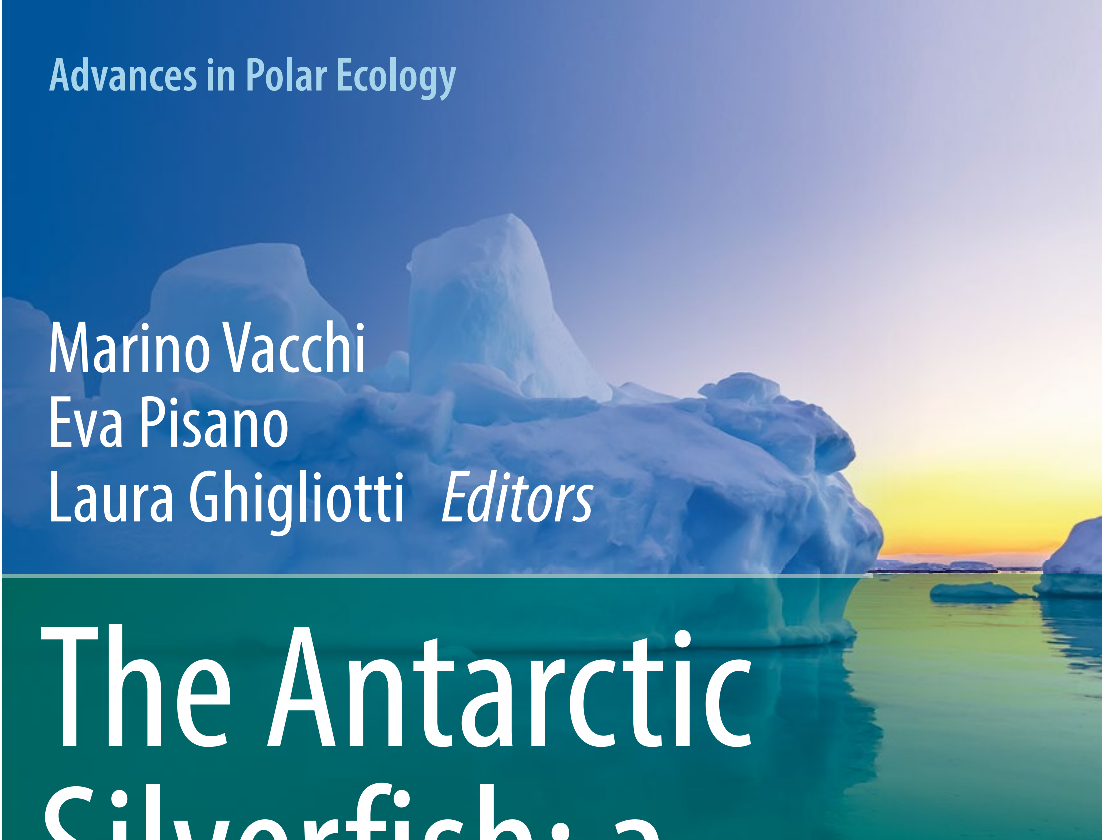
Advances in Polar Ecology



Marino Vacchi
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The Antarctic Silverfish: a Keystone Species in a Changing Ecosystem

Springer

Advances in Polar Ecology

Volume 3

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Advances in Polar Ecology

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Foreword

Implementation of the CCAMLR (Commission for the Conservation of Antarctic Marine Living Resources) ecosystem approach to the management of the marine living resources and environment of the Southern Ocean requires a thorough understanding of the biology and ecology of exploited, dependent and related species. CCAMLR came into force in 1982, primarily because of scientific, political and environmental concerns surrounding the potential detrimental impact of an expanding krill fishery upon the Southern Ocean ecosystem. Since the outset, a major scientific focus has been on Antarctic krill, *Euphausia superba*, and krill-dependent predators predominantly in the Atlantic sector of the Southern Ocean, a region where the krill fishery has traditionally operated.

However, in recent decades, the development of deep-water longline fisheries for toothfish (*Dissostichus* spp.) has led to an increasing scientific focus on other regions of the Southern Ocean where Antarctic krill are less abundant and play a reduced role in the ecosystem. In such regions, other macro zooplankton species or pelagic fish are important prey species in place of krill. Within the circum-Antarctic, high-latitude pelagic realm, the Antarctic silverfish, *Pleuragramma antarctica*, is a key prey species forming a significant part of the diet of a range of air-breathing and fish predators including the Antarctic toothfish (*Dissostichus mawsoni*). As the fishery for this species has developed at high latitudes in the Southern Ocean, there has been an increasing need to understand the ecology of the key species in the ecosystems within which the fishery operates.

The 13 chapters of this book provide, for the first time in a single volume, a comprehensive overview of the current state of knowledge of *P. antarctica*, a species that was once, itself, the target of a small exploratory fishery and until recently remained relatively poorly studied. The chapters cover a range of scientific disciplines including evolutionary adaptation, ecophysiology, trophic ecology and reproductive and population ecology, falling within three broad themes, namely, evolution and adaptations, ecology and life history and challenges and conservation perspectives. The broad circumpolar distribution of the Antarctic silverfish coupled with its key role in the Antarctic continental shelf pelagic ecosystem and physiological adaptation to living in a hostile environment makes understanding the species' likely response to environmental change a key component in the future functioning of the regional ecosystem. Additionally, a detailed understanding of the abundance and trophic interactions of such a dominant keystone species is a vital element of informing the development of marine spatial planning and marine protected areas in the Antarctic continental shelf region.

The editors are to be congratulated for bringing together the impressive list of contributors who have provided such a broad diversity of expertise relating to the ecology of *P. antarctica*. This book will undoubtedly be a key publication not only for those interested in the ecology of this keystone species but also more broadly for those interested in the conservation of Antarctic marine living resources and the impacts of climate change upon them.

Chair of the CCAMLR Scientific Committee Mark Belchier
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Preface

Soon after the description of the species *Pleuragramma antarcticum* (Antarctic silverfish) by Boulenger in 1902, data collected during the first German and English South Polar expeditions in the early twentieth century (Pappenheim 1912, Regan 1916) suggested an important role for this small fish in the pelagic Antarctic system. The bulk of ecological information that has accumulated in subsequent years, especially in the 1970s and 1980s, confirmed the dominance of the Antarctic silverfish in the Antarctic shelf waters, where it plays a crucial role as the prevalent mid-trophic level fish species.

The ecological success of this pelagic swim-bladder-less teleost is the result of a long evolutionary journey shared with a handful of Antarctic fish relatives, an amazing story of organismal adaptive capability that attracted and still attracts the interest of a wide community of Antarctic researchers.

And yet, despite the increasing scientific interest toward the Antarctic silverfish, information on this species remains point and patchy, thus limiting our understanding of the potential of this Antarctic endemic fish to face climatic perturbations as individual species and limiting us from making sound predictions on the impact of the ongoing climate change on the Antarctic ecosystem. The need of filling gaps in the ecology and life history of the Antarctic silverfish, necessary to support regulation and protection initiatives in the Southern Ocean, emerged also from several reports by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) and by the Antarctic Treaty Consultative Meeting (ATCM).

In accepting to edit this Springer volume, we thought the time was ripe to gather and organize the sparse information on Antarctic silverfish. This book was then conceived with the main aim to provide the large community of scientists and Institutions interested in Antarctic marine sciences and conservation with authoritative and comprehensive reviews useful to highlight current knowledge and gaps and hopefully to direct further research to specific and critical issues.

The book includes 13 thematic chapters arranged in the 3 subject areas (parts):

1. Evolution and adaptations
2. Ecology and life history
3. Challenges and conservation perspectives

Part I focuses on adaptive characteristics of the Antarctic silverfish including the astonishing evolutionary reshaping of its skeleton structure (Chap. 1), freezing avoidance capability (Chap. 2), peculiarities of the oxygen-transport system (Chap. 3) and antioxidant system responsiveness (Chap. 4).

Part II deals with fundamental ecological issues such as diet and trophic ecology in adults (Chap. 5) and early stages (Chap. 6), lipid accumulation (Chap. 7), bioenergetics and lifestyle (Chap. 8). The readers will also find information here on the species' reproduction (Chap. 9) as well as on the population structure and life history connectivity (Chap. 10).

A review on acoustic methods useful for improving the monitoring of the species (Chap. 11) is opening Part III, followed by a chapter approaching the potential vulnerability of the Antarctic silverfish using the Western Antarctic Peninsula (WAP) as a model system of warming shelf waters (Chap. 12). In the context of protection initiatives, Chap. 13, by underlining the role of this midtrophic fish as sentinel of changes in the Antarctic marine pelagic ecosystem, stresses the importance of conducting research and monitoring as well as preserving essential habitats of the species' sensitive ecophases.

The editing of the book took longer than expected due to several reasons, including the commitment in field activities of authors, editors and reviewers. However, as a bright side of the coin, this delay allowed the almost concurrent publication of the volume with the coming into force of the newly designated Ross Sea Region Marine Protected Area, a historical step toward the conservation of Antarctic marine ecosystems. Such a momentous decision rewards the efforts of researchers engaged in Antarctic research, including all contributors to the present volume, and encourages the thought that even greater environmental challenges may be addressed in the future.

Note on Taxonomy and Nomenclature

In this book, the taxonomic status of *Pleuragramma* follows the classification by Nelson (2006) with the genus included in the Antarctic notothenioid family Nototheniidae. The use of the specific name *antarctica* instead of the former *antarcticum* follows the change in specific nomenclature currently acknowledged (http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp) and adopted in the *Biogeographic Atlas of the Southern Ocean* (2014).

Genoa, Italy

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Part I
Evolution and Adaptations

Chapter 1

Evolution Reshaped Life for the Water Column: The Skeleton of the Antarctic Silverfish *Pleuragramma antarctica* Boulenger, 1902

Olga Voskoboinikova, H. William Detrich III, R. Craig Albertson, John H. Postlethwait, Laura Ghigliotti, and Eva Pisano

Abstract The Antarctic silverfish is a neutrally buoyant notothenioid fish that lives in resource-rich pelagic habitats through all life history stages. Given the ancestral benthic origin of notothenioids, the ability of this species to live in the water column required extensive evolutionary adjustments centered on buoyancy, a process referred to as secondary pelagization. Many of these adaptive changes are found in the silverfish skeleton, including partial or total reduction of bony elements, which reduces skeletal mass and body density. Other novel skeletal traits, such as lengthening of bones of the ethmoidal portion of the neurocranium and jaws, are related to foraging in the water column and planctophagia.

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Reconfiguration of silverfish skeletal traits occurred via paedomorphosis (the retention of characters in adults that are juvenile traits in outgroup species) and reflects heterochronic shifts in gene expression during development.

In this chapter we describe the skeletal anatomy of silverfish larvae, juveniles and adults, including evidence for ontogenetic changes that relate to pelagic life. We then present the molecular basis of skeletal reduction as revealed by analysis of craniofacial gene expression during early skeletogenesis.

Keywords Secondary pelagization • Skeletogenesis • Bone mineralization

1.1 Introduction

1.1.1 Secondary Pelagization, a Major Step in the Evolutionary History of the Antarctic Silverfish

The Antarctic silverfish, *Pleuragramma antarctica* Boulenger, 1902, is a species of great ecological success among the endemic fish inhabiting Antarctic waters, a conclusion supported by a substantial literature of ecological and morphological data (e.g. DeWitt 1962; Andriashev 1965, 1976, 1987; DeWitt and Hopkins 1977; DeVries and Eastman 1978; Eastman and DeVries 1981, 1982; Voskoboinikova 1982, 1993, 2010; Hubold 1985; Kellermann 1986; DeWitt et al. 1990; Eastman 1993, 1997, 2005; Voskoboinikova et al. 1994; Eastman and Lannoo 2011; Vacchi et al. 2004, 2012a, b; La Mesa and Eastman 2012). The silverfish has a circum-Antarctic distribution along the continental shelf (Duhamel et al. 2014), is abundant as shown by the high values reported for its biomass (DeWitt 1970; Hubold and Ekau 1987; Hubold 1990; La Mesa and Eastman 2012), and plays a major role in the Antarctic coastal ecosystem (Eastman 1985; La Mesa et al. 2004). There is little doubt that the ecological success of the Antarctic silverfish relates to its use of resource-rich pelagic habitats throughout its life cycle (Marshall 1953; Andriashev 1965; Eastman and DeVries 1982; Hubold and Ekau 1990; North 1991; Eastman 1993; Voskoboinikova 2001, 2007).

The ability of the silverfish to live primarily in the water column arose as part of the evolution of the perciform suborder Notothenioidei in the Southern Ocean (e.g. Anderson 1990; Eastman and Clarke 1998; Balushkin 2000; Eastman 2005; Voskoboinikova 2010). Acquisition of antifreeze glycoproteins (AFGPs), estimated by Near et al. (2012) as occurring between 42 and 22 million years ago (Ma), was an important innovation that enabled Antarctic notothenioids to survive in the cooling, and eventually icy, marine waters surrounding the Antarctic continent (Cheng and Detrich 2007). As near-shore fish taxa lacking AFGPs became locally extinct, numerous ecological niches opened to notothenioids, which share a negatively buoyant benthic ancestor (Eastman and Clarke 1998). Subsequently, the remarkable morphological and ecological disparity among Antarctic notothenioids as a whole (Hu et al. 2016), and within various subclades, was achieved during multiple, independent diversification events after the onset of widespread sea ice in the Late Miocene (11.6–5.3 Ma) (Near et al. 2012). Along the buoyancy axis, species within several notothenioid subclades independently evolved to colonize benthic, epibenthic, semipelagic, cryopelagic and pelagic niches (Eastman 1993; Klingenberg and Ekau 1996; Near et al. 2012; Eastman et al. 2014).

Given the benthic origin of notothenioids, the presence of modern notothenioid species in niches throughout the water column must be based on evolutionary selection driving important morphological and physiological modifications during species diversification. Organismal re-shaping for life in the water column, occurring on an evolutionary timescale, is referred to as secondary pelagization (Nybelin 1947; Andriashev 1965, 1987; Klingenberg and Ekau 1996; Eastman 1997; Montgomery and Clements 2000). During secondary pelagization, natural selection favors reduction of body density to change buoyancy from negative values toward neutrality. Typically, fish enhance buoyancy by divergence in skeletal features, including reduction of bone mineralization (Eastman 1997; Eastman et al. 2014), and/or by corporeal lipid accumulation (e.g. Eastman 1993; DeVries and Eastman 1978).

Among notothenioids, the Antarctic silverfish can be placed at the pelagic extreme of the evolutionary/ecological axis from benthic to secondarily pelagic life style because it is neutrally buoyant (Eastman 1993, 1997; Eastman et al. 2014), a condition shared with four other notothenioid species (Near et al. 2007): *Aethotaxis mitopteryx*, *Dissostichus eleginoides, D. mawsoni,* and *Gvozdarus svetovidovi.* These five species belong to the subclade Pleuragrammatinae (Andersen and Hureau 1979; Balushkin 1992), consistent with the hypothesis that complete neutral buoyancy has a single evolutionary origin in the Notothenioidei (Near et al. 2007). The Antarctic silverfish is also unique among notothenioids as the only known holopelagic species, with all life history stages, from embryo through adult, living in the water column (e.g. DeWitt et al. 1990; Vacchi et al. 2012b). Distinctive characters of adult silverfish that have been associated with secondary pelagization include its haemoglobin system (reviewed in di Prisco and Verde 2017), its red-fibred lateralis superficialis muscle, its silver coloration and its laterally compressed body form [for details see Eastman (1997), Table 1; Montgomery and Clements (2000), Table 1]. Many of those characters are considered paedomorphic (Balushkin 1984; Voskoboinikova 1994, 2001; Clarke and Johnston 1996; Eastman 1997, Montgomery and Clements 2000).

Paedomorphosis (retention of ancestral juvenile traits by adults) is a major evolutionary process in notothenioids (Balushkin 1984, 1989, 2000; Voskoboinikova 1994, 2001, 2010; Albertson et al. 2010; Eastman et al. 2014) and implies heterochrony in the schedule of developmental events during ontogeny [for a review of terminology and concepts, see Klingenberg (1998)]. The Antarctic silverfish is among the few notothenioid fish in which heterochronic events leading to paedomorphic skeletal traits have been studied during early development through molecular analysis of gene expression (Albertson et al. 2010). In *Pleuragramma*, delayed branchial and cranial bone development results from heterochronic shifts in skeletal gene expression, leading to the prolongation of the chondrogenic developmental pathway compared to benthic Antarctic notothenioids (Albertson et al. 2010; Detrich and Amemiya 2010; Postlethwait et al. 2016).

In the following sections of this chapter, we provide: (a) an update of silverfish skeletal anatomy, based on published descriptions and on unpublished data (including larvae, juveniles and adults) prepared according to the bone and cartilage staining methods by Potthoff (1984), with an emphasis on paedomorphic skeletal traits; and (b) a summary of the molecular evidence for heterochrony during early skeletal development.

1.1.2 Note on Taxonomy and Nomenclature

In this review, the taxonomic status of Nototheniodei and *Pleuragramma* follows the classification by Nelson (2006) with the genus included in the Antarctic notothenioid family Nototheniidae. Within Nototheniidae, *Pleuragramma* is part of the subfamily Pleuragrammatinae (Andersen and Hureau 1979) along with *Aethotaxis*, *Dissostichus* spp. and *Gvozdarus* (Balushkin 1992). The use of the specific name *antarctica*, instead of the former *antarcticum*, follows Duhamel et al. (2014) and the currently acknowledged taxonomic nomenclature (http://research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp).

1.2 Anatomical Features of the Silverfish Skeleton

The skeletal anatomy of *P. antarctica* has been analyzed as part of systematic studies of the notothenioids (DeVries and Eastman 1981; Eastman and DeVries 1982; Voskoboinikova 1982, 1993, 2001, 2010; Andersen 1984; Balushkin 1989; DeWitt et al. 1990; Miller 1993). Many of these studies were targeted at assessing the taxonomic position of the species within the family Nototheniidae and at evaluating skeletal features related to life in the water column (Regan 1913; Norman 1938; Andersen and Hureau, 1979; Andersen 1984; Balushkin 1984, 1989; Voskoboinikova 1982, 1993). Overall, coverage of skeletal elements in adult nototheniids is incomplete. For example, the structure of the neurocranium has been described only for members of the subfamily Nototheniinae (Balushkin 1984, 1989), and little information (Eastman et al. 2014) has been provided for *Pleuragramma* prior to this report.

1.2.1 The Neurocranium

The neurocranium of adult *P. antarctica* (Fig. 1.1) is elongated compared to other nototheniids, especially compared to benthic species (Balushkin 1989, Figs. 6–14). Elongation is mainly due to lengthening of bones of the ethmoid region (i.e. the mesethmoid, lateral ethmoid, and vomer) and the elongation of bones that form the interorbital region (the frontals and parasphenoid) on the anterior/posterior (AP)

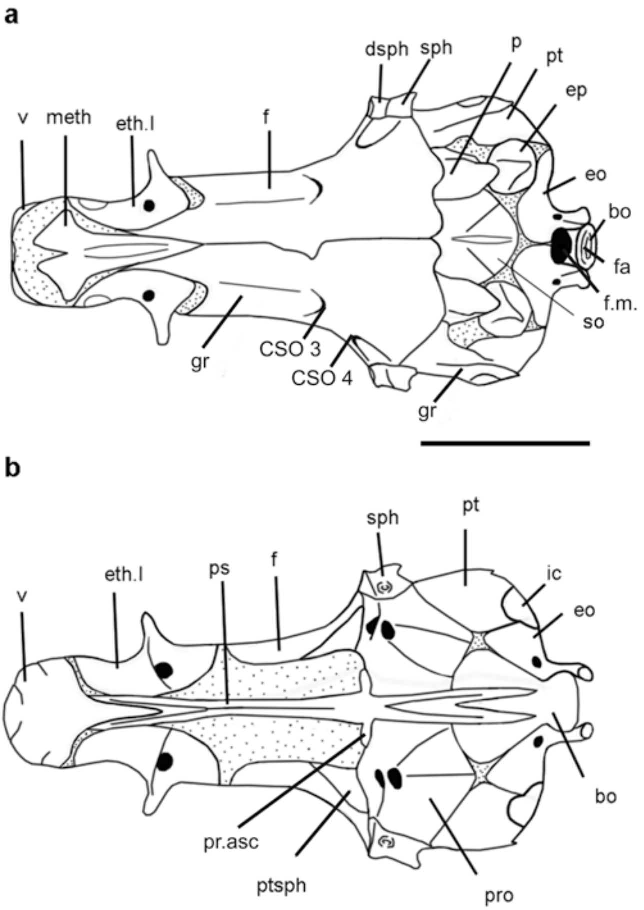


Fig. 1.1 The neurocranium of adult *P. antarctica* (specimen of SL 147.8 mm). (**a**) Dorsal view, (**b**) ventral view. *bo* basioccipital, *CSO3, CSO4* supraorbital canal pores, *sph* dermosphenoid, *eth.l* lateral ethmoid, *eo* exoccipital, *ep* epiotic, *f* frontal, *fa* articulating facet, *fm* foramen magnum, *gr* groove, *ic* intercalary, *meth* mesethmoid, *p* parietal, *pr.asc* ascending process of the parasphenoid, *ps* parasphenoid, *pro* prootic, *pt* pterotic, *ptsph* pterosphenoid, *so* supraoccipital, *sph* sphenotic, *v* vomer. Cartilage stippled. Scale bar = 10 mm

axis. The preponderance of cartilage in the ethmoid region and the skull, the thin lateral ethmoid, and the modest width of the interorbital space are also notable traits. Several features of other bones are noteworthy, including the unusual two-bladed shape of the anterior end of the mesethmoid that covers the upper surface of the ethmoidal region dorsally (probably with a protective function) and the elongated projections of the lateral ethmoid (Fig. 1.1a). The rectangular vomer has an extended anterior/posterior arm that reaches the middle of the lateral ethmoid (Fig. 1.1). The canals of the seismosensory system on the frontal, sphenotic and pterotic are almost completely lost but the longitudinal grooves on these bones indicate their antecedent location during development. The exception is the nasal, which forms a small tube through which an anterior segment of the supraorbital canal passes, as well as a small portion of the channel between the pores CSO3 and CSO4 on the frontal (Fig. 1.1a).

Ventrally, the slender parasphenoid (Fig. 1.1b) is situated within an elongated anterior region. Posteriorly, the parasphenoid forms a shortened ascending process and a posterior branch rostral to the basioccipital. The shape of bones forming the base of the skull – the basioccipitals and exoccipitals – differ from those of other nototheniids (see Balushkin 1989, Figs. 6–14) due to a laterally elongated exoccipital and the large size of the articular facet and foramen magnum. The infraorbital ring in silverfish adults includes the lacrimal, the infraorbital 4 and dermosphenotic similar to those of other nototheniids. In contrast to most other nototheniids (Jakubowski 1971; Andersen 1984), infraorbitals 2 and 3 are absent (see Fig. 1.3a), leaving a gap in the corresponding seismosensory canal.

