, Somoza GM (2006) Evolution of GnRH ligands and receptors in gnathostomata. Comp Biochem Physiol A Mol Integr Physiol 144:272–283

Guillemin R (1978) Peptides in the brain: the new endocrinology of the neuron. Science 202:390–402

Hagelin L-O, Steffner N (1958) Notes on the spawning habits of the river lamprey (*Petromyzon fluviatilis*). Oikos 9:221–238

Hall JA, Decatur WA, Daukss DM et al (2013) Expression of three GnRH receptors in specific tissues in male and female sea lampreys *Petromyzon marinus* at three distinct life stages. Front Neurosci 28:7–88

Hardisty MW, Potter IC, Hilliard RW (1986) Gonadogenesis and sex differentiation in the southern hemisphere lamprey, *Geotria australis*. J Zool Lond (A) 209:477–499

Harvey B, Carolsfeld J (1993) Induced breeding in tropical fish culture. International Development Research Centre, Ottawa

Heding A, Vrecl M, Bogerd J et al (1998) Gonadotropin-releasing hormone receptors with intracellular carboxyl-terminal tails undergo acute desensitization of total inositol phosphate production and exhibit accelerated internalization kinetics. J Biol Chem 273:11472–11477

Heimberg A, Cowper-Sallari R, Sémon M, Donoghue P, Peterson K (2010) microRNAs reveal the interrelationships of hagfish, lampreys, and gnathostomes and the nature of the ancestral vertebrate. Proc Natl Acad Sci U S A 107:19379–19383

Heinig JA, Keeley FW, Robson P, Sower SA, Youson JH (1995) The appearance of proopiomelanocortin early in vertebrate evolution: cloning and sequencing of POMC from a lamprey pituitary cDNA library. Gen Comp Endocrinol 99:137–144

Hinuma S, Habata Y, Fujii R et al (1998) A prolactin-releasing peptide in the brain. Nature 393:272–276

Hislop J, Caunt C, Sedgley K et al (2005) Internalization of gonadotropin-releasing hormone receptors (GnRHRs): does arrestin binding to the C-terminal tail target GnRHRs for dynamin-dependent internalization? J Mol Endocrinol 35:177–189

Ho SM, Press D, Liang LC, Sower S (1987) Identification of an estrogen receptor in the testis of the sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 67:119–125

Holland PW, Garcia-Fernandez J, Williams NA, Sidow A (1994) Gene duplications and the origins of vertebrate development. Development 1994(Suppl):125–133

Huhtaniemi I (2005) Focus on gonadotrophin signalling. Reproduction 130:261–262

Illing N, Troskie BE, Nahorniak CS et al (1999) Two gonadotropin-releasing hormone receptor subtypes with distinct ligand selectivity and differential distribution in brain and pituitary in the goldfish (*Carassius auratus*). Proc Natl Acad Sci U S A 96:2526–2531

Jackson IM, Reichlin S (1977) Thyrotropin-releasing hormone: abundance in the skin of the frog, *Rana pipiens*. Science 198:414–415

Jang M-H, Lucas MC (2005) Reproductive ecology of the river lamprey. J Fish Biol 66:499–512

Janvier P (2010) microRNAs revive old views about jawless vertebrate divergence and evolution. Proc Natl Acad Sci U S A 107:19137–19138

Joseph NT, Aquilina-Beck A, MacDonald C et al (2012) Molecular cloning and pharmacological characterization of two novel GnRH receptors in the lamprey (*Petromyzon marinus*). Endocrinology 153:3345–3356

Kah O, Dufour S (2010) Conserved and divergent features of reproductive neuroendocrinology in teleost fishes. In: Norris DO, Lopez KH (eds) Hormones and reproduction of vertebrates, vol 1, fishes. Academic Press, Boston, pp 15–42

Kah O, Dubourg P, Martinoli MG et al (1987) Central GABAergic innervation of the pituitary in goldfish: a radioautographic and immunocytochemical study at the electron microscope level. Gen Comp Endocrinol 67:324–332

Kah O, Trudeau VL, Sloley BD et al (1992) Influence of GABA on gonadotrophin release in the goldfish. Neuroendocrinology 55:396–404

Kah O, Lethimonier C, Somoza G et al (2007) GnRH and GnRH receptors in metazoa: a historical, comparative, and evolutive perspective. Gen Comp Endocrinol 153:346–364
Kauffman AS, Rissman EF (2004a) A critical role for the evolutionarily conserved gonadotropin-releasing hormone II: mediation of energy status and female sexual behavior. Endocrinology 145:3639–3646
Kauffman AS, Rissman EF (2004b) The evolutionarily conserved gonadotropin-releasing hormone II modifies food intake. Endocrinology 145:686–691
Kavanaugh SI, Nozaki M, Sower SA (2008) Origins of gonadotropin-releasing hormone (GnRH) in vertebrates: identification of a novel GnRH in a basal vertebrate, the sea lamprey. Endocrinology 149:3860–3869
Kawauchi H, Sower SA (2006) The dawn and evolution of hormones in the adenohypophysis. Gen Comp Endocrinol 148:3–14
Kawauchi H, Suzuki K, Itho H et al (1989) The duality of teleost gonadotropins. Fish Physiol Biochem 7:29–38
Kawauchi H, Suzuki K, Yamazaki T et al (2002) Identification of growth hormone in the sea lamprey, an extant representative of a group of the most ancient vertebrates. Endocrinology 143:4916–4921
Kim DK, Cho EB, Moon MJ et al (2011) Revisiting the evolution of gonadotropin-releasing hormones and their receptors in vertebrates: secrets hidden in genomes. Gen Comp Endocrinol 170:68–78
King JA, Millar RP (1991) Gonadotropin-releasing hormones. In: Pang PKT, Schreibman MP (eds) Vertebrate endocrinology: fundamentals and biomedical implications, vol 4. Academic Press, San Diego, pp 1–32
King JC, Sower SA, Anthony EL (1988) Neuronal systems immunoreactive with antiserum to lamprey gonadotropin- releasing hormone in the brain of *Petromyzon marinus*. Cell Tissue Res 253:1–8
Kitahashi T, Ogawa S, Parhar IS (2009) Cloning and expression of kiss2 in the zebrafish and medaka. Endocrinology 150:821–831
Kleinau G, Krause G (2009) Thyrotropin and homologous glycoprotein hormone receptors: structural and functional aspects of extracellular signaling mechanisms. Endocr Rev 30:133–151
Knox CJ, Boyd SK, Sower SA (1994) Characterization and localization of gonadotropin-releasing hormone receptors in the adult female sea lamprey, *Petromyzon marinus*. Endocrinology 134:492–498
Koda A, Ukena K, Teranishi H et al (2002) A novel amphibian hypothalamic neuropeptide: isolation, localization, and biological activity. Endocrinology 143:411–419
Kosugi T, Sower SA (2010) A role of Histidine^{151} in the lamprey gonadotropin-releasing hormone receptor-1 (lGnRHR-1): functional insight of diverse amino acid residues in the position of Tyr of the DRY motif in GnRHR from an ancestral type II receptor. Gen Comp Endocrinol 166:498–503
Kotani M, Detheux M, Vandenbogaerde A et al (2001) The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem 276:34631–34636
Kuo MW, Lou SW, Postlethwait J, Chung BC (2005) Chromosomal organization, evolutionary relationship, and expression of zebrafish GnRH family members. J Biomed Sci 12:629–639
Kuraku S, Meyer A, Kuratani S (2009) Timing of genome duplications relative to the origin of the vertebrates: did cyclostomes diverge before or after? Mol Biol Evol 26:47–59
Kuratani S, Kuraku S, Murakami Y (2002) Lamprey as an evo-devo model: lessons from comparative embryology and molecular phylogenetics. Genesis 34:175–183
Kusano K, Fueshko S, Gainer H, Wray S (1995) Electrical and synaptic properties of embryonic luteinizing hormone- releasing hormone neurons in explant cultures. Proc Natl Acad Sci U S A 92:3918–3922
Lagios MD (1970) The median eminence of the bowfin, *Amia calva* L. Gen Comp Endocrinol 15:453–463
Larhammar D (1996) Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. Regul Pept 62:1–11

Lariviere K, MacEachern L, Greco V et al (2002) GAD_{65} and GAD_{67} isoforms of the glutamic acid decarboxylase gene originated before the divergence of cartilaginous fishes. Mol Biol Evol 19:2325–2329

Larsen LO (1965) Effects of hypophysectomy in the cyclostome, *Lampetra fluviatilis* (L.) Gray. Gen Comp Endocrinol 5:16–30

Larsen LO (1973) Development in adult, freshwater river lampreys and its hormonal control: starvation, sexual maturation and natural death. PhD thesis, University of Copenhagen, Copenhagen

Lauder JM (1993) Neurotransmitters as growth regulatory signals: role of receptors and second messengers. Trends Neurosci 16:233–240

Lauder JM, Han VK, Henderson P, Verdoorn T, Towle AC (1986) Prenatal ontogeny of the GABAergic system in the rat brain: an immunocytochemical study. Neuroscience 19:465–493

Lee YR, Tsunekawa K, Moon MJ et al (2009) Molecular evolution of multiple forms of kisspeptins and GPR54 receptors in vertebrates. Endocrinology 150:2837–2846

Leranth C, MacLusky NJ, Sakamoto H, Shanabrough M, Naftolin F (1985) Glutamic acid decarboxylase-containing axons synapse on LHRH neurons in the rat medial preoptic area. Neuroendocrinology 40:536–539

Linville JE, Hanson LH, Sower SA (1987) Endocrine events associated with spawning behavior in the sea lamprey (*Petromyzon marinus*). Horm Behav 21:105–117

Liu F, Usui I, Evans LG et al (2002) Involvement of both $G_{q/11}$ and G_s proteins in gonadotropin-releasing hormone receptor-mediated signaling in LβT2 cells. J Biol Chem 277:32099–32108

Lovejoy DA, Fischer WH, Ngamvongchon S et al (1992) Distinct sequence of gonadotropin-releasing hormone (GnRH) in dogfish brain provides insight into GnRH evolution. Proc Natl Acad Sci U S A 89:6373–6377

MacIntyre JK, Chase C, Tobet SA, Sower SA (1997) The interrelationship of PMY, GABA, and GnRH in the sea lamprey, *Petromyzon marinus*. XIII Int Congr Comp Endocrinol 1:721–724

Manion PJ, Hanson LH (1980) Spawning behavior and fecundity of lampreys from the upper three Great Lakes. Can J Fish Aquat Sci 37:1635–1640

Marchant TA, Chang JP, Nahorniak CS, Peter RE (1989) Evidence that gonadotropin-releasing hormone also functions as a growth hormone-releasing factor in the goldfish. Endocrinology 124:2509–2518

Martinoli M, Dubourg P, Geffard M, Calas A, Kah O (1990) Distribution of GABA-immunoreactive neurons in the forebrain of the goldfish, *Carassius auratus*. Cell Tissue Res 260:77–84

Materne OL, Gazourian L, Chase C et al (1997) Characterization of the gonadotropin-releasing hormone binding sites in the pituitary and gonads of the sexually maturing adult lamprey. XIII Int Congr Comp Endocrinol 1:743–746

McArdle CA, Davidson JS, Willars GB (1999) The tail of the gonadotrophin-releasing hormone receptor: desensitization at, and distal to, G protein-coupled receptors. Mol Cell Endocrinol 151:129–136

Medina M, Reperant J, Dufour S et al (1994) The distribution of GABA-immunoreactive neurons in the brain of the silver eel (*Anguilla anguilla* L.). Anat Embryol 189:25–39

Melendez-Ferro M, Perez-Costas E, Rodriguez-Munoz R et al (2001) GABA immunoreactivity in the olfactory bulbs of the adult sea lamprey *Petromyzon marinus* L. Brain Res 893:253–260

Melendez-Ferro M, Villar-Cheda B, Manoel Abalo X et al (2002) Early development of the retina and pineal complex in the sea lamprey: comparative immunocytochemical study. J Comp Neurol 442:250–265

Mesa M, Bayer J, Bryan M, Sower S (2010) Annual sex steroid and other physiological profiles of Pacific lampreys (*Entosphenus tridentatus*). Comp Biochem Physiol A 155:56–63

Millar RP, King JA (1987) Structural and functional evolution of gonadotropin-releasing hormone. Int Rev Cytol 106:149–182

Millar R, Lowe S, Conklin D et al (2001) A novel mammalian receptor for the evolutionarily conserved type II GnRH. Proc Natl Acad Sci U S A 98:9636–9641

Millar RP, Lu ZL, Pawson AJ et al (2004) Gonadotropin-releasing hormone receptors. Endocr Rev 25:235–275

Moriyama S, Ito T, Takahashi A et al (2002) A homolog of mammalian PRL-releasing peptide (fish arginyl-phenylalanyl-amide peptide) is a major hypothalamic peptide of PRL release in teleost fish. Endocrinology 143:2071–2079
Moyle WR, Xing Y, Lin W et al (2004) Model of glycoprotein hormone receptor ligand binding and signaling. J Biol Chem 279:44442–44459
Moyle WR, Lin W, Myers RV et al (2005) Models of glycoprotein hormone receptor interaction. Endocrine 26:189–205
Murakami S, Arai Y (1994) Direct evidence for the migration of LHRH neurons from the nasal region to the forebrain in the chick embryo: a carbocyanine dye analysis. Neurosci Res 19:331–338
Murakami S, Kikuyama S, Arai Y (1992) The origin of the luteinizing hormone-releasing hormone (LHRH) neurons in newts (*Cynops pyrrhogaster*): the effect of olfactory placode ablation. Cell Tissue Res 269:21–27
Muske LE (1993) Evolution of gonadotropin-releasing hormone (GnRH) neuronal system. Brain Behav Evol 42:224–230
Myburgh DB, Millar RP, Hapgood JP (1998) Alanine-261 in intracellular loop III of the human gonadotropin-releasing hormone receptor is crucial for G-protein coupling and receptor internalization. Biochem J 331:893–896
Nakabayashi K, Matsumi H, Bhalla A et al (2002) Thyrostimulin, a heterodimer of two new human glycoprotein hormone subunits, activates the thyroid-stimulating hormone receptor. J Clin Invest 109:1445–1452
Near TJ (2009) Conflict and resolution between phylogenies inferred from molecular and phenotypic data sets for hagfish, lampreys, and gnathostomes. J Exp Zool Mol Dev Evol 312B:749–761
Neill JD, Duck LW, Sellers JC, Musgrove LC (2001) A gonadotropin-releasing hormone (GnRH) receptor specific for GnRH II in primates. Biochem Biophys Res Commun 282:1012–1018
Nieuwenhuys R (1977) The brain of the lamprey in a comparative perspective. Ann N Y Acad Sci 299:97–145
Norgren RB, Jr, Gao C (1994) LHRH neuronal subtypes have multiple origins in chickens. Dev Biol 165:735–738
Norris DO, Carr JA (2013) Vertebrate endocrinology, 5th edn. Academic Press, Waltham
Northcutt RG, Muske LE (1994) Multiple embryonic origins of gonadotropin-releasing hormone (GnRH) immunoreactive neurons. Dev Brain Res 78:279–290
Nozaki M, Gorbman A (1984) Distribution of immunoreactive sites for several components of proopiocortin in the pituitary and brain of adult lampreys, *Petromyzon marinus* and *Entosphenus tridentatus*. Gen Comp Endocrinol 53:335–352
Nozaki M, Kobayashi H (1979) Distribution of LHRH-like substance in the vertebrate brain as revealed by immunohistochemistry. Arch Histol Jpn 42:201–219
Nozaki M, Fernholm B, Kobayashi H (1975) Ependymal absorption of peroxidase in the third ventricle of the hagfish *Eptatretus burgeri* (Girard). Acta Zool 56:265–269
Nozaki M, Tsukahara T, Kobayashi H (1984) An immunocytological study of the distribution of neuropeptides in the brain of fish. Biomed Res-Tokyo 4(Suppl S):135–145
Nozaki M, Gorbman A, Sower SA (1994) Diffusion between the neurohypophysis and the adenohypophysis of lampreys, *Petromyzon marinus*. Gen Comp Endocrinol 96:385–391
Nozaki M, Takahashi A, Amemiya Y, Kawauchi H, Sower SA (1995) Distribution of lamprey adrenocorticotropin and melanotropins in the pituitary of the adult sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 98:147–156
Nozaki M, Ominato K, Takahashi A, Kawauchi H, Sower SA (1999) Possible gonadotropin cells in the lamprey pituitary: colocalization of mammalian LH-like immunoreactivity and glycoconjugate in adult sea lampreys (*Petromyzon marinus*). Gen Comp Endocrinol 113:23–31
Nozaki M, Ominato K, Gorbman A, Sower SA (2000) The distribution of lamprey GnRH-III in brains of adult sea lampreys (*Petromyzon marinus*). Gen Comp Endocrinol 118:57–67

Nozaki M, Ominato K, Takahashi A, Kawauchi H, Sower SA (2001) Adenohypophysial cell types in the lamprey pituitary: current state of the art. Comp Biochem Physiol B Biochem Mol Biol 129:303–309

Nozaki M, Ominato K, Shimotani T et al (2008) Identity and distribution of immunoreactive adenohypophysial cells in the pituitary during the life cycle of sea lampreys, *Petromyzon marinus*. Gen Comp Endocrinol 155:403–412

Oh DY, Song JA, Moon JS et al (2005) Membrane-proximal region of the carboxyl terminus of the gonadotropin-releasing hormone receptor (GnRHR) confers differential signal transduction between mammalian and nonmammalian GnRHRs. Mol Endocrinol 19:722–731

Ohno S (1970) Evolution by gene duplication. Allen and Unwin Springer, New York

Ohtaki T, Shintani Y, Honda S et al (2001) Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature 411:613–617

Okubo K, Nagahama Y (2008) Structural and functional evolution of gonadotropin-releasing hormone in vertebrates. Acta Physiol 193:3–15

Okubo K, Nagata S, Ko R et al (2001) Identification and characterization of two distinct GnRH receptor subtypes in a teleost, the medaka *Oryzias latipes*. Endocrinology 142:4729–4739

Osugi T, Ukena K, Bentley GE et al (2004) Gonadotropin-inhibitory hormone in Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*): cDNA identification, transcript localization and functional effects in laboratory and field experiments. J Endocrinol 182:33–42

Osugi T, Ukena K, Sower SA, Kawauchi H, Tsutsui K (2006) Evolutionary origin and divergence of PQRFamide peptides and LPXRFamide peptides in the RFamide peptide family: insights from novel lamprey RFamide peptides. FEBS J 273:1731–1743

Osugi T, Daukss D, Gazda K et al (2012) Evolutionary origin of the structure and function of gonadotropin-inhibitory hormone: insights from lampreys. Endocrinology 153:2362–2374

Ota KG, Kuraku S, Kuratani S (2007) Hagfish embryology with reference to the evolution of the neural crest. Nature 446:672–675

Pagelson G, Zohar Y (1992) Characterization of gonadotropin-releasing hormone binding to pituitary receptors in the gilthead seabream (*Sparus aurata*). Biol Reprod 47:1004–1008

Paris M, Pettersson K, Schubert M et al (2008) An amphioxus orthologue of the estrogen receptor that does not bind estradiol: insights into estrogen receptor evolution. Curr Biol 18:825–830

Pawson AJ, Katz A, Sun YM et al (1998) Contrasting internalization kinetics of human and chicken gonadotropin- releasing hormone receptors mediated by C-terminal tail. J Endocrinol 156:R9–R12

Peter RE, Paulencu CR (1980) Involvement of the preoptic region in gonadotropin release-inhibition in goldfish, *Carassius auratus*. Neuroendocrinology 31:133–141

Peter RE, Crim LW, Goos H, Crim JW (1978) Lesioning studies on the gravid goldfish: neuroendocrine regulation of ovulation. Gen Comp Endocrinol 35:391–401

Peter RE, Yu KL, Marchant TA, Rosenblum PM (1990) Direct neural regulation of the teleost adenophypophysis. J Exp Zool Suppl 4:84–89

Pombal MA, Puelles L (1999) Prosomeric map of the lamprey forebrain based on calretinin immunocytochemistry, Nissl stain, and ancillary markers. J Comp Neurol 414:391–422

Pombal MA, El Manira A, Grillner S (1997) Organization of the lamprey striatum—transmitters and projections. Brain Res 766:249–254

Quérat B, Sellouk A, Salmon C (2000) Phylogenetic analysis of the vertebrate glycoprotein hormone family including new sequences of sturgeon (*Acipenser baeri*) β-subunits of the two gonadotropins and the thyroid-stimulating hormone. Biol Reprod 63:222–228

Quérat B, Tonnerre-Doncarli C, Genies F, Salmon C (2001) Duality of gonadotropins in gnathostomes. Gen Comp Endocrinol 124:308–314

Quérat B, Arai Y, Henry A et al (2004) Pituitary glycoprotein hormone β subunits in the Australian lungfish and estimation of the relative evolution rate of these subunits within vertebrates. Biol Reprod 70:356–363

Reed KL, MacIntyre JK, Tobet SA et al (2002) The spatial relationship of gamma-aminobutyric acid (GABA) neurons and gonadotropin-releasing hormone (GnRH) neurons in larval and adult sea lamprey, *Petromyzon marinus*. Brain Behav Evol 60:1–12

Rissman EF, Alones VE, Craig-Veit CB, Millam JR (1995) Distribution of chicken-II gonadotropin-releasing hormone in mammalian brain. J Comp Neurol 357:524–531
Roa J, Castellano JM, Navarro VM et al (2009) Kisspeptins and the control of gonadotropin secretion in male and female rodents. Peptides 30:57–66
Roberts A, Dale N, Ottersen OP, Storm-Mathisen J (1987) The early development of neurons with GABA immunoreactivity in the CNS of *Xenopus laevis* embryos. J Comp Neurol 261:435–449
Root AR, Sanford JD, Kavanaugh SI, Sower SA (2004) *In vitro* and *in vivo* effects of GABA, muscimol, and bicuculline on lamprey GnRH concentration in the brain of the sea lamprey (*Petromyzon marinus*). Comp Biochem Physiol A Mol Integr Physiol 138:493–501
Root AR, Nucci NV, Sanford JD et al (2005) *In situ* characterization of gonadotropin-releasing hormone-I, -III, and glutamic acid decarboxylase expression in the brain of the sea lamprey, *Petromyzon marinus*. Brain Behav Evol 65:60–70
Rovati GE, Capra V, Neubig RR (2007) The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. Mol Pharmacol 71:959–964
Rowe ML, Elphick MR (2012) The neuropeptide transcriptome of a model echinoderm, the sea urchin *Strongylocentrous purpuratus*. Gen Comp Endocrinol 179:331–344
Satake H, Hisada M, Kawada T et al (2001) Characterization of a cDNA encoding a novel avian hypothalamic neuropeptide exerting an inhibitory effect on gonadotropin release. Biochem J 354:379–385
Sawada K, Ukena K, Satake H et al (2002) Novel fish hypothalamic neuropeptide. Eur J Biochem 269:6000–6008
Schally AV (1978) Aspects of hypothalamic regulation of the pituitary gland. Science 202:18–28
Schwanzel-Fukuda M, Pfaff DW (1989) Origin of luteinizing hormone-releasing hormone neurons. Nature 338:161–164
Seale AP, Itoh T, Moriyama S et al (2002) Isolation and characterization of a homologue of mammalian prolactin-releasing peptide from the tilapia brain and its effect on prolactin release from the tilapia pituitary. Gen Comp Endocrinol 125:328–339
Seminara S, Crowley J, WF (2008) Kisspeptin and GPR54: discovery of a novel pathway in reproduction. J Neuroendocrinol 20:727–731
Seminara S, Messager S, Chatzidaki E et al (2003) The GPR54 gene as a regulator of puberty. N Engl J Med 349:16114–16127
Seong JY, Wang L, Oh da Y et al (2003) Ala/Thr^{201} in extracellular loop 2 and Leu/Phe^{290} in transmembrane domain 6 of type 1 frog gonadotropin-releasing hormone receptor confer differential ligand sensitivity and signal transduction. Endocrinology 144:454–466
Sherwood NM, Sower SA, Marshak DR, Fraser BA, Brownstein MJ (1986) Primary structure of gonadotropin-releasing hormone in lamprey brain. J Biol Chem 15:4812–4819
Sidow A (1996) Gen(om)e duplications in the evolution of early vertebrates. Curr Opin Genet Dev 6:715–722
Silver MR, Sower SA (2006) Functional characterization and kinetic studies of an ancestral lamprey GnRH-III selective type II GnRH receptor from the sea lamprey, *Petromyzon marinus*. J Mol Endocrinol 36:601–610
Silver MR, Kawauchi H, Nozaki M, Sower SA (2004) Cloning and analysis of the lamprey GnRH-III cDNA from eight species of lamprey representing the three families of Petromyzoniformes. Gen Comp Endocrinol 139:85–94
Silver MR, Nucci NV, Root AR, Reed KL, Sower SA (2005) Cloning and characterization of a functional type II gonadotropin-releasing hormone receptor with a lengthy carboxy-terminal tail from an ancestral vertebrate, the sea lamprey. Endocrinology 146:3351–3361
Sloley BD, Kah O, Trudeau VL, Dulka JG, Peter RE (1992) Amino acid neurotransmitters and dopamine in brain and pituitary of the goldfish: involvement in the regulation of gonadotropin secretion. J Neurochem 58:2254–2262
Smith JJ, Kuraku S, Holt C et al (2013) Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. Nat Genet 45:415–421