The shape of the neurocranium in *Pleuragramma* differs from those of other nototheniids even in early stages of development (Voskoboinikova et al. 1994; Voskoboinikova and Kellermann 1997; Voskoboinikova 2010). As shown in Fig. 1.2,

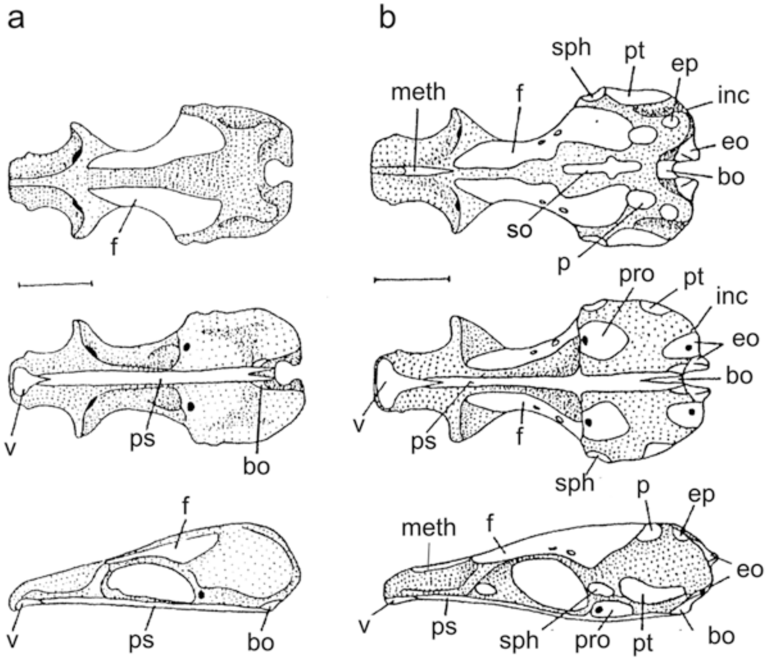


Fig. 1.2 Development of the neurocranium of *P. antarctica* showing dorsal (*upper*), ventral (*middle*) and left lateral (*bottom*) views. (**a**) specimen of SL 33.0 mm, (**b**) specimen of SL 44.9 mm. *bo* basioccipital, *eo* exoccipital, *ep* epiotic, *f* frontal, *inc* intercalar, *meth* mesethmoid, *p* parietal, *ps* parasphenoid, *pro* prootic, *pt* pterotic, *so* supraoccipital, *sph* sphenotic, *v* vomer. Cartilage stipped, ossifying white. Scale bars = 1 mm

the neurocranium of a young silverfish of 33 mm SL (approximately 9–10 months old) is already rather elongated, with a long and low ethmoidal region, a broad interorbital space, and convex dorsal profile of the skull (Fig. 1.2a). In a specimen of SL 44.9 mm (approximately 2 years old), the relative length and height of the ethmoidal region and the width of the interorbital space remain constant, the dorsal profile of the skull is straightened and the ventral profile becomes strongly bent (Fig. 1.2b), as also occurs in *A. mitopteryx* (Voskoboinikova and Kellermann 1997).

Later in development (from specimens at SL 72.1 mm), the longitudinal ridge along the mesethmoideum is formed which later develops two small lateral plates anteriorly divided by a narrow notch. In adults, such plates spread and touch laterally the margins of the ethmoidal region and the notch becomes deeper (Voskoboinikova et al. 1994). The most notable ontogenetic changes occur in the basioccipital and exoccipital. In the basioccipital, an articular facet of unusually large size compared to other nototheniids begins to form in specimens of 30 mm SL (Fig. 1.2a; see also Voskoboinikova et al. (1994)). In specimens of SL ≥ 40.0 mm, the exoccipital occurs as a small ossification lateral to the foramen magnum and closer to the upper than the temporal edge of the endocranium (not shown). From 44.0 mm, SL the exoccipital forms the well-developed condyle for the first vertebra (Fig. 1.2b). Later in development the condyle descends to the level of the temporal edge of the skull and its articular surface turns downward. In adults, the structure of these bones become similar to those of other nototheniids. Thus, their early features and changes during development are related to the large dimensions of the larval vertebral centra, which become relatively much smaller in adults.

1.2.2 The Splanchnocranium

In *P. antarctica* adults, elongation of the jaws, bones of the palatoquadrate, hyoid and gill arches and opercular bones are pronounced (Figs. 1.3 b, b′, b″ and 1.4). Most of these bony elements have structural features unique to nototheniids (Voskoboinikova 1982, 1993). Small and villiform teeth are arranged in 1–2 rows on the premaxillae and dentary (Fig. 1.3b). One tooth from the inner row at the symphysis of the premaxillae and 1–2 teeth in the middle of the first row of the dentary appear significantly enlarged relative to the teeth in other nototheniids. The mouth is slightly protractile because the ascending process of the premaxillae constitutes about one-third of the bone and the postmaxillar process is large. The posterior end of the maxillae forms an incisure, a common trait in Pleuragrammatinae.

The suspensorium (Fig 1.3b′) shows a number of prominent specific characters (Voskoboinikova 1993). The bones of this skeletal part are much thinner with respect to those of other nototheniids, and the elongate palatine directs forward and up, covering only a quarter to a third of the anterior edge of the pterygoid. The mesopterygoid does not lean on the quadrate medially. The posterior process of the quadrate is longer and higher than in other nototheniids. On the hyomandibulae, the channel of the facial nerve on the outer surface is sharply reduced and a foramen for

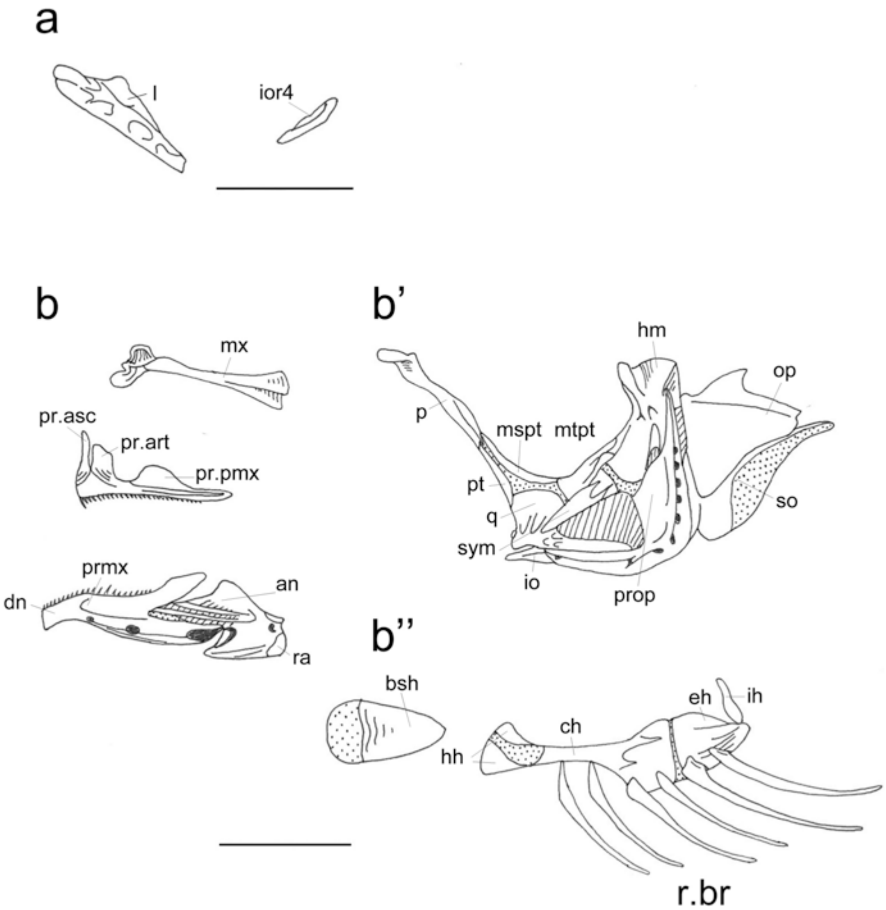


Fig. 1.3 The circumorbital ring (**a**) and the splanchnocranium (**b**, **b′**, **b″**) of adult *P. antarctica.* Specimen of SL 147.8 mm. (**a**) Circumorbital ring: *l* lacrimal, *ior4* interorbital; (**b**) Jaws: *an* anguloarticular, *dn* dentary, *mx* maxillae, *pr.art* articular process, *pr.asc* ascending process, *pr.pmx* postmaxillar process, *prmx* premaxillae, *ra* retroarticulare; (**b′**) Suspensorium: *hm* hyomandibular, *io* interopercle, *mspt* mesopterygoid, *mtpt* metapterygoid, *op* opercle, *p* palatine, *prop* preopercle, *pt* pterygoid, *q* quadratum, *so* subopercle, *sym* symplecticum; (**b″**) Hyoid arch, ventral part: *bsh* basihyal, *ch* ceratohyal, *eh* epihyal, *hh* hypohyals, *ih* interhyal, *r.br* radii branchiostegi. Scale bars = 10 mm

the facial nerve is located at the base of the anterior upper process of this bone. The front edge of the hyomandibulae and an intermediate portion of the preopercle are greatly reduced, and the latter does not reach the symplectic. Although the intermediate portion of the preopercle is reduced, this bone as a whole is unusually wide with very large pores of the seismosensory canal. The opercle has an elongated posterior end. The upper process of the opercle forms a small triangle that differs from the higher processes present in other nototheniids. Reduction compensatory ridges are located on the opercle, symplectic, epi- and ceratohyals. Other opercular

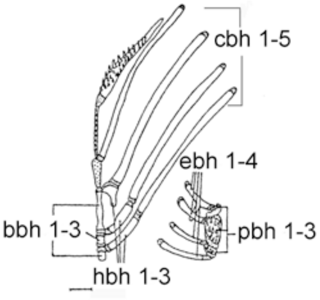


Fig. 1.4 Gill arches of *P. antarctica,* specimen of SL 83.1 mm. *bbh* basibranchials, *hbh* hypobranchials, *cbh* ceratobranchials, *ebh* epibranchials, *pbh* pharyngobranchials. Scale bar = 1 mm

bones are smooth. The presence, in the hyoid arch (Fig. 1.3b″), of a large basihyal of almost oval shape and of truncated branchiostegal rays with relatively enlarged heads is also unusual for the nototheniids. The bones of the gill arches (Fig. 1.4) are lengthened and considerably thinned. Pharyngeal teeth on pharyngobranchials 2–4 and ceratobranchial 5 are sharp, conical, and much larger than the maxillary teeth. Gill rakers on the cerato-, hypo-, and epibranchials of the first gill arch are greatly elongated compared to other nototheniids.

As for the neurocranium, ontogeny of the splanchnocranium of *P. antarctica* differs with respect to other nototheniids. Like other Pleuragrammatinae, many facial bones of larvae and juveniles are elongated (e.g., jaws, the palatine, etc.), and most of them are reduced (e.g. upper process of the opercle, radii branchiostegi), and comparison of juveniles and adults shows that many specific cranial traits persist relatively unchanged throughout the *Pleuragramma* life cycle (see for details Voskoboinikova et al. 1994; Voskoboinikova and Kellermann 1997).

1.2.3 The Shoulder Girdle Skeleton

The shoulder girdle of adult silverfish shares general basic features with other nototheniids (see Balushkin 1989, Figs. 18, 19). Specific characteristics include the elongated anterior process of the coracoid and the striking extension of the upper end of the cleithrum, which is considerably higher than the dorsal edge of the scapula (Fig. 1.5a). In other nototheniids, in contrast, the posterior portion of the upper lobe of the cleithrum occurs at the same level as the dorsal edge of the scapula. The presence of small compensatory longitudinal ridges on the cleithrum is another characteristic trait, possibly related to reduction of the pectoral fin skeleton. A deep notch is situated between the anterior process of the coracoid and the main part of this bone. The posttemporal and supracleithrum are elongated but otherwise similar to those of other nototheniids.

Enhancement of the upper cleithral extension is observed during early silverfish development (Fig. 1.5b, c). In other Pleuragrammatinae, including *Aethotaxis,* similar extensions occur, but they are not preserved in adults. The presence of three radials in the shoulder girdle is a diagnostic character for the suborder Notothenioidei

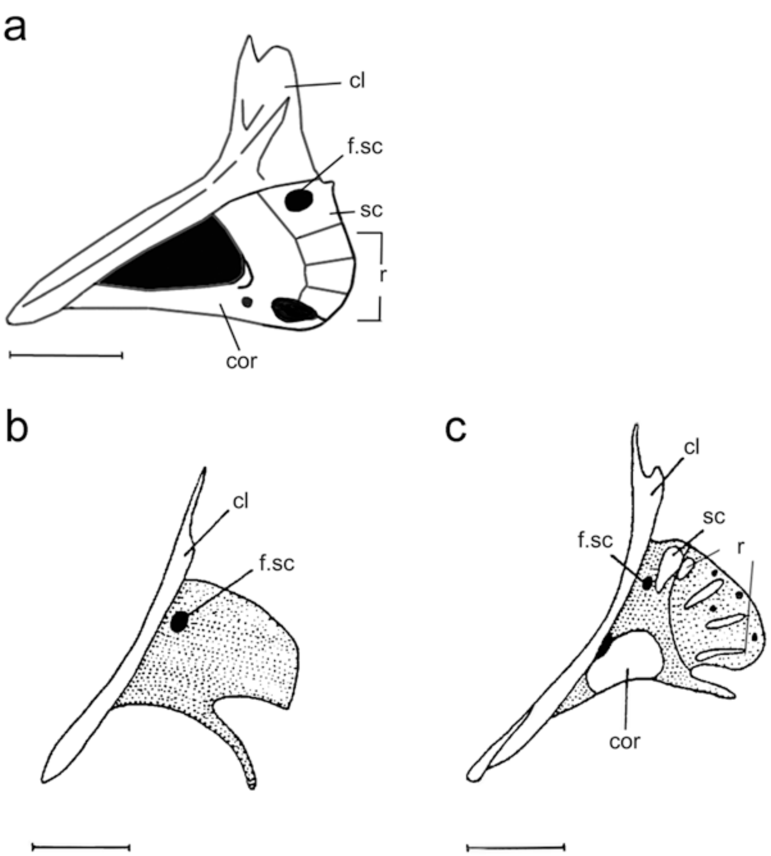


Fig. 1.5 The pectoral girdle of *P. antarctica.* (**a**) Adult specimen of SL 147.8 mm; (**b**) specimen of SL 33.0 mm; (**c**) specimen of SL 44.0 mm. *cl* cleithrum, *cor* coracoid; *f.sc* scapular foramen, *r* radials, *sc* scapula. *Black spots*: foramina; *stippled areas*: cartilaginous tissue. Scale bars: **a** = 10 mm; **b**, **c** = 1 mm

(e.g. Regan 1913, 1914; Norman 1938) and *P. antarctica* adults (Fig. 1.5a) share this feature with the other nototheniids. The three adult radials arise from four radial bone anlagen in the radial cartilage plate of the shoulder girdle of juveniles (Fig. 1.5c), the uppermost radial anlage fuses with the scapula as development proceeds (Andriashev 1987; Voskoboinikova et al. 1994; Voskoboinikova and Kellermann 1997).

The presence of a scapular foramen in the scapula is considered as an apomorphic feature in nototheniids (Voskoboinikova and Kellermann 1997). Many authors (Regan 1913, 1914; Pappenheim 1914; Norman 1937, 1938; Andriashev 1965; Balushkin 1984, 1992, 2000) assign considerable significance to the position of the scapular foramen, which currently serves as a diagnostic character for the subfamilies Nototheniinae and Trematominae. In *P. antarctica*, the foramen is in the scapula (Fig. 1.5a), as in Trematominae, but it forms in a different manner during ontogeny.

In all nototheniids, the anlage of the scapula initially grow down, creating a cavity between the anterior and posterior portions of the bone. In the trematomins (Voskoboinikova and Kellermann 1997), the posterior portion of the scapula grows and bends down and anteriorly, producing an edge below the cavity. This extension then fuses with the anterior upper portion of the bone, thus closing the cavity as the scapular foramen. In contrast, in *P. antarctica*, downward growth of the anterior and posterior portions of the scapula leads to closing at the bottom of the cavity to form the scapular foramen.

As shown by Balushkin (1984, 1989) for nototheniins, the number of small interradial foramina in nototheniids tends to decrease during evolution. Various developmental patterns lead to the establishment of the specific final number of these structures (Voskoboinikova and Kellermann 1997). *Pleuragramma* larvae initially have five interradial foramina (Fig. 1.5c); later in ontogeny the foramina between the scapula and radial 1, between radials 1 and 2, and between radials 2 and 3 sequentially close. In adults (Fig. 1.5a), only the lowermost hole remains, or it may be absent.

1.2.4 The Axial Skeleton

According to Norman (1938), the axial skeleton of the Antarctic silverfish is formed by 53 vertebrae (19 precaudal + 34 caudal), whereas DeWitt et al. (1990) reported 52–56. Recent counts indicate 17–20 precaudal + 34–37 caudal = 53–55 vertebrae (Balushkin, personal communication).

One of the most striking features of adults is the vertebral centrum (DeVries and Eastman 1978; Eastman and DeVries 1981, 1982; DeWitt et al. 1990; Eastman et al. 2014). Each centrum (Fig. 1.6a) appears as a thin bony collar surrounding a large hole, the notochordal canal, which is filled, *in vivo*, by a persistent notochord. Comparison of the relative diameter of the notochordal canal (diameter of notochordal canal/diameter of vertebral centrum × 100) in 38 species spanning the notothenioid phylogeny showed that the Antarctic silverfish possesses the relative largest canal (Eastman et al. 2014). The next largest was found in the close relative *A. mitopteryx.* Maintenance into adulthood of wide vertebral canals (and consequent reduction of axial bone mass), coupled with persistence of the low-density notochord, contributes substantially to the neutral buoyancy of the Antarctic silverfish.

The appearance of ossified vertebrae occurs late, during juvenile stages from 40.0 to 89.0 mm SL (Voskoboinikova et al. 1994), with consequent underdevelopment of the centra and persistence of the notochord in adults. Moreover, given the limited bone growth, the notochord canal remains large, and the vertebral centra do not become completely amphicoelous (Totton 1914; Eastman and DeVries 1981, 1982; DeWitt et al. 1990; Voskoboinikova et al. 1994; Voskoboinikova 2001; Eastman et al. 2014). The vertebral centra form from two small oval ossified plates lateral to the notochord (Fig. 1.7). The bony plates lie usually two by two lateral to the notochord dorsally and ventrally and join medially. Then the plates of both sides

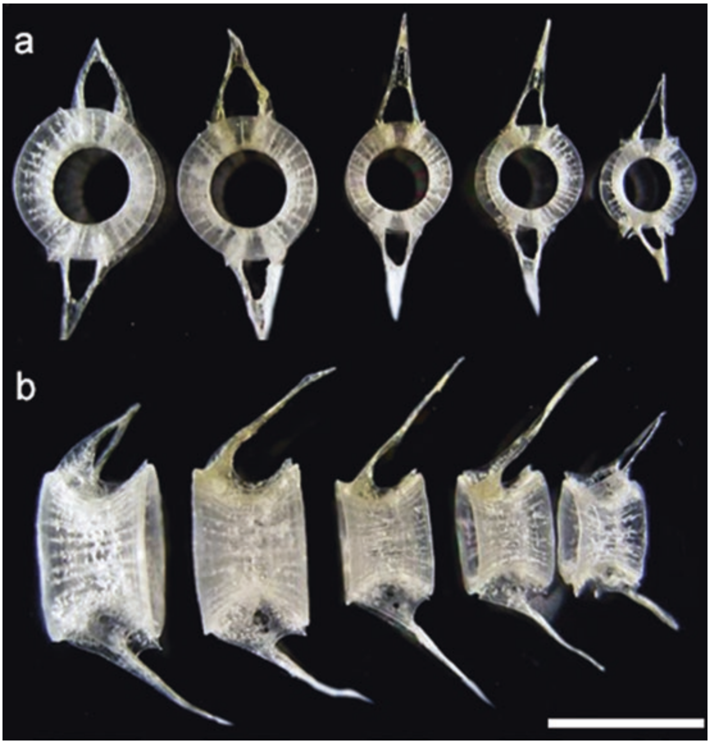
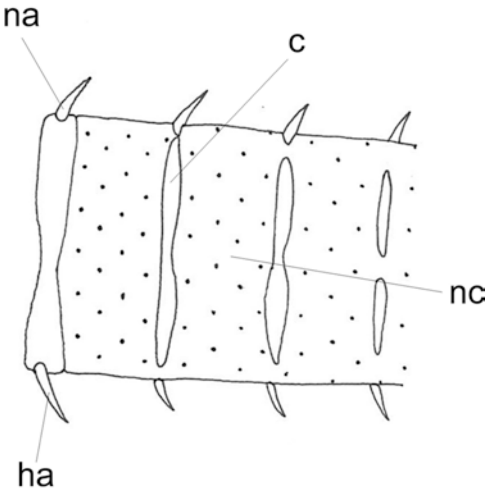


Fig. 1.6 Vertebrae of *P. antarctica,* adult specimen of SL 184.6 mm. (**a**) Superior view, (**b**) lateral view. Scale bar = 5 mm (Modified from Busekist et al. 2007, ANFIBO Base)

Fig. 1.7 Vertebral centra in formation in *P. antarctica* (specimen of SL 48.6 mm), middle region of the vertebral column. *c* centrum, *na* neural arch, *ha* haemal arch, *nc* notochord



join dorsally and ventrally and form the bony ring around the notochord (Voskoboinikova et al. 1994). The vertebrae form from the urostyle forward by spreading of two preurostilar centra; then the centra appear from the basioccipital and spread in a caudal direction. In other nototheniids, by contrast, the centra begin with the appearance of saddle plates at the base of the haemal arches (nototheniins) or the haemal and neural arches (trematomins and *A. mitopteryx*). The centra of the nototheniins, trematomins, and *A. mitopteryx* form simultaneously from the basioccipital caudally and from the urostyle rostrally.

Other vertebral features provide additional anatomical characters specific to the Antarctic silverfish. The neural and haemal arches are poorly developed, with corresponding spines greatly shortened (Fig. 1.6) (Norman 1938; Balushkin 1984, 1989; DeWitt et al. 1990; Voskoboinikova et al. 1994). According to available studies, epipleural ribs attached to the centra of the first eight vertebrae are first found in specimens of 51 mm SL; these increase to 10 ribs in adults. At 51.7 mm, six pleural ribs are attached to the parapophyses of vertebrae 4–9. Subsequently, their number gradually increases to 9–12 in adult fish, but the last 3–4 ribs are not attached to parapophyses and lie freely in myosepta. The presence of floating ribs is a diagnostic feature of the suborder Notothenioidei (Balushkin 1992, 2000; Hastings 1993; Near et al. 2015). As is typical for Pleuragrammatinae, the first pleural rib attaches to centrum 4, rather than to the third centrum (Balushkin 1992; Voskoboinikova et al. 1994). Such a variation of the attachment position of the first pleural rib could have functional implications by increasing head mobility during feeding.

1.2.5 Unpaired Fins and the Caudal Fin

The skeleton supporting the unpaired fins consists of the serial pterygiofores, including proximal and dorsal elements. The proximal pterygiofores develop a special dorsal articular surface for the attachment of the distal pterygiofores, which remain almost entirely cartilaginous except for a small pair of bony plates on their lateral surfaces. The first appearance of the proximal pterygiofores occurs in the middle of the second dorsal fin in specimens of 43.7 mm and in the middle of the anal fin in specimens of 40 mm. Subsequently, ossification of the pterygiofores extends forward and backward. Ossification of the pterygiofores of the first dorsal fin occurs later. Full development of the pterygiofores is observed in specimens of 62.1 mm LS. The sequence of appearance of unpaired fin pterygiofores in *P. antarctica* is similar to that of other nototheniids (Voskoboinikova and Kellermann 1997).

With respect to the caudal fin, *P. antarctica* (Fig. 1.8a), together with *Gobionotothen gibberifrons* (Nototheniinae), possesses the greatest number of apomorphic features within Nototheniidae (Totton 1914; Balushkin 1984, 1989; Voskoboinikova et al. 1994; Voskoboinikova and Kellermann 1997; Voskoboinikova 2010). During development, one hypaxial hypural 1 + 2 cartilage generates one hypural 1 + 2 bone (Fig. 1.8b, c); two cartilaginous epaxial hypurals fuse (cartilaginous hypural 3 + 4), and then produce hypural bones 3 and 4; these later fuse to

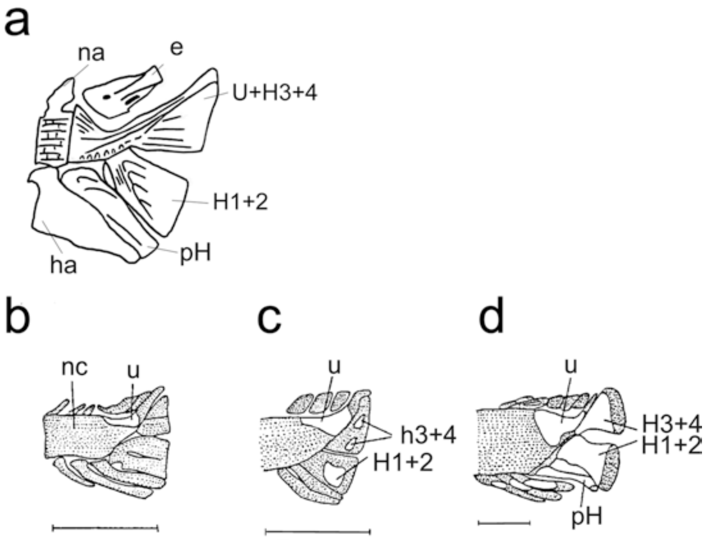


Fig. 1.8 Major steps in the development of the skeleton of the caudal fin of *P. antarctica*. (**a**) Specimen of SL 48.6 mm; (**b–d**) specimens of SL 18.9 mm, 33.0 mm, and 44.9 mm, respectively. *ha* haemal arch, *na* neural arch, *e* epural, *h* hypural, *H* complex hypural, *nc* notochord, *pH* parhypural, *u* ural center, *U* + *H* ural center + complex hypural. Scale bars = 1 mm

form one bone (hypural 3 + 4; Fig. 1.8c, d). As in trematomins, the epaxial hypural plate 3 + 4 is fused with the ural center. Hypural 5 is absent, as is the uronrural, which is present in all other nototheniids. Due to the loss of a number of early stages of development of the caudal fin skeleton the later stages in *P. antarctica* begin earlier than in other nototheniids, resulting in the most advanced structure of the caudal fin skeleton of the *P. antarctica* (Eakin 1981; Balushkin 1984; Voskoboinikova 2010).

1.3 Antarctic Silverfish Skeletal Characteristics and Pelagic Life

Studies of specimens at different developmental stages (Voskoboinikova et al. 1994; Voskoboinikova and Kellermann 1997; Voskoboinikova 2010, present work) indicate that silverfish develop almost all elements of the notothenioid bony skeleton. However, most skeletal elements are thin and have smooth surfaces, and many of them are reduced in size, compared to other notothenioids and to species of the same family Nototheniidae. Thinning and reduction in size of bony elements, together with low bone mineral density, result in reduced skeletal mass, as documented by the low weights of ashed silverfish skeletons (DeVries and Eastman 1978). The bone reduction, combined with the persistence of low-density tissues (cartilage and notochord), are major skeletal changes contributing to the acquisition of neutral buoyancy during the evolutionary re-shaping of the Antarctic silverfish for life in the pelagic habitat (see Sect. 1.1.1).

A trend towards the reduction of the bony skeleton, through partial reduction or elimination of bones and sometimes associated with the replacement of bone tissue with connective, occurs in various notothenioid lineages (Eakin 1981; Voskoboinikova 1982, 1993, 2001; Iwami and Abe 1984; Iwami 1985; Balushkin 1992, 2000; Balushkin and Voskoboinikova 1995; Rutschmann et al. 2011). In a comparative skeletal analysis of 20 notothenioid species, Eastman et al. (2014) related the reduction in skeletal mass to species/specific pelagic capabilities. In the taxa they examined, percentage skeletal mass (mass of dried skeleton/body mass × 100) ranged from 3.42% in the non-Antarctic and benthic *Bovichthus diacanthus* to 1.09% in the Antarctic and secondarily pelagic channichthyid *Champsocephalus gunnari*. *P. antarctica* was not included in that study, but its pleuragrammatin relative *D. mawsoni* showed a low skeletal mass value, 1.47% (Eastman et al. 2014). The skeletal mass of the non-Antarctic secondarily bentho-pelagic notothenioid *Eleginops maclovinus* (Eleginopsidae) was also light (<2%).

The ecological advantages of skeletal reduction and increased buoyancy for exploiting the rich food resources of the water column have been extensively described (e.g. Nybelin 1947; Andriashev 1965, 1976, 1978, 1987; Voskoboinikova 1982, 1993, 2001, 2010; Slosarczyk 1983; Eastman 1993, 1997; Miller 1993; Klingenberg and Ekau 1996). The importance of bone mass reduction in the adaptive radiation of Antarctic notothenioid lineages *per se* has been questioned by Eastman et al. (2014) because it is a synapomorphy shared with the non-Antarctic sister group Eleginopsidae. Rather, they posit that paedomorphic truncation of bone development in larvae and juveniles (Albertson et al. 2010) provides the density-reducing traits that enabled Antarctic notothenioids to expand into vacant ecological niches and to differentiate adaptively to generate new species.

Table 1.1 summarizes the main characters of the skeleton of the Antarctic silverfish that are functionally important with respect to pelagic life. Most features are recognized as paedomorphic. Some of them (e.g. partial or total reduction of skeletal elements) are directly related to the acquisition of buoyancy through reduction of skeletal mass and body density, whereas others (e.g. lengthening of bones of the ethmoidal portion of the neurocranium and jaws) are related to foraging in the water column and planctophagia. Interestingly, some paedomorphic traits seem functionally neutral or even potentially disadvantageous. For example, the absence or incomplete formation of canals in the seismosensory system, possibly a "side effect" of heterochronic events in bone skeletal development, leaves the canal neuromasts exposed on the body surface, without protection from low-frequency, self-generated noise (Montgomery and Clements 2000). This apparent disadvantage of exposed neuromasts appears to have been evolutionary compensated by the predatory behavior of the silverfish, which seems to produce little self-induced noise in the water column, and by means of hypertrophy of specific brain regions (the eminentia and crista), which are involved in filtering self-induced noise during central sensory processing (Montgomery and Bodznick 1994; Eastman 1997; Montgomery and Clements 2000).

Table 1.1 Main skeletal traits of adult *P. antarctica* relevant to pelagic life[a]

Skeletal trait	Predicted consequence	Main references
Partial/total reduction of skeletal elements	Density reduction	[1] [2] [3] [4] [5] [6]
Thin, deciduous scales; scales of the lateral line without canal	Density reduction	[7] [8] [9] [10]
Gaps in seismosensory canals	Density reduction at the cost of less noise reduction	[11] [12]
Thin and smooth bone strengthened by ridges	Maintained strength in face of density reduction	[3] [4] [5] [6]
Lengthening of bones of the ethmoidal portion of the neurocranium, and of the jaws	Improved planktivory and facultative carnivory	[3]
Lengthening of bones of the interorbital portion of the neurocranium and suspensorium related to enlarged eyes	Improved planktivory and facultative carnivory	[3] [4]
Enlargement of some jaw teeth; lengthening of the gill rakers	Improved planktivory and facultative carnivory	[3] [4]
Striking bone reduction in vertebral centra; large notochordal canal	Density reduction	[13]

References are as follows: [1] Eastman and DeVries (1981), [2] Eastman and DeVries (1982), [3] Voskoboinikova (1982), [4] Voskoboinikova (1993), [5] Voskoboinikova (2001), [6] Voskoboinikova (2010), [7] DeWitt (1962), [8] Andriashev (1965), [9] DeWitt and Hopkins (1977), [10] DeWitt et al. (1990), [11] Jakubowski (1971), [12] Montgomery and Bodznick (1994), [13] Eastman et al. (2014)

[a] As noted in Sect. 1.1.2, numerous non-skeletal traits are also related to secondary pelagization (Eastman 1997; Montgomery and Clements 2000)

1.4 Skeletal Formation

The onset of different osteological traits in nototheniids results from an ontogenetic slowing of skeletal formation (Voskoboinikova 1994). Slowing of skeletal development is related to the total duration of development, the size of the larvae passing to juvenile stages, and the size of young fish when they achieve a full set of bones. Among nototheniids, *P. antarctica* is characterized by a very slow rate of skeletal formation. After hatching at ~9 mm in length (Kellermann 1989; Hubold and Tomo 1989; Bottaro et al. 2009, silverfish larvae show recognizable bony elements from SL 17 mm (the smallest in our samples) through ~44 mm. Fin rays are present by 40 mm, and their complete ossification occurs at a length of ~77 mm. Most bony elements appear by 6 months after hatching, and their development is complete by the end of the first year of life. *P. antarctica* attains its full complement of bones by the end of the second year (Voskoboinikova 1994).

As in other Pleuragrammatinae, *P. antarctica* larvae and juveniles reach large sizes before most (~30–45 mm) or all (~75–80 mm) bones are formed. Consistent retardation (postdisplacement) in the appearance, and slowing in the rate of formation (neoteny) of bony elements demonstrate the role of heterochronic processes during development (Helfam et al. 2009) and together lead to retention of paedomorphic traits.

The late appearance of bony elements in larvae and the consequent prolongation of the larval stage in the winter months has be viewed as an ecological advantage to the silverfish by reducing interspecific competition for feeding on summer zooplankton (Marshall 1953; Andriashev 1964; North 1991; Eastman 1993; Voskoboinikova 2001, 2007 among others).

Progenesis (early sexual maturation compared to somatic development) could also be considered as a process related to the peculiar skeletal development in *Pleuragramma*. Among the Pleuragrammatinae, *P. antarctica* has the smallest body size (TL 330 mm) compared to *A. mitopteryx* (490 mm) (Voskoboinikova 2010), *G. svetovidovi* (650 mm), *D. mawsoni* (1750 mm) and *D. eleginoides* (2150 mm) (DeWitt et al. 1990). *P. antarctica* becomes sexually mature at the relatively short length of 125 mm (DeWitt et al. 1990) at the age of 4–6 years. For comparison available data on *D. mawsoni* indicate spawning at 16.6 years for females (1332 mm) and 12.8 years for males (1204 mm) (Parker and Gremes 2010) and *D. eleginoides* first spawns at 7–12 years (Laptikhovsky et al. 2006).

Based on the totality of evidence, we conclude that the Antarctic silverfish possesses a very high degree of skeletal modification (reduction) among adult Nototheniidae (see also Eastman and DeVries 1981, 1982; Voskoboinikova 1982, 1993; Balushkin 1984, 1989; DeWitt et al. 1990) due to multiple heterochronic processes. We turn now to molecular analyses to develop a mechanistic understanding of skeletal reduction in the Antarctic silverfish.

1.5 Molecular Basis of Reduced Bone Formation in *P. antarctica*

Advances in developmental genetics using a number of different laboratory model organisms have deepened our understanding of the molecular mechanisms that mediate the formation, growth and remodeling of the skeletal system. Bone forms by either endochrondral ossification, whereby it develops around and ultimately replaces a cartilage scaffold, or by intramembranous ossification, in which bone develops directly within the dermis without a cartilage template (dermal bone). Both mechanisms follow the same basic progression from mesenchyme condensation to bone cell differentiation to matrix deposition.

A variety of molecules regulate each step of skeletogenesis. The bone morphogenic protein (Bmp) signal transduction pathway plays important roles during condensation stages (Ekanayake and Hall 1997; Ying and Zhao 2001; Lim et al. 2015), and altered Bmp signaling in both zebrafish and mice leads to notable skeletal deficiencies (Kishimoto et al. 1997; Lim et al. 2015). Differentiation of cartilage cells requires the transcription factor *Sox9*, whereas differentiation of osteoprogenitor cells requires the transcription factor *Runx2* (Yan et al. 2002). Bmp signaling induces *Runx2* expression (Nakashima et al. 2002), providing a direct molecular link between these two stages of development. *Sox9* and *Runx2* can be expressed in the same cell, and it is the relative amount of each factor that determines its fate (cartilage or bone, Eames et al. 2004). *Sox9* induces *col2a1,* which determines cartilage fate (Yan et al. 2005). *Runx2* induces *col10a1* and *ihh* expression in chondrocytes (Iwamoto et al. 2003), which leads to cell hypertrophy and apoptosis of chondrocytes, a critical step in endochrondral ossification. *Ihh* signals back to osteoprogenitor cells to induce further differentiation of bone cells, which express *col1a1*. Type I collagen is a vital component of bone extracellular matrix that subsequently mineralizes via the accumulation of hydroxyapatite. In addition to these specific molecular interactions, mature bone cell differentiation requires input from other major developmental signal transduction pathways, including Wnt, Notch, and Fgf signaling (reviewed by Long 2011). Thus, skeletal development is an exquisitely complex and intricate process.

Perturbation of any stage (or pathway) contributing to skeletal differentiation could underlie reduced bone formation in *P. antarctica.* As a first step in trying to understand low levels of bone mineralization in Antarctic fish at the molecular level, skeletal development was examined in *P. antarctica* larvae and compared to that in two laboratory fish species (Albertson et al. 2010). Specifically, the expression of one cartilage gene, *col2a1,* and two bone genes, *col10a1* and *col1a1*, were surveyed in the craniofacial region. The prediction from the hypothesis raised by the morphological studies reported above is that skeletal reduction in *P. antarctica* adults can be traced to delayed bone development in *P. antarctica* larvae. Results are summarized in Fig. 1.9. Delayed osteogenic gene expression was indeed noted in the splanchnocranium of pelagic notothenioids. Specifically, *P. antarctica* larvae exhibited strong expression of the cartilage gene *col2a1* throughout the ceratohyal cartilage (Fig. 1.9a), whereas in comparably staged teleost outgroup species i.e., stickleback (*Gasterosteus aculeatus*) and zebrafish (*Danio rerio*), *col2a1* expression was restricted to the distal ends of this element (Fig. 1.9d, g). Expression of the bone gene *col10a1* was completely absent from the ceratohyal cartilage in *P. antarctica* (Fig. 1.9b), but was strongly expressed in the medial, hypertrophic domain of the cartilage in both outgroup species (Fig. 1.9e, h). Finally, the osteogenic marker *col1a1* was strongly expressed in the perichondrium surrounding the ceratohyal cartilage in stickleback and zebrafish (Fig. 1.9f, i), whereas only very weak expression was noted in *P. antarctica* (Fig. 1.9c). Together, these data are consistent with the retention of the chondrogenic program and delayed deployment of the osteogenic program in *P. antarctica* larvae. The specific mutations that precipitate these developmental shifts remain to be identified. However, data from this study suggest that early acting factors underlie at least some aspects of this phenotype.

Future studies should also investigate the possible contribution of later developmental events. Bone continues to grow and remodel itself over the life of an animal, and bone homeostasis is maintained via a balance between bone-forming progenitor cells (i.e., osteoblasts) and bone-resorbing cells (i.e., osteoclasts). When this balance is disrupted in humans, it can lead to osteoporosis. It is possible that the reduced skeletal formation in *P. antarctica* that begins early in development could be exasperated later in development due to altered osteoblast and/or osteoclast activity.

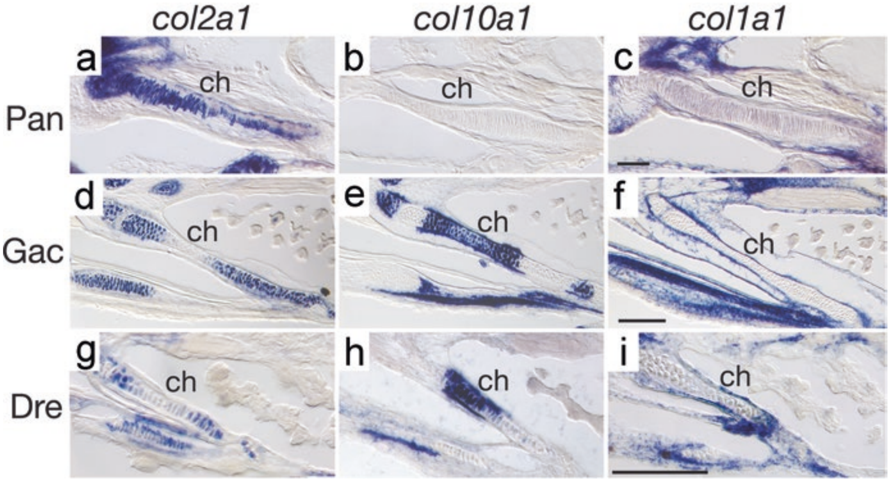


Fig. 1.9 The developmental genetic basis of reduced skeletal formation in *P. antarctica.* Collagen gene expression in the ceratohyal cartilage of comparably staged *P. antarctica* ('Pan', **a**–**c**), *G. aculeatus* ('Gac', **d**–**f**), and *D. rerio* ('Dre', **g**–**i**) larvae. *P. antarctica* exhibits strong and ubiquitous expression of the cartilage gene, *col2a1* (**a**) but nearly absent expression of both osteogenic markers, *col10a1* (**b**) and *col1a1* (**c**). A markedly different pattern is observed in both *G. aculeatus* and *D. rerio*. In these teleost outgroups, expression of *col2a1* is restricted to the distal ends of the ceratohyal (**d**, **g**), *col10a1* is expressed in hypertrophic chondrocytes (**e**, **h**), and *col1a1* is expressed in the perichondrium surrounding the cartilage (**f**, **i**). Scale bars = 100 μm (Modified from Albertson et al. 2010)

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Chapter 2
Coping with Ice: Freeze Avoidance in the Antarctic Silverfish (*Pleuragramma antarctica*) from Egg to Adult

Clive W. Evans and Arthur L. DeVries

Abstract The Antarctic silverfish survives in a hostile environment that includes hatching into a zone laden with platelet ice. Embryonated eggs and hatchling larvae lack adequate levels of antifreeze to survive in this environment, but they are afforded physical protection against freezing by the presence of a resistant chorion (around the embryonated eggs) and a resistant external epithelium (around the larvae). Adult Antarctic silverfish also have low levels of antifreeze, but they are less likely to tolerate freezing conditions than their eggs or larvae because of damage to their external epithelium suffered during their lifetime allowing for ice entry. Like most other notothenioids, the Antarctic silverfish synthesises antifreeze glycoproteins (AFGPs), primarily in acinar cells of the exocrine pancreas. From here they are secreted directly into the digestive tract, ultimately dispersing throughout the body after uptake in the rectum and transfer into the blood circulatory system. Surprisingly, the Antarctic silverfish lacks the full range of AFGP isoforms (AFGP1-8), having instead a single dominant ~20 kDa form with some minor AFGP6 variants. The total serum AFGP concentration is relatively low, providing about 0.2 °C thermal hysteresis. Total serum hysteresis, however, is ~1.3 °C, the increase being provided by a novel antifreeze protein that behaves akin to the antifreeze potentiating protein (AFPP) described in other notothenioids. Nonetheless, this level of protection is below that required for survival in a freezing environment and thus adult Antarctic silverfish can only survive in locales free of ice crystals.

Keywords AFGP • AFP • AFPP • Antifreeze • Hysteresis • Exocrine pancreas • Superheating

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2.1 Introduction

The life cycle of the Antarctic silverfish (*Pleuragramma antarctica*) encompasses a number of significant challenges, foremost among which is the need to survive and thrive in a hostile, frigid environment. Although there are some uncertainties with respect to the early life history of this species, nearing the time of hatch buoyant embryonated eggs in the vicinity of Terra Nova Bay, Antarctica may be found concentrated in "nurseries" that are intimately associated with the presence of platelet ice (Vacchi et al. 2012). As development proceeds, the buoyancy of embryonated eggs in nursery environments decreases through the mobilization of energy reserves and the conversion of lipid stores to more dense body tissues (Evans et al. 2012b). However, many embryonated eggs are physically trapped in interstices within the platelet ice and, failing to sink below this icy surface layer, they are unable to utilise any potential benefits from a change in density. On hatching, escape from the icy surface layer is potentially aided by two behavioural responses, positive gravitaxis and negative phototaxis, which together could help direct newly-hatched larvae to deeper water. Again, despite their capacity to do so, observations suggest that many newly-hatched larvae are thwarted in their attempts to escape the icy surface layer because of its three-dimensional complexity. Inevitably, many larvae must hatch into ice-laden surface waters and, being unable to escape immediately, their survival requires the ability to overcome the challenges presented by the presence of abundant environmental ice.

2.2 Coping with Ice: Freeze Tolerance and Freeze Avoidance

Survival in icy waters requires the evolution of strategies to deal with the potential for the freezing of body tissues and fluids. Organisms have met this challenge in two ways, by either surviving internal freezing (freeze tolerance) or by avoiding it (freeze avoidance). Organisms that depend on freeze tolerance have had to evolve strategies that allow them to cope with the accumulation of internal ice. The loss of liquid water into ice, usually initiated in the extracellular volume, concentrates soluble body constituents, establishing an osmotic imbalance that results in the loss of intracellular water. This loss leads to cellular collapse, which must be protected against if the freeze tolerant organism is to survive (Mazur 1970). Large, multicellular organisms generally cannot survive cellular collapse under freezing conditions and those that inhabit frigid environments, including *P. antarctica*, have instead evolved freeze avoidance strategies.

Freeze avoidant organisms utilise both colligative and non-colligative properties of their body fluids to survive in frigid environments (Cheng 2003; Duman 2014). Colligative-based strategies depend on the concentration of molecules or ions in the body fluids, and not upon the identity of the solute. In colligative-based freeze avoidance, a freezing point depression is usually achieved by increasing the concentration of a variety of molecules (colligative cryoprotectants), often sugars or polyalcohols such as trehalose in insects (Thompson 2003) and glycerol in some polar fishes (Raymond 1992). Non-colligative strategies depend on a unique capacity of the body fluids, namely the presence of specific antifreeze molecules that lower the freezing point with little colligative impact. This latter approach is the one utilised by notothenioid fishes, such as *P. antarctica*, that survive in frigid conditions.

Organisms that utilise a non-colligative freeze avoidance strategy survive in a supercooled state, with their body fluids remaining liquid at a temperature below their equilibrium freezing point (reflecting the concentration of solutes in the body fluids). The supercooled state is a metastable condition, with body fluids remaining liquid until some nucleating event that triggers ice crystal growth is initiated. Nucleation is either homogeneous or heterogeneous, the former requiring a precise structural clustering of atoms in the fluid itself while the latter involves a specific entity in the fluid, such as certain macromolecules or particulates.

For most notothenioids, the equilibrium (colligative) freezing/melting temperature of their sera (as a proxy for entire body fluids) is between −1.1 and −0.93 °C, with an approximate average of −1.04 °C (Cziko et al. 2014). If an ice nucleator is present at or below the equilibrium freezing point, and ice itself – usually as a minute crystal – is a common nucleator, then some agent that stops ice crystal growth must be present or the organism will freeze. These agents are commonly referred to as biological antifreezes (DeVries 1971, 1988; Peck 2015). In the absence of external ice, adult Antarctic notothenioids free of internal ice fail to freeze at temperatures as low as −8 °C, indicating that they lack an endogenous capacity to nucleate under these conditions (DeVries, personal observation). Given that such low temperatures are never reached under natural conditions, it follows that ice or other particulates introduced into the fish from the environment (heterogeneous nucleation) is the main challenge facing notothenioid fishes under freezing conditions.

2.2.1 Two Classes of Biological Antifreeze

A number of different antifreezes have been identified in a diversity of organisms (Davies 2014). In Antarctic notothenioids the antifreezes consist of two different classes of molecules, the antifreeze glycoproteins (AFGPs) and the antifreeze proteins (AFPs). Collectively, biological antifreezes bind to ice crystals to inhibit their growth, and in doing so they act as ice-structuring molecules, changing ice crystal shape when the temperature is dropped (usually only experimentally) to that required for active growth.