Sower SA (1987) Biological action of lamprey gonadotropin-releasing hormone in lampreys. Proceedings of the 3rd international symposium of the reproductive physiology of fish, St. John's, NL, pp 40–41
Sower SA (1989) Effects of lamprey gonadotropin-releasing hormone and analogs on steroidogenesis and spermiation in male sea lampreys. Fish Physiol Biochem 7:101–107
Sower SA (1990) Neuroendocrine control of reproduction in lampreys. Fish Phys Biochem 8:365–374
Sower SA (1997) Evolution of GnRH in fish of ancient origins. In: Parhar IS, Sakuma Y (eds) GnRH neurons: gene to behavior. Brain Shuppan, Tokyo
Sower SA (2003) The endocrinology of reproduction in lampreys and applications for male lamprey sterilization. J Great Lakes Res 29(Suppl 1):50–65
Sower SA, Baron MP (2011) The interrelationship of estrogen receptor and GnRH in a basal vertebrate, the sea lamprey. Front Endocrinol 2:58
Sower SA, Hanson LH (1992) Vertebrate sex determination/differentiation workshop. Report to the Great Lakes Fishery Commission. Ann Arbor, MI
Sower SA, Kawauchi K (2011) Reproduction in agnathan fishes: lampreys and hagfishes. In: Norris DO, Lopez KH (eds) Hormones and reproduction of vertebrates, vol 1. Academic Press, New York, pp 193–208
Sower SA, Larsen LO (1991) Plasma estradiol and progesterone after hypophysectomy and substitution with pituitary in female sea lampreys (*Petromyzon marinus*). Gen Comp Endocrinol 81:93–96
Sower SA, Dickhoff WW, Gorbman A, Rivier JE, Vale WW (1983) Ovulatory and steroidal responses in the lamprey following administration of salmon gonadotropin and agonistic and antagonistic analogues of GnRH. Can J Zool 61:2653–2659
Sower SA, Plisetskaya E, Gorbman A (1985a) Changes in plasma steroid and thyroid hormones and insulin during final maturation and spawning of the sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 58:259–269
Sower SA, Plisetskaya E, Gorbman A (1985b) Steroid and thyroid hormone profiles following a single injection of partly purified salmon gonadotropin or GnRH analogues in male and female sea lamprey. J Exp Zool 235:403–408
Sower SA, King JA, Millar RP, Sherwood NM, Marshak DR (1987) Comparative biological properties of lamprey gonadotropin-releasing hormone in vertebrates. Endocrinology 120:773–779
Sower SA, Chiang YC, Lovas S, Conlon JM (1993) Primary structure and biological activity of a third gonadotropin-releasing hormone from lamprey brain. Endocrinology 132:1125–1131
Sower SA, Nozaki M, Knox CJ, Gorbman A (1995a) The occurrence and distribution of GnRH in the brain of Atlantic hagfish, an agnatha, determined by chromatography and immunocytochemistry. Gen Comp Endocrinol 97:300–307
Sower SA, Goodman M, Nishiuchi Y (1995b) *In vivo* effects of lamprey GnRH-I and cyclized analogs: a structure-activity study. Neuropeptides 29:151–156
Sower SA, McGregor AJ, Materne OL et al (2000) Evidence for lamprey GnRH-I and -III-like molecules in the brains of the southern hemisphere lampreys *Geotria australis* and *Mordacia mordax*. Gen Comp Endocrinol 120:168–175
Sower SA, Nucci NV, Silver MR (2004) Family of gonadotropin-releasing hormone. In: Martini L (ed) Encyclopedia of endocrine diseases, vol 2. Elsevier, Amsterdam, pp 306–316
Sower SA, Moriyama S, Kasahara M et al (2006) Identification of sea lamprey GTHβ-like cDNA and its evolutionary implications. Gen Comp Endocrinol 148:22–32
Sower SA, Freamat M, Kavanaugh SI (2009) The origins of the vertebrate hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) endocrine systems: new insights from lampreys. Gen Comp Endocrinol 161:20–29
Sower SA, Balz E, Aquilina-Beck A, Kavanaugh SI (2011) Seasonal changes of brain GnRH-I, -II, and -III during the final reproductive period in adult male and female sea lamprey. Gen Comp Endocrinol 170:276–282

Stanislaus D, Ponder S, Ji TH, Conn PM (1998) Gonadotropin-releasing hormone receptor couples to multiple G proteins in rat gonadotrophs and in GGH3 cells: evidence from palmitoylation and overexpression of G proteins. Biol Reprod 59:579–586

Stevenson TJ, Bentley GE, Ubuka T et al (2008) Effects of social cues on GnRH-I, GnRH-II, and reproductive physiology in female house sparrows (*Passer domesticus*). Gen Comp Endocrinol 156:385–394

Struthers RS, Rivier J, Hagler AT (1985) Molecular dynamics and minimum energy conformations of GnRH and analogs. A methodology for computer-aided drug design. Ann N Y Acad Sci 439:81–96

Sudo S, Kuwabara Y, Park JI, Hsu SY, Hsueh AJ (2005) Heterodimeric fly glycoprotein hormone-α2 (GPA2) and glycoprotein hormone-β5 (GPB5) activate fly leucine-rich repeat-containing G protein-coupled receptor-1 (DLGR1) and stimulation of human thyrotropin receptors by chimeric fly GPA2 and human GPB5. Endocrinology 146:3596–3604

Sun B, Kavanaugh SI, Tsai P-S (2012) Gonadotropin-releasing hormone in protostomes: insights from functional studies on *Aplysia californica*. Gen Comp Endocrinol 176:321–326

Suzuki K, Kawauchi H, Nagahama Y (1988) Isolation and characterization of subunits from two distinct salmon gonadotropins. Gen Comp Endocrinol 71:302–306

Suzuki K, Gamble RL, Sower SA (2000) Multiple transcripts encoding lamprey gonadotropin releasing hormone-I precursors. J Mol Endocrinol 24:365–376

Swanson P, Suzuki K, Kawauchi H, Dickhoff WW (1991) Isolation and characterization of two coho salmon gonadotropins, GTH I and GTH II. Biol Reprod 44:29–38

Takahashi A, Amemiya Y, Nozaki M et al (1995a) Isolation and characterization of melanotropins from lamprey pituitary glands. Int J Pept Protein Res 46:197–204

Takahashi A, Amemiya Y, Sarashi M, Sower SA, Kawauchi H (1995b) Melanotropin and corticotropin are encoded on two distinct genes in the lamprey, the earliest evolved extant vertebrate. Biochem Biophys Res Commun 213:490–498

Tando Y, Kubokawa K (2009) Expression of the gene for ancestral glycoprotein hormone β subunit in the nerve cord of amphioxus. Gen Comp Endocrinol 162:329–339

Temple JL, Millar RP, Rissman EF (2003) An evolutionarily conserved form of gonadotropin-releasing hormone coordinates energy and reproductive behavior. Endocrinology 144:13–19

Thornton JW (2001) Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci U S A 98:5671–5676

Tobet SA, Schwarting GA (2006) Minireview: recent progress in gonadotropin-releasing hormone neuronal migration. Endocrinology 147:1159–1165

Tobet SA, Nozaki M, Youson JH, Sower SA (1995) Distribution of lamprey gonadotropin-releasing hormone-III (GnRH-III) in brains of larval lampreys (*Petromyzon marinus)*. Cell Tissue Res 279:261–270

Tobet SA, Chickering TW, Sower SA (1996) Relationship of gonadotropin-releasing hormone (GnRH) neurons to the olfactory system in developing lamprey (*Petromyzon marinus*). J Comp Neurol 376:97–111

Tobet SA, Sower SA, Schwarting GA (1997) Gonadotropin-releasing hormone containing neurons and olfactory fibers during development: from lamprey to mammals. Brain Res Bull 44:479–486

Tostivint H (2011) Evolution of the gonadotropin-releasing hormone (GnRH) gene family in relation to vertebrate tetraploidizations. Gen Comp Endocrinol 170:575–581

Trudeau VL, Sloley BD, Peter RE (1993a) GABA stimulation of gonadotropin-II release in goldfish: involvement of GABAA receptors, dopamine, and sex steroids. Am J Physiol 265:R348–R355

Trudeau VL, Sloley BD, Peter RE (1993b) Norepinephrine turnover in the goldfish brain is modulated by sex steroids and GABA. Brain Res 624:29–34

Trudeau VL, Sloley BD, Peter RE (1993c) Testosterone enhances GABA and taurine but not N-methyl-D, L-aspartate stimulation of gonadotropin secretion in the goldfish: possible sex steroid feedback mechanisms. J Neuroendocrinol 5:129–136

Trudeau VL, Spanswick D, Fraser EJ et al (2000) The role of amino acid neurotransmitters in the regulation of pituitary gonadotropin release in fish. Biochem Cell Biol 78:241–259
Tsai PS, Zhang L (2008) The emergence and loss of gonadotropin-releasing hormone (GnRH) in protostomes: orthology, phylogeny, structure, and function. Biol Reprod 79:798–805
Tsukahara T, Gorbman A, Kobayashi H (1986) Median eminence equivalence of the neurohypophysis of the hagfish, *Eptatretus burgeri*. Gen Comp Endocrinol 61:348–354
Tsuneki K (1988) Neurohypophysis of cyclostomes as a primitive hypothalamic center of vertebrates. Zool Sci 5:21–32
Tsuneki K, Gorbman A (1975) Ultrastructure of the anterior neurohypophysis and the pars distalis of the lamprey, *Lampetra tridentata*. Gen Comp Endocrinol 25:487–508
Tsuneki K, Nozaki M (1989) Histological and immunohistochemical studies of the neurohypophysis of primitive teleosts, the Osteoglossidae. Acta Zool 70:47–52
Tsutsui K, Saigoh E, Ukena K et al (2000) A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem Biophys Res Commun 275:661–667
Tsutsui K, Bentley G, Ubukab T et al (2007) The general and comparative biology of gonadotropin-inhibitory hormone (GnIH). Gen Comp Endocrinol 153:365–370
Tsutsumi M, Zhou W, Millar RP et al (1992) Cloning and functional expression of a mouse gonadotropin-releasing hormone receptor. Mol Endocrinol 6:1163–1169
Ubuka T, Ueno M, Ukena K, Tsutsui K (2003) Developmental changes in gonadotropin-inhibitory hormone in the Japanese quail (*Coturnix japonica*) hypothalamo-hypophysial system. J Endocrinol 178:311–318
Uchida K, Moriyama S, Chiba H et al (2010) Evolutionary origin of a functional gonadotropin in the pituitary of the most primitive vertebrate, hagfish. Proc Natl Acad Sci U S A 107:15832–15837
Uchida K, Moriyama S, Sower SA, Nozaki M (2013) Glycoprotein hormone in the pituitary of hagfish and its evolutionary implications. Fish Physiol Biochem 39:75–83
Ukena K, Iwakoshi E, Minakata H, Tsutsui K (2002) A novel rat hypothalamic RFamide-related peptide identified by immunoaffinity chromatography and mass spectrometry. FEBS Lett 512:255–258
Ukena K, Koda A, Yamamoto K et al (2003a) Novel neuropeptides related to frog growth hormone-releasing peptide: isolation, sequence, and functional analysis. Endocrinology 144:3879–3884
Ukena K, Ubuka T, Tsutsui K (2003b) Distribution of a novel avian gonadotropin-inhibitory hormone in the quail brain. Cell Tissue Res 312:73–79
Vandepoele K, De Vos W, Taylor JS, Meyer A, Van de Peer Y (2004) Major events in the genome evolution of vertebrates: paranome age and size differ considerably between ray-finned fishes and land vertebrates. Proc Natl Acad Sci U S A 101:1638–1643
von Bartheld CS, Rubel EW (1989) Transient GABA immunoreactivity in cranial nerves of the chick embryo. J Comp Neurol 286:456–471
Vrecl M, Heding A, Hanyaloglu A, Taylor PL, Eidne KA (2000) Internalization kinetics of the gonadotropin-releasing hormone (GnRH) receptor. Pflugers Arch 439:R19–R20
Wald U, Selzer ME, Krieger NR (1981) Glutamic acid decarboxylase in sea lamprey (*Petromyzon marinus*): characterization, localization, and developmental changes. J Neurochem 36:363–368
Wang L, Bogerd J, Choi HS et al (2001) Three distinct types of GnRH receptor characterized in the bullfrog. Proc Natl Acad Sci U S A 98:361–366
Willars GB, Heding A, Vrecl M et al (1999) Lack of a C-terminal tail in the mammalian gonadotropin-releasing hormone receptor confers resistance to agonist-dependent phosphorylation and rapid desensitization. J Biol Chem 274:30146–30153
Wray S, Grant P, Gainer H (1989a) Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. Proc Natl Acad Sci U S A 86:8132–8136
Wray S, Nieburgs A, Elkabes S (1989b) Spatiotemporal cell expression of luteinizing hormone-releasing hormone in the prenatal mouse: evidence for an embryonic origin in the olfactory placode. Brain Res Dev Brain Res 46:309–318

Wright DE, McBurney KM, Youson JH, Sower SA (1994) Distribution of lamprey gonadotropin-releasing hormone in the brain and pituitary gland of larval, metamorphic, and adult sea lampreys, *Petromyzon marinus*. Can J Zool 72:48–53

Yang H-YT, Martin RM (1995) Isolation and characterization of a neuropeptide FF-like peptide from brain and spinal cord of rat. Soc Neurosci Abstr 21:760

Yang HY, Fratta W, Majane EA, Costa E (1985) Isolation, sequencing, synthesis, and pharmacological characterization of two brain neuropeptides that modulate the action of morphine. Proc Natl Acad Sci U S A 82:7757–7761

Yoshida H, Habata Y, Hosoya M et al (2003) Molecular properties of endogenous RFamide-related peptide-3 and its interaction with receptors. Biochim Biophys Acta 1593:151–157

Young BA, Bryan MB, Sower SA, Scott AP, Li W (2004) 15α-hydroxytestosterone induction by GnRH I and GnRH III in Atlantic and Great Lakes sea lamprey (*Petromyzon marinus* L.). Gen Comp Endocrinol 136:276–281

Youngs LJ, Winokur A, Selzer ME (1985) Thyrotropin-releasing hormone in lamprey central nervous system. Brain Res 338:177–180

Youson JH, Sower SA (1991) Concentration of brain gonadotropin-releasing hormone during metamorphosis in the lamprey, *Petromyzon marinus*. J Exp Zool 259:399–404

Youson JH, Sower SA (2001) Theory on the evolutionary history of lamprey metamorphosis: role of reproductive and thyroid axes. Comp Biochem Physiol B Biochem Mol Biol 129:337–345

Youson JH, Docker M, Sower SA (1995) Concentration of gonadotropin-releasing hormones in brain of larval and metamorphosing lampreys of two species with different adult life histories. In: Goetz FW, Thomas P (eds) 5th International Symposium of the Reproductive Physiology of Fish, Austin, TX, p 83

Youson JH, Heinig JA, Khanam SF et al (2006) Patterns of proopiomelanotropin and proopiocortin gene expression and of immunohistochemistry for gonadotropin-releasing hormones (lGnRH-I and III) during the life cycle of a nonparasitic lamprey: relationship to this adult life history type. Gen Comp Endocrinol 148:54–71

Yu KL, He ML, Chik CC et al (1998) mRNA expression of gonadotropin-releasing hormones (GnRHs) and GnRH receptor in goldfish. Gen Comp Endocrinol 112:303–311

Zhang L, Tello JA, Zhang W, Tsai PS (2008) Molecular cloning, expression pattern, and immunocytochemical localization of a gonadotropin releasing hormone-like molecule in the gastropod mollusk, *Aplysia californica*. Gen Comp Endocrinol 156:201–209

Chapter 8
Conservation of Native Lampreys

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Abstract Forty-four species of lampreys (Petromyzontidae) are currently recognized: (a) nine species are anadromous and parasitic (i.e., feeding on actinopterygian fishes after metamorphosis); (b) nine species are freshwater resident and parasitic; and (c) 26 species are freshwater resident and non-parasitic (i.e., do not feed at all following metamorphosis). To date, the conservation status of 33 of these species (75 %) has been assessed at a global scale. Of those assessed, at least 12 are deemed at risk. Lampreys are at risk from a number of anthropogenic pressures, most notably pollution, habitat destruction (e.g., dredging of depositional habitats essential to larval lampreys), engineering works (particularly dams that act as barriers to migration and alter natural stream flow regimes), overharvest, and changes to their prey base. Legislation has been brought forward in recent years, most notably in North America and Europe, to give some protection to lampreys and their habitat. At least 16 species now receive legal protection in at least a portion of their range at the national (or European Union) level; others are protected by laws at the subnational level. A number of projects across the world are focusing on the protection and conservation of some populations of lampreys (particularly those harvested by humans); examples of these are described. Taxonomic uncertainty remains an

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impediment to the conservation of some lampreys, however, and there is also a need to explain and resolve disagreements between the global (IUCN) and national lists; better coordination and consultation should be developed to prevent confusion.

Keywords Conservation · Dams · Dredging · Habitat degradation · Legislation · Native · Overharvest · Petromyzontidae · Pollution · Threats · Translocation

8.1 Introduction

Worldwide, Hardisty (2006) lists 38 species of lampreys in 10 genera. Of these species, only four are found in the Southern Hemisphere, with the majority occurring in the Northern Hemisphere. Five additional species have since been described from the Northern Hemisphere (Naseka et al. 2009; Renaud and Economidis 2010; Mateus et al. 2013a), and a sixth has been resurrected (Holčík and Šorić 2004), bringing the total to 44 (but see Chap. 2, in which only 41 species are recognized). The International Union for Conservation of Nature (IUCN) Red List (2013) includes 30 lamprey species: over half of these are categorized globally as of Least Concern (20); the remaining 10 are listed as Data Deficient (3), Near Threatened (1), Vulnerable (3), Critically Endangered (2), or Extinct (1) (Table 8.1). However, one of the 30 species on the IUCN list, *Eudontomyzon* sp. nov. "migratory," which was last recorded around the end of the nineteenth century, has never been formally described (see Kottelat et al. 2005). Similarly, Vladykov's brook lamprey *Eudontomyzon vladykovi*, considered a synonym of the Ukrainian brook lamprey *E. mariae* pending a revision of the species over its wide range (Holčík and Renaud 1986), is not treated as a distinct species in several recent taxonomic lists (e.g., Renaud 2011; see Chap. 2). Thus, only 28 of the 44 lamprey species recognized here as distinct species are listed by the IUCN. A further four species have been assessed at a global scale by NatureServe (2013) (Table 8.1) and Jelks et al. (2008) evaluated one additional species (the Mexican brook lamprey *Tetrapleurodon geminis*) at the North American scale, which is equivalent to a global scale treatment because the species is endemic to Mexico (Table 8.1). So far, therefore, the conservation status of 33 of the 44 species (75 %) has been assessed at a global scale.

Speciation in lampreys has resulted in different ecological strategies among, and sometimes within, species. Although all species have a burrowing worm-like larval stage (known as the ammocoete stage), post-metamorphic lampreys adopt different migratory or feeding types (see Chap. 2; Docker and Potter in press). Nine species are anadromous and parasitic, migrating to the marine environment to feed mainly on teleost fishes and returning to fresh waters where appropriate habitat for reproduction and larval development is available. However, the majority of lamprey species (26) are freshwater-resident, non-parasitic species that do not migrate between the sea and fresh waters and do not feed during their brief adult lives. The remaining nine species belong to a third ecological type, intermediate between the first two—these are purely freshwater parasitic species that migrate to large lakes or rivers to feed on actinopterygian fishes. The anadromous lampreys, although they have

Table 8.1 Conservation status categories or ranks of lampreys at global (IUCN, NatureServe) and North American (American Fisheries Society, Jelks et al. 2008) scales; legal protection within the European Union (EU) or at national levels is also given. Table includes the 41 species recognized by Potter et al. (see Chap. 2); two undescribed species of *Lethenteron* from Japan (*Lethenteron* sp. N and sp. S; Yamazaki et al. 2006) and three recently described *Lampetra* species from Portugal (Mateus et al. 2013a) are not included (but see Sects. 8.4.3 and 8.4.2.3, respectively). Twelve species (names in bold) are deemed at risk on a global scale (see Sect. 8.3); 16 species (with asterisk) receive legal protection at the EU or national level in at least a portion of their range (protection at subnational levels is not listed). (Information is from IUCN (2013), NatureServe (2013), and Jelks et al. (2008) unless otherwise indicated.)

Species	Common name	IUCN (2013)[a]	NatureServe (2013)[b]	Jelks et al. (2008)[c]	Threats	Legal Protection	Comments
*Caspiomyzon wagneri**	Caspian lamprey	Near Threatened			Dams constructed on rivers of the Caspian Sea basin in the 1950s and 1960s prevented access to spawning grounds upriver; new spawning grounds below the dams are at risk from drought	Listed as declining according to the 2001 Red Data Book of the Russian Federation[d]	
Entosphenus folletti	Northern California brook lamprey		G1G2Q		Very small range is highly modified by dams, diversions, pollution		Taxonomic uncertainty based on Nelson et al. (2004); now recognized as distinct from *E. lethophagus* (Page et al. 2013) Also known as Modoc brook lamprey
Entosphenus lethophagus	Pit-Klamath brook lamprey		G3G4	Vulnerable	Dams have caused declines in abundance; narrowly restricted range		
Entosphenus macrostomus*	Vancouver lamprey	Data Deficient	G1G2	Threatened	Narrowly restricted range	Listed as Threatened under Schedule 1 of SARA[e]	Also known as Cowichan lamprey
Entosphenus minimus	Miller Lake lamprey	Vulnerable	G3	Endangered	Extirpated from part of range (Miller Lake, Oregon) through state-sanctioned poisoning in 1958; narrowly restricted range		

Table 8.1 (continued)

Species	Common name	IUCN (2013)[a]	NatureServe (2013)[b]	Jelks et al. (2008)[c]	Threats	Legal Protection	Comments
Entosphenus similis	Klamath lamprey		G3G4Q	Threatened	Narrowly restricted and highly modified range		Taxonomic uncertainty largely resolved; recognized as distinct from *E. tridentatus* and *E. folletti* (Page et al. 2013)
*Entosphenus tridentatus**	Pacific lamprey		G4	Vulnerable	Distribution and abundance have declined greatly in Washington, Oregon, Idaho, and California; at risk because of dams, stream regulation, stream and floodplain degradation, reduced water quality	Listed as Threatened by NOM[e]	The Goose Lake basin population in California and Oregon is considered by NatureServe as T1[f] and by AFS as Threatened; this population is at risk from drought, water diversion, and by virtue of its narrowly restricted range
*Eudontomyzon danfordi**	Carpathian lamprey	Least Concern			Locally at risk from damming of headwaters and pollution	Listed in Annex II EU Habitats Directive[g]	
*Eudontomyzon graecus**	Epirus brook lamprey					Listed in Appendix III Bern Convention and Annex II EU Habitats Directive[g]	See *Eudontomyzon hellenicus*
Eudontomyzon hellenicus*	Macedonia brook lamprey	Critically Endangered			Habitat destruction (including pollution), water extraction	Listed in Appendix III Bern Convention and Annex II EU Habitats Directive[g]	IUCN assessment was made before *E. graecus* was recognized as distinct from *E. hellenicus*; two species were evaluated together

Table 8.1 (continued)

Species	Common name	IUCN (2013)[a]	NatureServe (2013)[b]	Jelks et al. (2008)[c]	Threats	Legal Protection	Comments
*Eudontomyzon mariae**	Ukrainian brook lamprey	Least Concern			Locally at risk from damming of headwaters and pollution	Listed in Appendix III Bern Convention and Annex II EU Habitats Directive[g]; listed as declining according to the Red Data Book of the Russian Federation[d]	IUCN considers *E. vladykovi,* treated here as a synonym of *E. mariae,* as being Least Concern and with the same threats and protection applying
Eudontomyzon morii	Korean lamprey						
*Eudontomyzon stankokaramani**	Drin brook lamprey	Least Concern			No major threats known	Listed in Annex II EU Habitats Directive[g]	
Geotria australis	Pouched lamprey						
Ichthyomyzon bdellium	Ohio lamprey	Least Concern	G3G4		At risk from pollution, siltation, and hydrological alteration		
Ichthyomyzon castaneus	Chestnut lamprey	Least Concern	G4		No major threats known		
*Ichthyomyzon fossor**	Northern brook lamprey	Least Concern	G4		NatureServe considers the Canadian Great Lakes–Upper St. Lawrence populations as T3T4[f] due to non-specific action of lampricides used for sea lamprey control	Great Lakes-Upper St. Lawrence populations listed as Special Concern under Schedule 1 of SARA[e]	
Ichthyomyzon gagei	Southern brook lamprey	Least Concern	G5		No major threats known		

Table 8.1 (continued)

Species	Common name	IUCN (2013)[a]	NatureServe (2013)[b]	Jelks et al. (2008)[c]	Threats	Legal Protection	Comments
Ichthyomyzon greeleyi	Mountain brook lamprey	Least Concern	G4		Some populations extirpated from Ohio and Virginia as a result of pollution, siltation, and stream alteration		
Ichthyomyzon unicuspis	Silver lamprey	Least Concern	G5		Threats in Great Lakes-Upper St. Lawrence populations include lampricides and small dams used for sea lamprey control and pollution	SARA status of Great Lakes-Upper St. Lawrence populations under consideration[e]	
Lampetra aepyptera	Least brook lamprey	Least Concern	G5		No major threats known		
Lampetra ayresii	Western river lamprey	Least Concern	G4	Vulnerable	Habitat degradation and barriers to passage in Oregon and California		Also known as North American river lamprey
*Lampetra fluviatilis**	European river lamprey	Least Concern			No major threats known	Listed in Appendix III Bern Convention and Annexes II and V EU Habitats Directive[g] (except Finnish and Swedish populations)	
Lampetra hubbsi	Kern brook lamprey	Vulnerable	G1G2	Threatened	Habitat lost and degraded by dams, channelization, and diversions; populations fragmented by diversions; narrowly restricted range; poisoning associated with fisheries management; impacts of nonindigenous fishes		Originally referred to *Entosphenus* (Vladykov and Kott 1976) but now recognized as *Lampetra hubbsi* (Page et al. 2013)

Table 8.1 (continued)

Species	Common name	IUCN (2013)[a]	NatureServe (2013)[b]	Jelks et al. (2008)[c]	Threats	Legal Protection	Comments
Lampetra lanceolata	Turkish brook lamprey						
Lampetra pacifica	Pacific brook lamprey						
*Lampetra planeri**	European brook lamprey	Least Concern			No major threats known	Listed in Appendix III Bern Convention and Annex II EU Habitats Directive[g] (except Estonian, Finnish, and Swedish populations)	
*Lampetra richardsoni**	Western brook lamprey	Least Concern	G4G5		No major threats known	The trophically-dimorphic Morrison Creek population on Vancouver Island, Canada (sometimes known as the "marifuga" variety) is listed as Endangered under Schedule 1 of SARA[e]	Morrison Creek population is considered by NatureServe as T1Q[f] and by AFS as Endangered; at risk due to narrowly restricted range and from rapid residential development along stretches of important spawning and rearing habitat
*Lampetra zanandreai**	Po brook lamprey	Least Concern			Water extraction, pollution, and drought; major declines in Slovenia	Listed in Appendices II and III Bern Convention and Annexes II and V EU Habitats Directive[f]	Also known as Lombardy brook lamprey and *Lethenteron zanandreai* (see Chap. 2)
Lethenteron alaskense	Alaskan brook lamprey	Data Deficient	G3Q				Distinctiveness to American brook lamprey and Arctic lamprey questioned (Mecklenburg et al. 2002)

Table 8.1 (continued)

Species	Common name	IUCN (2013)[a]	NatureServe (2013)[b]	Jelks et al. (2008)[c]	Threats	Legal Protection	Comments
Lethenteron appendix	American brook lamprey	Least Concern	G4		No major threats known		
Lethenteron camtschaticum	Arctic lamprey	Least Concern	G4		Threats to spawning habitat including pollution, water flow regulation, or dams		
Lethenteron kessleri	Siberian brook lamprey						
Lethenteron ninae	Western Transcaucasian brook lamprey						
Lethenteron reissneri	Far Eastern brook lamprey	Least Concern			No major threats known		
Mordacia lapicida	Chilean lamprey	Data Deficient					
Mordacia mordax	Short-headed lamprey						
Mordacia praecox	Precocious lamprey	Vulnerable			At risk because population is restricted in its area of occupancy (< 100 km^2) or number of locations (< 5)		

Table 8.1 (continued)

Species	Common name	IUCN (2013)[a]	NatureServe (2013)[b]	Jelks et al. (2008)[c]	Threats	Legal Protection	Comments
*Petromyzon marinus**	Sea lamprey	Least Concern	G5		No major threats known	Listed in Appendix III Bern Convention and Annex II EU Habitats Directiveg (except Swedish populations); listed in the OSPAR convention list; listed as under threat of extinction according to the 2001 Red Data Book of the Russian Federationd	Segregation demonstrated between North American and European populations (Rodríguez-Muñoz et al. 2004; Genner et al. 2012); European population at risk with habitat reduction generally identified as the major threat (Mateus et al. 2012)
Tetrapleurodon geminis*	Mexican brook lamprey			Threatened	Habitat degradation; narrowly restricted range	Listed as In Danger of Extinction by NOM[e]	Also known as Jacona lamprey
Tetrapleurodon spadiceus*	Mexican lamprey	Critically Endan-gered		Endan-gered	Severe habitat degradation caused by water extraction and pollution; narrowly restricted range; fish hosts at risk due to overharvesting	Listed as In Danger of Extinction by NOM[e]	Also known as Chapala lamprey

[a] IUCN (International Union for Conservation of Nature) categories in decreasing order of conservation risk: Extinct; Critically Endangered; Vulnerable; Near Threatened; Least Concern; Data Deficient

[b] NatureServe G (=global) ranks in decreasing order of conservation risk: 1=Critically Imperiled; 2=Imperiled; 3=Vulnerable; 4=Apparently Secure; 5=Secure; Q=Questionable Taxonomy

[c] Jelks et al. (2008) applied AFS (American Fisheries Society) categories in decreasing order of conservation risk: Extinct; Endangered; Threatened; Vulnerable

[d] See Sect. 8.3.3

[e] See Sect. 8.3.1

[f] NatureServe T (=infraspecific taxon) ranks in decreasing order of conservation risk: 1=Critically Imperiled; 2=Imperiled; 3=Vulnerable; 4=Apparently Secure; 5=Secure; Q=Questionable Taxonomy

[g] See Sect. 8.3.2

wider geographic ranges (Renaud 1997; see Chap. 2), are the ones with a higher propensity to become endangered due to numerous anthropogenic threats that block their migrations, both upstream and downstream (see Sect. 8.2.3.1). Many of the freshwater-resident species, with their more restricted distributions as the result of stochastic or anthropogenic events (e.g., five of six *Entosphenus* spp., Kern brook lamprey *Lampetra hubbsi*, precocious lamprey *Mordacia praecox*, the two *Tetrapleurodon* spp. (see Table 8.1 under Threats), and the three *Lampetra* spp. from Portugal), have also experienced population decline or extirpation in some watersheds.

Until relatively recently, little consideration was given to the concept of lamprey conservation. Indeed, many sectors (anglers, commercial fishermen) regarded them as pests and something to be eliminated wherever possible—"noncharismatic" (Close et al. 2009) being a very mild term for lampreys in their eyes. There was generally little distinction made between native lamprey species and the sea lamprey *Petromyzon marinus* in the Great Lakes, and the notion that millions of dollars are spent each year trying to control the sea lamprey in the Great Lakes while many countries in Europe are working hard to conserve the same species seems confusing to the layperson.

However, the situation is now changing, with proposals for lamprey conservation put forward by biologists in Europe (e.g., Maitland and Lyle 1992; Mateus et al. 2012; Ferreira et al. 2013) and in North America (e.g., Mesa and Copeland 2009; Moser and Mesa 2009; Moyle et al. 2009; Renaud et al. 2009; Swift and Howard 2009) in recent years. The outlook for these fascinating and valuable animals is now a much more optimistic one. The goal of this chapter is to outline the global threats to lampreys and to describe how conservation measures in a number of countries are being developed to save local lamprey populations, unique races, and even whole species from extinction.

8.2 Worldwide Threats to Lampreys

Lampreys are at risk due to a number of anthropogenic pressures. These pressures appear to relate especially to one or more of the three main stages in their life history that occur in fresh water—larval development, migration (downstream and upstream), and spawning. Larval development lasts for several years in freshwater sediments, so several cohorts are vulnerable over this long period to threats affecting this habitat (Kainua and Valtonen 1980; King et al. 2008; Streif 2009). Lampreys migrating upstream to spawn may spend many months on their journeys against the current and face many barriers and other threats (including exploitation by humans) during this phase (see Chap. 5). Travel downstream by newly metamorphosed lampreys is aided by the current and is usually faster and easier than upstream migration, but downstream migrants may become entrained in water diversion projects or turbine intakes (Streif 2009; see Chap. 3). At spawning time, whilst engaged in nest building and mating on open gravels in shallow water, the adults are exposed to a variety of predators (Sjöberg 1980; see Chap. 6). Threats, particularly

anthropogenic threats, to these three stages are described below. Rates and causes of mortality during the feeding phase of parasitic species, especially those that feed in the open ocean, are virtually unknown.

8.2.1 Pollution

Pollution in its various forms (e.g., toxic chemicals, organic sediments, deoxygenating discharges from domestic sewers) can completely eliminate lampreys from river systems. Because most polluting effluents are directed into running waters (and so to the sea), many rivers became grossly polluted in the past and lost their populations of anadromous lampreys. For example, pollution eliminated entire populations of sea lamprey and European river lamprey *Lampetra fluviatilis* from the River Thames in England and River Clyde in Scotland (Maitland 2003), and probably the River Ave in Portugal where the former species was once considered common (Quintella 2006). At their worst, both the Thames and Clyde were totally devoid of oxygen and comprised a lethal mixture of various industrial chemicals; some 20–30 fish species were extirpated from their lower reaches (Maitland 1987).

Pollution has also been implicated in lamprey population declines (see Table 8.1), and various types of pollution, either alone or in combination with other factors, appear to limit lamprey distribution. Morman et al. (1980), for example, reported that the streams in the southern half of Michigan's Lower Peninsula—subject to pollution from urbanization, agriculture, and industry—had fewer and more isolated larval sea lamprey populations compared to streams in the less-developed northern half of the peninsula where water quality is comparatively high. Stream pollution has also been thought to limit distribution in the Lake Erie basin and along the southwestern shore of Lake Ontario (Morman et al. 1980), and formation of methane in bottom habitats was considered to be the reason for the mortality and disappearance of sea lamprey larvae from areas in the Lake Champlain basin where they were formerly abundant (Wilson 1955).

Adult lampreys may be more tolerant of pollution than embryos or larvae. Morman et al. (1980) reported that adult landlocked sea lamprey have been observed migrating through heavily polluted mainstem rivers on the Lower Peninsula of Michigan and then spawning successfully in their relatively clear tributaries. In a heavily polluted section of the Tittabawassee River, Michigan, spawning was observed but no larvae were ever collected. Nevertheless, severe pollution in the estuaries (e.g., Wheeler 1979) or lower reaches of the rivers can prevent upstream migration of adults (and kill downstream migrants) in spite of the fact that there may be hundreds of kilometers upstream where the water quality is good and where there is plenty of good spawning and larval habitat. Even with non-anadromous lampreys, there may be significant migrations from the nursery or feeding areas in lakes or rivers to upstream spawning grounds (see Chap. 5), and one belt of pollution between these two habitats can have a major effect on lamprey populations in a river.

With the exception of lamprey-specific larvicides developed for sea lamprey control (see below), there is no evidence to suggest that lampreys are more sensitive

to most chemical pollutants than other fishes. Andersen et al. (2010) evaluated the acute toxicity of six chemicals on Pacific lamprey *Entosphenus tridentatus* larvae; although Pacific lampreys were relatively sensitive to one (pentachlorophenol), for the other five chemicals, they showed average (copper) or lower sensitivity relative to other fishes. However, it does appear that lamprey larvae—as long-lived filter feeders in direct contact with contaminated sediments—are exposed to and accumulate many of these pollutants at higher levels than other fishes. Renaud et al. (1998), Drevnick et al. (2006), and Bettaso and Goodman (2010) have reported high levels of mercury in larval lampreys (relative to mussels or teleost fishes) in the Châteauguay, Connecticut, and Trinity rivers, respectively. High levels of mercury have also been detected in lampreys following the adult feeding phase (e.g., MacEachen et al. 2000; Drevnick et al. 2006; Pedro et al. 2014), not surprisingly given that parasitic lampreys are top predators, feeding on the blood and tissues of fishes that may themselves be significantly contaminated with environmental pollutants. Mercury concentrations in the muscle tissue of upstream-migrating sea lamprey from the Great Lakes, for example, were up to ten times higher than lake trout *Salvelinus namaycush*, on which the sea lamprey feeds (MacEachen et al. 2000). Drevnick et al. (2006) also reported that sea lamprey show a higher rate of maternal transfer of mercury to eggs compared with teleosts. Unusually high concentrations of chlorinated persistent organic pollutants have also been detected in lamprey larvae (Soimasuo et al. 2004) and adults (MacEachen et al. 2000; Isosaari et al. 2006) in Europe and North America.

Relatively few studies, however, have examined whether these levels of contaminants are harmful to lampreys (e.g., Mallatt et al. 1986; Stinson and Mallatt 1989; Andersen et al. 2010). Many of the above-mentioned studies were conducted to determine whether human consumption of lampreys is safe; based on mercury and persistent organic pollutant levels, they generally are not (MacEachen et al. 2000; Soimasuo et al. 2004; Isosaari et al. 2006; but see Merivirta et al. 2001). It is well known that mercury and other pollutants have significant negative effects on the growth, behavior, and reproduction of aquatic and other organisms (e.g., Kidd and Batchelar 2012), and it is likely that these have negative effects on lampreys also. Average total mercury concentrations reported in larval lampreys (0.49 and 0.29–0.88 μg/g wet weight; Drevnick et al. 2006; Bettaso and Goodman 2010, respectively) exceed the threshold of 0.2 μg/g considered safe for juvenile and adult teleosts, and sublethal effects to embryonic and larval stages may occur at even lower concentrations (Beckvar et al. 2005).

The larvicide used in the control of sea lamprey in Great Lakes tributaries (3-trifluoromethyl-4-nitrophenol, TFM) was discovered to be lamprey-specific after more than 6,000 chemical compounds were screened (see Marsden and Siefkes in press), but it is not species-specific. In toxicity trials, native species (i.e., northern brook lamprey *Ichthyomyzon fossor* and American brook lamprey *Lethenteron appendix*) were less susceptible to the lampricide than sea lamprey larvae (King and Gabel 1985), but this difference is not sufficient to allow for selective control of sea lamprey without impacting native lampreys. Declines in native lampreys (particularly northern brook lamprey and silver lamprey *Ichthyomyzon unicuspis*) were

reported following the initiation of sea lamprey control. For example, TFM treatment began in tributaries to Lake Superior in 1958, and Schuldt and Goold (1980) reported that, of the 105 Lake Superior tributaries in which *Ichthyomyzon* larvae were found in 1953–1972, 81 (77%) received lampricide treatment. By 1973–1977, *Ichthyomyzon* larvae had disappeared from 41 (51%) of these tributaries. American brook lamprey were less affected (largely because they inhabit headwater areas not invaded by the sea lamprey), disappearing from only six of 42 (14%) treated streams (Schuldt and Goold 1980). Even where *Ichthyomyzon* larvae persist, density is much lower in treated than in untreated streams (COSEWIC 2007). In recent years, the number of streams with *Ichthyomyzon* larvae has stabilized—because most of the remaining streams are not inhabited by sea lamprey and thus are untreated (COSEWIC 2007)—but vulnerability to the non-specific action of lampricides is considered the major threat to both northern brook and silver lampreys in the Great Lakes basin (COSEWIC 2007, 2011; Renaud et al. 2009).

In addition to their direct toxic effects, pesticides and other pollutants can also affect food sources of larval lampreys. Renaud et al. (1995) suggested that the absence in the early 1990s of northern brook lamprey ammocoetes from the upper Yamaska River, Québec, relative to their high abundance in the 1940s (Vladykov 1952), was due in part to the herbicide atrazine (patented in 1960) leaching into the river from extensive corn fields during rain events, resulting in the destruction of the phytoplankton food source.

Eutrophication may also have negative effects on lampreys. The algal and bacterial production resulting from increased nutrients smothers both the spawning gravels (preventing spawning or killing eggs) and the larval rearing areas, creating anoxic conditions. Effects on spawning and embryonic development, however, may be greater than the effects on the filter feeding larvae. Morman et al. (1980) reported substantial numbers of larval lampreys in a lagoon heavily contaminated with raw untreated municipal sewage, and Nelson and Nelle (2007) likewise reported finding larval and metamorphosed Pacific lamprey in a pollution abatement pond in a salmon hatchery, suggesting that larvae may sometimes do well under these conditions.

Moderate amounts of sedimentation (e.g., associated with logging) may be beneficial for larval lampreys in high gradient streams or other sediment-poor areas (see Chap. 3), but excessive sediment inputs will likely have a negative impact. In most lamprey species, fine sand has been found to be the optimal substrate for larvae; clay and silt are more compact and difficult to burrow into, and could potentially smother existing burrows or clog the gill lamellae of the ammocoetes (see Chap. 3). Excessive sediment inputs also likely negatively impact spawning habitat (Beamish 2001; see Chap. 6).

Population recoveries have been reported following pollution abatement. Morman et al. (1980) reported that larval sea lamprey became re-established in at least one Lake Michigan tributary and increased in numbers in two creeks in Lake Erie following improvements to water quality. Populations of several lamprey species (e.g., sea and European river lampreys, European brook lamprey *Lampetra planeri*) that declined due to pollution in central and/or western Europe have been recovering since the 1980s (IUCN 2013). Following increased wastewater treatment efforts

and improved water quality in the Scheldt estuary, sea lamprey—considered Regionally Extinct in northern Belgium in the late 1990s—have occasionally been reported again (Verreycken et al. 2014). However, lampreys may sometimes take quite a long time to return naturally to rivers after pollution has been removed. For example, after substantial improvements to water quality were achieved in the River Clyde, Scotland, Atlantic salmon *Salmo salar* came back some 20 years before European river lamprey were first noted returning (Maitland 1987; O'Reilly 2000). Nevertheless, given that lampreys appear not to home to their natal streams—and instead are attracted, through pheromonal communication, to streams containing ammocoete populations (see Chap. 5), there is hope that recolonizations are possible once threats are removed (see Sect. 8.2.3.1).

8.2.2 Habitat Destruction

Given that a large proportion of the life cycle of lampreys is spent in burrows in depositional zones that consist primarily of a mixture of sand and fine organic matter (see Chap. 3), special attention must be paid to these areas in any consideration of the impact of development proposals on lamprey conservation. Such "silt beds" are not normally considered important fish habitat, and management for other species (e.g., dredging of depositional zones to create fishing pools for salmonids) may be detrimental to lampreys. Since multiple age classes of ammocoetes occur in these beds, their destruction can lead to the loss of several generations. Projects that cause physical disturbance to the substrate include dredging (e.g., for channel maintenance and mining), road-crossing modifications (e.g., culvert replacements), and instream structures for grade control (Streif 2009; King et al. 2011; Mateus et al. 2012; Thomas et al. 2013). Even temporary dewatering of river stretches (e.g., during instream work, for irrigation, or during flow regulation; see Sect. 8.2.3.2) can have a significant effect on larval lampreys; since they are unable to move quickly from a disturbed area, they are vulnerable to desiccation and temperature fluctuations (Streif 2009). In these situations, the ammocoetes often emerge from the sediment long after dredging or other operations cease and they are not salvaged.

Although receiving relatively little notice until recently, dredging appears to be responsible for both the direct (i.e., through their removal with the sediment) and indirect (i.e., through habitat destruction) loss of larval lampreys. In some cases, these losses can be substantial. Suction-dredge mining may be one of the reasons for the loss of lampreys in the John Day River basin in central and northeast Oregon (Klamath River Expert Panel 2010), and dredging for flood control and navigation also appears to be highly detrimental given their occurrence in the depositional areas targeted during channel maintenance. The Moy and Boyne catchments in Ireland, for example, were subjected to major arterial drainage schemes in the 1960s–1980s, largely in an attempt to control flooding (O'Connor 2004, 2006). Over a 17-year period in the Boyne catchment, roughly one tributary and a section of the main Boyne channel were drained each year and dredged to remove the silt. This dredging likely resulted in the death of a significant proportion of the lamprey larvae present in

the sediments and in almost complete destruction of larval and spawning habitat (O'Connor 2006). In the River Stonyford in the Boyne catchment, when areas of optimal lamprey habitat were targeted for maintenance, electrofishing surveys 7 weeks after maintenance showed that larval abundance in 18 1-m^2 plots was only 20% of the pre-maintenace abundance (King et al. 2008). Sampling of the sediment removed in the Silver River (also known as Tullamore), in the Shannon catchment, showed that 1–35 ammocoetes were removed per m^2 of substrate, with the majority of excavator bucket samples containing 5–10 ammocoetes/m^2 (King et al. 2008). Channelization to improve drainage also reduces habitat heterogeneity, eliminating or reducing the flow refugia and backwater habitats important to larval lampreys (O'Connor 2006; see Chap. 3). Similar dredging operations appear to be destroying larvae and larval habitat elsewhere. In Southland, New Zealand, over 1,200 km of waterways have been excavated to remove sediment and weeds, and such dredging occurs every 3 years to maintain flow (Cindy F. Baker, National Institute of Water and Atmospheric Research, Hamilton, NZ, personal communication, 2013). It is believed that some of New Zealand's densest populations of pouched lamprey *Geotria australis* occur in these small headwater streams in Southland, and that these dredging operations result in the destruction of both prime larval habitat and the physical removal and death of all larval age classes (Jane Kitson, Kitson Consulting, Invercargill, NZ, personal communication, 2013). Dredging of large rivers for navigation may result in an even more egregious loss of larval lampreys and habitat. Over the past century, the main navigation channel in the lower Willamette River in Oregon, for example, has been deepened from 6 to 13 m (to allow large shipping vessels to enter Portland Harbor), and is dredged every 2–5 years by the Army Corps of Engineers to maintain this depth. Jolley et al. (2012) demonstrated that multiple age classes of larval lampreys (Pacific lamprey and *Lampetra* sp.) occur in the lower Willamette River, and larval lampreys have been found in deep water in other large river systems (e.g., Beamish and Youson 1987; Taverny et al. 2012; see Chap. 3). The effect of large-scale channelization on larval lamprey habitat needs to be investigated.

Some efforts, however, are being made to mitigate the effects of channel maintenance on larval lampreys. Although an 80% reduction in larval density was observed in enclosures in the River Stonyford, Ireland, when dredging deliberately targeted areas of sediment deposition (as described above), other Ecological Impact Assessment surveys found fewer detrimental effects. In the River Deel, where sampling enclosures were spread over several kilometers and when a new Office of Public Works (OPW) 10-point environmental channel maintenance protocol was followed (e.g., leaving some sections untouched), no significant difference was detected in larval abundance pre- versus post-maintenance (King et al. 2008, 2011). These surveys also show the potential for colonization, particularly by small ammocoetes, of newly excavated sites (e.g., areas that were terrestrial prior to channel widening) and also suggest that, in some cases, periodic maintenance might prevent habitat loss that could result from excessive siltation and "terrestrialization" of existing depositional areas (King et al. 2008). Additional research into these and other mitigation measures is needed.

Sand extraction may also drastically modify the river bed and even be responsible for the complete destruction of ammocoete habitat; it is considered to be one of the reasons for the endangerment of the larval phase of lampreys in Portugal (Quintella et al. 2007). Similarly, engineering works that remove areas of riffle and associated spawning gravels may entirely eliminate lampreys from a river (Maitland 2003). Removal of riparian vegetation is likewise thought to contribute to lamprey declines (e.g., for northern brook lamprey; Fortin et al. 2007), since larval lamprey abundance is often positively correlated with the extent of riparian canopy (e.g., for Pacific lamprey; Claire 2003; Torgersen and Close 2004; but see Chap. 3). Other watershed changes (e.g., fire suppression, wetland drainage, intensive logging, urban development) almost certainly have had (and continue to have) negative effects on lampreys (e.g., Petersen Lewis 2009), but are poorly studied.