2.2.2 *Antifreeze Activity: Thermal Hysteresis*

Antifreeze functionality is measured in terms of thermal hysteresis activity, a characteristic feature of all biological antifreezes. Although water typically freezes and melts at a single temperature (the equilibrium freezing/melting point), in the presence of biological antifreeze the melting point and especially the freezing point diverge from the equilibrium value so that ice crystals melt at a slightly higher temperature and grow at a much lower temperature. The difference between these non-equilibrium freezing and melting points, often referred to as the hysteresis gap, defines the amount of freezing hysteresis activity.

Hysteresis activity is typically measured in a device such as the Clifton Nanolitre Osmometer, originally designed to measure the osmolarity of a solution by determining the melting point depression. In essence, a small sample suspended in immersion oil is first frozen at −20 °C and then melted back to a single ice crystal as viewed under a microscope (~200× total magnification). The equilibrium melting point (effectively equivalent to the equilibrium freezing point) is recorded when the ice is quickly melted to one small crystal (10-20 μm diameter). The sample is then slowly cooled until rapid growth in the form of spicules is observed at the non-equilibrium freezing point (Cziko et al. 2006).

2.3 Notothenioid Antifreeze Molecules

2.3.1 *Antifreeze Glycoproteins (AFGPs)*

The AFGPs identified in Antarctic notothenioids have been grouped into eight size classes, originally defined by their electrophoretic properties (reviewed in DeVries and Cheng 2005). All share a common element, namely the presence of a variable number of glycotripeptide repeats (4–50, yielding molar masses between 2.6 and 33.7 kDa). The repeating element is composed of an alanine-alanine-threonine (AAT) backbone in which the threonine is *O*-linked to a disaccharide, ß-D-galactosyl-(1, 3)-α-D-*N*-acetylgalactosamine (Gal-GalNac). In the two smallest electrophoretic size classes, AFGPs 7 and 8 (3.5 and 2.6 kDa respectively), the AAT glycotripeptides are occasionally replaced by proline-alanine-threonine (PAT) glycotripeptides, and their antifreeze activity is about two thirds that of the larger sized isoforms. AFGP8, for example, is a 14-residue glycopeptide that contains four disaccharides (on the threonine residues) and two prolines (AAT-AAT-PAT-AAT-PA) (Hsiao et al. 1990). The AFGPs are found in the blood and intestinal and coelomic fluids at concentrations up to ~40 mg mL^{-1}, but not in the urine, ocular fluids or endolymph (Ahlgren et al. 1988).

2.3.2 Antifreeze Potentiating Proteins (AFPPs)

As explained above, the *condicio sine qua non* defining antifreeze activity is the demonstration of thermal hysteresis. From a biological perspective, hysteresis activity must be demonstrable at antifreeze concentrations existing *in vivo*. Given this functional constraint, only one AFP has been identified to date. Although this AFP remains poorly defined and has been described in only a limited number of Antarctic notothenioids (Jin 2003; DeVries and Cheng 2005; Yang et al. 2013), it displays a distinctive functional property, namely the capacity to potentiate antifreeze activity wrought by the AFGPs, particularly the larger isoforms. With respect to its functionality, this specific AFP is referred to as antifreeze potentiating protein (AFPP). It is thought that AFPPs and AFGPs bind to different ice crystal faces (see below), and hence they complement each other's activity. AFPP activity is heat labile whereas AFGP activity is not, allowing functional separation of the two by the simple expedient of boiling. Fields and DeVries (2015) took advantage of the heat lability of AFPP to separate the contributions of the two types of antifreezes to hysteresis activity in notothenioids inhabiting the freezing waters of McMurdo Sound and those of the warmer Antarctic Peninsula waters.

2.4 How Do Biological Antifreezes Function?

Antifreezes inhibit ice crystal growth by binding essentially irreversibly to specific ice crystal faces, interfering with the subsequent attachment of water molecules to the ice crystal surface (Raymond and DeVries 1977; Celik et al. 2013). AFGPs, for example, bind primarily to prism faces, thereby inhibiting growth predominantly along the a-axis (Fig. 2.1). Bound AFGP molecules produce a puckered surface with regions of high curvature between them since water molecules can only be added to the ice crystal in the spaces between the bound AFGPs. The curved surfaces limit further water molecules from adding to the ice crystal through a phenomenon known as the Kelvin (Gibbs–Thompson) effect, and thus ice crystal growth ceases. Growth can only resume when the temperature is lowered to a new, depressed freezing point, the non-equilibrium freezing point, often simply referred to as the hysteresis freezing point. Should the temperature fall below this point, ice crystals with bound AFGPs will show explosive growth along the c-axis, giving rise to needle-shaped, bipyramidal crystals (Peltier et al. 2010).

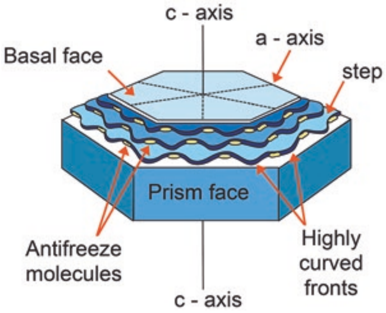

Fig. 2.1 Schematic representation of AFGP molecules binding to an ice crystal. Bound AFGPs inhibit access of water molecules to the nascent ice crystal. Crystal growth is thus restricted to areas between the AFGP molecules, forming curved fronts that become limiting through the Kelvin effect. The binding of AFGPs to ice is essentially irreversible. If the temperature drops below a critical point (the hysteresis freezing point) growth resumes explosively, predominantly along the c-axis for AFGPs

2.4.1 *Biological Antifreezes as Superheating Agents*

The essentially irreversible binding of antifreeze molecules to ice crystals leads to the slowing and ultimate inhibition of crystal growth by progressively inhibiting the opportunities for water molecules to add to the crystalline structure. Conversely, it might be expected that the same adsorbed molecules could inhibit the loss of water molecules from the crystal surface during melting, thus inducing a melting hysteresis as opposed to a freezing one. This superheating capacity of notothenioid AFGPs was first demonstrated by Knight and DeVries (1989). Later, Celik et al. (2010) showed the same phenomenon in AFPs from non-Antarctic fishes. These initial reports were based on laboratory analyses, leaving the potential biological implications uncertain until Cziko et al. (2014) showed that superheated ice occurs naturally within Antarctic notothenioids.

The key functional implication arising from the study by Cziko et al. (2014) is that in order to completely melt internal ice through passive warming, Antarctic fishes must be exposed to water temperatures well above the equilibrium freezing-melting point of their body fluids (−1.04 °C). In fact, these authors showed that individual specimens of *Trematomus bernacchii* can retain superheated ice in their bodies up to at least +0.15 °C, a value never reached in a decade long record of sea water temperature in McMurdo Sound.

The inevitable conclusion is that through this superheating effect, antifreeze molecules will limit opportunities for the passive melting of internal ice, which will thus accumulate in a fish's body over its lifetime. Since the accumulation of internal ice is potentially life threatening, antifreeze molecules present a novel example of antagonistic pleiotropy (Williams 1957), in which a gene influences different traits that are beneficial on the one hand and detrimental on the other. In the present example, AFGPs provide a beneficial effect by preventing ice crystals from growing, but they also have a detrimental effect by preventing ice crystals from melting, thus allowing potentially lethal ice to accumulate.

2.4.2 Antifreeze Glycoproteins Have a Pancreatic Origin

Antifreeze glycoproteins in Antarctic notothenioid fishes are synthesised primarily in acinar cells of the exocrine pancreas, as revealed by both molecular genetic and immunocytochemical techniques (Cheng et al. 2006). This result is not unexpected, given the evolutionary origin of AFGP from a pancreatic trypsinogen-like protease pre-cursor (Chen et al. 1997; Cheng and Chen 1999), although previously AFGPs were thought to be synthesised in the liver (Hudson et al. 1979). Since the pancreas is a diffuse organ in notothenioid fishes (Section 2.4.3) it is likely that the earlier interpretation was a consequence of the presence of ectopic exocrine pancreatic tissue within the liver sample.

In fishes, as in other vertebrates, the acinar cells also produce a suite of digestive enzymes, particularly the proteases trypsin and chymotrypsin (as inactive precursors), and pancreatic lipase and amylase. These enzymes are discharged first into an intercalated duct, which lies at the centre of a cluster of acinar cells. From here they pass into the anterior part of the intestine (typically the duodenum) via yet more ducts that progressively increase in internal diameter. Immunocytochemistry shows AFGP molecules to be concentrated in the acinar cell secretory vesicles (zymogen granules) that also enclose the pancreatic digestive enzymes or their inactive precursors (Cheng et al. 2006). Since secretions from the pancreas are believed to be released directly and exclusively into the digestive tract, it follows that AFGPs will take the same route from the exocrine pancreas to the gut. The endocrine pancreas, involved in the synthesis and secretion into the blood of hormones including insulin and glucagon, does not contribute to AFGP production. Thus all the AFGPs synthesised in the exocrine pancreas are presumed to pass into the gut on their release, and not the blood.

2.4.3 How Do Antifreeze Glycoproteins Reach the Blood?

Antifreeze glycoproteins are found in the blood of Antarctic notothenioids at concentrations up to ~40 mg mL^{-1}, depending on the species (Ahlgren et al. 1988). If AFGPs are synthesised exclusively in the exocrine pancreas and released directly into the gastrointestinal (GI) tract, then some avenue must exist for their transport to the blood where they can accumulate to significant concentration. Several scenarios present themselves:

1. Other cells/organs also produce AFGPs and these can be released into the blood.
2. In fishes, the exocrine pancreas has a unique and hitherto undescribed anatomical connection with the circulatory system.

3. The exocrine pancreas in tissues is “leaky”, with AFGPs traversing the intercellular space into blood vessels.
4. AFGPs are reabsorbed from the GI tract and transferred to the circulatory system.

Northern blot analysis using a probe from the AFGP gene coding region confirmed expression of AFGPs in pancreatic tissue from a variety of nototheniod fishes (Cheng et al. 2006). Expression was also found in anterior stomach tissue immediately rostral to the oesophageal-stomach junction. Secretion of AFGPs synthesised in the anterior stomach is presumed to be directly into the stomach lumen, reflecting the requirement of high levels of AFGPs in the GI tract to counter ice imbibed along with feeding and drinking. The results of northern blot analyses in the absence of a full suite of controls to confirm tissue specificity need to be interpreted with caution, however. This is particularly so for studies of the exocrine pancreas, which is widely dispersed in many species, including notothenioid fishes. Indeed, using microscopical methods we have found AFGP-secreting exocrine pancreatic tissue deeply embedded in a variety of body locations, especially in the wall of the GI tract (Fig. 2.2). At many of these sites we have been able to identify ducts that penetrate the wall of the GI tract, enabling discharge of AFGPs directly into its lumen. Thus it remains conceivable that AFGPs detected within the anterior stomach wall by northern blot analysis have an exocrine pancreatic origin. Irrespective of this possibility, however, all lines of evidence suggest that all likely sources of AFGPs discharge directly into the gut, not the circulatory system. Furthermore, detailed microscopical examination of nototheniid exocrine tissue has not offered any evidence for unique anatomical connections between the acinar cells and the blood, the former secreting AFGPs in secretory granules from their apical surfaces, which open into a duct and not a blood vessel. Leakage of AFGPs from secreting cells directly into the extracellular fluids, from where they can reach the blood, cannot be rigorously excluded. However, histological evidence showing an extensive ductal system likely to confine AFGPs suggests that leakage would play only a minor role in AFGP accumulation in the blood, if at all.

2.4.4 AFGPS Are Reabsorbed in the Rectum and Recycled via the Bile

Several lines of evidence suggest that AFGPs, synthesised in exocrine pancreatic tissue and discharged directly into the GI lumen, can reach the blood by reabsorption through the rectum and transfer into the circulatory system. Significantly, AFGP molecules remain intact as they transit the GI tract, presumably because of the presence of protective sugars and the lack of cleavage sites for known digestive proteases (Cheng et al. 2006).

The possibility of rectal uptake of large macromolecules by Antarctic notothenioid fishes was shown initially by Hernandez-Blazquez and Cunha da Silva (1998)

Fig. 2.2 Ectopic exocrine pancreas lying in the intestinal body wall of a *P. antarctica* larva, between the outer and inner muscle layers. Haematoxylin and eosin

who traced the fate of ferritin introduced into the GI tract of *Notothenia neglecta*. Evans et al. (2012a) later showed that AFGPs within the gut of *Trematomus* (*Pagothenia*) *borchgrevinki* can be reabsorbed through the rectal epithelium, transported into the blood, and recycled via the bile. Further support for recycling is derived from the electrophoretic profiles of AFGPs in the intestinal fluid and serum of individual fishes, which are nearly identical suggesting that they may be interconnected.

2.4.5 What Happens to Ice Crystals that Reach the Circulatory System?

Endogenous ice nucleators that function at physiologically relevant temperatures appear to be lacking in both the tissues and body fluids of Antarctic notothenioids (Praebel et al. 2009). The only obvious nucleator is ice itself, which is abundant in the freezing environment of high latitudinal Antarctic fishes. Ice can be imbibed along with drinking and eating (in which case it enters the GI tract, where it can interact with intestinal AFGPs before being purged along with the faeces). Additionally, ice can enter the fish by penetrating its protective epithelial barriers. The most likely situation under which the latter will happen will be as a consequence of wounding.

Ice crystals that are internalised through wounds will immediately interact with AFGPs (and AFPP, where present) that circulate in the blood and body fluids, and crystal growth *in vivo* will be effectively inhibited. Ice crystals stabilized with adsorbed antifreeze molecules that reach the circulation remain potentially hazardous, however, because they may lodge in small vessels and interfere with blood flow, leading to tissue damage and possibly death. An obvious way to minimize this risk is to remove the offending ice crystals from the circulation, a task accomplished primarily by ellipsoid macrophages in the spleen (Evans et al. 2011, 2012a). Although removed from the circulation, the ice will remain inside the spleen until it can be melted or purged in some way (Praebel et al. 2009).

2.4.6 Removal of Internal Ice

Phagocytised ice crystals with absorbed antifreeze will presumably remain relatively stable inside spleen macrophages within the temperature range defining the hysteresis gap, where ice crystals neither grow nor melt. Assuming ice crystals cannot be disposed directly by macrophages (by transfer to the gut, for example, or by concentrating salts in ice-containing phagocytic vesicles) the only way to remove the ice is to await passive thermal melting. For an ectothermal species, this will require environmental warming until the internal temperature exceeds the critical melting point for ice with adsorbed antifreeze. As explained above, however, all internal ice will not be removed until the hysteresis melting point defined by the superheating capacity of the antifreeze is attained (Cziko et al. 2014). Since this critical temperature may never be reached in at least some fishes in the high latitudinal waters of McMurdo Sound, ice must accumulate throughout their lifetime, possibly contributing to their death.

2.5 Freeze Avoidance in the Antarctic Silverfish

Most Antarctic notothenioids have AFGP isoforms ranging in size from 2.6 to 33.7 kDa, with serum levels up to 40 mg mL^{-1}. In a re-evaluation of the antifreeze content of *P. antarctica,* we found serum AFGP levels to be much lower (~1 mg mL^{-1}) and dominated by a single large isoform (~20 kDa), with trace amounts of the six variants of AFGP6. This is one of the few examples of a notothenioid that lacks AFGPs 7 and 8. Native *P. antarctica* serum yields about 1.35 °C hysteresis activity, of which all but 0.23 °C is lost on boiling and thus attributable to heat stable AFGP (Table 2.1). In other notothenioids AFGP-associated hysteresis activity ranges from 1 to 1.3 °C. The remaining 1.12 °C of hysteresis activity in *P. antarctica* serum is due to a heat labile protein similar in behaviour to AFPP, but much larger in size. This novel AFPP-like molecule in *P. antarctica* remains to be characterized in detail.

An unusual AFGP, known as *Pleuragramma* antifreeze glycoprotein (PAGP) was identified in the Antarctic silverfish by Wöhrmann (1996). PAGP was initially thought to be the glycosaminoglycan hyaluronan (a repetitive dimer of glucuronic acid and *N*-acetyl glucosamine) covalently bound at both ends to peptides through

Table 2.1 Freezing point depressions and hysteresis due to different antifreeze molecules

Species	Native serum MPD (°C)	AFGP (°C)	AFPP (°C)	Native serum FPD (°C)	Native serum hysteresis (°C)
Trematomus loennbergii	1.03	0.99	0.47	2.48	1.46
Trematomus borchgrevinki	1.09	1.26	0.98	3.32	2.24
Pleuragramma antarctica	0.86	0.23	1.12	2.21	1.35

AFGP is heat stable, whereas AFPP is heat labile. Native = not boiled
AFGP (°C) = Boiled MP – Boiled FP; AFPP (°C) = Native MP – Boiled FP
MPD melting point depression, *AFGP* anti-freeze glycoprotein, *AFPP* anti-freeze potentiating protein, *FPD* freezing point depression

N-glycosidic linkages at asparagine residues (Wöhrmann 1997). This speculative interpretation was soon dropped, however, in favour of a glycine-rich (30.1 mol %) glycoprotein with a single dominant sugar, *N*-acetyl-glucosamine (Wöhrmann et al. 1997). PAGP (MW ~150 kDa) was determined to be present in roughly the same concentration as AFGP, with the combined antifreezes comprising 2.46 mg mL^{-1} of adult serum (although content varied with age of the fish). AFGP in *P. antarctica* displayed a maximum freezing hysteresis activity of 1.19 °C (20 mg mL^{-1}), while the maximum for PAGP was 1.23 °C (30 mg mL^{-1}). The hysteresis activity of serum (with less antifreeze) was determined to be 1.06 °C which, when added to the estimated colligative effects (0.89 °C), yielded a freezing point of −1.95 °C, above the coldest water temperature likely experienced by adult *P. antarctica* (<−2.1 °C, according to Wöhrmann et al. 1997). The authors argued that adult *P. antarctica* could survive in a supercooled state at depth in the absence of environmental ice, but not in surface waters where they were likely to come into contact with ice crystals. Cziko et al. (2006) reported similar hysteresis and freezing point values for sera from adult *P. antarctica* (0.91 °C and −1.84 °C respectively), but unfortunately independent studies have failed to confirm the existence of the novel PAGP.

The possible existence of a further AFP in notothenioids was described by Lee et al. (2011). These authors identified sequences in *P. antarctica* (and *Notothenia coriiceps*) closely related to the type IV AFP described previously in the longhorn sculpin, *Myoxocephalus octodecemspinosus* (Deng et al. 1997). Recombinant forms of the notothenioid type IV AFPs expressed in *E. coli* by Lee et al. (2011) were found to have ice-binding activity, inducing "star-shaped" morphology in nascent ice crystals. The thermal hysteresis activities of both were extremely low (0.08 °C at 500 μg mL^{-1}), however, and unlikely to be biologically relevant. Indeed, Gauthier et al. (2008) had previously questioned the functional relevance of type IV AFPs, arguing that although they might show antifreeze activity *in vitro*, this was likely coincidental to their primary role. More recently, Xiao et al. (2014) have identified two type IV AFP isoforms (AFP4a and AFP4b) in the goldfish and the zebrafish, and demonstrated their involvement in regulating gastrulation in the latter. Given that neither of these two fish species is polar in origin or distribution, it would seem that a primary antifreeze role for these proteins is indeed unlikely. Although both type IV AFP isoforms are expressed in zebrafish development, only AFP4a is expressed in the adult (primarily in the liver and digestive tract), where its role remains uncertain. A possibly different AFP in *P. antarctica*, identified tentatively by Wöhrmann (1996), was not pursued by the author and no further evidence has emerged in support.

2.5.1 **P. antarctica** *Hatchlings Have Functionally Inadequate Levels of Antifreeze*

The eggs and larvae of some high latitudinal notothenioid species (including *P. antarctica*) are found in frigid environments, often in intimate association with ice. Given the assumed critical importance of antifreeze in protecting adult nototheniids from freezing, it would seem logical to conclude that their early developmental stages would show a similar dependency, but this has not proven always to be the case.

In a study of larvae from three notothenioid species (*Gymnodraco acuticeps*, *P. antarctica*, *T. borchgrevinki*), Cziko et al. (2006) found that only new hatchlings from the cryopelagic *T. borchgrevinki* (examined at <1 day old) had levels of antifreeze sufficient to protect them in their natural environment. The hatchlings from *T. borchgrevinki* were from eggs found within a crevice of a grounded iceberg near Cape Evans, while those from *P. antarctica* were from eggs collected from the sub-ice platelet zone in Terra Nova Bay, both of which are demonstrably frigid environments. Hatchlings from *G. acuticeps* were from eggs collected from a rocky substrate near McMurdo Station at 15–35 m depth. Although this site was potentially impacted by anchor ice, the eggs may have developed in an ice-free environment. However, on hatching *in situ*, *G. acuticeps* larvae were noted to immediately swim upwards to the overlying sea ice, where they are presumably afforded protection from predators within its interstices while having access to abundant food (Evans et al. 2005). As a consequence, within minutes of hatching the larvae of *G. acuticeps* are also exposed to ice-laden water.

2.5.2 Physical Barriers to Ice Propagation

Nototheniid eggs are protected by a pervious chorion (egg envelope), through which seawater and small molecules can penetrate to reach the perivitelline space (the gap between the chorion and the egg membrane). The perivitelline fluid is thus isosmotic with seawater and is without hysteresis activity; indeed, the freezing and melting points of perivitelline fluid from near-term *P. antarctica* eggs were both measured at −1.88 °C (Cziko et al. 2006). Although pervious, the chorion nonetheless provides a significant physical barrier to ice propagation (Davenport et al. 1979; Harvey and Ashwood-Smith 1982; Aarset and Jørgensen 1988; Valerio et al. 1992a; Cziko et al. 2006). Near-term *P. antarctica* embryonated eggs, for example, can survive cooling to below −9.6 °C in the presence of ice as long as their chorion is intact (Cziko et al. 2006). Although this temperature is unlikely to be experienced in their natural environment, intact *P. antarctica* eggs and near-term embryos (both with protective chorions) must be able to avoid freezing in the sub-ice platelet zone (−1.91 °C) in which they are found. Resistance to freezing at such a low temperature also confirms that *P. antarctica* eggs lack endogenous nucleators.

The average freezing point of homogenates from newly hatched larvae of *P. antarctica* has been measured at −0.99 °C (Cziko et al. 2006). How can these hatchlings survive in ice-laden seawater with a typical freezing temperature of −1.91 °C? Just as the chorion provides protection for the egg, the larvae of *P. antarctica* must also have a physical barrier to ice propagation. This is presumably provided by the multi-layered integument (Valerio et al. 1992b), but what unique features of the integument contribute to this property are unknown. Although not as proficient as the egg chorion in protecting against freezing, the larval integument can still protect hatchlings against externally applied ice down to −2.7 °C, a temperature again not reached under natural conditions (Cziko et al. 2006). Thus larvae too can survive in the sub-ice platelet zone, as long as their integument is intact.

The skin of some fishes is known to secrete AFPs (Evans and Fletcher 2004). Although this is not the case in notothenioids, their mucus (at least in *G. acuticeps*, in which it coats the integument extensively) does contain AFGPs, although at low concentration (Evans et al. 2011). In adults, the integument is absent in regions such as the gills, in which a single layer of epithelial cells is in direct contact with the environment. Remarkably, this single cell layer must protect against ice propagation, and presumably does so unless physically damaged. The situation in larvae with respect to the gills is somewhat different, however, since in hatchlings the gills are not yet fully formed. In fact, in 1 day-old *P. antarctica* larvae the gills completely lack even rudimentary filaments and thus the possibility of ice damage through these respiratory organs is significantly reduced (Cziko et al. 2006). Presumably young *P. antarctica* larvae are capable of cutaneous respiration and this must suffice until the gills are fully developed.

2.5.3 *Dynamics of Antifreeze Production in Developing* **P. antarctica**

Newly spawned eggs of *G. acuticeps* contain antifreeze activity (Cziko et al. 2006). Since the mid-blastula transition (in which zygotic genes are activated) occurs much later, the antifreeze content of *G. acuticeps* eggs (providing a freezing hysteresis of 1.02 °C) is presumably maternally derived. The capacity for maternal deposition of antifreeze activity is unlikely to be unique to *G. acuticeps* amongst the nototheniids, but further studies are required. Although newly spawned *P. antarctica* eggs have not been available for analysis, if maternal deposition of antifreeze proves to be the case in this species then antifreeze activity must decline during embryogenesis since hatchlings have very little antifreeze activity, displaying a thermal hysteresis (whole larval homogenate) of 0.11 °C (Cziko et al. 2006). Freezing hysteresis activity must then rise during larval and/or post larval development, subsequently reaching adult levels (adult serum hysteresis 0.91 °C). In *G. acuticeps*, freezing hysteresis activity decreases from 1.02 °C in newly spawned eggs to 0.44 °C in hatchlings (whole larval homogenate), subsequently rising to adult levels (serum hysteresis 1.55 °C) at 147 days post hatch (Cziko et al. 2006).

2.5.4 Development of the Pancreas and Antifreeze Synthesis in **P. antarctica**

Immunohistochemical staining of *P. antarctica* yolk sac larvae shows antifreeze localised in acinar cells of the exocrine pancreas (Fig. 2.3a). Early in larval development the pancreas assumes a significant proportion relative to the rest of the digestive tract and its associated organs (Fig. 2.3b). The precise timing of initial antifreeze synthesis in *P. antarctica* is uncertain, but may well be associated with the first appearance of secretory granules in differentiated pancreatic acinar cells (Fig. 2.3c). Interestingly, on hatch the pharynx appears to be sealed with a thin membrane indicating that external feeding is not initiated immediately. Presumably early in development the pancreatic enzymes assist in the breakdown of yolk nutrients. As a generalization, secretion from the exocrine pancreas develops progressively in teleost larvae (Zambonino Infante and Cahu 2001), and thus antifreeze levels might be expected to progressively increase (as shown by Cziko et al. 2006).

In the zebrafish *Danio rerio*, which serves as a model for vertebrate development, the pancreas arises from two separate endodermal structures, the dorsal and ventral buds (Field et al. 2003; Tiso et al. 2009). The dorsal bud appears first in development at 24 hours post fertilization (hpf) and gives rise to endocrine cells that cluster to form the primary islet, which later repositions to the right side of the embryo. The ventral bud appears anterior to the dorsal bud around 34 hpf, and its component cells (primarily giving rise to acinar and duct cells) subsequently migrate to position themselves around the primary islet cells of the dorsal bud. The exocrine component then expands rostrocaudally along the intestine as the ductal system develops to connect it to the gut (Wan et al. 2006). The primary islet of the endocrine component continues to expand during development and a number of considerably smaller secondary islets also appear (Chen et al. 2007).

Double immunostaining of yolk sac larvae of *P. antarctica* with antibodies directed against insulin and nototheniid AFGP shows exocrine cells positioned around primary islet (endocrine) cells of the dorsal bud, as described in the zebrafish

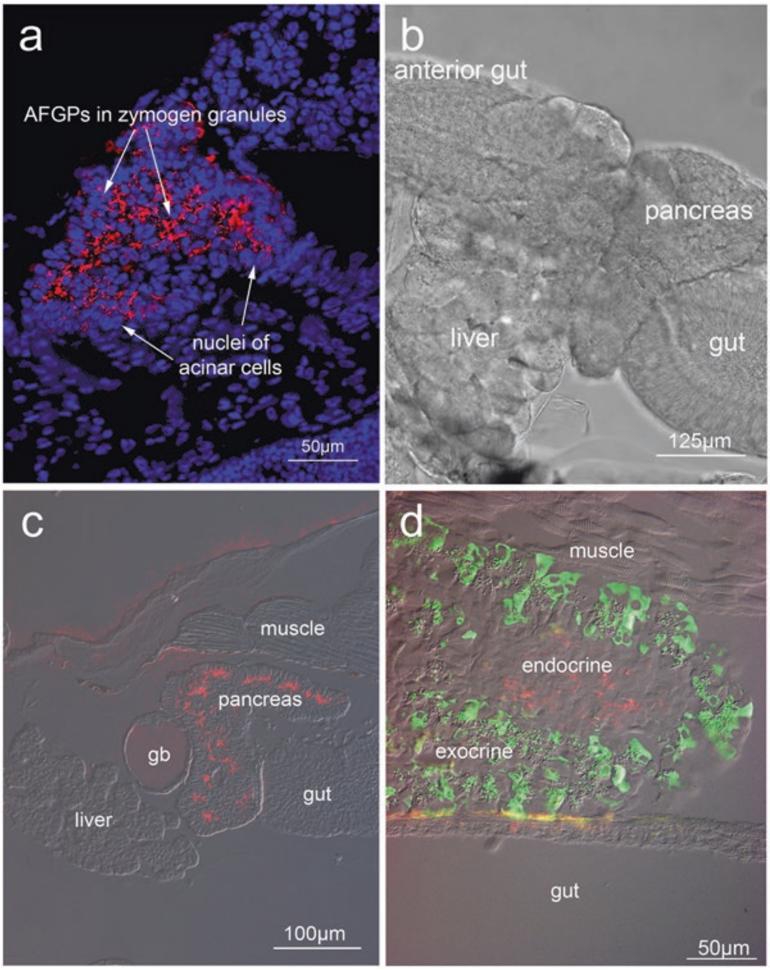

Fig. 2.3 Development of the pancreas and antifreeze synthesis in *P. antarctica*. (**a**) Immunostained AFGPs (red) are present in zymogen granules in the apical regions of acinar cells, the basal nuclei of which are readily apparent (blue). Immunostained with anti-AFGP (primary) and a secondary antibody labelled with Alexa Fluor 594 dye (Life Technologies). Nuclei counterstained with DAPI. (**b**) Freshly excised portion of the digestive tract of young larvae, the pancreas is a relatively large organ compared with the liver. As development proceeds the pancreas forms a collar (from fusion of ventral and dorsal pancreatic buds) to almost encircle the gut, before the latter undergoes a significant relative increase in size. The lobulated structure of the liver is obvious. Phase contrast (unfixed). (**c**) Immunostained AFGPs (red) are present in the exocrine pancreas at an early stage of larval development. The liver, gut (intestinal bulb) and skeletal muscle are negative for AFGP. Note slight immunoreactivity with the outer surface of the skin epithelium (upper surface) and remaining luminal contents of the gall bladder (AFGP recirculates via the bile). Merged immunostained and differential interference contrast (DIC) images. *Gb* gall bladder. (**d**) Early in the development of *P. antarctica*, exocrine pancreatic cells come to be positioned around primary islet (endocrine) cells of the dorsal bud (positioned posteriorly). The endocrine cells express insulin (red), while the exocrine cells secrete AFGPs (green). Note that not all zymogen granules contain AFGPs, suggesting at least some differential packaging after synthesis. Mammalian anti-insulin and nototheniid anti-AFGP antibodies have been stained with Alexa Fluor 594 and Alexa Fluor 488 labelled secondary antibodies respectively. The immunostained image is merged with the corresponding DIC image

(Fig. 2.3d). The pancreas (actively synthesising AFGPs) extends both rostrocaudally and ventrally to form a collar around the developing digestive tract, at the junction between the oesophagus and the intestinal tube. At this stage of development there is no clear anatomical demarcation of the stomach. As development proceeds, cells of the exocrine pancreas come to surround the gall bladder and disperse along the gut tube and associated mesenteries. These dispersed components of exocrine pancreatic tissue can develop substantial ductal systems that can penetrate the wall of the digestive tube and presumably deliver both digestive enzymes and AFGPs direct into the gut, independently of the normally positioned pancreatic duct. As explained previously, the dispersed nature of the pancreas in the notothenioid adult, with ectopic tissue buried in the wall of the digestive tract, can yield false positives for antifreeze synthesis in northern blot analyses unless the tissue sample is also examined histologically. Although AFP production in Arctic fishes is seasonal and apparently regulated through hormonal changes (Fletcher et al. 2001), in Antarctic notothenioids the production of AFGP appears to be constitutive. Thus once initiated early in notothenioid development, synthesis of this antifreeze will likely continue through the lifetime of the fish.

2.6 Can Adult *P. antarctica* Survive in an Ice-Laden Environment?

P. antarctica has a circumpolar distribution and is found in some of the coldest waters of the Southern Ocean. Adult Antarctic silverfish, for example, have been trawled adjacent the Weddell and Ross Sea ice shelves, where often plumes of supercooled water are present in the upper water column (McGuinness et al. 2009; Robinson et al. 2014). Nonetheless, adult *P. antarctica* tolerate cold water only if they avoid contact with ice crystals. The presence of frozen adult *P. antarctica* floating in ice fishing holes as well as in seal access holes in the ice of McMurdo Sound strongly suggests that they froze in the upper water column when they came into contact with suspended ice crystals. Normally *P. antarctica* adults are not found in the surface water, but when pursued by seals they sometimes are chased towards the surface where they encounter ice crystals in the upper water column and freeze, becoming positively buoyant. The presence of floating carcasses in ice fishing holes covered by a heated hut is consistent with this scenario. This is in contrast with observations that the cryopelagic *T. borchgrevinki* often rests on ice platelets and ledges that form in the fishing holes, but do not freeze. Because live specimens of *P. antarctica* are rarely caught it has not been possible to perform controlled freezing experiments to determine their resistance to freezing in the presence of ice and thus it can only be inferred from the observations of frozen specimens floating in the ice-laden surface waters. However, the organismal freezing point of adult *P. antarctica* can also be inferred from a possible generally applicable relationship between the serum and organismal freezing points for notothenioids. In McMurdo Sound notothenioid fishes, the serum freezing point is a close estimate of the temperature at which the fish will freeze in the presence of ice. *T. borchgrevinki* inhabits the ice laden surface waters of McMurdo Sound and its freezes at −2.6 °C, but ice will not grow in its serum above −3.3 °C. A deep water fish, *T. loennbergii*, freezes at −2.0 °C in the presence of ice and its serum freezing point is −2.5 °C. If the same relationship holds for *P. antarctica*, then with a serum freezing point of −2.2 °C (obtained when a small seed crystal is used and it is annealed for several minutes at a temperature slightly above its growth temperature) the adult freezing temperature for this species is about −1.75 °C.

2.7 Ecological Considerations

Despite containing inadequate levels of antifreeze, *P. antarctica* embryonated eggs and hatchling larvae in the vicinity of Terra Nova Bay are found in intimate association with the sub-ice platelet zone. The significance of this relationship is uncertain, but hatching into such an environment may provide crevices for protection from predators and ready access to microorganismal food resources on the underside of the sea ice. Given the relationship with platelet ice, a key question relates to the potential consequences of climate warming that may alter the abundance and/or distribution of platelet ice. As with the adults of other Antarctic notothenioid fishes, larval *P. antarctica* display a critical thermal maximum (CT_{max}) above their normal environmental temperature. The CT_{max} in fishes is a measure of heat tolerance determined as the temperature at which constant warming visibly incapacitates a specimen, either through the loss of equilibrium or the onset of opercular spasms (Bilyk et al. 2012). When *P. antarctica* larvae (n = 20) were tested at a warming rate of ~0.35 °C min^{-1}, their CT_{max} (assessed by loss of equilibrium) was determined to be 14.55 ± 0.87 °C (± s.d.). All larvae recovered their equilibria within a few minutes after immersion in seawater at +0.5 °C. This result is within the CT_{max} range determined for adult notothenioids (Bilyk et al. 2012) but is elevated compared to that from larvae of *G. acuticeps* ($CT_{max} = 13.24 \pm 1.05$; n = 13; $p < 0.001$). The ecological relevance of CT_{max} measurements is uncertain, but thermotolerance assays remain an important tool for understanding and predicting species responses to environmental change (Terblanche et al. 2011). Further analyses employing a wide range of adult and larval notothenioids are required.

The susceptibility of adult *P. antarctica* to freezing is puzzling given that it is a common inhabitant of the coldest bodies of water in the Southern Ocean and is often in close proximity to ice crystals. It is present in the water column in McMurdo Sound throughout the winter, for example, when the seawater is very close to its freezing point. Presumably it escapes freezing in this environment by avoiding the ice-laden surface waters. In locations where it spawns beneath the sub-ice platelet layer, it may release its eggs at depths where small ice crystals are absent because of the depression of the freezing point by hydrostatic pressure.

A key question that arises is why is this fish so abundant in near freezing waters where the potential for freezing from contacting ice crystals is possible. It may be that there is a favourable trade-off between the risk of freezing and access to an abundant food resource. Indeed, the mid water column in these seas is enriched in copepods and krill and the risk of occasional freezing of a small fraction of the *P. antarctica* population thus may well be offset by access to an abundant food resource.

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This manuscript is dedicated to the memory of John A Macdonald, our friend and colleague who was a respected and much liked long-term member of the Antarctic scientific community.

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Chapter 3
The Unique Haemoglobin System of Migratory *Pleuragramma antarctica*: Correlation of Haematological and Biochemical Adaptations with Mode of Life

Guido di Prisco and Cinzia Verde

Abstract In the Southern Ocean the suborder Notothenioidei is dominant. Most notothenioids are benthic and sedentary; we have studied haemoglobin structure/function in search of correlations with mode of life and evolution.

In the Antarctic shelf *Pleuragramma antarctica* (0–900 m) is dominant in abundance and biomass. It has circum-Antarctic distribution, and is the only fully pelagic notothenioid. Being the best example of notothenioid adaptation to pelagic habitats, *P. antarctica* calls for studies on adaptive strategies.

In notothenioids, evolution has developed blood adaptations, such as reduction of erythrocyte number and haemoglobin concentration/multiplicity, reaching the extreme of eliminating haemoglobin in Channichthyidae. Species of the red-blooded families generally only have one haemoglobin (95–99% of the total). In contrast, *P. antarctica* has three major haemoglobins. As this species performs seasonal migrations through water masses that may have different and fluctuating temperatures, during evolution it developed adaptations suitable to allow optimal energy savings during the oxygenation-deoxygenation cycle, producing haemoglobins displaying wide differences in thermodynamic behaviour. The expression of multiple genes, typical of juveniles, remains high also in the adult stage. This oxygen-transport system is remarkably unique and appears designed to fit an unusual mode of life through refined adaptation strategies.

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In the phylogenetic trees, the α^a chain of *P. antarctica* haemoglobins falls into the clade of major Antarctic haemoglobins; the same applies to the β^a chains. The α^b chain is in a basal position with respect to the clade of Antarctic minor Hbs; the same applies to the β^b chain. All this appears congruent with the phylogenetic evidence.

Population dynamics and ecophysiological adaptations of *P. antarctica* are worth investigating to identify strategies of resilience to current climate changes.

Keywords Oxygen-transport system • Haemoglobin • Adaptation

3.1 Introduction

The high-Antarctic shelf seas are characterised by low and constant temperatures (−1.6 to −2.1 °C), oxygen contents of more than 95% saturation (Hellmer and Bersch 1985) and extreme seasonality in ice conditions and plankton production. As in all seas, additional abiotic components, such as a complex system of currents and ice drift, summer stratification due to melting processes and advection and a specific distribution of water masses on the shelf, e.g. in the Weddell Sea (Hubold 1991), govern fish life.

In the Southern Ocean, most Antarctic notothenioids, in the adult stage, are benthic, sedentary fish. Some species show larval and early juvenile pelagic stages that undergo passive transport by strong currents (Matschiner et al. 2009), e.g. the powerful clockwise-flowing Antarctic Circumpolar Current (ACC), and, closer to the continent, the anticlockwise Coastal Current.

Life history, ecophysiology, adaptation and ocean circulation influence the marine ichthyofauna. The suborder Notothenioidei is largely dominant, accounting for 77% of the shelf-fish diversity and 91% of biomass (Eastman 1993, 2005). Eight families (Pseudaphritidae, Bovichtidae, Nototheniidae, Eleginopsidae, Bathydraconidae, Harpagiferidae, Artedidraconidae, Channichthyidae) encompass 139 species, 32 of which (23%) are non-Antarctic (see http://www.oucom.ohiou.edu/dbms-eastman/), i.e. sub-Antarctic or temperate. Seven families have haemoglobin (Hb)-containing erythrocytes, whereas the species of the family Channichthyidae are devoid of Hb (Ruud 1954) and erythrocytes capable of binding oxygen. Bovichtidae (nine species), monotypic Pseudaphritidae and Eleginopsidae became established in sub-Antarctic and temperate waters, e.g. around New Zealand, Australia and high-latitude South America. Notothenioids thriving south of the Antarctic Polar Front (APF) have developed mechanisms of adaptation to extreme life conditions.

In Antarctic shelf areas *Pleuragramma antarctica* Boulenger 1902 (family Nototheniidae; see Eschmeyer 2014 for species designation) is dominant in terms of abundance and biomass (La Mesa and Eastman 2012) and may be the key species in the midwater ecosystem and food web of the shelf (Hubold 1984). It has a circum-Antarctic distribution and is the only fully pelagic notothenioid of the high-Antarctic shelf systems (Hubold 1985). It migrates across different water masses (Andersen 1984; Hubold 1985; Kunzmann 1990).

P. antarctica combines the general adaptations of all Antarctic notothenioids with specialisations necessary for life in the water column (Wöhrmann et al. 1997). It is an important component of the trophic web of the Antarctic marine ecosystem, being one of the principal consumers of zooplankton. It is found in most shelf areas around the continent at depths of 0–700 m (DeWitt et al. 1990) or more, and the developmental stages display stratification by depth (La Mesa et al. 2010). Adults are generally found deeper than 400 m and migrate vertically in the presence of seasonal light (Lancraft et al. 2004). They have an extremely slow growth (Ekau 1988) and a very low-energy-consuming mode of life combined with sluggish and pelagic or benthopelagic behaviour (DeWitt 1970; Johnston 1989; Kunzmann 1990).

Because among notothenioids the life history and ecology of this species is exceptional (Hubold 1985) and due to its unique mode of life and great biological significance in the pelagic systems of the high Antarctic, *P. antarctica* is a suitable target for studies on adaptation to extreme environmental conditions, being the best example of notothenioid adaptation to pelagic habitats (see also Evans and De Vries 2017; Benedetti et al. 2017).

In an adaptive feature typical of Notothenioidei, blood has a reduced number of erythrocytes, counterbalancing the viscosity increase due to low temperature, and a lower haemoglobin (Hb) content (Everson and Ralph 1968; Hureau et al. 1977; Wells et al. 1980). The number of Hb components is also reduced. Species of the red-blooded families generally have a single Hb accounting for 95–99% of the total (Hb 1), and often a minor component, Hb 2 (di Prisco and D'Avino 1989; di Prisco et al. 1990).

Our studies on the biochemistry of oxygen transport in Antarctic fish – at the origin of this overview – have focussed on the molecular structure and oxygen-binding properties of Hbs in search of correlations with mode of life and evolution of Notothenioidei (di Prisco and Tamburrini 1992). The Hbs of many species have been functionally characterised, and their amino-acid sequence has been established (di Prisco et al. 1991a, b; di Prisco and Verde 2015, and references therein).

The fish respiratory system is an excellent model for such studies (Powers 1980), encompassing morphological, physiological and molecular levels. Several haematological parameters can be regulated according to environmental conditions (Val et al. 1990). Integration of haematology with Hb structure and function is a useful approach to gain insight into the development of adaptations of the oxygen-transport system of Antarctic fish.

Molecular investigations on the oxygen-transport system of *P. antarctica,* with special attention to mode of life and evolution in relation to the habitat, revealed that, among notothenioids, *P. antarctica* is the only one having three major Hbs.

Their amino-acid sequence, oxygen-binding properties, and thermodynamic features have been investigated (Tamburrini et al. 1996, 1997; Tamburrini and di Prisco 2000).

3.2 The Blood Parameters and the Structure/Function Relationship of the Hbs of *P. antarctica*

3.2.1 Blood Parameters

Table 3.1 summarises the values of the blood parameters of *P. antarctica*, together with relevant statistical information; erythrocytes were of similar size (11.5 µm in longitudinal direction) and shape (ellipsoid) as those of other nototheniids (Kunzmann 1991).

In comparison with other nototheniids (Kunzmann et al. 1992), the values of haematocrit, erythrocyte number and Hb concentration are at the lower end of the range of values known for red-blooded Antarctic fishes and very similar to those of *Aethotatis mitopteryx*, a closely related species of the same family. From the values of MCHC and MCH and of oxygen solubility in plasma (0.8 vol%) (Grigg 1967), a total blood O_2-CC of 4.32 vol% is calculated, falling within the ranges reported for this ecologically important parameter in other Antarctic notothenioids (Kunzmann 1991). The values of Po_2 and Pco_2 are likely to be affected by stress due to capture and handling, but the difficulty to keep specimens alive for long periods of time prevents measurement on animals recovered from stress.

Although it is difficult to establish an unambiguous correlation between haematological parameters and mode of life, the data are in agreement with the sluggish (although migratory) behaviour of *P. antarctica*.

Table 3.1 Blood parameters of *P. antarctica*

Parameter	Value	SD
Hematocrit	16.6%	9.8
Erythrocytes	0.43×10^{12}/l	0.17
Haemoglobin	26.5 g/l	8.1
MCHC	159.6 g/l	–
MCH	61.6 pg	
Blood O_2-CC	4.32 vol%	
pH	7.66	0.07
P_{O2}	29.6 mm Hg	8.9
P_{CO2}	3.0 mm Hg	1.6

MCHC means corpuscular Hb concentration, *MCH* means cellular Hb content, O_2-CC carrying capacity for oxygen, P_{O2} *and* P_{CO2} partial pressure of oxygen and carbon dioxide, respectively, *SD* standard deviation

3.2.2 Hb Multiplicity

The haemolysate of red-blooded notothenioids generally contains a single major Hb (Hb 1) and often a minor component (Hb 2, approximately 5% of the total). The two Hbs have the β chain in common (di Prisco 1988; di Prisco and D'Avino 1989; di Prisco et al. 1991a, b), with the only exception of the bathydraconid *Cygnodraco mawsoni*, in which Hb 1 and Hb 2 share the α chain (Caruso et al. 1991). A cathodal Hb (Hb C), with the α chain in common with Hb 1, is present in trace amounts in adult fish, except in the nototheniid *Trematomus newnesi*, in which it accounts for 20–25% of the total (di Prisco et al. 1991a).

Electrophoretic analysis on the haemolysate shows that *P. antarctica* has three major components (Hb 1, Hb 2 and Hb 3), which were purified by ion-exchange chromatography. Trace amounts of a fourth component coeluted with Hb 3. The globin chains were isolated by reverse-phase chromatography of the purified Hbs (D'Avino and di Prisco 1989). Their elution times, amino-acid composition, and migration in SDS-polyacrylamide gel electrophoresis indicated that Hb 1 has the α chain in common with Hb 2 and the β chain in common with Hb 3. Hb 2 and Hb 3 have no chain in common. Thus, the Hb system of *P. antarctica* is made of two α and two β chains. The derived chain composition of Hb 1, Hb 2, and Hb 3 is, respectively, $\alpha^a{}_2\beta^a{}_2$, $\alpha^a{}_2\beta^b{}_2$ and $\alpha^b{}_2\ \beta^a{}_2$.