Although management for other fish species may be counterproductive to management for lamprey larvae—and, as discussed above, there generally has been little attention paid to larval habitats—the reverse may be true for the spawning phase. Habitat requirements for spawning lampreys (i.e., gravel substrate and unidirectional flow of water; see Chap. 6) is similar to those for salmonids—in fact, lampreys may use nests constructed several months earlier by sympatric salmonids (Nika and Virbickas 2010)—and the restoration of spawning gravels for salmonids usually also benefits lampreys (Streif 2009).

8.2.3 Dams and Other Engineering Works

8.2.3.1 Barriers to Passage

The construction of large dams for hydroelectric power production, weirs (ornamental weirs, mill weirs, and hydrological regulating weirs), and other man-made barriers, severely restricts the overall riverine habitat available to migratory lampreys throughout the world (Lucas et al. 2009; Russon et al. 2011; Table 8.1). Dams are ubiquitous in modern waterways: for example, including smaller multi-purpose dams (for flood control, irrigation, transportation, and recreation), over 200 dams have been constructed within the Columbia River drainage (Gelfenbaum and Kaminsky 2010) and 113 dams have been constructed in the Penobscot River watershed in Maine (Gardner et al. 2012). Large hydroelectric dams have the most rapid and dramatic negative effects on diadromous fishes (Nunn and Cowx 2012), impeding upstream migration by adults and downstream movement of recently-metamorphosed juveniles, but the cumulative effects of large numbers of smaller barriers can also be significant (Lucas et al. 2009). Although many such barriers can easily be overcome by migrating salmonids, particularly with the aid of fish ladders designed with them in mind, they are often impassable by lampreys (e.g., Moser and Mesa 2009; Foulds and Lucas 2013; see Chap. 5). Likewise, because lampreys generally travel deeper in the water column than salmonids (due largely to their lack of a swim bladder), traditional spill gates may also block passage (Streif 2009). Furthermore, relatively little attention has been paid to the impact of smaller barriers

(e.g., culverts, sluice gates) on lamprey distribution. Using a fish barrier dataset maintained by the state of Oregon, Starcevich and Clements (2013) identified over 4,000 potential barriers that may be blocking Pacific lamprey access to spawning and rearing habitat along the Oregon coast alone. Such barriers also concentrate large numbers of spawners in the areas immediately downstream, making adult lampreys easy targets for poachers (Quintella 2006) and predators (see Sect. 8.2.4).

Barriers to upstream migration are probably the single greatest threat to sea lamprey populations, in Portugal (Assis 1990; Almeida et al. 2000, 2002; Cabral et al. 2005; Quintella 2006; Andrade et al. 2007; Mateus et al. 2012) and elsewhere in Europe where this species is naturally distributed (Lelek 1987; Renaud 1997; Doadrio 2001; Kelly and King 2001). Based on historical records of sea lamprey occurrence in the upper reaches of the main Iberian Peninsula river basins, the construction of insurmountable obstacles in the lower reaches caused a contraction of 69–96 % of the original area (Mateus et al. 2012). Dams have also restricted the upstream distribution of anadromous sea lamprey in eastern North America (Gardner et al. 2012; Hogg et al. 2013).

In western North America, studies have shown that fewer than 50 % of adult Pacific lamprey encountering the lower Columbia River hydroelectric dams were able to negotiate the fishways (Moser et al. 2002a, b; Keefer et al. 2013), and dams in some tributaries (e.g., the Umatilla and Snake rivers) and the upper Columbia River are largely impassable to lampreys (see Jackson and Moser 2012; Ward et al. 2012). In the Yakima River subbasin in south-central Oregon, for example, an average of only 20 adult lamprey are now observed returning per year (Patrick Luke, Yakama Nation, Toppenish, WA, personal communication, 2013). Pacific lamprey management efforts in these tributaries are currently dependent on translocation past these dams (see Sect. 8.4.1.2). The Methow River, in northern Washington state, is accessible only to Pacific lamprey that successfully pass nine dams during their upstream migration, and only a small number of spawning adults have been observed in recent years (see Spice et al. 2012). The Columbia River mainstem dams have thus effectively reduced spawning escapement of Pacific lamprey, and reduced the available habitat for spawning and rearing. This has probably resulted in extirpation of larval populations in tributaries located in the catchments above these dams (Moser and Close 2003). Unlike some other anadromous species that may become freshwater resident above impassable barriers (see Docker and Potter in press), Pacific lamprey populations persist for only a few years above such barriers before becoming extirpated (Wallace and Ball 1978; Beamish and Northcote 1989).

There has been a huge decline in the numbers of upstream migrating pouched lamprey in southwestern Australia and Tasmania and the same is true for the short-headed lamprey *Mordacia mordax* in southeastern mainland Australia and Tasmania (Ian C. Potter, Murdoch University, Western Australia, personal communication, 2011, 2012). These declines, which have not been documented properly, are almost certainly related to the construction of numerous dams on many river systems in these areas. Likewise, according to Holčík (1986), the collapse of the Caspian lamprey *Caspiomyzon wagneri* fisheries on the Volga (Russia) and Kura (Azerbaijan) rivers is due to water regulation projects preventing access to areas above the

Volgograd and Mingechaur reservoirs, respectively. Hydroelectric dams are similarly considered one of the main reasons for the decline of European river lamprey in areas around the Baltic Sea. In the River Perhonjoki in Finland, for example, a dam restricts lamprey migration to the lower 32 km of the river (and much of this lower region is largely uninhabitable to larvae as the result of dredging and water regulation; Sjöberg 2011). A ten-fold decrease in lamprey numbers was observed in this river in the decade following dam construction (Ojutkangas et al. 1995). Mitigation efforts (e.g., transportation of lampreys above dams, habitat modification) are underway, however, in many of these rivers (see Sect. 8.4.2.2).

Knowledge of the way in which barrier dams can effectively block the upstream migration of lampreys while permitting passage of most other fishes has been used for several decades to control sea lamprey in the Great Lakes (see Marsden and Siefkes in press); fortunately, this knowledge is now also being used to help improve passage of anadromous lampreys of conservation concern. Unlike teleost fishes (particularly salmonids), lampreys are relatively poor swimmers (Mesa et al. 2003) and use their oral disc to attach and rest in high velocity situations (e.g., Quintella et al. 2004; Keefer et al. 2010), although climbing abilities differ among species (Moser et al. 2011). Improvements to passage structures are now being implemented in some areas to improve lamprey passage efficiency (e.g., Keefer et al. 2011; Moser et al. 2011; see Chap. 5).

In other situations, the outright removal of dams (responsible for blocking migration in other anadromous fishes) is also benefitting lamprey conservation. In 2009, the lowermost dam in the Penobscot River was removed in an attempt to restore marine–freshwater connectivity in this system. In 2010 and 2011, spawning-phase anadromous sea lamprey moved past the site of the former dam and penetrated into the upstream reaches (Hogg et al. 2013). Abundance of adults and nests increased at least four-fold following dam removal, and movement into the upstream reaches occurred more rapidly in 2011 than 2010 (Hogg et al. 2013), suggesting that spawners may have been attracted to pheromones produced by the previous year's larvae (see Sect. 8.2.1). Relatively rapid colonization or recolonization has also been observed under natural conditions. Farlinger and Beamish (1984) reported that Pacific lamprey colonized new habitats in the Babine Lake system in British Columbia within 10 years following the removal of a rockslide barrier. Likewise, after the 1980 Mount St. Helens eruption devastated the Toutle River in Washington state, adult Pacific lamprey were found to have recolonized the North Fork Toutle within 10 years (Lin et al. 2008a, b). Thus, we are cautiously optimistic that lampreys would return to the upper reaches of river systems if barriers can be surmounted or removed.

Although knowledge of and interest in the passage needs of anadromous lampreys is increasing, relatively little is known about the effects of dams and other structures on potamodromous lampreys, that is, on the nine freshwater-resident parasitic and 26 non-parasitic lamprey species (see Chap. 5; Docker and Potter in press). Although these species migrate entirely within fresh water, there is evidence that they are also negatively impacted by barriers to migration. Upstream migrating chestnut lamprey *Ichthyomyzon castaneus*, for example, have frequently been

captured at dams, implying that these structures impede their migrations (COSEWIC 2010). Barriers to migration (including dams intended to block migration of sea lamprey in the Great Lakes) are also considered a threat to silver lamprey (COSEWIC 2011). Dams are also implicated in the declines of some non-parasitic species (e.g., Pit-Klamath brook lamprey *Entosphenus lethophagus* and Kern brook lamprey; Table 8.1), and channelization and the installation of sluice gates in rice paddy fields in Japan has been shown to restrict gene flow among subpopulations of the brook lamprey *Lethenteron* sp. N (Yamazaki et al. 2011; see Sect. 8.4.3).

In addition to blocking upstream migration of the spawning-phase adults, dams and other engineering works may also disrupt the downstream migration of recently-metamorphosed individuals (see Chap. 3). This is generally considered less of a problem (Clay 1995), but passage through hydraulic turbines and spillways can cause physical damage and high mortality rates to downstream migrating lampreys (Travade and Larinier 1992). Downstream migrating lampreys are relatively robust and have been shown to pass through low-head turbines, especially Archimedes screw designs, with little or no damage (Bracken and Lucas 2013), and to show little or no ill effects from simulated turbine shear stress and pressure fluctuations (Moursund et al. 2003). However, screens intended to prevent juvenile salmonids from entering the turbines appear to represent greater hazards to downstream migrating lampreys than the turbines themselves (Moursund et al. 2003). Due to their smaller size and weaker swimming ability, they are frequently impinged on these screens and, because their tails are narrower than the rest of their bodies, these often become wedged in the bars. Moursund et al. (2003), for example, observed that 70% of downstream migrating Pacific lamprey became impinged on bar screens with 0.32 cm square mesh within 1 min of exposure to water velocities of 0.46 m/s. Higher diversion flows at night may also be particularly problematic for lampreys, given the higher rate of downstream migration at night (Streif 2009; Bracken and Lucas 2013; see Chap. 3). Furthermore, even when lampreys entrained in turbines survive the pressure differences and passage through the screens, they are sometimes disorientated and fall prey to fishes and birds in the tailraces of the dams. Predatory fishes have been observed to congregate downstream of turbine outflows (Lucas and Baras 2001).

8.2.3.2 Dewatering and Other Stream Flow Alterations

Dams and other engineering works have also resulted in significant alterations to natural flow regimes. Hydropeaking (i.e., the rapid increase or decrease in the release of water from hydroelectric reservoirs in response to fluctuations in the demand for power) changes the flow regime in the rivers downstream of the dam, and extensive water extraction for irrigation or hydropower production can substantially decrease flow. Low and unstable flows were considered by Morman (1979) to be two of the major limiting factors for the absence or scarcity of larvae in many streams in the Great Lakes basin, and hydrological alteration, including water extraction, is considered a threat to the survival of at least five lamprey species on a

global or North American scale (Table 8.1). Rapid reductions in water levels can result in the dewatering of spawning sites and larval lamprey habitat, which will result in the desiccation of nests and the stranding of larvae. This can be particularly problematic since prime larval habitat is often located at stream margins in areas of low velocity, which are the regions first affected when stream flows decrease (Hardisty 1979; Almeida and Quintella 2002). Karuk tribal community members in the Klamath River basin in northern California recall finding hundreds or even thousands of ammocoetes stranded in dry sand bars when water release from Iron Gate Dam was drastically decreased; some people refer to these incidents as "invisible fish kills" (Petersen Lewis 2009). As with other forms of habitat destruction, a single dewatering event can obliterate several age classes of lampreys and it is recommended that those involved in salvage efforts be cognizant of the fact that larvae may continue to emerge from the substrate long after the event (Streif 2009; see Sect. 8.2.2). Rapid increases in flow can also have disastrous effects on the survival of eggs and ammocoetes, resulting in them being swept downstream.

Rapid changes in flow can also be detrimental to post-metamorphic stages; unusually high flows, for example, can result in downstream migrants being impinged on turbine screens (Sect. 8.2.3.1) and severe reductions in flow during the spawning migration can prevent movement upstream to high-quality spawning habitat. In the Umatilla River in Oregon, abstractions of water for irrigation are so severe that the river is virtually dewatered during the peak adult lamprey migration, thus severely limiting access to spawning habitat (Close et al. 2009). In the Klamath basin, water diversion for agriculture in the upper basin reduces water flow in the lower basin to the extent that water in the tributaries is no longer high enough to support spawning (Petersen Lewis 2009). Likewise, a reduction in river discharge during the migration period due to dams and weirs can diminish the attractive potential of the river and hence the numbers of spawners entering it. According to Oliveira et al. (2004), the present minimum instream flow for the River Tagus in Portugal downstream from the first impassable obstacle, Belver Dam, results in a significant loss of spawning habitat for sea lamprey.

Another consequence of dam construction is the creation of reservoirs, as the result of river impoundment, and this leads to the transformation of lotic habitats to lentic ones. Independently of free passage problems, lampreys spawn in relatively fast flowing reaches (Hardisty and Potter 1971), and the absence of such habitat, upstream of the barrier, prevents successful spawning. With the exception of a few species (e.g., Vancouver or Cowichan lamprey *Entosphenus macrostomus*), lampreys rarely spawn in the absence of a unidirectional current (see Chap. 6). During their spawning migration, lampreys are rheophilic swimmers and their passage through a large body of water with low currents may disorient the adults or increase passage time. Additionally, in large impoundments, the effect of larval pheromones may be lost and no longer guide adult lampreys to spawning streams (Li et al. 1995; Vrieze and Sorensen 2001; Fine et al. 2004; Sorensen et al. 2005; see Chap. 5).

Alterations in discharge and temperature regimes may also interfere with the timing and success of migration and spawning. Discharge and temperature regimes are the two most important environmental factors affecting the lamprey spawning

migration (Hardisty and Potter 1971). The largest catches of sea lamprey are made generally when the water level is high (Hardisty and Potter 1971; Duarte et al. 2003; Masters et al. 2006), and when water temperatures reach between 10 and 19 °C (Applegate 1950; Beamish 1980). In a study by Santos et al. (2005) in the Lima River in Portugal, sea lamprey were found to migrate at temperatures above 11 °C and showed pronounced activity at 12–16 °C. Binder et al. (2010) found migratory activity in sea lamprey to peak at temperatures of c. 15 °C (see Chap. 5). Initiation of spawning is likewise highly dependent on temperature (see Chap. 6). Temperatures below optimal values are thought to reduce migration and spawning intensity (Beamish 1980), and hypolimnetic coldwater releases from dams may provoke high amplitude changes in temperature regimes. Unusually high discharge is also thought to inhibit migration (Masters et al. 2006) and spawning (Hogg et al. 2013) activity.

Likewise, embryonic development in lampreys is highly dependent on temperature. In sea lamprey in Spain, for example, survival from fertilization to hatching was 61, 89, 91 and 89 % at 11, 15, 19 and 23 °C, respectively, and survival from hatching to burrowing was 58, 70 and 70 % at 15, 19 and 23 °C, respectively (Rodríguez-Muñoz et al. 2001). Hypolimnetic releases from dams would therefore be expected to decrease embryonic survival. Mortality likewise increases with higher than optimal temperatures. For example, in Pacific lamprey, the optimal temperature for survival of early life stages is 18–19 °C; at higher temperatures (22 °C), survival was significantly reduced and developmental abnormalities increased (Meeuwig et al. 2005). This means that the successful conditions at which embryos develop and hatch are not compatible with sudden changes in temperature.

Impoundments change flow regimes of streams and rivers and can severely alter ecological processes (Larinier 2001). Food production and the transit of suspended material may be diminished, whereas erosion of the margins normally increases. An important geomorphological feature downstream of many dams is the erosion of steep sandy river banks and the eventual creation of gently sloping grassy shores; these are more resistant to erosion but not as adequate for lamprey larvae. Reduction of sediment deposition downstream of impoundments typically reduces the habitat available for ammocoetes (Klamath River Expert Panel 2010).

Dams and other anthropogenic modifications to the rivers and streams in which lampreys spend the majority of their life cycle are ubiquitous in modern waterways, and it is clear that the effects of these modifications on lampreys are myriad. Far more research is required, however, to understand the extent of these threats to different lamprey species worldwide and the ways in which they might be remediated.

8.2.4 Predators, Parasites, and Prey

It is known that lamprey eggs and ammocoete larvae are eaten by a variety of predators such as teleost fishes, including minnows (Cyprinidae), sticklebacks (Gasterosteidae), eels (Anguillidae), sculpins (Cottidae), perch (Percidae), and bullheads or catfishes (Ictaluridae) (Hardisty 1961a, b; Heard 1966; Manion 1968; Tuunainen et al. 1980; Maitland 2003). Cochran (2009) argued that assemblages of predators

on lampreys are changed by human activities such as stocking and harvest of fishes, citing as an example the many streams in Minnesota that are stocked with and managed for exotic brown trout *Salmo trutta*. Brown trout are much larger than most native fish species, few of which are large enough to feed on large ammocoetes or adult lampreys, and may be a significant predator on several species of lampreys. In southern California, the introduction of numerous non-native piscivorous fishes and those that disrupt soft sediments have probably contributed to the decline of Pacific lamprey (Moyle et al. 2009).

Other vertebrates also prey on adult lampreys, for example, birds such as herons (*Ardea*), ducks (*Mergus*), and seagulls (*Larus*) (Sjöberg 1980), and mammals like otters (*Lutra*), especially at spawning time (Andrade et al. 2007). Predation is also likely to be high in downstream migrants. There is concern that dams and other engineering works, by delaying or disrupting passage of downstream or upstream migrating lampreys, may make these life stages more vulnerable to these predators (see Sect. 8.2.3.1).

Relative to other aspects of lamprey biology, little has been written about their parasites—and even less is known about their effect on the host. As with studies on contaminant levels in lampreys (see Sect. 8.2.1), many of the studies examining parasites and other pathogens in lampreys have largely focused on their impact on human health and their potential role in the transmission of diseases to commercially-important fishes (e.g., Eissa et al. 2006; Faisal et al. 2007; Gadd et al. 2010; Bao et al. 2013). Appy and Anderson (1981) listed 70 distinct parasite species that have been found in larval and adult lampreys. The list includes five species of bacteria (including one *Rickettsia* species; *Aeromonas salmonicida*, the causative agent of furunculosis in cultured salmonids; and *Aeromonas hydrophila*, which causes infectious enteritis in humans), one identified and one unidentified fungi, two protozoans belonging to the phylum Ciliophora, 39 species from the phylum Platyhelminthes, six from the phylum Acanthocephala, nine from the phylum Aschelminthes, two from the phylum Mollusca, two from the phylum Annelida, and three from the phylum Arthropoda. More recently, *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in salmonids, has been found in lampreys (Eissa et al. 2006), as well as the virus causing viral hemorrhagic septicemia (VHS; Gadd et al. 2010). Bell and Traxler (1986), however, demonstrated that adult Pacific lamprey were not susceptible to *R. salmoninarum*, and Kurath et al. (2013) have shown larval Pacific lamprey were not susceptible to the virus causing VHS or infectious hematopoietic necrosis (IHN). Since 2011, the prevalence of finding dead or dying pouched lamprey during their upstream migration in Southland, New Zealand, has increased (Cindy F. Baker, National Institute of Water and Atmospheric Research, Hamilton, NZ, personal communication, 2013). These lamprey had lesions around their fins and gills and unusual red skin markings, but the cause of this Lamprey Reddening Syndrome (i.e., whether bacterial, viral, or due to some environmental factor) is not yet known (see Sect. 8.4.3). As mentioned above, the impact of these parasites and pathogens on the health of lampreys is generally unknown. However, *A. salmonicida* does appear to produce furuncle-like lesions in Great Lakes sea lamprey (Faisal et al. 2007), and incidence of this bacterium in sexually mature Pacific

lamprey has raised questions about its negative impact on reproduction, particularly at warm temperatures (Clemens et al. 2009).

Although poorly studied to date (particularly in anadromous species), it is probably safe to say that prey/host availability has an effect on and can limit lamprey abundance. There is some evidence that a reduction in the size of a host population can affect lamprey numbers, as well as body size and sex ratio (Hardisty 2006). A decline in the abundance of coho salmon *Oncorhynchus kisutch* in Cowichan Lake on Vancouver Island, British Columbia, is believed to directly affect the abundance of the Vancouver lamprey (COSEWIC 2008). Lyons et al. (1994) have indicated that Mexican lamprey *Tetrapleurodon spadiceus* has been negatively impacted by major declines in several of its primary host species. In Lake Superior, the body size of sea lamprey started to decline after their numbers reached a peak in the mid-1950s (Heinrich et al. 1980). However, body size started to increase again in the 1960s, possibly due to successful restocking with lake trout. Pacific lamprey counts at Bonneville Dam on the Columbia River were high immediately before the 1976/1977 ocean climate shift that affected the abundances of many marine fishes (Anderson and Piatt 1999; Benson and Trites 2002), and then significantly lower during the first available counts after the regime shift (Close et al. 1995). Murauskas et al. (2013) found significant positive correlations between Pacific lamprey returns in the Columbia River between 1997 and 2010 and the abundance indices of five host species in the marine environment: Pacific hake *Merluccius productus*, walleye pollock *Gadus chalcogrammus*, Pacific cod *G. macrocephalus*, Chinook salmon *Oncorhynchus tshawytscha*, and Pacific herring *Clupea pallasii*.

8.2.5 Exploitation by Humans

Lampreys have long been valued as a food source (see Chap. 1), and Renaud (2011) lists seven species which are or have been subjected to commercial fishing within the past century. Although most of the widespread threats listed in Table 8.1 are related to habitat destruction and barriers to passage, exploitation in the past has been significant for some species. Although catches—in virtually all species and locations—declined around the middle of the last century, probably as the result of pollution and barriers to migration (Hardisty 1986a; Close et al. 2002; Sjöberg 2011), exploitation continues to be high at a local level for some species and overexploitation may represent a significant ongoing threat in some regions. Furthermore, although recent harvest levels are lower (dramatically so, in most cases) than historic levels, we cannot assume that current fishing pressures are sustainable; relative exploitation levels (i.e., the proportion of the spawning population that is being harvested) need to be estimated (Valtonen 1980; Masters et al. 2006). Catching lampreys during their upstream migration can result in high levels of exploitation, particularly if traps are associated with physical bottlenecks that concentrate the spawning population, and catchability can remain high even as population sizes decrease (thus masking such decreases). Furthermore, semelparity may make lampreys particularly at risk to overexploitation since all lampreys caught will never

have had the opportunity to spawn (Masters et al. 2006). Below, we review historical and current harvest levels of the most heavily exploited lamprey species (particularly European river and sea lampreys), briefly discuss fishing restrictions (if they exist) and, where possible, make inferences regarding the effects of exploitation on the species in question.

The European river lamprey was formerly fished extensively in the River Severn and several other rivers in Britain (Wheeler 1979; Masters et al. 2006). For example, for several centuries, prior to the increasing pollution in the River Thames in the nineteenth century, river lamprey were trapped in very large numbers; the annual catch in just one section of the river in 1791 was estimated at c. 500,000 lamprey (Wheeler 1979). In addition to fishing for human consumption, river lamprey were also used as bait for cod fishing. Up until the early 1900s, line fishermen in the Netherlands imported c. 700,000 river lamprey annually from the River Thames fishermen for use as bait (Lanzing 1959). Commercial fisheries for this species also operated in the Ouse, Derwent, and Trent catchments in northeast England (Masters et al. 2006). In the tidal River Ouse, total catches ranged from 25,500 to 54,500 lamprey per year in 1908–1914. This level of exploitation stopped when deepsea trawling replaced long lining in the North Sea but, in recent years, river lamprey have again been caught in the River Ouse and sold to anglers for use as bait; 9,100 to 31,000 lamprey were caught annually between 2000 and 2004 (Masters et al. 2006). Using mark-recapture methods, Masters et al. (2006) estimated that between 9.9 and 12.0% of the spawning migrants were captured in this intercept fishery. These lamprey were originally considered by-catch in a licensed eel *anguilla* fishery, but concern regarding the possible impact of these unregulated fishing efforts led to the introduction of lamprey-specific legislation (e.g., a cap on fishing licenses, catch quotas, and restricted fishing seasons) in 2011 (Foulds and Lucas 2014). However, given the lack of reliable data on demographic processes (e.g., annual recruitment, natural and anthropogenic-related mortality) and the difficulties experienced in establishing an accurate exploitation rate, it is not yet known what would constitute a sustainable catch level in this river (Masters et al. 2006; Foulds and Lucas 2014; Richard A. Noble, Hull International Fisheries Institute, University of Hull, Hull, U.K., personal communication, 2014).