The experimental details of the purification and elucidation of the amino-acid sequences of the three Hbs are outlined in Tamburrini et al. (1996, 1997).

3.2.3 Amino-Acid Sequence

The N terminus of the α chain in common between Hb 1 and Hb 2 (α^a) and of the α chain of Hb 3 (α^b) is not available to Edman degradation. Similar to all teleost Hbs sequenced to date, the blocking group is acetyl, as shown by fast-atom-bombardment mass spectrometry of the N-terminal tryptic peptides.

Cleavage of peptide bonds of α^a, α^b, β^a and β^b was achieved by trypsin digestion and cleavage of an internal Asp-Pro bond with *o*-phthalaldehyde. Tryptic peptides were purified by reverse-phase HPLC. Alignment of the tryptic peptides was obtained by overlaps and by homology with other known fish Hb sequences.

The complete sequence of the two α and two β chains (142 and 146 residues, respectively) constituting the three Hbs of *P. antarctica* is reported in Fig. 3.1. A fourth component, isolated in trace amounts, differs from Hb 3 only in having Glu instead of Gln at position 94 of the β chain, suggesting microheterogeneity.

Table 3.2 summarises the degree of sequence identity between the globin chains of *P. antarctica* Hbs and those of Hbs of a selection of Antarctic and non-Antarctic notothenioids, Arctic and temperate species. In α chains, high identity was observed between the chain in common in Hb 1 and Hb 2 and those of the main Hbs of notothenioids, and between the chain of Hb 3 and those of minor Hb 2 of notothenioids.

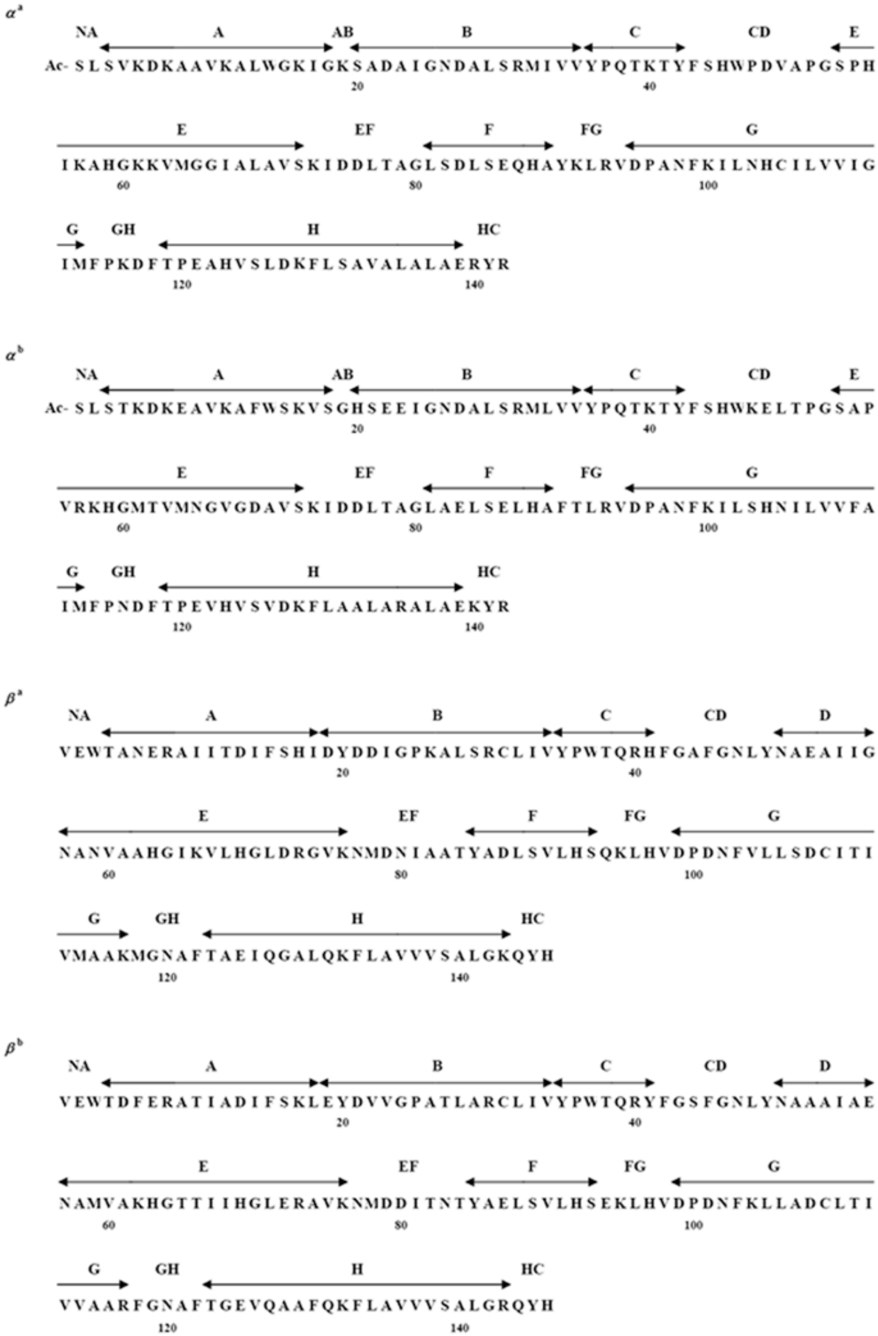


Fig. 3.1 Amino-acid sequences of the α and β chains of the Hbs of *P. antarctica*. α^a is identical in Hb 1 and Hb 2; α^b is the α chain of Hb 3. β^a is identical in Hb 1 and Hb 3; β^b is the β chain of Hb 2

Table 3.2 Chain sequence identity (%) of *P. antarctica* (*Pa*) Hbs with Hbs of a selection of notothenioids of several families with Antarctic and non-Antarctic (n-a) distribution, Arctic (arc) and temperate (temp) species

α chains																		
Species	*Ss*	*Aa*	*Tt*	*Gm*	*Am*	*Am*	*Pa*	*Tn*	*Gg*	*Nc*	*Ga*	*Ao*	*Pu*	*Gg*	*Na*	*Nc*	*Pa*	*Tn*
Hb		*A*		*2*	*1*	*2,3*	*3*	*2*	*2*	*2*			*1,2*	*1*	*1*	*1*	*1,2*	*1,C*
T. bernacchii Hb1,C	54	54	76	59	66	74	67	62	64	66	90	87	91	94	96	89	91	97
T. newnesi Hb1,C	63	53	76	60	64	72	66	63	64	66	91	85	76	92	93	87	88	
P. antarctica Hb1,2	58	56	77	57	68	75	69	64	66	66	84	88	80	91	93	86		
N. coriiceps Hb1	51	50	71	56	62	68	64	61	61	62	81	83	71	86	90			
$^{\text{n-a}}$ *N. angustata Hb1*	57	54	76	59	67	75	69	64	65	66	88	90	77	95				
G. gibberifrons Hb1	55	53	74	61	69	74	71	65	67	68	89	86	78					
$^{\text{n-a}}$ *P. urvillii Hb1,2*	58	54	76	57	66	76	64	63	64	65	75	74						
A. orianae	58	56	73	58	66	73	69	64	65	66	81							
G. acuticeps	54	52	74	59	64	71	68	65	64	66								
N. coriiceps Hb2	61	56	66	66	80	68	92	93	95									
G. gibberifrons Hb2	62	57	65	66	80	68	92	95										
T. newnesi Hb2	63	57	64	66	57	66	90											
P. antarctica Hb3	64	57	66	67	79	69												
$^{\text{arc}}$ *A. minor Hb2,3*	62	57	73	58	72													
$^{\text{arc}}$ *A. minor Hb1*	69	57	68	65														
$^{\text{arc}}$ *G. morhua Hb2*	58	52	61															
$^{\text{temp}}$ *T. thynnus*	59	54																
$^{\text{temp}}$ *A. anguilla HbA*	57																	
$^{\text{temp}}$ *S. salar*																		

β chains																		
Species	*Aa*	*Tt*	*Gm*	*Am*	*Am*	*Tb*	*Tn*	*Pu*	*Pa*	*Gg*	*Ga*	*Ao*	*Pu*	*Gg*	*Na*	*Nc*	*Pa*	*Tn*
Hb	*C*		*2,3*	*3*	*1,2*	*C*	*C*	*2*	*2*	*2*			*1*	*1*	*1,2*	*1,2*	*1,3*	*1,2*
T. bernacchii Hb1	60	67	71	76	75	69	69	69	71	68	82	80	81	95	91	88	90	93
T. newnesi Hb1,2	57	65	70	72	75	67	67	67	68	66	80	78	77	89	84	84	83	
P. antarctica Hb1,3	62	67	71	74	72	69	69	69	71	69	79	76	77	91	87	85		
N. coriiceps Hb1,2	60	66	69	75	78	70	70	69	71	68	80	82	78	89	93			
$^{\text{n-a}}$*N. angustata Hb1,2*	60	65	71	76	78	70	70	70	72	69	80	83	80	93				
G. gibberifrons Hb1	60	67	71	77	76	71	71	71	73	69	82	81	78					
$^{\text{n-a}}$*P. urvillii Hb1*	60	69	71	71	77	69	68	68	69	67	74	73						
A. orianae	58	65	69	71	74	67	67	67	70	68	77							
G. acuticeps	58	63	65	70	71	66	67	65	68	65								
G. gibberifrons Hb2	58	62	73	82	67	91	89	87	90									
P. antarctica Hb2	60	65	71	84	68	91	91	84										
$^{\text{n-a}}$*P. urvillii Hb2*	60	63	71	84	68	88	86											
T.newnesi HbC	60	60	68	81	66	95												
T. bernacchii HbC	58	62	70	82	75													
$^{\text{arc}}$*A. minor Hb1,2*	59	71	71	73														
$^{\text{arc}}$*A. minor Hb3*	58	65	72															
$^{\text{arc}}$*G. morhua Hb2,3*	58	69																
$^{\text{temp}}$*T. thynnus*	60																	
$^{\text{temp}}$*A. anguilla HbC*																		

T. bernacchii (*Tb*), *Trematomus bernacchii*; *T. newnesi* (*Tn*), *Trematomus newnesi*; *N. coriiceps* (*Nc*), *Notothenia coriiceps*; *N. angustata* (*Na*), *Notothenia angustata*; *G. gibberifrons* (*Gg*), *Gobionotothen gibberifrons*; *P. urvillii* (*Pu*), *Pseudaphritis urvillii*; *A. orianae* (*Ao*), *Artedidraco orianae*; *G. acuticeps* (*Ga*), *Gymnodraco acuticeps*; *A. minor* (*Am*), *Anarhichas minor*; *G. morhua* (*Gm*), *Gadus morhua*; *T. thynnus* (*Tt*), *Thunnus thynnus*; *A. anguilla* (*Aa*), *Anguilla anguilla*; *S. salar* (*Ss*), *Salmo salar*. Grey areas: chains of major and minor Hbs

In β chains, high identity was observed between the chain in common in Hb 1 and Hb 3 and those of the main Hbs of notothenioids, and between the chain of Hb 2 and those of the minor components of notothenioids. Similar to other notothenioids, *P. antarctica* Hbs have low identity with Hbs of temperate fish, but show higher identity with Hbs of the Arctic zoarcoid *Anarhichas minor* (Verde et al. 2002). This observation is reflected in the phylogenetic trees (see below).

3.2.4 Considerations on the Primary Structures

The oxygen affinity (see below) of the three Hbs is much higher than that of the other notothenioids. In Hb 2, the substitution ValβE11 → Ile, reported to be responsible for lower affinity in engineered human Hb at alkaline pH (Nagai et al. 1987; Mathews et al. 1989), does not decrease the affinity in comparison with Hb 1 (in which the α chain is identical). The substitution GluFG1 → Gln in the β^a chain, identical in Hb 1 and Hb 3, does not hinder a strong Root effect. Thus, in these Hbs, the Root effect must be due to other mechanisms than formation of a salt bridge between GluβFG1 and HisβHC3, as suggested by Ito et al. (1995) for the Root-effect Hb of the Antarctic teleost *Trematomus bernacchii*, because no such salt bridge is possible in the T state. In *T. bernacchii*, AspαG1, AspβG3, and AspβG1 interact with each other, and half of the Root effect has been ascribed to these interactions (Ito et al. 1995); these residues are also found in the Hbs of *P. antarctica.* Within the positive charge cluster proposed by Mylvaganam et al. (1996) to be responsible for the other half of the Root effect in Spot HbCO, ValβNA1, LysβH21, and HisβHC3 are conserved, but LysβEF6 is not, similar to all antarctic Hbs except in one minor component (di Prisco et al. 1991a). The constraints that stabilise the positive charge cluster once again cannot include the bond between GlnβHC1 and GluβFG1 in Hb 1 and Hb 3, because in FG1 Glu is replaced by Gln.

Among the other residues suggested by Perutz and Brunori (1982) to be involved in the molecular mechanism of the Bohr and Root effects in fish Hbs, LysαC5, SerβF9, GlnβHC1, and HisβHC3 are conserved in the three Hbs. In the phosphate binding site, AspβNA2 is conservatively replaced by Glu; in the β^a chain, ArgβH21 is conservatively replaced by Lys, similar to all other Hbs of Antarctic fish (di Prisco et al. 1991a), and LysβEF6 is replaced by Ala and Thr in the β^a and β^b chain, respectively. The substitution of LysβEF6 with nonpolar or neutral residues is frequently found in the Hbs of Antarctic fish and does not decrease the effect of organophosphates on the oxygen affinity of Bohr- and Root-effect Hbs (di Prisco et al. 1991a).

3.2.5 Oxygen-Binding and Effect of Temperature

Hb 1, Hb 2, and Hb 3 showed a strong, effector-enhanced dependence of oxygen affinity on pH (alkaline Bohr effect). In comparison with Hb 1 and Hb 2, the Hill coefficient n_H of Hb 3 denoted lower cooperativity (enhanced by organophosphates)

Table 3.3 Oxygen affinity (P_{50}) of the Hbs of *P. antarctica,* at 2 °C, in the absence (−) and presence (+) of the physiological effectors

100 mM NaCl, 3 mM ATP		P_{50} pH 6.5	pH 7.0	pH 7.5	pH 8.0
Hb 1	−	43.65	19.27	3.98	1.97
	+	51.29	38.02	6.31	2.14
Hb 2	−	42.66	20.89	6.31	2.99
	+	53.70	40.73	7.94	2.75
Hb 3	−	30.90	24.55	5.62	2.45
	+	51.29	40.74	10.00	3.80

Table 3.4 Heat of oxygenation of *P. antarctica* Hbs

100 mM NaCl, 3 mM ATP		ΔH (kcal/mol O_2) pH 7.0	pH 8.0
Hb 1	−	−12.8	−15.3
	+	−8.6	−17.4
Hb 2	−	−3.6	−6.4
	+	−1.8	−8.1
Hb 3	−	−0.1	−16.5
	+	−4.1	−7.6

of oxygen binding. At pH 6.5, the values of n_H were close to one in all components, indicative of Root effect.

The Root effect (Root 1931; Brittain 1987) was in fact displayed by the three components, and its amplitude was enhanced by the effectors, indicating a strong pH dependence of Hb oxygenation in air. *P. antarctica*, as all Antarctic notothenioids, lacks a swimbladder, and Root-effect Hbs may be associated with the occurrence of a choroid *rete* in the eye that, being poorly vascularised in fish, depends on the diffusion of oxygen from other tissues.

Details on the Bohr and Root effects in the absence and presence of the effectors are outlined in Tamburrini et al. (1996, 1997).

The oxygen affinity of the three Hbs is very high, P_{50} at pH 8.0 ranging from 1.97 to 2.99 mm of Hg in the absence and from 2.14 to 3.8 mm of Hg in the presence of the physiological effectors. Additional data showing the influence of effectors on the oxygen affinity are reported in Table 3.3. Although low oxygen affinities have been found in most of the investigated species of Antarctic fish that live in waters that can reach over 95% oxygen saturation (Macdonald et al. 1987; Kunzmann 1991; di Prisco et al. 1991a), Hbs with high oxygen affinity may be of advantage because *P. antarctica* lives in the water column, where different water bodies with considerably lower oxygen concentration can easily occur.

The effect of temperature on the oxygen affinity shows that Hb 1, Hb 2, and Hb 3 have different values of heat of oxygenation, in both the absence and presence of the effectors (Table 3.4). Moreover, in their absence, Hb 3 shows the largest ΔH variation between pH 7.0 and 8.0 (16.4 kcal/mol; Hb 1 and Hb 2 have 2.5 and 2.8 kcal/mol, respectively), whereas in their presence, it shows the smallest variation (3.5 kcal/mol; Hb 1 and Hb 2, showing the opposite trend, have 8.8 and 6.3 kcal/mol, respectively).

Some implications of these functional features are analysed below.

3.2.6 Thermodynamics and Mode of Life

The Hb system of pelagic, sluggish but migratory *P. antarctica* is made of three high-affinity major components (see Table 3.3). It displays the highest multiplicity among Notothenioidei. Most species of this suborder have a single major Hb, generally regulated by pH, chloride and organophosphates (di Prisco et al. 1991a), except two active cryopelagic nototheniids, i.e. *T. newnesi*, having two (D'Avino et al. 1994), and *Trematomus borchgrevinki* (for re-naming, see Eschmeyer 2014), having five (Riccio et al. 2000).

The three Hbs of *P. antarctica* are similar in several oxygen-binding features (e.g. they all display the Bohr and Root effects), but they show differences and peculiarities that deserve some comments. They have similar oxygen affinities, higher than those of the other notothenioids. Organophosphates enhance cooperativity at all pH values higher than 6.5, and lower the oxygen affinity also at pH 8.0 in Hb 3 only, indicating strong interaction with the binding site even under alkaline conditions.

However, attention should mainly be addressed to the thermodynamic differentiation of the three components (see Table 3.4). Hb 1 and Hb 3 show a very strong enthalpy change at pH 8.0, further enhanced by the effectors in the former, but drastically decreased in the latter; the heat of oxygenation of Hb 2, in the presence and absence of effectors, is much lower (Tamburrini et al. 1996, 1997). A dramatic decrease is observed at lower pH in Hb 3 and Hb 2 (ΔH approaches zero in both); in contrast, Hb 1 retains high oxygenation enthalpy (especially without effectors). These observations indicate a stronger Bohr effect at physiological temperatures in Hb 1 (in the presence of effectors) and Hb 3 (also in their absence). The moderate effect of temperature on Hb 2 in the pH range 7.0–8.0 and on Hb 3 at pH 7.0 is indicative of energy-saving mechanisms of oxygen loading and unloading.

The ensemble of thermodynamic features of the three components is likely to reflect highly refined energy-saving molecular mechanisms of adaptation to a pelagic mode of life.

3.2.7 Phylogeny

The Neighbour Joining trees inferred from the α- and β-globin amino-acid sequences of Hbs from Antarctic and sub-Antarctic notothenioids, and several Arctic and temperate fish species (used as markers of species phylogeny) are shown in Figs. 3.2 and 3.3, respectively. The obtained topology is in general agreement with the trees

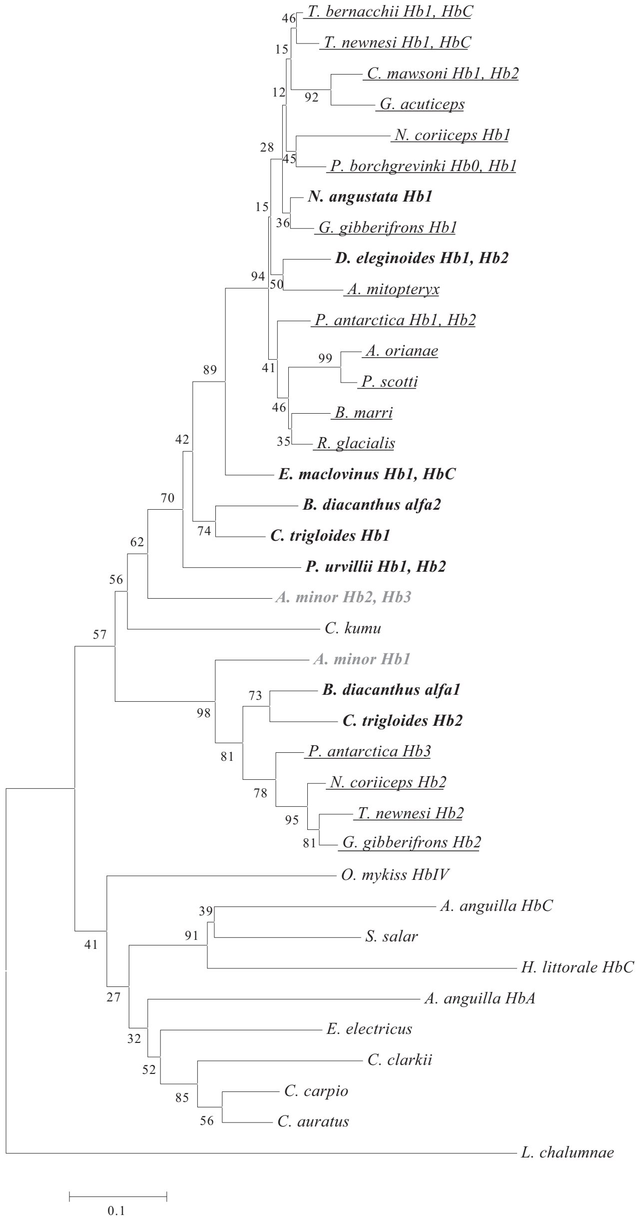
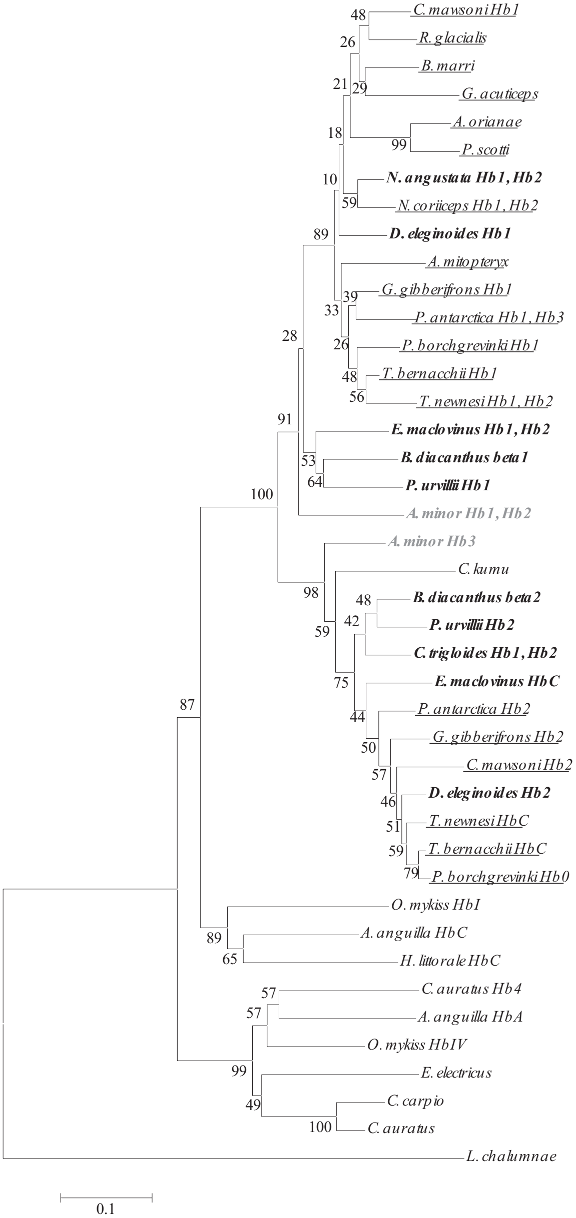


Fig. 3.2 Phylogenetic tree of amino-acid sequences of α chains of Notothenioidei having Antarctic distribution (*underlined*; *R. glacialis*, *Racovitsia glacialis*; *B. marri*, *Bathydraco marri*; *P. scotti*, *Pogonophryne scotti*), Notothenioidei having non-Antarctic distribution (*black bold*; *D. eleginoides*, *Dissostichus eleginoides*; *E. maclovinus*, *Eleginops maclovinus*; *B. diacanthus*, *Bovichthus diacanthus*; *C. trigloides*, *Cottoperca trigloides*), Arctic (*grey bold*), and temperate (*black*; *C. kumu*, *Chelidonichthys kumu*; *O. mykiss*, *Oncorhynchus mykiss*; *H. littorale*, *Hoplosternum littorale*; *E. electricus*, *Electrophorus electricus*; *C. clarkii*, *Catostomus clarkii*; *C. carpio*, *Cyprinus carpio*; *C. auratus*, *Carassius auratus*) fish Hbs. Bootstrap values above 50 (percentage of 10,000 replicates) are given at the nodes

Fig. 3.3 Phylogenetic tree of amino-acid sequences of β chains of Arctic, Antarctic and temperate fish Hbs. See Fig. 3.2 for details



inferred by a Bayesian method (di Prisco et al. 2007) and trees obtained by maximum likelihood analysis (Verde et al. 2006; di Prisco and Verde 2015, and references therein). Although recent work indicates that Nototheniidae and Bathydraconidae are paraphyletic (Dettaï et al. 2012), we are provisionally retaining the traditional family names. The molecular evolution of Hb in polar fish and the relations with mode of life are discussed in Verde et al. (2012).

Multiple alignments of α- and β-globin sequences were performed with the programme CLUSTAL X (Thompson et al. 1997; Verde et al. 2006). The sequence of *Latimeria chalumnae* was included in the analysis as out-group. Phylogenetic trees of globin sequences were constructed using the Neighbour Joining (NJ) method implemented in the programme MEGA 3 (Kumar et al. 2004). The genetic distances were measured according to the *p*-distance model to evaluate to what extent the resulting tree differs from the expected interrelationships among species in a given cluster of orthologs (i.e. gene copies diversified by speciation). Robustness of the NJ trees was assessed by bootstrap analysis with 10,000 replications.

The topologies shown in the trees indicate that the globin chains of major and minor notothenioid Hbs cluster in two well separated, strongly supported monophyletic groups, and the globins of temperate fish Hbs constitute the most ancestral clade. The time of the gene duplication event that gave origin to the two paralogous groups of major and minor Hbs suggests that they diverged long before the first stock of ancestral notothenioids.

The availability of reliable phylogeny data for Antarctic notothenioids allows inferences to be made on which factor may have promoted species diversification within the suborder. In fact, by jointly considering the phylogenetic relationships and the geographic distributions of several taxa, it appears that speciation occurred repeatedly across both high-Antarctic and sub-Antarctic biogeographic zones (Bargelloni et al. 2000).

The obtained topology is in general agreement with the maximum-likelihood method (Giordano et al. 2006). The α^a chain of *P. antarctica*, shared by Hb 1 and Hb 2, falls into the clade of major Antarctic Hbs, and the same applies to the β^a chain shared by Hb 1 and Hb 3. The α^b chain of *P. antarctica* Hb 3 is in a basal position with respect to the clade of Antarctic minor Hbs and the same applies to the β^b chain of Hb 2. The position of the globins of *P. antarctica* appears congruent with the phylogenetic evidence from nuclear and mitochondrial genes (Bargelloni et al. 2000).

The globin trees also include Arctic species. The Arctic globins occupy variable positions in both trees, suggesting independent evolutionary histories, with the exception of *A. minor*, an Arctic zoarcoid fish, which is close to the notothenioid clades, in agreement with the teleostean phylogeny (Verde et al. 2006).

3.3 Concluding Remarks

The respiratory properties of blood, especially the oxygen-carrying capacity and oxygen affinity of Hb, respond to evolutionary selective pressures. In Antarctic notothenioids, evolution has developed a variety of adaptations in the oxygen-transport system, such as reduction of erythrocyte number, Hb concentration and multiplicity (di Prisco et al. 1991a; Wells et al. 1980), reaching the extreme of eliminating Hb and erythrocytes capable of binding oxygen in icefishes (Ruud 1954).

Among the investigated species of Nototheniidae and of the other red-blooded families of the suborder Notothenioidei, pelagic *P. antarctica* is the only one having higher multiplicity: three major components, characterised by strong Bohr and Root effects and unusually high oxygen affinity, and regulated by temperature to widely different extents.

T. newnesi also has three Hbs: Hb C, Hb 1, and Hb 2 (D'Avino et al. 1994). However, the latter is a minor component, and only one (Hb C) of the two major components displays the Bohr and Root effects. The mode of life of these two notothenioids is widely different. The Hb system of *T. newnesi* (an active, cryopelagic fish) must conceivably ensure oxygen delivery to tissues also in conditions of acidosis. In turn, *P. antarctica*, albeit a migratory, pelagic species, is considered sluggish (see Eastman 1993, p 218). The main adaptive feature of the Hb system of this fish should conceivably be the response to the need to save energy during migration across water regions where, due to the complex system of currents and gyres characterising all depths of the Antarctic oceanic system, the low temperature is likely to show significant differences and fluctuations, reflected in the oxygen-saturation percentage. In fact, as in *T. newnesi*, the selective advantage offered by multiple Hb genes appears clearly. The expression of multiple genes remains high in these two species also in the adult stage, in close similarity with juveniles (di Prisco et al., unpublished).

T. borchgrevinki, another active, cryopelagic fish, has five functionally different Hbs (Riccio et al. 2000).

Hence, while a single Hb (or none at all) appears sufficient to all notothenioids when they are sedentary bottom dwellers, *P. antarctica* can instead rely on three major Hbs with high oxygen affinity, which differ functionally in subunit cooperativity, phosphate regulation, and, above all, overall heat of oxygenation and influence of pH on temperature regulation of oxygen affinity. It is tempting to speculate that during evolution the oxygen-transport system of *P. antarctica* has developed physiological and biochemical adaptations suitable to allow optimal energy savings in the oxygenation-deoxygenation cycle during migrations largely driven by currents through water masses where the cold temperature may not keep constant, producing Hbs displaying the widest differences in the thermodynamic behaviour rather than in pH and organophosphate regulation. The possibility that each component (namely a fraction of the total Hb) becomes selectively functional may explain the need for high oxygen affinity.

A. mitopteryx, a very closely related benthopelagic species (Andersen 1984) with a very sluggish mode of life, has a single Hb with a moderate Bohr effect and no Root effect (D'Avino et al. 1992). These observations suggest that the oxygen demands of these two species are different, and likely to arise from the respective special environmental conditions.

In temperate and tropical fish, ratios between multiple Hbs can vary seasonally and synthesis on demand is possible (Love 1980). This may well be a typical feature of the oxygen-transport system of *P. antarctica.* This species performs seasonal migrations (Kunzmann 1991) through water masses that (within the range typical of the environment) may have different and fluctuating temperatures. Thus, the main adaptive feature of the Hb system of this fish should conceivably be the response to the need to save energy during migrations.

From this standpoint, and on the basis of their relative amounts and of the awareness that *P. antarctica* (as well as *A. mitopteryx*) is a modern species (Iwami 1985), none of the three Hbs of *P. antarctica*, unlike the minor components found in benthic notothenioids, can be considered as evolutionary (or larval) remnants devoid of physiological significance (di Prisco et al. 1991a), even though the sequence data reveal high phylogenetic distance between Hb 1 and the globins of Hb 2 and Hb 3 that are not in common (see Table 3.2). In this species, the expression of multiple genes, typical of juveniles (di Prisco et al., unpublished), remains high also in the adult stage, suggesting refined mechanisms of regulation within the gene family.

The loss of Hb expression in Hb-less Channichthyidae, in which retention in the genome of inactive α-globin-related sequences has been demonstrated (Cocca et al. 1995; di Prisco et al. 2002), is worth noting.

In conclusion, among Notothenioidei, the oxygen-transport system of *P. antarctica*, one of the most specialised ever found in fish, is remarkably unique and appears designed to fit the unusual mode of life of this fish through highly refined molecular mechanisms of adaptation in the thermodynamic features of each Hb.

The three Hbs may also serve different purposes during ontogeny, because young post-larvae, juveniles and adults have different environmental temperature preferences (Hubold 1985). The depth range of *P. antarctica* is from 0 to 900 m (Gerasimchuk 1986); hence, it is exposed to hydrostatic pressure changes, due to vertical displacement, which may act as major selective factors (Somero 1990), regulating biological activities in fish. Therefore, *P. antarctica* is a suitable target for studies on the effect of pressure on conformational changes in multisubunit proteins such as Hbs. These investigations may shed light on this critical point of fish physiology and are a promising area for future research.

No less important will be studies of population dynamics and ecophysiological adaptations of *P. antarctica*, in order to identify strategies of resilience to current climate changes. These issues are discussed in detail in other chapters of this Volume (Ashford et al. 2017; Mintenbeck and Torres 2017).

Climate changes in the Antarctic Peninsula deserve some short considerations. *P. antarctica* plays a major trophic role in coastal Antarctic waters as a predator of krill and as prey for penguins and seals. Agostini et al. (2015) investigated the population structure of *P. antarctica* along the shelf of the Antarctic Peninsula, and found that the eastern and western sides host genetically distinct populations. *P. antarctica* depends on sea ice for reproduction, however sea ice at the western side is decreasing because of warming. Sea-ice declines are causing migration of krill and, consequently, of *P. antarctica*. The lack of *P. antarctica* in the diet of Adélie penguins at Palmer Station and the closely linked penguin precipitous decline thus appear as impacts of climate change.

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Chapter 4
Pro-oxidant Challenges and Antioxidant Adaptation of *Pleuragramma antarctica* in Platelet Ice

Maura Benedetti, Maria Elisa Giuliani, and Francesco Regoli

Abstract Antarctic organisms developed specific adaptation mechanisms making these species able to survive to extreme environment conditions. Among fishes, *Pleuragramma antarctica* presents a specific peculiarity due to the occurrence of eggs with fully developed yolk-sac embryos below the platelet ice layer. This ice is an environment with strong pro-oxidant characteristics at the beginning of austral spring, when the rapid growth of algal ice communities, the massive release of nutrients and the photoactivation of dissolved organic carbon and nitrates represent an important sources for oxyradical formation. Such processes are concentrated in a short period of a few weeks, which overlaps with the final stage of development of *P. antarctica* embryos in platelet ice. For this reason, embryonated eggs of *P. antarctica*, before hatching, should possess adequate protection toward the marked and sudden increase of reactive oxygen species exposure. In this respect, molecular and functional characteristics of antioxidants in *P. antarctica* provide new insights on the modulation of the antioxidant defence pathway in response to varied environmental pro-oxidant challenge. To this aim, the main antioxidant components have been characterized in *P. antarctica* sampled from platelet ice in its nursery area in the Ross Sea, and data on nucleotide and protein sequences have been integrated with the analysis of regulation at transcriptional and functional levels. The results revealed a marked temporal increase of antioxidants in embryos of *P. antarctica* as adaptive counteracting response to oxidative conditions of platelet ice.

Keywords Antioxidant defence • Antarctic fishes • Catalase • Glutathione peroxidase

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4.1 Introduction

4.1.1 The Oxidative Stress Adaptation in Antarctic Organisms

Life in cold and oxygen-rich Antarctic waters has evolved through several adaptive mechanisms that allowed the optimal performance of cellular machineries and physiological processes in such environmental conditions. Molecular strategies observed in Antarctic fishes include the loss of specific genomic information (e.g. heat-shock response and oxygen transport proteins) (Hofmann et al. 2000; di Prisco et al. 2002), the appearance of genes with new functions (e.g. antifreeze proteins genes) (Fletcher et al. 2001), gene duplications (e.g. pepsin A, hepcidin and genes involved in mitochondrial function, protein and lipid metabolism) (Brier et al. 2007; Chen et al. 2008; Xu et al. 2008; Coppe et al. 2013), modifications in specific gene sequences that optimize the protein function at cold temperature (Kawall et al. 2002; Fields and Houseman 2004; Brier et al. 2007; Bilyk and Cheng 2013). In this respect, cold-adapted (or psychrophilic) enzymes exhibit higher efficiencies (*k*cat/*K*m) due to the increase of the catalytic rate constant (*k*cat) relative to the Michaelis-Menten constant (*K*m) at low temperatures (Feller and Gerday 1997). The understanding of molecular and biochemical mechanisms of adaptation has benefited from the growth of phylogenetic, x-ray crystallography and mutagenesis studies, since a direct correlation exists between the function of a protein, its three-dimensional structure, and the gene sequence (D'Amico et al. 2002; Marx et al. 2007). Comparative analyses between psychrophilic and mesophilic homologues demonstrated that the key residues in the active site, responsible for the catalytic mechanism, are strictly conserved: however, cold-active enzymes can present a few other structural modifications, which commonly result in a high conformational flexibility (i.e. decreased stability) of specific regions of the proteins, and a better accessibility of the active site. These features seem to be the main requirement for maintaining a high catalytic efficiency at low temperature (D'Amico et al. 2002). Similar molecular and kinetic characteristics have been observed also for antioxidant enzymes (Lorentzen et al. 2006, Riise et al. 2007; Wang et al. 2011), which represent a matter of growing interest for polar organisms, often characterized by a more elevated capability to neutralize toxic oxyradicals compared to similar temperate species.

As suggested by Shick and Dykens (1985), the basal efficiency of oxygen detoxification systems is influenced by the natural pro-oxidant pressure to which organisms are exposed, and the capability to modulate antioxidant defenses represents a typical adaptation mechanism in organisms that face fluctuating enhancements of pro-oxidant pressure during their life cycle. The antioxidant system consists of a highly sophisticated network (Fig. 4.1) containing antioxidant enzymes and low molecular weight scavengers, which interact with both direct and indirect effects (Regoli and Giuliani 2014). Among the enzymes, superoxide dismutase (SOD) is responsible for O_2^- removal, but it also represents a source of H_2O_2, promptly reduced to H_2O by catalase (CAT), glutathione peroxidases (GPx) and some

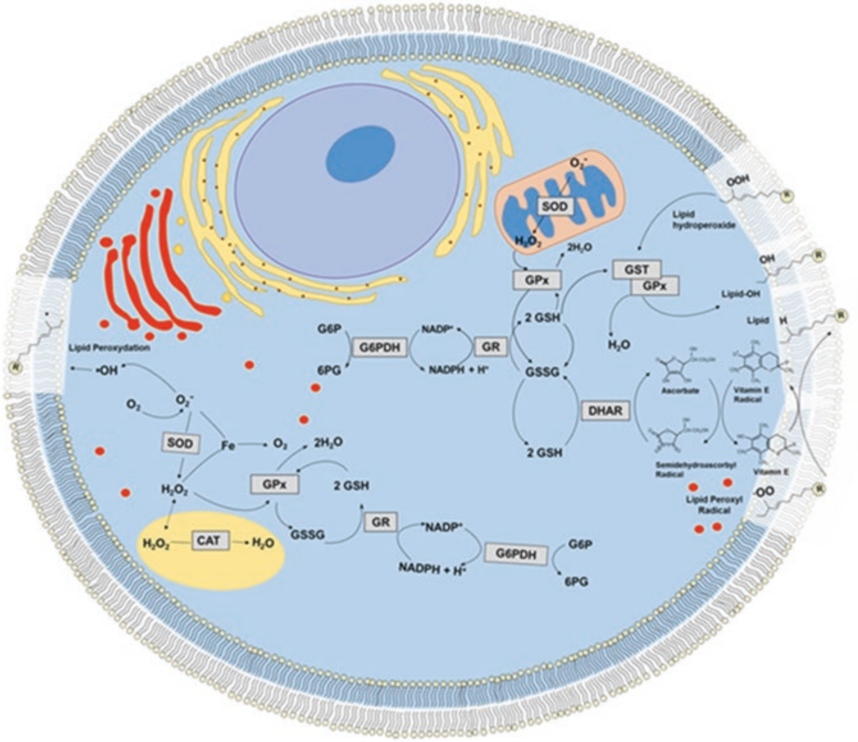


Fig. 4.1 Main cellular antioxidant defenses and antioxidant pathways. *CAT* catalase, *SOD* superoxide dismutase, *GPx* glutathione peroxidases, *GR* glutathione reductase, *GSH* reduced glutathione, *GSSG* oxidised glutathione, *GST* glutathione S-transferases, *DHAR* dehydroascorbate reductase, *G6PDH* glucose 6-phosphate dehydrogenase, *G6P* glucose 6-phosphate, *6PG* 6-phospogluconate

isoforms of glutathione S-transferases (GST). Although it is not a real antioxidant enzyme, glutathione reductase (GR) is nonetheless essential to maintain the correct GSH/GSSG ratio and the intracellular redox status. Scavengers of reactive oxygen species (ROS) include ascorbate, reduced glutathione (GSH) and α-tocopherol (vitamin E); the latter assumes a fundamental role in counteracting lipid peroxidation in Antarctic organisms, where elevated content of polyunsaturated fatty acids (PUFA) enhances the susceptibility of cellular membranes to oxidative stress conditions (Yamamoto et al. 2001; Abele and Puntarulo 2004).

The role of antioxidants in the adaptation to extreme environmental conditions has been widely documented for polar invertebrates. The Antarctic scallop *Adamussium colbecki* and the Arctic scallop *Chlamys islandica* exhibit higher activities of catalase, glutathione reductase and glutathione peroxidases compared to the Mediterranean species *Pecten jacobaeus* and *Mytilus galloprovincialis* (Viarengo et al. 1995; Regoli et al. 1997, 2000a). Similarly, polar molluscs (e.g. *A. colbecki*, *C. islandica*, *Laternula elliptica*, *Mya truncata*) have a greater adaptive antioxidant capability compared to temperate counterparts (*P. jacobaeus*, *Mya arenaria*), as demonstrated by the higher total oxyradical scavenging capacity (TOSC) toward peroxyl radicals (ROO·) and hydroxyl radicals (HO·) (Regoli et al. 1997, 2000a; Camus et al. 2005). Additional studies on the ice-associated Arctic amphipod *Gammarus wilkitzkii* have revealed a much stronger scavenging capacity towards ROO· and HO· during summer than in winter (Krapp et al. 2009), suggesting that antioxidant protection is adjusted to meet seasonal metabolic requirements and higher pro-oxidant challenge when organisms graze sea ice algae and copepods during the ice melting (Werner 2000; Scott et al. 2001). Levels of thiobarbituric acid reactive substances (TBARS) were also elevated during the summer in amphipods, indicating higher rates of lipid peroxidation when the sea ice cover is at its minimum (Krapp et al. 2009).

More limited and contrasting evidences are available on the involvement of antioxidants in adaptation of polar fishes. Higher activities of antioxidant enzymes have been measured in red-blooded Antarctic nototheniids compared to temperate teleosts and white-blooded channichthyids (the Antarctic icefish) (Witas et al. 1984; Cassini et al. 1993; Ansaldo et al. 2000; Nahrgang et al. 2010), while low molecular weight scavengers such as vitamin E were more abundant in the heart and muscle tissues of channichthyids than of nototheniids (Johnston et al. 1998). The latter evidence is probably attributed to remarkably high density of mitochondria typical in icefish tissues, resulting in enhanced intracellular oxygen flux and higher susceptibility to ROS-induced damage to cellular membranes (Filho et al. 1993).

Several hypotheses have been postulated to identify different pro-oxidant factors and the need of antioxidant adaptation in Antarctic organisms. Depending on species, physiological features, biological interactions and life cycle, major oxidative challenges can include: the elevated levels of dissolved oxygen in cold seawater, the membrane lipid composition to maintain fluidity at low temperatures, the high density of mitochondria in fish, algal symbionts and photosynthetically produced oxygen in several invertebrates, reduced respiration rate to compensate for low food supply and long periods of starvation, marked changes in food availability and feedings activities, efficient long term protection needed for RNA and proteins due to the extremely low turnover rate and high exposure to UV radiation and oxidative conditions during sea ice melting (Abele and Puntarulo 2004; Camus et al. 2005; Regoli et al. 2011).

4.1.2 **Pleuragramma antarctica** ***Pro-oxidant Challenges***

The Antarctic silverfish *Pleuragramma antarctica* represents an interesting model to investigate the complex interactions between cellular antioxidant machineries and adaptation to natural oxidative pressure. This notothenioid fish is widely distributed in Antarctic shelf waters, with a key role in the food webs of Southern Ocean, representing a major contribution to the diet of many vertebrates such as whales, seals, penguins, flying birds and benthic fishes (La Mesa et al. 2004). Among the few pelagic species derived from a benthic notothenioid ancestor, the entire life cycle of *P. antarctica*, from the eggs to the adult phase, occurs in the water column. After the spawning, pelagic eggs are thought to float freely and accumulate into the platelet ice packed under the sea ice where predation is greatly prevented by the inaccessibility of this unusual brooding site (La Mesa and Eastman 2012).

In November 2002, a hatching and nursery area of silverfish has been detected at Terra Nova Bay (TNB), Ross Sea, with huge amounts of embryonated eggs developing in the subsurface platelet ice (Vacchi et al. 2004). From this discovery, scientific effort has been directed to evaluate presence, abundance, geographical distribution and ecology of early life stage of *P. antarctica* in that region (Vacchi et al. 2004, 2012a, b; La Mesa et al. 2010; La Mesa and Eastman 2012; Guidetti et al. 2015).

The presence of Antarctic silverfish embryos in ice platelets during the last stage of development has also interesting implications in terms of physiological response to a rapid increase of environmental pro-oxidant pressure at the beginning of Antarctic spring. In fact, when sunlight returns, extremely high concentrations of organic matter, phosphates, nitrates, dissolved organic carbon (DOC), proteins, lipids, carbohydrates, bacteria biomass and diatoms are released from the lower layer of coastal sea ice. The photo-activation of DOC, nitrates and oxygen determines the formation of ROS, a phenomenon accentuated in polar regions due to ozone depletion (Hader et al. 2015). In addition, the rapid increase of algal growth and associated photosynthetic processes further contributes to oxygen oversaturation in the platelet ice environment (Gleitz et al. 1995; Thomas et al. 2001; Delille et al. 2007).

4.2 Molecular and Functional Adaptation of Antioxidants in *Pleuragramma antarctica*

The oxidative reactions in platelet ice are concentrated in a relatively short period, generally 3–6 weeks, ranging from the middle of October to the end of November. For such reason, embryonated eggs of *P. antarctica*, completing their development in this layer before the hatching, should possess adequate protection toward the marked and sudden increase of ROS exposure.

In this respect, investigations on molecular and functional characteristics of antioxidants in *P. antarctica* may provide new insights on the possibility for this species to modulate such intricate pathway of defenses in response to varied environmental pro-oxidant challenge. To this aim, the main antioxidant components have been characterized in *P. antarctica* sampled from platelet ice in its nursery ground in the Ross Sea in 2003 and 2013, and data on nucleotide and protein sequences have been integrated with the analysis of regulation at transcriptional and catalytic levels, as reported in present chapter.