The largest commercial fisheries for European river lamprey have been (and continue to be) in rivers that flow into the Baltic Sea (i.e., in Finland, Sweden, Latvia, Estonia, Lithuania, Poland, Germany, and Russia; Hardisty 1986a; Sjöberg 2011; Lajus et al. 2013). In Finland, where statistics on river lamprey catches are recorded, total catch ranged from approximately 1.8 to 3.0 million lamprey per year in the 1970s and 1980s, and approximately 0.6 to 1.8 million lamprey per year in 2000–2011 (Tuunainen et al. 1980; Dill 1990; Sjöberg 2011). A tagging study by Valtonen (1980) suggested that a significant portion of the spawning population was harvested in the fishery, estimating that 65 and 80% of the upstream migrants were being captured in the two Finnish rivers studied. In Sweden, approximately 150,000 lamprey were harvested in 2010–2011, much less than was harvested before hydroelectric dams blocked upstream migration in many rivers (e.g., c. 200,000 lamprey were harvested per year in 1942–1951 in just one of the approximately 25

rivers fished for this species; Sjöberg 2011). Lamprey harvest is also significant in Latvia, where mean catches per year have ranged from 8 to 270 t (in 1980 and 1975, respectively) or approximately 92,000 to 3.1 million lamprey; catches were still high in 2003 (112 t or approximately 1.3 million lamprey). Likewise, lamprey fishing is important in Estonia; although catches have decreased over the last 60–70 years (and, as in Latvia, fluctuate among years), up to 63 t per year were harvested between 1994 and 2005 (Sjöberg 2011). There are no general rules regulating lamprey fishing in Sweden but, in Latvia and Estonia, fishing is restricted during the latter part of the spawning run; in Finland, there are regulations regarding fishing gear (Sjöberg 2011). In Poland, annual catches in the Vistula River in 1930–1939 exceeded 100 t (sometimes during a one-week fishing period) but, by the late 1980s, harvests amounted to less than 1 t per year and fishing ceased; this drastic decline was attributed to pollution, dams, and overfishing (Witkowski 1996). Historically, annual river lamprey catches in Lithuania and Russia ranged from 8 to 53 t (Sjöberg 2011; Lajus et al. 2013), and 1 to 7 t per year in Germany (Hardisty 1986a; Sjöberg 2011). Fishing still occurs in Lithuania and Russia, but appears to have ceased in Germany (Sjöberg 2011; Lajus et al. 2013).

The anadromous sea lamprey was likewise formerly fished extensively in the River Severn and several other rivers in Britain, but it is now exploited only in a few rivers in Europe—notably in France, Spain, and Portugal (see below). In fact, despite the former abundance of sea lamprey in the River Severn (e.g., in centuries past, when the city of Gloucester would send a lamprey pie to the monarch every Christmas), it has been virtually extirpated from this river; sea lamprey had to be imported from the Great Lakes in order to make Queen Elizabeth II's lamprey pie for her Diamond Jubilee celebrations (see Chap. 1). There was also a fishery for anadromous sea lamprey in North America in the nineteenth century, principally on the Merrimack and Connecticut rivers in Massachusetts, before dams greatly reduced its abundance (Goode 1884). Fish lifts (e.g., at Holyoke Dam) and perhaps other fish passage facilities have helped restore sea lamprey runs in the Connecticut River in recent decades (Stier and Kynard 1986; Boyd Kynard, BK Riverfish LLC and Department of Environmental Conservation, University of Massachusetts, Amherst, MA, personal communication, 2014) but, notwithstanding this situation, there is no longer a market for sea lamprey in the United States (Flescher and Martini 2002). Modest commercial catches now only supply specimens for scientific research. During the 1970s and 1980s, up to 8,000 anadromous sea lamprey were caught for this purpose from the Sheepscot River in Maine, but the two companies recently permitted to harvest adult sea lamprey in Maine have been unable to meet their needs locally and import sea lamprey from Nova Scotia or the Great Lakes (Kircheis 2004).

The largest commercial fishery for sea lamprey in Europe occurs in the Garonne River basin in France, also known as the Gironde, the estuary where the Garonne and Dordogne rivers meet (Beaulaton et al. 2008). The annual landings from this basin (on average 72 t, or approximately 65,000 lamprey, per year between 1985 and 2003) are said to represent more than 50 % of the total production in France (Beaulaton et al. 2008). Annual landings ranged from approximately 40,000

lamprey in 1994 to 140,000 lamprey in 2000. Long-term monitoring of the catch per unit effort (CPUE) in the fishery suggests that sea lamprey abundance reached its peak between 1952 and 1970, but that the trend since 1973 has been relatively stable at 35–40 % of the peak abundance. Since the end of the 1990s, the CPUE has shown a strong increase; although CPUE is still below previous levels, this recent increase was considered by Beaulaton et al. (2008) to be a sign of an upward trend in lamprey abundance in this French river basin. Interestingly, mean length of harvested sea lamprey has also increased over this time period (Beaulaton et al. 2008). According to these authors, these results are encouraging but caution is still needed. Sea lamprey was also fished in the Rhone, Loire, and Adour rivers at the beginning of the twentieth century. Fishing has almost disappeared in the Rhone River but, in the past few decades, harvests of 58 and 8.5 t have been recorded for the Loire (1989) and Adour (mean for 1986–2004) rivers, respectively. In Spain, sea lamprey is targeted by commercial fisheries particularly in Galicia in the northwest (Gradín 2010).

In Portugal, where sea lamprey is regarded as a delicacy and fetches high prices, overexploitation for human consumption is considered one of the major threats to the conservation of this species in some Portuguese watersheds (Quintella 2006; Mateus et al. 2012). Annual harvest levels can be roughly estimated at 120,000–160,000 lamprey in the Minho River and 10,000–15,000 lamprey in the Tagus River. These numbers were obtained by multiplying the average number of captured lamprey per fisherman for the two systems (Suíssas 2010) by the total number of licensed fishermen in each river (Bernardo R. Quintella, personal observation). The gastronomic importance of sea lamprey is reflected by their high commercial value, which can easily reach €50 per lamprey during the peak of the season. The high economic value of the sea lamprey makes them the preferred target of both professional fishermen and poachers, creating a major threat to the sustainability and conservation of this species in Portugal (Quintella 2006; Mateus et al. 2012). In a study by Andrade et al. (2007) that was aimed at investigating the spawning migration of sea lamprey in the Vouga River basin via radio telemetry, 76 % of the tagged lampreys were recaptured by poachers. The study was conducted during two consecutive drought years, resulting perhaps in an overestimation of the poaching capture rate when extrapolating to a normal flow year; nevertheless, this percentage reflects the threat that poaching activities, if not properly policed, may pose to the survival of the exploited sea lamprey populations in Portuguese rivers.

Professional fisheries regulations in Portugal define in general the official fishing season for sea lamprey as between the beginning of January and the end of April, and capture is allowed in both estuaries and in designated areas in fresh water. Captures are limited to lamprey over 350 mm in body length and to a maximum of 30 individuals per day for each fisherman. In river basins where the species is less abundant, the quota is lower (e.g., six lamprey per day in the River Guadiana and 10 per day in the rivers Vouga and Cávado; Mateus et al. 2012). Sea lamprey upstream migration starts in December and peaks between February and March, with the bulk of the spawning occurring between April and June (Almeida et al. 2000). The fishing gears traditionally used by professional fishermen to harvest adult sea lamprey

in Portugal are drift trammel nets and fyke nets (Quintella 2006). The impact of this intense fishing effort is not negligible, but is difficult to quantify; sea lamprey are often sold directly to restaurants or intermediaries without being taxed, resulting in inaccurate official records of capture numbers. As an estimate, however, Duarte et al. (2003) assessed the catch rate of a large fyke net in the River Mondego between 6 January and 13 April 2002 as 555 lampreys—a catch rate of 7.4 individuals per tide (12 h). The same authors indicated that six local fishermen using six fyke nets between January and April 2002 captured 2,846 lamprey. Although the catch rate for trammel nets is not currently known, with at least 100 commercial fishing licenses issued in the River Mondego (Bernardo R. Quintella, personal observation), it is clear that, if not properly regulated, commercial fishing may constitute a significant threat to the survival of Portuguese sea lamprey populations.

There were important fisheries for the Caspian lamprey in Russia and Azerbaijan into the twentieth century (Berg 1948); the maximum recorded catches (in the early twentieth century) reached 2000 to 3000 t annually from the Volga River (Russia) alone (Holčík 1986). Total annual catches in the Volga fell to 500–850 t from 1930 to 1934 and to less than 100 t between 1941 and 1945 (although the Second World War perhaps contributed to a decrease in fishing effort). The commercial harvest in the Kura River (Azerbaijan) between 1930 and 1963 ranged from 10 to 269 t (Holčík 1986). Water regulation projects on these river basins had such profound negative effects on lamprey abundance that those fisheries are no longer commercially viable (Holčík 1986). There is no commercial fishing for this species in Iran (Kiabi et al. 1999).

In Japan, the Arctic lamprey *Lethenteron camtschaticum* is consumed by humans and is also highly valued as a medicine against night blindness (Honma 1960). However, there has been a dramatic decline in the catch in northern Japan from a high of about 200 t in 1988 to a low of less than 5 t annually between 2000 and 2009 (Greig and Hall 2011). During the nineteenth century (and most likely previously), native people in Alaska also consumed the Arctic lamprey and used its rendered oil as fuel for lamps (Turner 1886; Nelson 1887). There has been a recent renewed interest in starting a commercial fishery for upstream migrants in the Yukon River, Alaska. In addition to the traditional subsistence harvest, this fishery is aimed at the Asian market in the USA and abroad, and its 2003 quota was set at 20 t (Gay 2003).

Pacific lamprey have been fished by indigenous peoples in the Columbia and Klamath river basins of Oregon and California for thousands of years (Close et al. 2002; Petersen Lewis 2009). These subsistence fisheries used traditional harvesting techniques (e.g., dip nets, hooks, baskets, catching by hand) and collected lamprey only for food or ceremonial or medicinal purposes. In years when abundance was high (i.e., prior to the 1950s or 1960s) and at the peak, a single Yurok or Karuk "eeler," for example, might catch up to 5,000 lamprey in one night (or approximately 4.7 t; Petersen Lewis 2009). Over the course of the spawning run, and with hundreds of Karuk and Yurok villages located along the Klamath River, harvests within the basin could be on the order of tens of thousands of lamprey per year, but such harvests would be shared among people living in the vicinity and traditional ecological knowledge suggests that, at that time, Pacific lamprey were coming through

the Klamath River by the millions (Petersen Lewis 2009). A commercial fishery for Pacific lamprey operated in the Columbia River basin in the early twentieth century, harvesting lamprey largely for use in fishmeal (for cultured salmon, livestock, or poultry) or for vitamin oil (Close et al. 2002). At Willamette Falls, 24.5 t (c. 22,000 lamprey) were harvested in 1913 and an average of 105 t per year (c. 95,000 lamprey) was harvested between 1943 and 1949 (Close et al. 2002).

Commercial catches for Pacific lamprey in recent years have been more modest, and have largely been for human consumption (e.g., 1.8 t were exported to Europe in 1994) or to supply specimens for research or teaching purposes (Close et al. 2002). Tribal harvests also have been considerably reduced (due to the dramatic declines experienced in the 1960s; Petersen Lewis 2009); instead of a thousand or more Pacific lamprey per night, for example, a Kurok or Yurok eeler might now only be able to harvest 10–15 (Petersen Lewis 2009). Willamette Falls, located downstream of a number of dams that block passage within the Columbia River basin (see Ward et al. 2012), is now the source of the largest tribal harvest in Oregon; harvests are virtually non-existent in the upper Columbia River basin (see Sect. 8.2.3.1). Harvest for non-tribal commercial and personal use is also permitted at Willamette Falls, but is regulated (Kostow 2002). In 2001, the Oregon Fish and Wildlife Commission started to require permits for the harvest of Pacific lamprey and imposed regulations that included setting a season, limiting harvest to daylight hours, limiting the area around the falls that was open for harvest, and requiring that harvest be done by hand or with hand-operated equipment. In 2001, the harvest at Willamette Falls was approximately 15,500 lamprey (c. 14 t), about half the yearly average estimated over the past decade (Kostow 2002). Although Jelks et al. (2008) list overexploitation for commercial, recreational, scientific, or educational purposes as one of the criteria for listing Pacific lamprey as vulnerable to extinction, it appears that this is not the major threat to this species.

In New Zealand, the Māori catch the pouched lamprey at the beginning of their upstream spawning migration and use them for human consumption and ceremonial purposes (McDowall 1990). It appears, however, that this subsistence fishery is not the major threat to this species.

In the last century, some lamprey species were harvested as larvae, either commercially (e.g., western brook lamprey *Lampetra richardsoni* and American brook lamprey) or non-commercially (e.g., Carpathian lamprey *Eudontomyzon danfordi* and northern brook lamprey) for use as bait to catch sportfishes (Renaud 2011). In the late 1940s, for example, about 200,000 American brook lamprey ammocoetes were harvested annually for this purpose from the upper St. Maurice River, Québec (Vladykov 1973). It is not known what impact this might have had on their population numbers, but in a relatively long-lived semelparous species with low fecundity such as the American brook lamprey, it was undoubtedly significant. While the use of lamprey larvae as bait is now illegal in Québec (Fortin et al. 2007), their appeal as hardy live bait to catch sportfishes means that the practice may still be ongoing there and elsewhere, and it was ongoing in various parts of Québec in the early 1990s (Claude B. Renaud, personal observation).

8.2.6 Climate Change

Climate change is likely to affect the marine and freshwater distribution of aquatic organisms, including lampreys. Cheung et al. (2009) projected that there would be increases in extinctions and invasions of marine invertebrates and fishes at a global scale by 2050. Though it is uncertain how climate change will affect lampreys specifically, one scenario is that some species may disappear in the southern parts of their current range, but be able to inhabit rivers further north of their current distribution. For instance, under climate change scenarios, Lassalle et al. (2008) projected a disappearance of sea lamprey in river basins bordering the east coast of the Adriatic Sea, in most of the Italian river basins, and in the majority of the river basins in the Iberian Peninsula by the end of the twenty-first century. However, in the same study, conditions were predicted to remain suitable in the northern part of the present distribution area of this species and even waters in Iceland could become favorable to this species. However, although this may be true for anadromous species which can move in the sea to river systems further north, this will not be the case with isolated non-anadromous species in southerly areas, for example, freshwater-resident species in California (Moyle et al. 2009) and the brook lampreys belonging to the *Lampetra* genus from the Iberian Peninsula (Mateus et al. 2012, 2013a). Such species may only survive if translocations to suitable waters further north—a controversial issue for many reasons—are undertaken (Maitland 1991).

Changes in the distribution of their prey/host base are also likely to have an impact on parasitic lampreys; however, a lack of host specificity (see Renaud and Cochran in press) may buffer them to some extent. Thus, rather than being affected by the distribution of a particular prey/host species, parasitic lampreys are more likely to be affected by general changes in prey/host abundance. The findings of Murauskas et al. (2013) indicated that host abundance was the principal factor predicting Pacific lamprey returns in the Columbia River and that inclusion of oceanic conditions increased the precision of the model. The abundance of Pacific salmon *Oncorhynchus* spp. in the North Pacific Ocean has been clearly shown to fluctuate with the ocean-atmosphere climate (e.g., Mantua et al. 1997; Beamish et al. 1999), and long-term climate changes are expected to affect prey abundance for lampreys (Klamath River Expert Panel 2010). There are also predictions of climate-driven changes to the relative productivity of marine and freshwater systems (i.e., with suggestions that increases in terrestrial primary production are expected to increase primary production in lakes relative to the oceans) that may alter the prevalence and distribution of anadromy in salmonids (Finstad and Hein 2012) and perhaps of parasitic lampreys as well (see Docker and Potter in press).

Climate change is also expected to exacerbate many of the threats discussed above (e.g., alterations to flow regimes, disease). For example, streams in California are expected to be warmer and drier during the summer in response to reduced spring run-off and reduced summer precipitation (Klamath River Expert Panel 2010), intensifying the ill-effects already noted regarding decreases in water quantity (see Sect. 8.2.3.2). In other regions, drought in the summer is expected to be accompanied by periods of heavy rain in the autumn and winter (Maitland

1991). Rapid increases in temperature during embryonic development could lead to increased mortality (see Sect. 8.2.3.2); other negative effects could include an increase in the rate at which energy stores are mobilized during the non-feeding period of upstream migration and the extent to which they become depleted (Clemens et al. 2009). Nothing is known about the genetic capacity of lampreys to adapt to climate change over the projected time period (Klamath River Expert Panel 2010); adaptation and an ability to colonize new environments may mitigate some of the negative effects of climate change. Although our understanding of the threats to lamprey persistence under current climatic conditions is still incomplete, research into the impact of these threats under various climate change scenarios is needed.

8.3 Legislation Protecting Lampreys

Although widespread species whose distributions encompass numerous countries are evaluated at a global scale by the IUCN (2013) and NatureServe (2013), ultimately, their protection is mitigated at a national level. In the case of narrowly-distributed species, the national and global evaluations coincide. A species is said to be at risk and may qualify for protection if its designation is Threatened (i.e., Vulnerable, Endangered, or Critically Endangered) according to IUCN (2013) and Vulnerable (G3), Imperiled (G2), or Critically Imperiled (G1) according to the nomenclature of NatureServe (2013). Of the 33 recognized lamprey species assessed at a global scale—i.e., 32 species assessed by IUCN (2013) and/or NatureServe (2013) plus the Mexican brook lamprey assessed by Jelks et al. (2008)—12 are deemed at risk (Table 8.1). We include in this number three species that have a G3G4 NatureServe (2013) ranking (G4=Apparently Secure). Of these 12 species that qualify for protection, only four are protected throughout their ranges by legislation at the national or European Union (EU) level: Vancouver lamprey in Canada, Mexican lamprey and Mexican brook lamprey in Mexico (see Sect. 8.3.1), and Macedonia brook lamprey *Eudontomyzon hellenicus* in Europe (see Sect. 8.3.2). A fifth species, the Epirus brook lamprey *Eudontomyzon graecus*, could be added by default because it was recognized as distinct from *E. hellenicus* after the IUCN assessment of the latter. Of the remaining eight qualifying species (i.e., those deemed at risk but not protected at the national or EU level), it is worth noting that there has been some taxonomic uncertainty surrounding three of these species (Northern California brook lamprey *Entosphenus folletti*, Klamath lamprey *Entosphenus similis*, and Alaskan brook lamprey *Lethenteron alaskense*), and—although somewhat resolved (e.g., for Klamath lamprey; Table 8.1)—this undoubtedly has an effect on the application of protection to them. Twenty-one species have thus been assessed to be not at risk at a global scale (although this number includes the Caspian lamprey, considered Near Threatened). The remaining 11 species that have not yet been evaluated at a global scale are largely freshwater-resident species with relatively narrow distributions, but two (pouched lamprey and short-headed lamprey, both from the Southern Hemisphere) are anadromous species with wider ranges (see

Chap. 2); all need to be evaluated to ensure that they are receiving the protection that they may require.

In addition to species that are deemed at risk globally, four other species receive protection at the EU level (Po brook lamprey *Lampetra zanandreai* and the three other European *Eudontomyzon* species), and there is some protection afforded at a national level to at risk populations of more widespread species (see Sects. 8.3.1, 8.3.2, 8.3.3). Thus, at least 16 species now receive legal protection at the national or EU level in at least a portion of their range (Table 8.1). In addition, there are laws protecting species at the subnational level (e.g., in many provinces of Canada and many U.S. states; see Mesa and Copeland 2009), but dealing with these is beyond the scope of this review.

8.3.1 North America

Canada, the United States, and Mexico each have their own legislation concerning species at risk: the *Species at Risk Act* (SARA), the *Endangered Species Act* (ESA), and the *Norma Oficial Mexicana* (NOM), respectively. In order of enactment, the ESA (http://www.nmfs.noaa.gov/pr/pdfs/laws/esa.pdf) became law in 1973, the NOM (http://www.biodiversidad.gob.mx/pdf/NOM-059-ECOL-2001.pdf) in 1994 with a second version in 2001, and the SARA (http://www.sararegistry.gc.ca/approach/act/sara_e.pdf) in 2002.

As stated in section 6 of the SARA, the purposes of the Act "are to prevent wildlife species from being extirpated or becoming extinct, to provide for the recovery of wildlife species that are extirpated, endangered or threatened as a result of human activity and to manage species of special concern to prevent them from becoming endangered or threatened." In terms of equivalency to IUCN categories, Endangered, Threatened, and Special Concern are, respectively, equivalent to Critically Endangered/Endangered, Vulnerable, and Near Threatened. Section 14 establishes the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as an independent scientific advisory body to the Canadian government in matters of species assessments for conservation purposes (see Renaud et al. 2009). Under section 37, subsection (1) of the SARA, if a wildlife species is listed as an extirpated species, an endangered species, or a threatened species, the competent minister (the Minister of Canadian Heritage or the Minister of Fisheries and Oceans, or both) must prepare a strategy for its recovery. Under section 120, the Minister of the Environment must establish a public registry for the purpose of facilitating access to documents relating to matters under the SARA. Three lampreys are listed in the public registry under Schedule 1, the official list of wildlife species at risk: the Morrison Creek population of the western brook lamprey as Endangered, the Vancouver lamprey as Threatened, and the Great Lakes-Upper St. Lawrence populations of the northern brook lamprey as Special Concern (Renaud et al. 2009). The Great Lakes-Upper St. Lawrence populations of the silver lamprey have been designated Special Concern by COSEWIC (COSEWIC 2011), but its SARA status is still under consideration. Note that the SARA can list taxa below the species level.

Under section 4, subsection (d) of the ESA, for any species listed on the Federal Register as Endangered or Threatened, "the Secretary shall issue such regulations as he deems necessary and advisable to provide for the conservation of such species." At present, there is no lamprey listed in either of those categories. In 2003, a petition was sent to the U.S. Fish and Wildlife Service (USFWS) urging the listing of four lampreys—the Pacific lamprey, Kern brook lamprey, western river lamprey *Lampetra ayresii*, and western brook lamprey—under the ESA. The petition was rejected, however, because the USFWS determined that there was insufficient evidence to support such listing (United States Fish and Wildlife Service 2004).

The NOM lists species under four risk categories: Probably Extinct in the Wild, In Danger of Extinction (equivalent to IUCN categories Critically Endangered and Endangered), Threatened (equivalent to IUCN category Vulnerable), and Subject to Special Protection (equivalent to the IUCN Near Threatened). There are three lampreys on the NOM list: the Mexican lamprey and Mexican brook lamprey, both considered In Danger of Extinction, and the Pacific lamprey, which is Threatened. Once listed, the conservation of aquatic species is under the responsibility of the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (http://www.sagarpa.gob.mx) and the Federal Attorney for Environmental Protection (http://www.profepa.gob.mx).

8.3.2 Europe

Freyhof and Brooks (2011) provide an excellent overview of the freshwater fishes at risk in Europe and the legislation that applies to them. The two important pieces of legislation are the Bern Convention and the Habitats Directive. Most countries in Europe are members of the EU, at present comprising 28 states; all of them are signatories to the EU Habitats Directive (1992) (ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm), which is the main piece of legislation protecting wildlife across Europe. The EU Habitats Directive gives special protection to species identified as being threatened with extinction at the European level: all *Eudontomyzon* species, European river lamprey (except the Finnish and Swedish populations), European brook lamprey (except the Estonian, Finnish, and Swedish populations), Po brook lamprey, and sea lamprey (except the Swedish populations) are listed in Annex II; and European river lamprey and Po brook lamprey are also listed in Annex V (Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:01992L0043-20070101:EN:NOT). Annex II lists all species and subspecies for which core areas of their habitat (Special Areas of Conservation, SACs) must be protected and managed in accordance with the ecological requirements of the species; Annex V lists species and subspecies for which taking from the wild may be restricted.

Compulsion for member states to establish SACs is the most important practical element affecting lampreys in the Directive. Article 6.2 of the Directive states that member states shall take appropriate steps to avoid, in these SACs, the deterioration

of natural habitats and the habitats of species as well as disturbance of the species for which the areas have been designated. Article 6.3 states that any plan or project not directly connected with or necessary to the management of the site but likely to have a significant effect thereon, either individually or in combination with other plans or projects, shall be subject to appropriate assessment of its implications for the site in view of the site's conservation objectives. In the light of the conclusions of the assessment of the implications for the site, the competent national authorities shall agree to the plan or project only after having ascertained that it will not adversely affect the integrity of the site concerned.

In addition to protection at the EU level, sea, European river, and European brook lampreys are all given protection at a more local level in some countries (see Sects. 8.4.2.1 and 8.4.2.2).

8.3.3 Elsewhere

In Australia, the *Environment Protection and Biodiversity Conservation Act 1999* (http://www.environment.gov.au/biodiversity/legislation/index.html) became effective on 16 July 2000, and it is administered by the Australian Government Department of Sustainability, Environment, Water, Population and Communities. None of the three lamprey species (pouched, short-headed, and precocious lampreys) occurring in Australia are listed under the Act.

In New Zealand, the *Conservation Act 1987* came into force on 1 April 1987 (http://www.legislation.govt.nz/act/public/1987/0065/latest/DLM103610.html). It established the Department of Conservation (DOC), which administers the Act. A species threat classification system was developed by the New Zealand DOC in 1992, and was subsequently replaced (in 2002, with revisions in 2007) with a system that listed taxa purely according to risk of extinction (Townsend et al. 2008; Allibone et al. 2010). The pouched lamprey, the only lamprey species occurring in New Zealand, has been assessed as Declining (Allibone et al. 2010), but there is no legal protection under the Act.

The Red Data Book of the Russian Federation has six categories, but these are not readily equivalent to IUCN categories. They are: Probably extinct (0); Endangered (1); Decreasing number (2); Rare (3); Uncertain status (4); and Rehabilitated and rehabiliting (5). According to the 2001 Red Data Book of the Russian Federation, there are three lampreys listed nationally: sea lamprey as Endangered, and both Caspian lamprey and Ukrainian brook lamprey as Decreasing number (Alexander M. Naseka, Russian Academy of Sciences, St. Petersburg, Russia, personal communication, 2011). Two Russian laws protect listed species: the law enacted on 19 December 1991 "About protection of natural environment" and the second enacted on 5 May 1995 "About animal world" (Iliashenko and Iliashenko 2000). At the oblast' (=province) level, six lamprey species have been evaluated as follows: sea lamprey Rare in Leningrad Oblast'; Caspian lamprey Probably extinct from Nizhniy Novgorod Oblast'; Ukrainian brook lamprey Rare in Krasnodar Oblast' and Uncertain status in Saratov Oblast'; European river lamprey Uncertain status in Vologda

Oblast'; European brook lamprey Decreasing number in Yaroslavl' Oblast', Rare in Moscow Oblast', and Uncertain status in Nizhniy Novgorod Oblast'; and Arctic lamprey Uncertain status in Vologda Oblast' and in Tyumen' Oblast' (Alexander M. Naseka, personal communication, 2011).

Declines in the population size of Caspian lamprey have been reported in Iranian water bodies, primarily as the result of loss of spawning grounds (Kiabi et al. 1999), but this species receives no legal protection in Iran (Hassan Nazari, Shahid Beheshti University, Tehran, Iran, personal communication, 2013).

In Japan, five lamprey species (including the two as-yet-undescribed species of *Lethenteron*) have been listed—as Vulnerable (3), Near Threatened (1), and Threatened Local Population (1)—in the Red List of Threatened Fishes of Japan (http://www.biodic.go.jp/english/rdb/rdb_f.html; see Sect. 8.4.3). However, species listed in the Red Data Books are not given any legal status; in order to gain protection under the *Act on Conservation of Endangered Species of Wild Fauna and Flora* (ACES), enacted in 1992, a species must be listed by Cabinet Order (http://www.biodic.go.jp/english/biolaw/syu_e/; Takahashi 2009). To date, fewer than 3 % of the more than 3,000 species listed in the Red Data Books (and no lamprey species) are listed and protected by the ACES (Takahashi 2009).

8.4 Lamprey Conservation Efforts

A number of projects across the world are concerned with particular species or populations of lampreys, their protection, and conservation. That this number—and research in support of lamprey conservation efforts (e.g., Yamazaki et al. 2005, 2011, 2014; Yamazaki 2007; Beamish and Wade 2008; Lucas et al. 2009; Nazari and Abdoli 2010; Pereira et al. 2010; Docker et al. 2012; Stewart and Baker 2012; Taylor et al. 2012; Bracken and Lucas 2013; Ferreira et al. 2013)—appears to be growing yearly is cause for cautious optimism. The following case histories illustrate some examples of these efforts.

8.4.1 North America

8.4.1.1 Miller Lake Lamprey *Entosphenus minimus*

An example of population restoration is provided by the Miller Lake lamprey, the smallest known parasitic lamprey species, which is endemic to the upper Klamath basin, Oregon (Lorion et al. 2000). It was eradicated from Miller Lake through toxaphene poisoning conducted by the Oregon Game Commission on 16 September 1958 because of the deleterious impacts it was perceived to have on nonnative salmonid fisheries (Bond and Kan 1973; Kan and Bond 1981). The Miller

Lake lamprey was described as a distinct species by Bond and Kan (1973), based exclusively on material collected from Miller Lake in 1950–1952, and therefore, the species was assumed to be extinct. However, Miller Lake lamprey were collected in other parts of the upper Klamath basin between 1992 and 1999, namely, the lower reaches of Miller Creek, Jack Creek, the upper Williamson River, Long Creek, and the upper Sycan River above Sycan Marsh (Lorion et al. 2000).

A Miller Lake Lamprey Conservation Plan was adopted by the Oregon Fish and Wildlife Commission in 2005 to re-establish the species in Miller Lake, its type locality. The first action taken that same year was the removal of a man-made barrier installed on Miller Creek in 1959 by the Oregon Game Commission to prevent any recolonization of the lamprey into Miller Lake from downstream (Stewart B. Reid, Miller Lake Lamprey Technical Management Team, Ashland, OR, personal communication, 2010). Despite removal of this barrier in 2005, lamprey from Miller Creek had still not recolonized Miller Lake by 2010; thus, 698 ammocoetes (c. 10–140 mm total length) and two adult Miller Lake lamprey from Miller Creek were released at three sites upstream of the removed barrier on 4 August 2010 (Stewart B. Reid, personal communication, 2010). In 2011, 2012, and 2013, another c. 600 ammocoetes were again transferred from lower Miller Creek into the lake, just below the lake in upper Miller Creek, and in the primary tributary to the lake (c. 200 ammocoetes per site). Reproduction has not yet been confirmed in Miller Lake itself, but the ammocoetes appear to be surviving successfully (Stewart B. Reid, personal communication, 2013). No immediate threats are known to be acting upon the Miller Lake lamprey at the present time.

8.4.1.2 Pacific Lamprey *Entosphenus tridentatus*

The distribution and abundance of Pacific lamprey has declined greatly in Washington, Oregon, Idaho, and California in the past several decades and, recently, concern over this decline has prompted a number of collaborative conservation efforts (e.g., among federal, state, local, and tribal organizations). The idea of Pacific lamprey conservation, however, is relatively new for state and federal governments. During the late 1960s, for example, the USFWS and Oregon Department of Fish and Wildlife (ODFW) became concerned that "rough fish" distribution was moving upstream and might affect the steelhead *Oncorhynchus mykiss* population in the Umatilla River. Prior to steelhead supplementation, they used rotenone treatments to extirpate all fishes from the lower reaches to the headwaters in 1967 and 1974; after 1974, lamprey larvae were no longer observed in the fish traps located at entrances to the irrigation canals (Close et al. 2004).

However, the Pacific lamprey is a highly valued fish and holds cultural importance to the indigenous peoples of the Columbia River basin (Close et al. 2002); this long-held appreciation for Pacific lamprey has been the impetus for many of the conservation efforts and restoration plans for this species. By 1993, concerns about the declining numbers of Pacific lamprey throughout much of its range in the Columbia River basin (Close et al. 2002) led the State of Oregon to list it as a

sensitive species and it was given legal protection status as a Species of Concern in 1997 (Kostow 2002). In Idaho, the Pacific lamprey was classified as an Endangered species in 1993 (Idaho Administrative Code 2011). In 2003, conservation groups petitioned the USFWS to protect Pacific lamprey under the Federal *Endangered Species Act* (United States Fish and Wildlife Service 2004), but the listing was denied because of lack of information on population structure, distinct population units, and quantitative evidence of population declines. Nevertheless, the USFWS recognized the need for a comprehensive plan to conserve and restore Pacific lamprey, in collaboration with Native American tribes and other federal, state, and local agencies. In 2008, the USFWS established the Pacific Lamprey Conservation Initiative, a strategy to improve the status of Pacific lamprey throughout its range by helping to implement research and conservation actions (Luzier et al. 2009). In June 2012, a Lamprey Conservation Agreement was signed by a number of tribal, federal, and state partners (United States Fish and Wildlife Service 2012).

Specific restoration plans have been developed by tribal agencies within the Columbia River basin. The Confederated Tribes of the Umatilla Indian Reservation (CTUIR), for example, developed a restoration plan for Pacific lamprey in the Umatilla River in 1997 with the goal of re-establishing natural production that would provide a sustainable and harvestable abundance of adults. The Columbia River Inter-Tribal Fish Commission (CRITFC), which coordinates management policy and provides fisheries technical services for the Yakama, Warm Springs, Umatilla, and Nez Perce tribes, established its Tribal Pacific Lamprey Restoration Plan for the Columbia River Basin in 2011 (CRITFC 2011). The goal of this restoration plan is to immediately halt the decline of Pacific lamprey and ultimately restore its throughout its historic range in numbers that provide for ecological integrity and sustainable tribal harvest. The CRITFC plan addresses the need for resolving key uncertainties and identified threats on a number of issues related to Pacific lamprey restoration (e.g., mainstem and tributary passage, habitat quality, water quality). In the paragraphs below, we will focus on the ongoing efforts to re-establish natural production in the Columbia River basin through translocation.

Translocation involves the collection of adult Pacific lamprey from the mainstem lower Columbia River (e.g., using adults salvaged during dewatering operations at downstream dams), and release into a subbasin upstream where it is scarce or extirpated (Ward et al. 2012). Such translocation is intended as an interim measure while primary threats (particularly passage and habitat degradation) are addressed (Ward et al. 2012). Translocation of spawning adults is intended to increase the number of larvae present, which may—through pheromonal cues—eventually attract even more spawning adults (Yun et al. 2011; see Chap. 5). As part of the CTUIR restoration plan for Pacific lamprey in the Umatilla River, Oregon, over 2,600 adult lamprey were reintroduced into the upper Umatilla River between 1999 and 2007 (Close et al. 2009). Reintroduced lamprey were capable of finding spawning habitat, constructing nests, and producing larvae and, by 2005, larvae were distributed downstream to the middle reaches of the river. Further downstream distribution of larvae seemed to be limited by irrigation in the lower Umatilla River (Close et al. 2009). Low head diversion dams in the lower basin also appear to be a problem for adults during their spawning run; telemetry studies have shown that passage

efficiency for adult Pacific lamprey is less than 50 % at low head diversion dams in the Umatilla (Jackson and Moser 2012; see Sect. 8.2.3.1). In concert with translocation efforts, the CTUIR has also modified passage structures, and adult lamprey numbers have increased after several years of reintroduction efforts. In 2011, 129 lampreys entered the Umatilla River (Ward et al. 2012). In 2006, the Nez Perce Tribe similarly established a trial translocation program to move adult Pacific lamprey past mainstem dams in the Snake River basin; efforts to date have focused on augmenting natural lamprey production in the Clearwater (in Idaho) and Asotin (in Washington state) subbasins. From 2007–2010, 480 adult lamprey were released into four study streams and, as in the Umatilla River, successful spawning and larval production has been observed (Ward et al. 2012). Most recently (in 2010), Pacific lamprey have been translocated into the Yakima River subbasin, where abundance has been severely depressed for the past 13 years (Patrick Luke, Yakama Nation, Toppenish, WA, personal communication, 2013). The success of these translocation efforts continues to be monitored, including through a genetic monitoring program where all transplanted adults are being genotyped so that parentage analysis of ammocoetes can help evaluate reproductive success of each adult (Jon E. Hess, Columbia River Inter-Tribal Fish Commission, Hagerman, ID, personal communication, 2013).

The importance of genetic monitoring was recognized in the lamprey translocation guidelines outlined by the Columbia River Inter-Tribal Fish Commission (CRITFC 2011), since translocation can potentially disrupt population structure and local adaptation. It appears, however, that Pacific lamprey may be only weakly philopatric, although recent genetic studies have shown somewhat conflicting results. A genetic survey of larval Pacific lamprey using mitochondrial DNA markers showed little differentiation along the Pacific coast of North America (Goodman et al. 2008). However, another study using amplified fragment length polymorphism (AFLP) markers in adult Pacific lamprey found genetic differentiation among various rivers along the coast (Lin et al. 2008a, b). Spice et al. (2012), using microsatellite loci, found relatively low but often statistically significant genetic differentiation among locations from central British Columbia to central California and weak but significant isolation-by-distance; these authors therefore concurred with previous views that Pacific lamprey are not strongly philopatric (since natal homing tends to minimize gene flow among locations), but argued that they are not panmictic either, suggesting that some limitations to dispersal at sea prevent complete genetic homogeneity throughout their range. Spice et al. (2012) therefore cautioned that, although Pacific lamprey do not appear to form geographically distinct populations, translocations are likely to be more successful from geographically proximate sites. In the most recent study using single nucleotide polymorphism (SNP) loci, Hess et al. (2013) reconciled previous studies and showed evidence of adaptive genetic variation despite high gene flow, and identified a significant amount of variation among three broad populations, northern British Columbia, Columbia River–southern U.S. coast, and “dwarf” adults (i.e., Pacific lamprey which mature at lengths < 370 mm, presumably as the result of a shorter post-metamorphic feeding phase; see Docker and Potter in press). Hess et al. (2013) suggested that even though the results show

Table 8.2 Special Areas of Conservation (SACs) in Scotland which give protection to lampreys. In some, lampreys are the Priority Species (P); in others they are listed as Additional Qualifying Species (A) to the main Priority Species (e.g., Atlantic salmon) at that site

Site	European river lamprey	European brook lamprey	Sea lamprey
Endrick Water	P	P	–
River Tay	A	A	A
River Teith	P	P	P
Solway Firth	P	–	P
River Spey	–	–	P
River Tweed	A	A	A
Tweed Estuary	A	–	A

that Pacific lamprey have high gene flow throughout their range, it is important to maintain genetic diversity in each location so that adaptive variation is not lost. Further work is currently underway to better understand adaptive genetic variation in Pacific lamprey within the Columbia River basin.

8.4.2 Europe

8.4.2.1 Lamprey Conservation in Scotland

This case history is cited as an example of a conservation approach to all species of lampreys in one country. As indicated above (see Sect. 8.3.2), all 28 member states of the European Union are legally obliged to establish Special Areas of Conservation (SACs) for threatened species, including lampreys. Scotland may be cited as a good example of where positive action has taken place in recent years for the three species of lampreys which occur there—European river lamprey, European brook lamprey, and sea lamprey. So far seven SACs have been established for these lampreys (Table 8.2).

Scottish Natural Heritage (SNH) is the government organization with responsibility for nature conservation in Scotland. In addition to National Nature Reserves (NNRs), protected sites are graded at three levels: (1) Natura 2000 sites are internationally important sites which are designated as SACs or SPAs (Special Protection Areas) under European legislation (http://www.natura.org); (2) Sites of Special Scientific Interest (SSSIs) are nationally important sites designated by SNH via the provisions of the *Wildlife and Countryside Act 1981* (http://www.snh.gov.uk); and (3) locally important sites include Local Nature Reserves, Country Parks, and Regional Parks, which are designated by local authorities (http://www.snh.gov.uk). Lampreys occur in some of all three categories of protected site; for example, although no NNR has ever been declared because of lampreys, one NNR in Scotland contains sea lamprey (three in Great Britain in total), two in Scotland have European river lamprey (four in Great Britain), and nine in Scotland have European brook lamprey (12 in Great Britain) (Lyle and Maitland 1992).

In recognition of the importance of these designated protected sites in conserving Scotland's natural diversity, the Scottish Government set a Condition Target to achieve 95 % of natural features in "favourable condition" by March 2010 (http://www.snh.gov.uk). The condition of features on designated protected sites is determined by SNH's Site Condition Monitoring (SCM) program; the purpose of this program is to determine whether the habitat, species, or geological feature on a site is likely to maintain itself in the medium to longer term under the current site management regime. The framework for such a determination is the Common Standards Monitoring Guidance, a U.K.-wide approach which: provides a simple, quick, assessment of feature condition; is limited to protected sites; and is supported, when required, by more detailed research/monitoring (http://jncc.defra.gov.uk/page-2199). SCM is a reporting obligation under the European Habitats Directive. So far, member states have completed two reporting rounds: 1994–2000 and 2001–2006.

8.4.2.2 Conserving the European River Lamprey *Lampetra fluviatilis*

This case history is a good example of the conservation measures concerning a single species across its entire range. The European river lamprey is a parasitic species that occurs from the Gulf of Bothnia east, along the Baltic and North Sea coasts, to the Atlantic waters of the British Isles, France, and the Iberian Peninsula, where it has been reported as far south as the Tagus River (Hardisty 1986a; see Chap. 2). The species is mainly anadromous, but landlocked populations have been reported from Finland (Tuunainen et al. 1980), Russia (Lakes Ladoga and Onega) in the Baltic catchment (Hubbs and Potter 1971), and Loch Lomond in Scotland (Maitland 1980; Docker and Potter in press). In the Iberian Peninsula, an anadromous population is present only in the Tagus River basin (Cabral et al. 2005), where persistence may have been possible due to the size of its estuary, c. 300 km^2 (Mateus et al. 2011).

The current global status of this species is considered to be Least Concern according to the IUCN Red List of Threatened Species (IUCN 2013). This is due to a marked recovery following earlier pollution problems in central and western Europe (Freyhof 2011). However, in Portugal, following the same IUCN criteria, it was classified as Critically Endangered, being present only in the lower section of the Tagus River watershed (Cabral et al. 2005; Mateus et al. 2012). In France, the European river lamprey is considered Vulnerable (UICN France et al. 2010) and in Spain, Regionally Extinct (Doadrio 2001).

Existing management measures are being put in place because the European river lamprey is protected by Annexes II and V of the European Habitats Directive (whose conservation, as noted in Sect. 8.3.2, requires the designation of SACs) and Appendix III of the Bern Convention. In Portugal, the Tagus Estuary was listed in the Natura 2000 Habitats Directive due to, among other biodiversity features, the presence of this species (Mateus et al. 2012). In France, 49 Natura 2000 sites were designated for this species (Mateus et al. 2012).

Numerous activities have also been undertaken to improve the status of European river lamprey in countries around the western Baltic Sea. Barriers to migration

(as the result of hydroelectric dams)—and habitat degradation and water regulation in the reaches downstream of the dams—appear to be the main reason for the decline of this species in Finland and Sweden (Sjöberg 2011). Lamprey-friendly modifications to fish ladders in Finland appear promising, but mitigation efforts to date have mostly relied on transportation of adult and larval lampreys above migration barriers. In Finland, tens of thousands of adults are transported per year in each of several rivers (e.g., Kemijoki, Iijoki, and Oulujoki rivers). In the River Perhonjoki, where all but the lower 32 km of the river is inaccessible to upstream migrants (see Sect. 8.2.3.1), more than 575,000 adult European river lamprey were transported past the dam between 1981 and 2010 (see Sjöberg 2011). Transportation of adult lamprey around barriers has also been implemented in several rivers in Sweden, although on a smaller scale than in Finland (Sjöberg 2011). In addition, in Finland, Estonia, and Latvia, larvae are being reared in fish hatcheries and millions per year are being released above dams in each of several rivers (Sjöberg 2011). However, although larval densities have increased in at least one of the rivers that has been stocked, there has been no clear increase in the number of upstream-migrating adults returning to these rivers; perhaps this is due to a lack of homing (see Sjöberg 2011).

8.4.2.3 Iberian Endemic Cryptic *Lampetra* Species

The Iberian Peninsula seems to have played a major role as a glacial refugium for lampreys of the genus *Lampetra*. The high genetic diversity observed in Iberian *Lampetra* populations is probably the result of refugial persistence and subsequent accumulation of variation over several ice ages, whereas the low levels of genetic diversity observed in central and northern Europe likely reflect a rapid post-glacial colonization (Espanhol et al. 2007; Mateus et al. 2011). European river and brook lampreys are paired species; the larvae are morphologically similar but the adults adopt different life history types (Zanandrea 1959; Docker 2009). It is generally assumed that the brook lamprey has evolved from the migratory form and become non-parasitic (Zanandrea 1959; Hardisty 1986b). Glaciations may have promoted evolution of non-parasitic lampreys by blocking migratory routes and preventing anadromy (Hardisty 1986b; Docker and Potter in press). Molecular phylogeographical analysis revealed that European river and brook lampreys are not reciprocally monophyletic, suggesting that loss of the migratory ability has occurred multiple times (Espanhol et al. 2007).

In the Iberian Peninsula, brook lampreys confined to small, isolated river basins evolved in allopatry, giving rise to four evolutionary lineages. Combined genetic and morphological analyses suggested the existence of three new brook lamprey species of the genus *Lampetra* in Portugal. These species were recently described as *Lampetra alavariensis, L. auremensis*, and *L. lusitanica* from the Vouga-Esmoriz, Tagus, and Sado river basins, respectively (Mateus et al. 2013a); *L. planeri* shows a wider distribution in the Iberian Peninsula. Although Potter et al. 2014 (see Chap. 2) do not recognize these populations as specifically distinct from *L. planeri*—because

they were not compared to material of *L. planeri* from its type locality and because none of the Portuguese populations is morphologically diagnosable—we consider that the brook lampreys described in Mateus et al. (2013a) represent a complex of cryptic species. Genetic distinctiveness is evidence of a long history of local independent evolution (Mateus et al. 2011, 2013a) and, although not diagnostic, there are subtle morphological differences between the new species and *L. planeri*, especially those related to dentition (Mateus et al. 2013a). Each of these three species has a smaller geographic range than *L. planeri* sensu stricto. *Lampetra planeri* was already considered Critically Endangered and Critically Endangered/Vulnerable in Portugal and Spain, respectively (Mateus et al. 2012); consequently, each of the four species in the complex is highly susceptible to extinction.

In Portugal, a conservation plan was recently developed for lampreys in the genus *Lampetra*. The prime objective of this plan was to assess the distribution of these species in Portuguese waters and, at the same time, identify their macrohabitat preferences (Ferreira et al. 2013). About 400 sampling stations (approximately one station per 200 km^2) were selected across the country to detect the occurrence of *Lampetra* populations. Their presence was confirmed in eight distinct watersheds: Douro, Mangas, Vouga, Mondego, Lis, S. Pedro, Tagus, and Sado. The presence of *Lampetra* species was strongly related to five abiotic predictors (i.e., altitude, distance to coast, sand, maximum temperature of the warmest month, and precipitation of the driest month). A probability of occurrence model was built with the data gathered that explained the distribution of the genus *Lampetra* in Portugal. The distribution model was applied to build a map of probability of occurrence and was used as a baseline tool to prioritize rivers in terms of their level of importance for conservation (Ferreira et al. 2013). One of the promising applications of this type of information is the possibility of providing sound background information for the selection of rivers or river stretches as SACs for these species. A total of 31 river stretches from eight river basins were identified as having the potential to be designated as SACs (Ferreira et al. 2013). Within the Tagus basin, 10 locations have been selected to be proposed as SACs, of which eight presumably support populations of European brook and river lampreys, as well as sea lamprey, as no obstacle to the migration of anadromous species is known to occur.

The situation described here concerning the *Lampetra* species from the Iberian Peninsula reflects similar problems regarding the conservation of several other described (Renaud and Economidis 2010) and undescribed cryptic species (Yamazaki et al. 2006; Boguski et al. 2012) with restricted distributional ranges.

8.4.3 Elsewhere

In Japan, The National Biodiversity Strategy, administered by the Ministry of the Environment, is the national basic plan for the conservation of biodiversity and its sustainable use. The first such plan was made in 1995 and has been reviewed three times since: in 2002, 2007 and 2010. As mentioned in Sect. 8.3.3, there is no legal protection for any of the lamprey species found in this country. However, the

fourth edition of the Red List of Japan, produced by the Ministry of the Environment, contains the following five lamprey species: *Lethenteron* sp. N and sp. S (two undescribed species; Yamazaki et al. 2006) and Arctic lamprey are Vulnerable; Siberian brook lamprey *Lethenteron kessleri* is Near Threatened; and Pacific lamprey is listed as Threatened Local Population (Yuji Yamazaki, University of Toyama, Toyama, Japan, personal communication, 2011; http://www.biodic.go.jp/english/rdb/rdb_f.html).

There are no specific projects aimed at conserving lampreys in Australia (Ian C. Potter, Murdoch University, Western Australia, personal communication, 2011, 2012). In New Zealand, where the pouched lamprey is listed as Declining (see Sect. 8.3.3), a six-year Ministry of Business, Innovation and Employment-funded project on aquatic rehabilitation is being conducted by the National Institute of Water and Atmospheric Research (NIWA). This project includes conducting lamprey research aimed at understanding key aspects of their ecology (e.g., regarding spawning stream and habitat selection) and developing methods for estimating larval population size (e.g., Stewart and Baker 2012). Biologists are also working with iwi (i.e., Māori tribes) to understand more about their indigenous knowledge regarding pouched lamprey and identify tools for monitoring population trends for this species (Jane Kitson, Kitson Consulting, Invercargill, NZ, personal communication, 2013). Recently, the Ministry for Primary Industries (MPI) and other interested groups in New Zealand have begun investigating the Lamprey Reddening Syndrome, which was discovered in upstream migrating pouched lamprey in 2012 (see Sect. 8.2.4).

8.5 Conclusions: Knowledge and Legislative Gaps

Although it is promising that lampreys are now given protection in some parts of the world, notably North America and much of Europe, there are still many gaps and some inconsistencies in the approaches taken to the conservation of these animals internationally. For example, in Europe, most of the conservation effort appears to be directed at the three most common species there (European river lamprey, European brook lamprey, and sea lamprey; see Sect. 8.4.2). These species are relatively widespread and, though Vulnerable (or even Regionally Extinct) in some countries (Mateus et al. 2012), are all of Least Concern globally (IUCN 2013). Other species (e.g., the Caspian lamprey, which is Near Threatened, and the Macedonia brook lamprey, which is Critically Endangered) have largely been ignored. The Macedonia brook lamprey and the recently-described Epirus brook lamprey occur, respectively, in only three localities and one locality on the east and west sides of Greece. Their main threat is loss of habitat, caused largely by water abstraction, sedimentation, and pollution from sewage. Although all *Eudontomyzon* species in Europe receive formal legal protection (see Sect. 8.3.2), no one is studying these two species at the moment, conservation action is required (Panos S. Economidis, Aristotle University, Thessaloniki, Greece, personal communication, 2011), and

an awareness campaign is urgently needed; otherwise they face extinction. In the case of the Caspian lamprey, its current status is all the more dramatic when one considers that it was once sufficiently plentiful to sustain a commercial fishery in both Russia (at least until 1913) and Azerbaijan (at least until 1937) (Berg 1948; see Sect. 8.2.5). Conservation initiatives in North America (specifically the United States) are largely directed at the Pacific lamprey (see Sect. 8.4.1.2), although some others (e.g., the Vancouver lamprey, Mexican lamprey, and Mexican brook lamprey) do receive legal protection (Sect. 8.3.1). Thus, despite legal protection for a growing number of species (Table 8.1), there is still relatively little conservation concern for those species that are not harvested by humans.

Furthermore, for some species, there are discrepancies between the status assigned on global versus national lists. Helfman (2007) highlighted the need to explain the reasons for any disagreements between the global (IUCN) and national lists in order to prevent confusion. While he suggests that differences of scale and lag times between the re-assessments of those lists may in part explain the discrepancies, some of the differences that occur are harder to explain and require resolution. For lampreys, it is noteworthy that the Canadian endemic Vancouver lamprey is listed as Threatened by Canada but Data Deficient by IUCN and that the Mexican endemic Mexican brook lamprey is listed as In Danger of Extinction by Mexico but it is not even listed by IUCN (Table 8.1). This, we suggest, could be resolved with better coordination and consultation and would better serve the common cause of conservation.

Taxonomic uncertainty in some lampreys remains an impediment to their conservation. One cannot conserve something that cannot be properly identified. A number of species, such as *Lethenteron* sp. N and *Lethenteron* sp. S, remain undescribed (Yamazaki et al. 2006), and recent molecular data suggest that there may be other undescribed (and morphologically cryptic) brook lamprey species with restricted distributional ranges (Boguski et al. 2012). Even if these lampreys are not identified as distinct species, such data suggest the existence of divergent evolutionary lineages or non-interchangeable evolutionarily significant units (ESUs) for conservation (e.g., Martin and White 2008; Pereira et al. 2010). The paired species conundrum (i.e., whether closely-related parasitic and non-parasitic species pairs represent distinct species or ecotypes of a single species) likewise awaits resolution (Docker 2009). Renaud et al. (2009) suggested that this may be the most daunting question facing lamprey biologists; this debate has been ongoing for over a century (e.g., Enequist 1937; McPhail and Lindsey 1970) and has been raised again with evidence that some species or populations (e.g., silver and northern brook lampreys in the Great Lakes; Docker et al. 2012)—but not all (e.g., European river and brook lampreys in the Tagus River basin in Portugal; Mateus et al. 2013b)—show no evidence of genetic differentiation when they co-occur (see Docker and Potter in press). This issue has conservation implications, such as whether paired non-parasitic and parasitic lampreys should be treated as separate or single conservation units and whether it might be possible to rehabilitate or rescue populations of one feeding type that are at risk with the other (Docker 2009; Docker et al. 2012).

Finally, more research is needed to better understand the threats that affect lamprey survival. Although all the "usual suspects" (i.e., overharvest, barriers to passage, pollution and other forms of habitat degradation, and alteration of stream flow regimes) are generally cited as potential reasons for the decline of many lamprey species or populations, the causal relationships between these threats and the distribution and abundance of lampreys have not been well tested (Renaud et al. 2009). In this chapter, we have tried to include specific information, where available, so that the nature and magnitude of certain threats can be better evaluated, but more study is clearly needed. Key knowledge gaps have also been highlighted, and other recent reviews likewise have identified critical uncertainties that are hampering our efforts to conserve lampreys (e.g., Mesa and Copeland 2009; Moser and Mesa 2009; Moyle et al. 2009; Renaud et al. 2009). A better understanding of threats to lamprey survival is necessary in order for more accurate assessments of conservation status and more directed recovery actions.

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References

Allibone R, David B, Hitchmough R et al (2010) Conservation status of New Zealand freshwater fish, 2009. N Z J Mar Freshw Res 44:271–287

Almeida PR, Quintella BR (2002) Larval habitat of the sea lamprey (*Petromyzon marinus* L.) in the River Mondego (Portugal). In: Collares-Pereira MJ, Coelho MM, Cowx IG (eds) Freshwater fish conservation: options for the future. Fishing News Books, Blackwell, Oxford, pp 121–130

Almeida PR, Silva HT, Quintella BR (2000) The migratory behaviour of the sea lamprey *Petromyzon marinus* L., observed by acoustic telemetry in River Mondego (Portugal). In: Moore A, Russell I (eds) Advances in fish telemetry. CEFAS Lowestoft Laboratory, Lowestoft, pp 99–108

Almeida PR, Quintella BR, Dias NM (2002) Movement of radio-tagged anadromous sea lamprey during the spawning migration in the River Mondego (Portugal). Hydrobiologia 483:1–8

Andersen HB, Caldwell RS, Toll J, Do T, Saban L (2010) Sensitivity of lamprey ammocoetes to six chemicals. Arch Environ Contam Toxicol 59:622–631

Anderson PJ, Piatt JF (1999) Community reorganization in the Gulf of Alaska following ocean climate regime shift. Mar Ecol Prog Ser 189:117–123

Andrade NO, Quintella BR, Ferreira J et al (2007) Sea lamprey (*Petromyzon marinus* L.) spawning migration in the Vouga river basin (Portugal): poaching impact, preferential resting sites and spawning grounds. Hydrobiologia 582:121–132

Applegate VC (1950) Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. US Fish and Wildlife Service Special Scientific Report 55, Washington, DC

Appy RG, Anderson RC (1981) The parasites of lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 1–42
Assis CA (1990) Threats to the survival of anadromous fishes in the River Tagus, Portugal. J Fish Biol 37:225–226
Bao M, Garci ME, Antonio JM, Pascual S (2013) First report of *Anisakis simplex* (Nematoda, Anisakidae) in the sea lamprey (*Petromyzon marinus*). Food Control 33:81–86
Beamish FWH (1980) Biology of the North American anadromous sea lamprey, *Petromyzon marinus*. Can J Fish Aquat Sci 37:1924–1943
Beamish RJ (2001) Updated status of the Vancouver Island Lake Lamprey, *Lampetra macrostoma*, in Canada. Can Field-Nat 115:127–130
Beamish RJ, Northcote TG (1989) Extinction of a population of anadromous parasitic lamprey, *Lampetra tridentata*, upstream of an impassable dam. Can J Fish Aquat Sci 46:420–425
Beamish RJ, Wade J (2008) Critical habitat and the conservation ecology of the freshwater parasitic lamprey, *Lampetra macrostoma*. Can Field-Nat 122:327–337
Beamish RJ, Youson JH (1987) Life history and abundance of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the Strait of Georgia. Can J Fish Aquat Sci 44:525–537
Beamish RJ, Noakes DJ, McFarlane GA et al (1999) The regime concept and natural trends in the production of Pacific salmon. Can J Fish Aquat Sci 56:516–526
Beaulaton L, Taverny C, Castelnaud G (2008) Fishing, abundance and life history traits of the anadromous sea lamprey (*Petromyzon marinus*) in Europe. Fish Res 92:90–101
Beckvar N, Dillon TM, Read LB (2005) Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Environ Toxicol Chem 24:2094–2105
Bell GR, Traxler GS (1986) Resistance of the Pacific lamprey, *Lampetra tridentata* (Gairdner), to challenge by *Renibacterium salmoninarum*, the causative agent of kidney disease in salmonids. J Fish Dis 9:277–279
Benson AJ, Trites AW (2002) Ecological effects of regime shifts in the Bering Sea and eastern North Pacific Ocean. Fish Fish 3:95–113
Berg LS (1948) Freshwater fishes of the USSR and adjacent countries. Guide to the fauna of the USSR no 27, vol 1. Israel Program for Scientific Translations Ltd, Jerusalem
Bettaso JB, Goodman DH (2010) A comparison of mercury contamination in mussel and ammocoete filter feeders. J Fish Wildl Manag 1:142–145
Binder, TR, McLaughlin RL, McDonald DG (2010) Relative importance of water temperature, water level, and lunar cycle to migratory activity in spawning-phase sea lampreys in Lake Ontario. Trans Am Fish Soc 139:700–712
Boguski DA, Reid SB, Goodman DH, Docker MF (2012) Genetic diversity, endemism and phylogeny of lampreys within the genus *Lampetra sensu stricto* (Petromyzontiformes: Petromyzontidae) in western North America. J Fish Biol 81:1891–1914
Bond CE, Kan TT (1973) *Lampetra* (*Entosphenus*) *minima* n. sp., a dwarfed parasitic lamprey from Oregon. Copeia 1973:568–574
Bracken FSA, Lucas MC (2013) Potential impacts of small-scale hydroelectric power generation on downstream moving lampreys. River Res Appl 29:1073–1081
Cabral MJ, Almeida J, Almeida PR et al (eds) (2005) Livro vermelho dos vertebrados de Portugal. Instituto da Conservação da Natureza, Lisbon
Cheung WL, Lam VWY, Sarmiento JL et al (2009) Projecting global marine biodiversity impacts under climate change scenarios. Fish Fish 10:235–251
Claire CW (2003) Life history, habitat utilization, and distribution of Pacific lamprey in the South Fork Clearwater River drainage, Idaho. MS thesis, University of Idaho, Moscow, ID
Clay CH (1995) Design of fishways and other fish facilities, 2nd edn. CRC, Boca Raton
Clemens BJ, van de Wetering S, Kaufman J, Holt RA, Schreck CB (2009) Do summer temperatures trigger spring maturation in Pacific lamprey, *Entosphenus tridentatus?* Ecol Freshw Fish 18:418–426
Close DA, Fitzpatrick MS, Li HW et al (1995) Status report of the Pacific lamprey (*Lampetra tridentata*) in the Columbia River basin. Report to the Bonneville Power Administration, Contract 9SBI39067, Portland, OR

Close DA, Fitzpatrick MS, Li, HW (2002) The ecological and cultural importance of a species at risk of extinction, Pacific lamprey. Fisheries 27:19–25

Close DA, Jackson AD, Conner BP, Li HW (2004) Traditional ecological knowledge of Pacific lamprey (*Entosphenus tridentatus*) in northeastern Oregon and southeastern Washington from indigenous peoples of the Confederated Tribes of the Umatilla Indian Reservation. J Northw Anthropol 38:141–161

Close DA, Currens KP, Jackson A et al (2009) Lessons from the reintroduction of a noncharismatic, migratory fish: Pacific Lamprey in the Upper Umatilla River, Oregon. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 233–253

Cochran PA (2009) Predation on lampreys. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 139–151

COSEWIC (2007) COSEWIC assessment and update status report on the northern brook lamprey *Ichthyomyzon fossor* (Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson population) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

COSEWIC (2008) COSEWIC assessment and update status report on the Vancouver Lamprey *Lampetra macrostoma* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

COSEWIC (2010) COSEWIC assessment and status report on the Chestnut Lamprey *Ichthyomyzon castaneus* (Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson River populations) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

COSEWIC (2011) COSEWIC assessment and status report on the Silver Lamprey, Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson Rivers populations *Ichthyomyzon unicuspis* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

CRITFC (2011) Tribal Pacific lamprey restoration plan for the Columbia River basin, Columbia River Inter-Tribal Fish Commission, Portland. http://www.critfc.org/wp-content/uploads/2012/12/lamprey_plan.pdf. Accessed 12 Dec 2013

Dill WA (1990) Inland fisheries in Europe. EIFAC Technical Paper 52, FAO, Rome

Doadrio I (ed) (2001) Atlas y libro rojo de los peces continentales de España. Dirección General de Conservación de la Naturaleza, Museo Nacional de Ciencias Naturales, Madrid

Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 71–114

Docker MF, Potter IC (in press) Life history variation in lampreys: alternate feeding and migratory types. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Docker MF, Mandrak NE, Heath DD (2012) Contemporary gene flow between "paired" silver (*Ichthyomyzon unicuspis*) and northern brook (*I. fossor*) lampreys: implications for conservation. Conserv Genet 134:823–835

Drevnick PE, Horgan MJ, Oris JT, Kynard BE (2006) Ontogenetic dynamics of mercury accumulation in Northwest Atlantic sea lamprey (*Petromyzon marinus*). Can J Fish Aquat Sci 63:1058–1066

Duarte ACL, Jorge I, Sobral MP et al (2003) Rendimento do botirão usado na captura da lampreia *Petromyzon marinus* L. 1758 no estuário do Rio Mondego. Relatórios Científicos e Técnicos do IPIMAR, Série digital 8

Eissa AE, Elsayed EE, McDonald R, Faisal M (2006) First record of *Renibacterium salmoninarum* in the sea lamprey (*Petromyzon marinus*). J Wildl Dis 42:556–560

Enequist P (1937) Das Bachneunauge als Ökologische Modifikation des Flussneunauges—Über die Fluss- und Bachneunaugen Schwedens Vorläufige Mitteilung. Arkiv Zool 29:1–22

Espanhol R, Almeida PR, Alves MJ (2007) Evolutionary history of lamprey paired species *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) as inferred from mitochondrial DNA variation. Mol Ecol 16:1909–1924

Faisal M, Eissa AE, Elsayed EE (2007) Isolation of *Aeromonas salmonicida* from sea lamprey (*Petromyzon marinus*) with furuncle-like lesions in Lake Ontario. J Wildl Dis 43:618–622
Farlinger SP, Beamish RJ (1984) Recent colonization of a major salmon-producing lake in British Columbia by Pacific lamprey (*Lampetra tridentata*). Can J Fish Aquat Sci 41:278–285
Ferreira AF, Quintella BR, Maia C et al (2013) Influence of macrohabitat preferences on the distribution of European brook and river lampreys: implications for conservation and management. Biol Conserv 159:175–186
Fine JM, Vrieze LA, Sorensen PW (2004) Evidence that petromyzontid lampreys employ a common migratory pheromone that is partially comprised of bile acids. J Chem Ecol 30:2091–2110
Finstad AG, Hein CL (2012) Migrate or stay: terrestrial primary productivity and climate drive anadromy in Arctic char. Glob Chang Biol 18:2487–2497
Flescher D, Martini FH (2002) Lampreys. Order Petromyzontiformes. In: Collette BB, Klein-McPhee G (eds) Bigelow and Schroeder's Fishes of the Gulf of Maine. Smithsonian Institution Press, Washington, DC, pp 16–19
Fortin C, Ouellet M, Cartier I, Banville D, Renaud CB (2007) Biologie et situation de la lamproie du nord, *Ichthyomyzon fossor*, au Québec. Can Field-Nat 121:402–411
Foulds WL, Lucas MC (2013) Extreme inefficiency of two conventional, technical fishways used by European river lamprey (*Lampetra fluviatilis*). Ecol Eng 58:423–433
Foulds WL, Lucas MC (2014) Paradoxical exploitation of protected fishes as bait for anglers: evaluating the lamprey bait market in Europe and developing sustainable and ethical solutions. PLoS ONE 9:e99617
Freyhof J (2011) *Lampetra fluviatilis*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. http://www.iucnredlist.org. Accessed 18 Feb 2014
Freyhof J, Brooks E (2011) European Red List of freshwater fishes. Publications Office of the European Union, Luxembourg. http://ec.europa.eu/environment/nature/conservation/species/redlist/downloads/European_freshwater_fishes.pdf. Accessed 17 Jan 2014
Gadd T, Jakava-Viljanen M, Einer-Jensen K et al (2010) Viral haemorrhagic septicaemia virus (VHSV) genotype II isolated from European river lamprey *Lampetra fluviatilis* in Finland during surveillance from 1999 to 2008. Dis Aquat Org 88:189–198
Gardner C, Coghlan SM, Zydlewski J (2012) Distribution and abundance of anadromous sea lamprey spawners in a fragmented stream: current status and potential range expansion following barrier removal. Northeast Nat 19:99–110
Gay J (2003) Eels under Yukon ice turn into cash for villagers. Anchorage Daily News, 30 Nov 2003, pp A–1, A–12
Gelfenbaum G, Kaminsky GM (2010) Large-scale coastal change in the Columbia River littoral cell: an overview. Mar Geol 273:1–10
Genner MJ, Hillman R, McHugh M, Hawkins SJ, Lucas MC (2012) Contrasting demographic histories of European and North American sea lamprey (*Petromyzon marinus*) populations inferred from mitochondrial DNA sequence variation. Mar Fresh Res 63:827–833
Goode GB (1884) The lampreys— Petromyzontidae. The fisheries and fishery industries of the United States, section I, part III. US Fish Commission at Central Station, Washington DC, pp 677–681
Goodman DH, Reid SB, Docker MF, Hass GR, Kinziger AP (2008) Mitochondrial DNA evidence for high levels of gene flow among populations of a widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae). J Fish Biol 72:400–417
Gradín C (2010) Estado de conservación da lamprea mariña (*Petromyzon marinus* Linnaeus 1758) en ríos de Galicia. Xunta de Galicia. Cansellería do Media Rural. Dirección Xeral de Conservación da Natureza, Santiago de Compostela, Spain
Greig L, Hall A (2011) First international forum on the recovery and propagation of lamprey: workshop report. Prepared by ESSA Technologies Ltd, Vancouver BC, for the Columbia River Inter-Tribal Fish Commission, Portland, OR
Hardisty MW (1961a) Studies on an isolated spawning population of brook lampreys (*Lampetra planeri*). J Anim Ecol 30:339–355
Hardisty MW (1961b) The growth of larval lampreys. J Anim Ecol 30:357–371

Hardisty MW (1979) Biology of the cyclostomes. Chapman and Hall, London
Hardisty MW (1986a) *Lampetra fluviatilis* (Linnaeus, 1758). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part I—Petromyzontiformes, Aula, Wiesbaden, pp 249–278
Hardisty MW (1986b) General introduction to lampreys. In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part I—Petromyzontiformes. Aula, Wiesbaden, pp 19–83
Hardisty MW (2006) Lampreys: life without jaws. Forrest Text, Tresaith
Hardisty MW, Potter IC (1971) The general biology of adult lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 127–206
Heard WR (1966) Observations on lampreys in the Naknek River system of southwest Alaska. Copeia 1966:332–339
Heinrich JW, Weise JG, Smith BR (1980) Changes in biological characteristics of the sea lamprey (*Petromyzon marinus*) as related to lamprey abundance, prey abundance, and sea lamprey control. Can J Fish Aquat Sci 37:1861–1871
Helfman GS (2007) Fish conservation: a guide to understanding and restoring global aquatic biodiversity and fishery resources. Island Press, Washington, DC
Hess JE, Campbell NR, Close DA, Docker MF, Narum SR (2013) Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. Mol Ecol 22:2898–2916
Hogg R, Coghlan SM, Zydlewski J (2013) Anadromous Sea Lampreys recolonize a Maine coastal river tributary after dam removal. Trans Am Fish Soc 142:1381–1394
Holčík J (1986) *Caspiomyzon wagneri* (Kessler, 1870). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part I, Petromyzontiformes. Aula, Wiesbaden, pp 119–142
Holčík J, Renaud CB (1986) *Eudontomyzon mariae* (Berg, 1931). In: Holčík J (ed) The freshwater fishes of Europe, vol 1, part I, Petromyzontiformes. Aula, Wiesbaden, pp 165–185
Holčík J, Šorić V (2004) Redescription of *Eudontomyzon stankokaramani* (Petromyzontes, Petromyzontidae)—a little known lamprey from the Drin River drainage, Adriatic Sea basin. Folia Zool 53:399–410
Honma Y (1960) Sado awashima kinkai no okeru kuromekura unagi to kawayatsume. Saishu Shiiku 22:34–36
Hubbs CL, Potter IC (1971) Distribution, phylogeny and taxonomy. In: Hardisty MW, Potter IC (eds) The biology of lampreys, vol 1. Academic Press, London, pp 1–65
Idaho Administrative Code (2011) Rules governing classification and protection of wildlife: 13.01.06—Threatened or endangered species, Pacific lamprey (*Lampetra tridentata*), 7-1-93
Iliashenko VY, Iliashenko EI (2000) Krasnaya kniga Rossi: Pravovye akty. State Committee of the Russian Federation for Environmental Protection, Moscow
Isosaari P, Hallikainen A, Kiviranta H et al (2006) Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, naphthalenes and polybrominated diphenyl ethers in the edible fish caught from the Baltic Sea and lakes in Finland. Environ Pollut 141:213–225
IUCN (2013) IUCN Red List of threatened species. Version 2013.2. http://www.iucnredlist.org. Accessed 17 Jan 2014
Jackson A, Moser M (2012) Low-elevation dams are impediments to adult Pacific lamprey spawning migration in the Umatilla River, Oregon. N Am J Fish Manag 32:548–556
Jelks HL, Walsh SJ, Burkhead NM et al (2008) Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372–407
Jolley JC, Silver GS, Whitesel TA (2012) Occupancy and detection of larval Pacific lampreys and *Lampetra* spp. in a large river: the lower Willamette River. Trans Am Fish Soc 141:305–312
Kainua K, Valtonen T (1980) Distribution and abundance of European river lamprey (*Lampetra fluviatilis*) larvae in three rivers running into Bothnian Bay, Finland. Can J Fish Aquat Sci 37:1960–1966
Kan TT, Bond CE (1981) Notes on the biology of the Miller Lake lamprey *Lampetra (Entosphenus) minima*. Northwest Sci 55:70–74
Keefer ML, Daigle WR, Peery CA et al (2010) Testing adult Pacific lamprey performance at structural challenges in fishways. N Am J Fish Manag 30:376–385
Keefer ML, Peery CA, Lee SR, Daigle WR, Johnson EL (2011) Behaviour of adult Pacific lamprey in near-field flow and fishway design experiments. Fish Manag Ecol 18:177–189

Keefer ML, Caudill CC, Clabough TS et al (2013) Fishway passage bottleneck identification and prioritization: a case study of Pacific lamprey at Bonneville Dam. Can J Fish Aquat Sci 70:1551–1565

Kelly FL, King, JJ (2001) A review of the ecology and distribution of three lamprey species, *Lampetra fluviatilis* (L.), *Lampetra planeri* (Bloch) and *Petromyzon marinus* (L.): a context for conservation and biodiversity considerations in Ireland. Proc R Irish Acad 101B:165–185

Kiabi BH, Abdoli A, Naderi M (1999) Status of the fish fauna in the south Caspian basin of Iran. Zool Middle East 18:57–65

Kidd K, Batchelar K (2012) Mercury. In: Wood CM, Farrell AP, Brauner CJ (eds) Fish physiology: homeostasis and toxicology of non-essential metals, vol 31B. Academic Press, New York, pp 237–295

King EL Jr, Gabel JA (1985) Comparative toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol to ammocoetes of three species of lampreys. Great Lakes Fishery Commission, Technical Report 47, Ann Arbor

King JJ, Hanna G, Wightman GD (2008) Ecological impact assessment of the effects of statutory arterial drainage maintenance activities on three lamprey species (*Lampetra planeri* Bloch, *Lampetra fluviatilis* L. and *Petromyzon marinus* L.). Series of Ecological Assessments on Arterial Drainage Maintenance No 9, Office of Public Works, Headford, Ireland. http://www.opw.ie/media/Issue%20No.%209%20EcIA%203%20Lamprey%20Species.pdf. Accessed 23 May 2014

King JJ, Lehane BM, Wightman GD, Dooley R, Gilligan N (2011) Development and implementation of environmental protocols in river maintenance in Ireland. Water Environ J 25:422–428

Kircheis FW (2004) Sea lamprey *Petromyzon marinus* Linnaeus 1758. FW Kircheis LLC, Carmel ME. http://www.fws.gov/GOMCP/pdfs/lampreyreport.pdf. Accessed 6 June 2014

Klamath River Expert Panel (2010) Scientific assessment of two dam removal alternatives on lamprey, final report. http://northamerica.atkinsglobal.com/~/media/Files/A/Atkins-North-America/Attachments/klamath-river/Lamprey%20Final%20EP%20Report%201-14-11.pdf. Accessed 20 Dec 2013

Kostow K (2002) Oregon lampreys: natural history, status, and analysis of management issues. Oregon Department of Fish and Wildlife, Portland

Kottelat M, Bogutskaya NG, Freyhof J (2005) On the migratory Black Sea lamprey and the nomenclature of the ludoga, Peipsi and ripus whitefishes (Agnatha: Petromyzontidae; Teleostei: Coregonidae). Zoosyst Rossica 14:181–186

Kurath G, Jolley JC, Thompson TM et al (2013) Ammocoetes of Pacific lamprey are not susceptible to common fish rhabdoviruses of the U.S. Pacific Northwest. J Aquat Anim Health 25:274–280

Lajus J, Kraikovski A, Lajus D (2013) Coastal fisheries in the eastern Baltic Sea (Gulf of Finland) and its basin from the 15 to the early 20th centuries. PLoS ONE 8:e77059

Lanzing WJR (1959) Studies on the river lamprey, *Lampetra fluviatilis*, during its anadromous migration. Uitgeversmaatschappij, Utrecht

Larinier M (2001) Environmental issues, dams and fish migration. In: Marmulla G (ed) Dams, fish and fisheries. Opportunities, challenges and conflict resolution. FAO Fisheries Technical Paper No 419, FAO, Rome, pp 45–90

Lassalle G, Béguer M, Beaulaton L, Rochard E (2008) Diadromous fish conservation plans need to consider global warming issues: an approach using biogeographical models. Biol Conserv 141:1105–1118

Lelek A (1987) The freshwater fishes of Europe, vol 9. Threatened fishes of Europe. Aula, Wiesbaden

Li W, Sorensen PW, Gallaher DD (1995) The olfactory system of migratory adult sea lamprey (*Petromyzon marinus*) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. J Gen Physiol 105:569–587

Lin B, Zhang Z, Wang Y et al (2008a) Amplified fragment length polymorphism assessment of genetic diversity in Pacific lampreys. N Am J Fish Manag 28:1182–1193

Lin B, Zhang Z, Wang Y et al (2008b) Erratum: amplified fragment length polymorphism assessment of genetic diversity in Pacific lampreys. N Am J Fish Manag 28:1648

Lorion CM, Markle DF, Reid SB, Docker MF (2000) Redescription of the presumed-extinct Miller Lake lamprey, *Lampetra minima*. Copeia 2000:1019–1028

Lucas MC, Baras E (2001) Migration of freshwater fishes. Blackwell, Oxford
Lucas MC, Bubb DH, Jang M-H, Ha K, Masters JEG (2009) Availability of and access to critical habitats in regulated rivers: effects of low-head barriers on threatened lampreys. Freshw Biol 54:621–634
Luzier CW, Brostom J, Cook-Tabor C et al (2009) Proceedings of the Pacific Lamprey Conservation Initiative Work Session—28–29 Oct 2008. US Fish and Wildlife Service, Portland, OR. http:www.fws.gov/columbiariver/publications/Lamprey_Conservation_Proceedings_Final_09.pdf. Accessed 12 Dec 2013
Lyle AA, Maitland PS (1992) Conservation of freshwater fish in the British Isles: the status of fish in National Nature Reserves. Aquat Conserv Mar Freshw Ecosyst 2:19–34
Lyons J, Cochran PA, Polaco OJ, Merino-Nambo E (1994) Distribution and abundance of the Mexican lampreys (Petromyzontidae: *Lampetra*: subgenus *Tetrapleurodon*). Southwest Nat 39:105–113
MacEachen DC, Russell RW, Whittle DM (2000) Spatial distribution of mercury and organochlorine contaminants in Great Lakes sea lamprey (*Petromyzon marinus*). J Great Lakes Res 26:112–119
Maitland PS (1980) Review of the ecology of lampreys in northern Europe. Can J Fish Aquat Sci 37:1944–1952
Maitland PS (1987) Fish in the Clyde and Leven systems—a changing scenario. Proc Inst Fish Manag Conf 1987:13–20
Maitland PS (1991) Climate change and fish in northern Europe: some possible scenarios. Proc Inst Fish Manag, Annu Stud Course 22:97–110
Maitland PS (2003) Ecology of the river, brook and sea lamprey. Conserving Natura 2000, Rivers Ecology Series No 5. English Nature, Peterborough
Maitland PS, Lyle AA (1992) Conservation of freshwater fish in the British Isles: proposals for management. Aquat Conserv 2:165–183
Mallatt J, Barron MG, McDonough C (1986) Acute toxicity of methyl mercury to the larval lamprey, *Petromyzon marinus*. Bull Environ Contam Toxicol 37:281–288
Manion, PJ (1968) Production of sea lamprey larvae from nests in two Lake Superior streams. Trans Am Fish Soc 97:484–486
Mantua NJ, Hare SR, Zhang Y, Wallace JM, Francis RC (1997) A Pacific interdecadal climate oscillation with impacts on salmon production. Bull Am Meteorol Soc 78:1069–1079
Marsden JE, Siefkes MJ (in press) Sea lamprey control in the Great Lakes, Finger Lakes, and Lake Champlain. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht
Martin H, White MM (2008) Intraspecific phylogeography of the least brook lamprey (*Lampetra aepyptera*). Copeia 2008:579–585
Masters JEG, Jang MH, Ha K et al (2006) The commercial exploitation of a protected anadromous species, the river lamprey (*Lampetra fluviatilis* (L.)), in the tidal River Ouse, north-east England. Aquat Conserv Mar Freshw Ecosyst 16:77–92
Mateus CS, Almeida PR, Quintella BR, Alves MJ (2011) mtDNA markers reveal the existence of allopatric evolutionary lineages in the threatened lampreys *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) in the Iberian glacial refugium. Conserv Genet 12:1061–1074
Mateus CS, Rodríguez-Muñoz R, Quintella BR, Alves MJ, Almeida PR (2012) Lampreys of the Iberian Peninsula: distribution, population status and conservation. Endanger Species Res 16:183–198
Mateus CS, Alves MJ, Quintella BR, Almeida PR (2013a) Three new cryptic species of the lamprey genus *Lampetra* Bonnaterre, 1788 (Petromyzontiformes: Petromyzontidae) from the Iberian Peninsula. Contrib Zool 82:37–53
Mateus CS, Stange M, Berner D et al (2013b) Strong genome-wide divergence between sympatric European river and brook lampreys. Curr Biol 23:R649–R650
McDowall RM (1990) New Zealand freshwater fishes—a natural history and guide. Heinemann Reed, Auckland
McPhail JD, Lindsey CC (1970) Freshwater fishes of northwestern Canada and Alaska. Bulletin Fisheries Research Board of Canada 173, Ottawa

Mecklenburg CW, Mecklenburg TA, Thorsteinson LK (2002) Fishes of Alaska. American Fisheries Society, Bethesda

Meeuwig MH, Bayer JM, Seelye JG (2005) Effects of temperature on survival and development of early life stage Pacific and western brook lampreys. Trans Am Fish Soc 134:19–27

Merivirta LO, Nordlund J, Korkeala HJ (2001) Cadmium, mercury and lead content of river lamprey caught in Finnish rivers. Arch Lebensmittelhyg 52:69–71

Mesa MG, Copeland ES (2009) Critical uncertainties and research needs for the restoration and conservation of native lampreys in North America. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. Amercian Fisheries Society, Symposium 72, Bethesda, pp 311–321

Mesa MG, Bayer JM, Seelye JG (2003) Swimming performance and physiological responses to exhaustive exercise in radio-tagged and untagged Pacific lampreys. Trans Am Fish Soc 132:483–492

Morman RH (1979) Distribution and ecology of lampreys in the Lower Peninsula of Michigan, 1957–1975. Great Lakes Fishery Commission, Technical Report 33, Ann Arbor

Morman RH, Cuddy DW, Rugen PC (1980) Factors influencing the distribution of sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can J Fish Aquat Sci 37:1811–1826

Moser ML, Close DA (2003) Assessing Pacific lamprey status in the Columbia River basin. Northwest Sci 77:116–125

Moser ML, Mesa MG (2009) Passage considerations for anadromous lampreys. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 115–124

Moser ML, Ocker PA, Stuehrenberg LC, Bjornn TC (2002a) Passage efficiency of adult Pacific lampreys at hydropower dams on the lower Columbia River, USA. Trans Am Fish Soc 131:956–965

Moser ML, Matter AL, Stuehrenberg LC, Bjornn TC (2002b) Use of an extensive radio receiver network to document Pacific lamprey (*Lampetra tridentata*) entrance efficiency at fishways in the lower Columbia River, USA. Hydrobiologia 483:45–53

Moser ML, Keefer ML, Pennington HT, Ogden DA, Simonson JE (2011) Development of Pacific lamprey fishways at a hydropower dam. Fish Manag Ecol 18:190–200

Moursund RA, Dauble DD, Langeslay M (2003) Turbine intake diversion screens: investigating effects on Pacific lamprey. Hydro Rev 22:40–46

Moyle, PB, Brown LR, Chase SD, Quiñones RM (2009) Status and conservation of lampreys in California. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 279–292

Murauskas JG, Orlov AM, Siwicke KA (2013) Relationships between the abundance of Pacific Lamprey in the Columbia River and their common hosts in the marine environment. Trans Am Fish Soc 142:143–155

Naseka AM, Tuniyev SB, Renaud CB (2009) *Lethenteron ninae*, a new nonparasitic lamprey species from the north-eastern Black Sea basin (Petromyzontiformes: Petromyzontidae). Zootaxa 2198:16–26

NatureServe (2013) NatureServe Explorer: an online encyclopedia of life [web application]. Version 7.1. http://www.natureserve.org/explorer. Accessed 17 Jan 2014

Nazari H, Abdoli A (2010) Some reproductive characteristics of endangered Caspian Lamprey (*Caspiomyzon wagneri* Kessler, 1870) in the Shirud River southern Caspian Sea, Iran. Environ Biol Fish 88:87–96

Nelson EW (1887) Field notes on Alaskan fishes, part III. The Miscellaneous Documents of the Senate of the United States for the first session of the 49th Congress, Washington, DC, vol 8, Misc Doc No 156, pp 299–322

Nelson MC, Nelle RD (2007) Juvenile Pacific lamprey use of a pollution abatement pond on the Entiat National Fish Hatchery. Final Report, US Fish and Wildlife Service, Leavenworth

Nelson JS, Crossman EJ, Espinosa-Pérez H et al (2004) Common and scientific names of fishes from the United States, Canada, and Mexico, 6th edn. American Fisheries Society, Special Publication 29, Bethesda

Nika N, Virbickas T (2010) Brown trout *Salmo trutta* redd superimposition by spawning *Lampetra* species in a lowland stream. J Fish Biol 77:2358–2372

Nunn AD, Cowx IG (2012) Restoring river connectivity: prioritizing passage improvements for diadromous fishes and lampreys. Ambio 41:402–409

O'Connor W (2004) A survey of juvenile lamprey populations in the Moy catchment. Ir Wildl Man 15:1–36

O'Connor W (2006) A survey of juvenile lamprey populations in the Boyne catchment. Ir Wildl Man 24:1–45

Ojutkangas E, Aronen K, Laukkanen E (1995) Distribution and abundance of river lamprey (*Lampetra fluviatilis*) ammocoetes in the regulated River Perhonjoki. Regul Rivers: Res Manag 10:239–245

Oliveira JM, Ferreira MT, Pinheiro AN, Bochechas JH (2004) A simple method for assessing minimum flows in regulated rivers: the case of sea lamprey reproduction. Aquat Conserv Mar Freshw Ecosyst 14:481–489

O'Reilly M (2000) Migrating river lampreys at the tidal weir, Glasgow. Glasgow Nat 23:57–58

Page LM, Espinosa-Pérez H, Findley LT et al (2013) Common and scientific names of fishes from the United States, Canada, and Mexico, 7th edn. American Fisheries Society, Special Publicatin 34, Bethesda

Pedro S, Caçador I, Quintella BR, Lança MJ, Almeida PR (2014) Trace element accumulation in anadromous sea lamprey spawners. Ecol Freshw Fish 23:193–207

Pereira AM, Robalo JI, Freyhof J et al (2010) Phylogeographical analysis reveals multiple conservation units in brook lampreys *Lampetra planeri* of Portuguese streams. J Fish Biol 77:361–371

Petersen Lewis RS (2009) Yurok and Karuk traditional ecological knowledge: insights into Pacific lamprey populations of the Lower Klamath Basin. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 1–39

Quintella BR (2006) Biology and conservation of sea lamprey (*Petromyzon marinus* L.) in Portugal. PhD thesis, University of Lisbon, Lisbon

Quintella BR, Andrade NO, Koed A, Almeida PR (2004) Behavioural patterns of sea lampreys' spawning migration through difficult passage areas, studied by electromyogram telemetry. J Fish Biol 65:961–972

Quintella BR, Andrade NO, Dias NM, Almeida PR (2007) Laboratory assessment of sea lamprey larvae burrowing performance. Ecol Freshw Fish 16:177–182

Renaud CB (1997) Conservation status of Northern Hemisphere lampreys (Petromyzontidae). J Appl Ichthyol 13:143–148

Renaud CB (2011) Lampreys of the world. An annotated and illustrated catalogue of lamprey species known to date. FAO Species Catalogue for Fishery Purposes 5, FAO, Rome

Renaud CB, Cochran PA (in press) Post-metamorphic feeding in lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 2. Springer, Dordrecht

Renaud CB, Economidis PS (2010) *Eudontomyzon graecus*, a new nonparasitic lamprey species from Greece (Petromyzontiformes: Petromyzontidae). Zootaxa 2477:37–48

Renaud CB, Kaiser KLE, Comba ME (1995) Historical versus recent levels of organochlorine contaminants in lamprey larvae of the St. Lawrence River basin, Québec. Can J Fish Aquat Sci 52:268–275

Renaud CB, Wong HKT, Metcalfe-Smith JL (1998) Trace metal levels in benthic biota from four tributaries to the St. Lawrence River, Quebec. Water Qual Res 33:595–610

Renaud, CB, Docker, MF, Mandrak NE (2009) Taxonomy, distribution and conservation of lampreys in Canada. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 293–309

Rodríguez-Muñoz R, Nicieza AG, Braña F (2001) Effects of temperature on developmental performance, survival and growth of sea lamprey embryos. J Fish Biol 58:475–486

Rodríguez-Muñoz R, Waldman JR, Grunwald C, Roy NK, Wirgin I (2004) Absence of shared mitochondrial DNA haplotypes between sea lamprey from North American and Spanish rivers. J Fish Biol 64:783–787

Russon IJ, Kemp PS, Lucas MC (2011) Gauging weirs impede the upstream migration of adult river lamprey *Lampetra fluviatilis*. Fish Manag Ecol 18:201–210
Santos JM, Ferreira MT, Godinho FN, Bochechas J (2005) Efficacy of a nature-like bypass channel in a Portuguese lowland river. J Appl Ichthyol 21:381–388
Schuldt RJ, Goold R (1980) Changes in the distribution of native lampreys in Lake Superior tributaries in response to sea lamprey (*Petromyzon marinus*) control, 1953–1977. Can J Fish Aquat Sci 37:1872–1885
Sjöberg K (1980) Ecology of the European river lamprey (*Lampetra fluviatilis*) in northern Sweden. Can J Fish Aquat Sci 37:1974–1980
Sjöberg K (2011) River lamprey *Lampetra fluviatilis* (L.) fishing in the area around the Baltic Sea. J North Stud 5:51–86
Soimasuo M, Kervinen J, Sinkkonen S, Paasivirta J (2004) Bioaccumulation of POPs from contaminated sediment to lamprey (*Lampetra fluviatilis* L.) larva. J Soils Sediments 4:75–83
Sorensen PW, Fine JM, Dvornikovs V et al (2005) Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. Nat Chem Biol 1:324–328
Spice EK, Goodman DH, Reid SB, Docker MF (2012) Neither philopatric nor panmictic: microsatellite and mtDNA evidence suggests lack of natal homing but limits to dispersal in Pacific lamprey. Mol Ecol 21:2916–2930
Starcevich S, Clements S (2013) Larval lamprey distribution and habitat use in small stream channels on the Oregon coast. Oregon Department of Fish and Wildlife, Progress Report, Corvallis
Stewart M, Baker CF (2012) A sensitive analytical method for quantifying petromyzonol sulfate in water as a potential tool for population monitoring of the southern pouched lamprey, *Geotria australis*, in New Zealand streams. J Chem Ecol 38:135–144
Stier K, Kynard B (1986) Abundance, size, and sex ratio of adult sea-run sea lampreys, *Petromyzon marinus*, in the Connecticut River. Fish Bull 84:476–480
Stinson CM, Mallatt J (1989) Branchial ion fluxes and toxicant extraction efficiency in lamprey (*Petromyzon marinus*) exposed to methylmercury. Aquat Toxicol 15:237–251
Streif B (2009) Considering Pacific lampreys when implementing instream activities. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 255–268
Suíssas C (2010) Assessing the feasibility of commercial exploitation of sea lamprey (*Petromyzon marinus*) in Minho and Tagus river basins. Master's thesis, University of Évora, Évora, Portugal [in Portuguese, with English abstract]
Swift CC, Howard SR (2009) Current status and distribution of the Pacific lamprey south of Point Conception, southern coastal California, USA. In: Brown LR, Chase SD, Mesa MG, Beamish RJ, Moyle PB (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, pp 269–278
Takahashi MA (2009) Overview of the structure and the challenges of Japanese wildlife law and policy. Biol Conserv 142:1958–1964
Taverny C, Lassalle G, Ortusi I et al (2012) From shallow to deep waters: habitats used by larval lampreys (genus *Petromyzon* and *Lampetra*) over a western European basin. Ecol Freshw Fish 21:87–99
Taylor EB, Harris LN, Spice EK, Docker MF (2012) Microsatellite DNA analysis of parapatric lamprey (*Entosphenus* spp.) populations: implications for evolution, taxonomy, and conservation of a Canadian endemic. Can J Zool 90:291–303
Thomas JT, Culler ME, Dermisis DC et al (2013) Effects of grade control structures on fish passage, biological assemblages and hydraulic environments in western Iowa streams: a multidisciplinary review. River Res Appl 29:389–398
Torgersen CE, Close DA (2004) Influence of habitat heterogeneity on the distribution of larval Pacific lamprey (*Lampetra tridentata*) at two spatial scales. Freshw Biol 49:614–630
Townsend AJ, de Lange PJ, Duffy CAJ et al (2008) New Zealand threat classification system manual. Dep Conserv, Wellington. http://www.doc.govt.nz/upload/documents/science-and-technical/sap244.pdf. Accessed 12 Dec 2013
Travade F, Larinier M (1992) La migration de dévalaison: problèmes et dispositifs. Bull Fr Pêche Piscic 326/327:165–206

Turner LM (1886) Contributions to the natural history of Alaska, part IV, Fishes. The Miscellaneous Documents of the Senate of the United States for the first session of the 49th Congress, Washington, DC, vol 8, Misc Doc No 155, pp 87–113

Tuunainen P, Ikonen E, Auvinen H (1980) Lampreys and lamprey fisheries in Finland. Can J Fish Aquat Sci 37:1953–1959

UICN France, MNHN, SFI and ONEMA (2010) La liste rouge des espèces menacées en France—Chapitre Poissons d'eau douce de France métropolitaine, Paris. http://www.uicn.fr/Liste-rouge-France.html. Accessed 18 Feb 2014

United States Fish and Wildlife Service (2004) Endangered and threatened wildlife and plants; 90-day finding on a petition to list three species of lampreys as threatened or endangered. Fed Regist 69:77158–77167

United States Fish and Wildlife Service (2012) Conservation agreement for Pacific lamprey (*Entosphenus tridentatus*) in the states of Alaska, Washington, Idaho, Oregon, and California. http://www.fws.gov/pacific/fisheries/sphabcon/lamprey/pdf/Pacific_Lamprey_CI.pdf. Accessed 27 May 2014

Valtonen T (1980) European river lamprey (*Lampetra fluviatilis*) fishing and lamprey populations in some rivers running into Bothnian Bay, Finland. Can J Fish Aquat Sci 37:1967–1973

Verreycken H, Belpaire C, Van Thuyne G et al (2014) IUCN Red List of freshwater fishes and lampreys in Flanders (north Belgium). Fish Manag Ecol 21:122–132

Vladykov VD (1952) Distribution des lamproies (Petromyzonidae) dans la province de Québec. Nat Can (Qué) 79:85–120

Vladykov VD (1973) North American nonparasitic lampreys of the family Petromyzonidae must be protected. Can Field-Nat 87:235–239

Vladykov VD, Kott E (1976) A new nonparasitic species of lamprey of the genus *Entosphenus* Gill, 1862 (Petromyzonidae) from south central California. Bull South Calif Acad Sci 75:60–67

Vrieze LA, Sorensen PW (2001) Laboratory assessment of the role of a larval pheromone and natural stream odor in spawning stream localization by migratory sea lamprey (*Petromyzon marinus*). Can J Fish Aquat Sci 58:2374–2385

Wallace RL, Ball KW (1978) Landlocked parasitic Pacific lamprey in Dworshak Reservoir, Idaho. Copeia 1978:545–546

Ward DL, Clemens BJ, Clugston D et al (2012) Translocating adult Pacific lamprey within the Columbia River basin: state of the science. Fisheries 37:351–361

Wheeler A (1979) The tidal Thames. Routledge and Keegan Paul, London

Wilson FH (1955) Lampreys in the Lake Champlain basin. Am Midl Nat 54:163–172

Witkowski A (1996) Distribution of the river lamprey *Lampetra fluviatilis* (Linnaeus, 1758) in inland waters of Poland and reasons for the species decline. Bull Lampetra 2:77–82

Yamazaki Y (2007) Microhabitat use by the larvae of cryptic lamprey species in *Lethenteron reissneri* in a sympatric area. Ichthyol Res 54:24–31

Yamazaki Y, Fukutomi N, Oda N et al (2005) Occurrence of larval Pacific lamprey *Entosphenus tridentatus* from Japan, detected by random amplified polymorphic DNA (RAPD) analysis. Ichthyol Res 52:297–301

Yamazaki Y, Yokoyama R, Nishida M, Goto A (2006) Taxonomy and molecular phylogeny of *Lethenteron* lampreys in eastern Eurasia. J Fish Biol 68(Suppl B):251–269

Yamazaki Y, Yamano A, Oura K (2011) Recent microscale disturbance of gene flow in threatened fluvial lamprey, *Lethenteron* sp. N, living in a paddy water system. Conserv Genet 12:1373–1377

Yamazaki Y, Yokoyama R, Nagai T, Goto A (2014) Population structure and gene flow among anadromous Arctic lamprey (*Lethenteron camtschaticum*) populations deduced from polymorphic microsatellite loci. Environ Biol Fish 97:43–52

Yun SS, Wildbill AJ, Siefkes MJ et al (2011) Identification of putative migratory pheromones from Pacific lamprey (*Lampetra tridentata*). Can J Fish Aquat Sci 68:2194–2203

Zanandrea G (1959) Speciation among lampreys. Nature 184:380

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