Table 4.1 Homology of *P. antarctica* Cat amino acid sequence with catalase from other representative species

Species	Identity	Accession number
Antarctic fishes		
Pagothenia borchgrevinki	100%	AHJ37522
Trematomus bernacchii	98%	Deduced from GBXS01045998
Other fishes		
Oplegnathus fasciatus	91%	AAU44617
Siniperca chuatsi	91%	AIA98428
Sparus aurata	90%	AFV39797
Oryzias melastigma	88%	AGE44248
Takifugu obscurus	87%	ABV24056
Danio rerio	86%	NP_570987
Salmo salar	85%	ACN11170
Other vertebrates models		
Mus musculus	80%	NP_033934
Rattus norvegicus	80%	NP_036652
Bos taurus	80%	NP_001030463
Xenopus laevis	79%	NP_001080544
Homo sapiens	78%	NP_001743

4.2.1 Nucleotide and Amino Acid Sequences of Antioxidants in Pleuragramma antarctica

Despite the lack of specific crystallographic and kinetic studies for *P. antarctica* antioxidant enzymes, the nucleotide sequences of catalase (*cat*) and glutathione peroxidases (*gpx*) were obtained for the first time from total RNA isolated from the embryonated eggs developing at Terra Nova Bay. Primers designed on homologous genes have allowed amplification, cloning and subsequent sequencing of *cat* and *gpx*. Sequence alignment (BLAST) demonstrated that *P. antarctica cat* belongs to the clade III of monofunctional heme-containing catalases, which includes small subunit catalases from bacteria, archaea, fungi and higher eukaryotes.

Comparative analysis performed on the deduced amino acid sequence through ClustalW multiple alignment tool, showed that *P. antarctica* Cat is highly conserved with catalase sequences from other vertebrate species. The amino acid sequence revealed a 98% and 100% identity when compared with catalase of *Pagothenia borchgrevinki* and *Trematomus bernacchii* (the only Antarctic fishes in which this antioxidant has been sequenced), 85–91% identity with catalase from other temperate fishes (marine and freshwater), 78–80% identity with other vertebrate models (from the amphibian *Xenopus laevis* to mammals, such as *Mus musculus*, *Rattus norvegicus*, *Bos taurus*, *Homo sapiens*) (Table 4.1). The extremely high conservation among Cat sequences from *P. antarctica*, *P. borchgrevinki* and *T. bernacchii* reflects the taxonomical proximity of these species, all belonging to the Nototheniidae family.

Monofunctional heme-containing catalases are homotetrameric enzymes with a high degree of similarity in the three-dimensional structure. Each subunit contains four domains (the N-terminal arm, the β-barrel domain, the wrapping domain and the α-helical domain) and a heme prosthetic group. The heme-active site is located deep in the β-barrel domain and a major channel provides access of the substrate from the surface to the heme group. Several catalases also bind NADPH, whose role is likely to prevent formation of inactive reaction intermediates (Fita and Rossmann 1985; Díaz et al. 2012).

The multiple alignment of *P. antarctica* Cat and the search for conserved domains have highlighted several conserved structural features in monofunctional heme-containing catalase of Antarctic silverfish (Fig. 4.2). Both the proximal heme-ligand signature, RLFSYPDTH, and the proximal active site signature, FDRERIPERVVHAKGGG, (Prosite database, http://prosite.expasy.org) are present in *P. antarctica* Cat (Fig. 4.1). Amino acid residues involved in the active site (like the essential histine His75, its nearest neighbour Ser114 and the coessential asparagine Asn148 – *Bos taurus* numbering) are perfectly conserved (Fig. 4.2; Fita and Rossmann 1985; Díaz et al. 2012), suggesting that the catalytic mechanism is preserved. The residues lining the major substrate channel are also conserved (Gerhard et al. 2000), as well as the NADPH binding residues (Lorentzen et al. 2006), thus indicating the ability of Cat to bind such cofactor. A few, non-conservative amino acid substitutions were found in Cat, which appear peculiar for the three Antarctic fishes catalase: a Tyr replaces Val440 in the α10 helix, which is involved in NADPH binding; a Gly is found instead of Val247, located in the outermost part of the major channel (Fita and Rossmann 1985); a semi-conservative substitution is found in position 322, at the connection between the β-barrel and the wrapping domain, where an alanine residue substitutes a proline, strictly conserved in the other species (Fig. 4.2). These substitutions may potentially alter some conformational aspects of the three-dimensional structure of the protein. Tyrosine is an acidic residue, usually in contact with the solvent and exposed to the surface, while valine is hydrophobic and tends to be buried in the inside portion of the protein, protected from water. Valine has also a big lateral chain, unlike glycine which is the smallest amino acid and provide high flexibility to the protein structure. Proline has the opposite effect, providing rigidity due to rotational constrains of its ring structure; thus it has a great influence on protein architecture. The unique presence of these substitutions limited to *P. antarctica*, *T. bernacchii* and *P. borchgrevinki* suggests their involvement in the functioning of catalase in these Antarctic species.

Another key antioxidant characterized in embryos of *P. antarctica* is the glutathione peroxidase (*gpx*). The identification of the nucleotide sequence and the alignment of the deduced amino acid sequence showed a significant homology with the isoform 1 of the Gpx family (Gpx1) from other vertebrate species, highlighting a high conservation across different classes (Table 4.2).

The most elevated identity (98–99%) has been found with species from the same family Nototheniidae (*T. lepidorhinus*, *T. bernacchii*, *T. eulepidotus*) and with *Cygnodraco mawsoni*, family Bathydraconidae. This is consistent with the close relationship between Antarctic notothenioid lineages and the strong selective

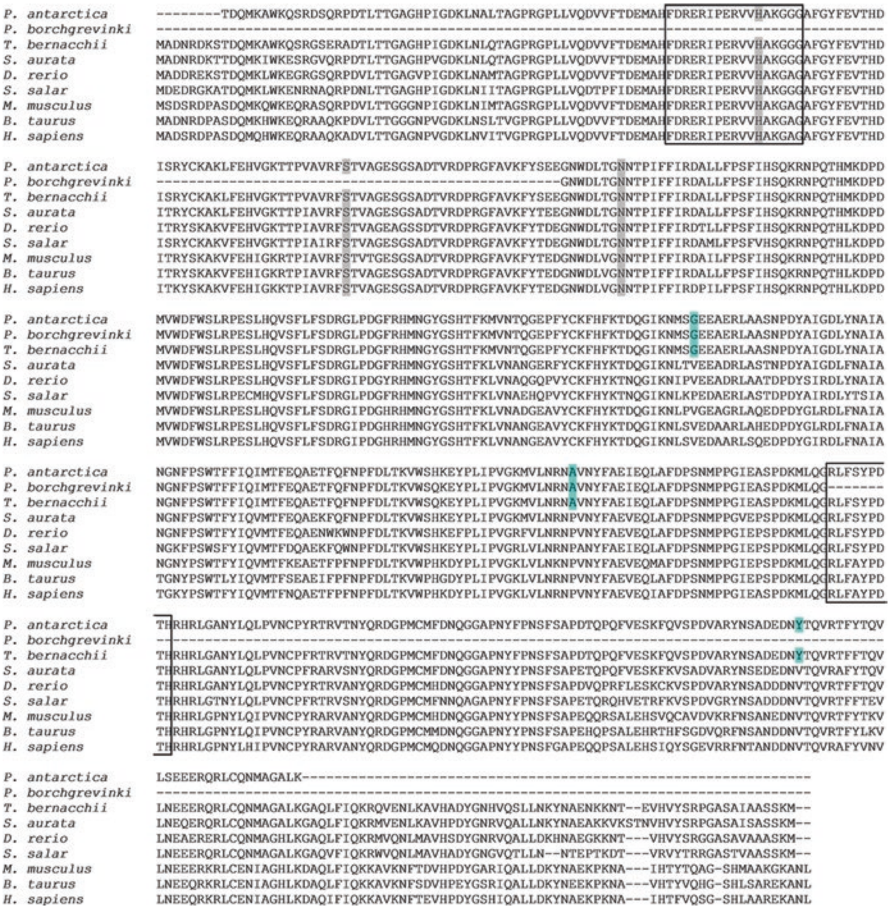


Fig. 4.2 Multiple alignment of *P. antarctica* Cat with amino acid sequences from Antarctic fishes (*T. bernacchii* and *P. borchgrevinki*), other fish species (*S. aurata*, *D. rerio, S. salar*) and mammalian models (*M. musculus, B. taurus, H. sapiens*). The proximal active site signature (FDRERIPERVVHAKGGG) and the proximal heme-ligand signature (RLFSYPDTH) are boxed. Amino acid residues involved in the active site are highlighted in *grey*. Residues peculiar of Antarctic species (G247, A322, Y440 – *B. taurus* numbering) are shown in *blue*

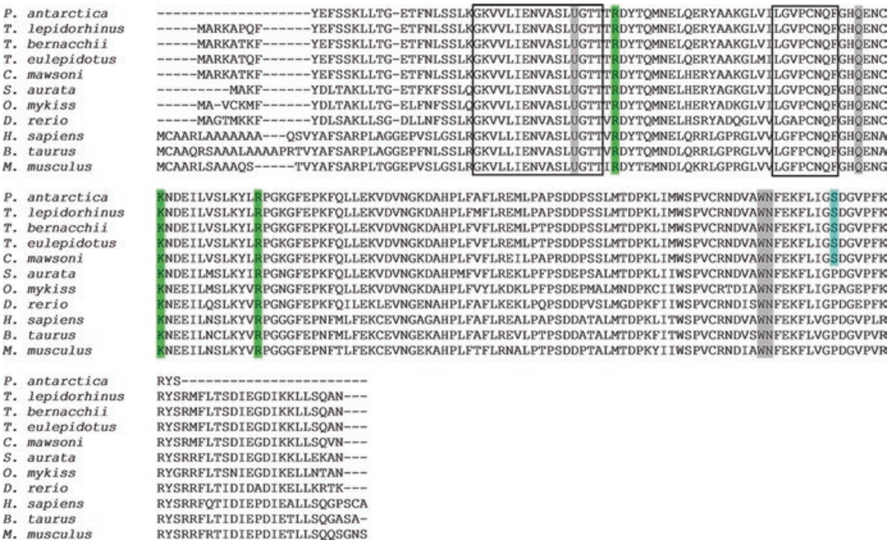
pressure (due to extreme environmental condition), which drove the evolution of a highly adapted fauna. *P. antarctica* Gpx shows a relatively high percentage of identity also with Gpx1 proteins from other marine and freshwater fishes (from 77% to 88%), and with Gpx1 from various mammalian models – primates, rodents and other mammals (72–75% identity) (Table 4.2).

The analysis of the open reading frame performed on *P. antarctica gpx1* nucleotide sequence has highlighted an inframe stop codon (TGA), which is known to encode for the selenocysteine residue (Sec or U; Fig. 4.3) when a selenocysteine insertion sequence (SECIS) is present in the 3′ UTR of the mRNAs (Walczak et al.

Table 4.2 Homology of *P. antarctica* Gpx1 amino acid sequence with glutathione peroxidase (Gpx1) from other representative species

Species	Identity	Accession number
Antarctic fishes		
Trematomus lepidorhinus	99%	ADV03913
Trematomus bernacchii	99%	ADV03914
Trematomus eulepidotus	98%	ADV03912
Cygnodraco mawsoni	98%	ADV03915
Other fishes		
Sparus aurata	88%	AFY97791
Siniperca chuatsi	87%	AIA98427
Thunnus maccoyii	83%	ABO38817
Ictalurus punctatus	83%	NP_001187670
Seriola lalandi	82%	AEI91048
Anguilla japonica	82%	ACN78878
Megalobrama amblycephala	81%	AHJ80778
Oncorhynchus mykiss	81%	NP_001117997
Danio rerio	78%	NP_001007282
Oreochromis niloticus	77%	NP_001266640
Mammalian models		
Homo sapiens	75%	NP_000572
Bos taurus	74%	NP_776501
Mus musculus	72%	NP_032186
Rattus norvegicus	72%	NP_110453

1996). The selenocysteine, the 21st amino acid, is an important residue in the active site of selenium-dependent isoforms of the Gpx family, since it is necessary for maximal catalytic activity (Epp et al. 1983; Rocher et al. 1992). Besides the Sec residue, additional conserved features of Gpx1 protein have been found in *P. antarctica* Gpx1 after multiple alignments with homologous sequences (Fig. 4.3). The Gpx1 amino acid sequence contains three conserved residues (Gln87, Trp165 and Asn166; *B. taurus* numbering) which are located in the active site of the enzyme (Fig. 4.3; Epp et al. 1983; Ursini et al. 1995): homology and mutational studies in *Drosophila*, *Escherichia coli* and mammals, suggested those residues as essential for Gpx catalytic efficiency (Ursini et al. 1995; Brigelius-Flohé and Maiorino 2013) and, together with the active Sec residue, they are referred to as the "catalytic tetrad" of the Gpx family (Tosatto et al. 2008). *P. antarctica* Gpx1 also contains two highly conserved arginine and a lysine residues (Arg57, Arg103 and Lys91) (Fig. 4.3), which are probably involved in the binding of glutathione molecules, as deduced from x-ray and docking experiments (Epp et al. 1983; Brigelius-Flohé and Maiorino 2013). Proline 174 (*B. taurus* numbering), well conserved in temperate species, is replaced by a serine residue in Gpx1 of *P. antarctica* and of other Antarctic fishes (Fig. 4.3). Such amino acid substitution, known to improve the protein flexibility, is placed between two β-sheets (β4 and β5), and exposed on the protein surface, oppo-



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P. antarctica    -------------------YEFSSKLLTG-ETFNLSSLKGKVVLIENVASLUGTTTRDYTQMNELQERYAAKGLVILGVPCNQFGHQENC
T. lepidorhinus  -----MARKAPQF------YEFSSKLLTG-ETFNLSSLKGKVVLIENVASLUGTTTRDYTQMNELQERYAAKGLVILGVPCNQFGHQENC
T. bernacchii    -----MARKATKF------YEFSSKLLTG-ETFNLSSLKGKVVLIENVASLUGTTTRDYTQMNELQERYAAKGLVILGVPCNQFGHQENC
T. eulepidotus   -----MARKATQF------YEFSSKLLTG-ETFNLSSLKGKVVLIENVASLUGTTTRDYTQMNELQERYAAKGLMILGVPCNQFGHQENC
C. mawsoni       -----MARKATKF------YEFSSKLLTG-ETFNLSSLKGKVVLIENVASLUGTTTRDYTQMNELHERYAAKGLVILGVPCNQFGHQENC
S. aurata        ---------MAKF------YDLTAKLLTG-ETFKFSSLQGKVVLIENVASLUGTTTRDYTQMNELHERYAGKGLVILGVPCNQFGHQENC
O. mykiss        -----MA-VCKMF------YDLTAKLLTG-ELFNFSSLQGKVVLIENVASLUGTTTRDYTQMNELHERYADKGLVILGVPCNQFGHQENC
D. rerio         -----MAGTMKKF------YDLSAKLLSG-DLLNFSSLKGKVVLIENVASLUGTTVRDYTQMNELHSRYADQGLVVLGAPCNQFGHQENC
H. sapiens       MCAARLAAAAAAA---QSVYAFSARPLAGGEPVSLGSLRGKVLLIENVASLUGTTVRDYTQMNELQRRLGPRGLVVLGFPCNQFGHQENA
B. taurus        MCAAQRSAAALAAAAPRTVYAFSARPLAGGEPFNLSSLRGKVLLIENVASLUGTTVRDYTQMNDLQRRLGPRGLVVLGFPCNQFGHQENA
M. musculus      MCAARLSAAAQS-----TVYAFSARPLTGGEPVSLGSLRGKVLLIENVASLUGTTIRDYTEMNDLQKRLGPRGLVVLGFPCNQFGHQENG

P. antarctica    KNDEILVSLKYLRPGKGFEPKFQLLEKVDVNGKDAHPLFAFLREMLPAPSDDPSSLMTDPKLIMWSPVCRNDVAWNFEKFLIGSDGVPFK
T. lepidorhinus  KNDEILVSLKYLRPGKGFEPKFQLLEKVDVNGKDAHPLFMFLREMLPAPSDDPSSLMTDPKLIMWSPVCRNDVAWNFEKFLIGSDGVPFK
T. bernacchii    KNDEILVSLKYLRPGKGFEPKFQLLEKVDVNGKDAHPLFVFLREMLPTPSDDPSSLMTDPKLIMWSPVCRNDVAWNFEKFLIGSDGVPFK
T. eulepidotus   KNDEILVSLKYLRPGKGFEPKFQLLEKVDVNGKDAHPLFVFLREMLPTPSDDPSSLMTDPKLIMWSPVCRNDVAWNFEKFLIGSDGVPFK
C. mawsoni       KNDEILVSLKYLRPGKGFEPKFQLLEKVDVNGKDAHPLFVFLREILPAPRDDPSSLMTDPKLIMWSPVCRNDVAWNFEKFLIGSDGVPFK
S. aurata        KNDEILMSLKYIRPGNGFEPKFQLLEKVDVNGKDAHPMFVFLREKLPFPSDEPSALMTDPKLIIWSPVCRNDVAWNFEKFLIGPDGVPFK
O. mykiss        KNEEILMSLKYVRPGNGFEPKFQLLEKVDVNGKDAHPLFVYLKDKLPFPSDEPMALMNDPKCIIWSPVCRTDIAWNFEKFLIGPAGEPFK
D. rerio         KNEEILQSLKYVRPGNGFEPKFQILEKLEVNGENAHPLFAFLKEKLPQPSDDPVSLMGDPKFIIWSPVCRNDISWNFEKFLIGPDGEPFK
H. sapiens       KNEEILNSLKYVRPGGGFEPNFMLFEKCEVNGAGAHPLFAFLREALPAPSDDATALMTDPKLITWSPVCRNDVAWNFEKFLVGPDGVPLR
B. taurus        KNEEILNCLKYVRPGGGFEPNFMLFEKCEVNGEKAHPLFAFLREVLPTPSDDATALMTDPKFITWSPVCRNDVSWNFEKFLVGPDGVPVR
M. musculus      KNEEILNSLKYVRPGGGFEPNFTLFEKCEVNGEKAHPLFTFLRNALPTPSDDPTALMTDPKYIIWSPVCRNDIAWNFEKFLVGPDGVPVR

P. antarctica    RYS-----------------------
T. lepidorhinus  RYSRMFLTSDIEGDIKKLLSQAN---
T. bernacchii    RYSRMFLTSDIEGDIKKLLSQAN---
T. eulepidotus   RYSRMFLTSDIEGDIKKLLSQAN---
C. mawsoni       RYSRMFLTSDIEGDIKKLLSQVN---
S. aurata        RYSRRFLTSDIEGDIKKLLEKAN---
O. mykiss        RYGRRFLTSNIEGDIKELLNTAN---
D. rerio         RYSRRFLTIDIDADIKELLKRTK---
H. sapiens       RYSRRFQTIDIEPDIEALLSQGPSCA
B. taurus        RYSRRFLTIDIEPDIETLLSQGASA-
M. musculus      RYSRRFRTIDIEPDIETLLSQQSGNS
```

Fig. 4.3 Multiple alignment of *P. antarctica* Gpx1 with amino acid sequences from Antarctic fishes (*T. lepidorhinus*, *T. bernacchii*, *T. eulepidotus* and *C. mawsoni*), other fish species (*S. aurata*, *O. mykiss*, *D. rerio*) and mammalian models (*H. sapiens*, *B. taurus*, *M. musculus*). Key residues are highlighted: the catalytic tetrad in *grey* (U52, Q57, W165, N166); the glutathione binding residues in *green* (R57, K91, R103); the serine residue (S174) peculiar of Antarctic species in *blue*. The Gpx active site motif and the Gpx signature 2 motif are *boxed*

site to the selenocysteine active-site (Epp et al. 1983; Ren et al. 1997). Moreover, some typical motifs are also present in *P. antarctica* Gpx1: the Gpx active site motif (GKVVLIENVASLUGTT) and the Gpx signature 2 motif (LGVPCNQF), with a Val replacing the Phe, similarly to all other fish sequences (Fig. 4.3; Prosite database, http://prosite.expasy.org).

A key role in modulation of the oxidative stress response is assumed by the nuclear factor erythroid 2-related factor 2 (Nrf2), transcription factor regulating the expression of genes under the control of the antioxidant response element (ARE); these include antioxidant genes, those catalysing biotransformation and conjugating reactions, metabolism of glutathione, and production of reducing equivalents (Kobayashi and Yamamoto 2006). Under basal non-stressed conditions, Nrf2 is mainly localized in the cytosol, associated with its repressor Keap1 (Kelch like-ECH-associated protein 1) and targeted to proteasomal degradation (Cullinan et al. 2004; Tong et al. 2006). The increase of intracellular ROS levels induces the dissociation of Nrf2 from Keap1 and the translocation of Nrf2 to the nucleus, with consequent activation of expression of target genes (Dinkova-Kostova et al. 2005). The system returns to its basal state when the redox homeostasis is restored. The Nrf2-Keap1 pathway is still relatively unexplored in marine organisms. Homologous genes of *nrf2* have been characterized in a few fish species (i.e. the Atlantic salmon (*Salmo salar*), the zebrafish (*Danio rerio*), the European eel (*Anguilla anguilla*), and the mummichog (*Fundulus heteroclitus*)), which highlighted an elevated

Table 4.3 Homology of *P. antarctica* Nrf2 amino acid sequence with Nrf2 from other representative species

Species	Identity	Accession number
Antarctic fishes		
Pagothenia borchgrevinki	99%	AHJ37517
Trematomus bernacchii	97%	Deduced from GBXS01081376
Notothenia coriiceps	96%	XP_010792797
Other fishes		
Oplegnathus fasciatus	84%	BAM36384
Takifugu rubripes	67%	ABF22469
Salmo salar	60%	NP_001133279
Danio rerio	52%	NP_878309
Silurus lanzhouensis	50%	ABG90498
Other vertebrate models		
Homo sapiens	40%	NP_006155
Rattus norvegicus	39%	NP_113977
Bos taurus	39%	NP_001011678
Mus musculus	39%	NP_035032
Xenopus laevis	36%	NP_001086307

percentage of nucleotide (74–78%) and amino acid identity (76–77%) (Giuliani and Regoli 2014).

Due to the importance of the Nrf2 pathway in the cytoprotection against oxidative stress, a preliminary characterization of the Nrf2 gene has been performed in *P. antarctica*: a portion of *nrf2* transcript has been sequenced for the first time in this species. From the comparative analysis, the deduced amino acid sequence (corresponding to the N-terminal portion of the Nrf2 protein) appeared extremely conserved among the nototheniid species *P. borchgrevinki*, *T. bernacchii* and *Notothenia coriiceps* (96–99% identity). On the other hand, the identity was 50–60% with sequences from fish like *D. rerio* and *S. salar*, and even lower with mammalian models (39–40% identity) (Table 4.3).

Despite the limited homology of this sequence across evolution, the search for conserved domains demonstrated that *P. antarctica* Nrf2 sequence contains several features typical of this transcription factor. Indeed, three Neh (Nrf2-ECH homology) domains have been identified in *P. antarctica* Nrf2: Neh2 for the interaction with the repressor Keap1, Neh4 and Neh5 for the transcriptional activation (Fig. 4.4). The ETGE motif, responsible for Keap1 binding, is present within the Neh2 domain, and perfectly conserved among vertebrate species (Fig. 4.4; Kobayashi et al. 2002). The TRAM-binding motif in the Neh4 domain has also been reported in other species (Fig. 4.4; Katoh et al. 2001), while the actin-related motif, (D/E)(M/I/L)EXXW in the Neh5 domain is critical for Neh5-mediated transactivation (Fig. 4.4; Zhang et al. 2007). Based on such few molecular information, it can be hypothesized that the mechanisms of repression and transactivation modulate the Nrf2 system in *P. antarctica*. Nevertheless, the question is still open, whether polar organisms have

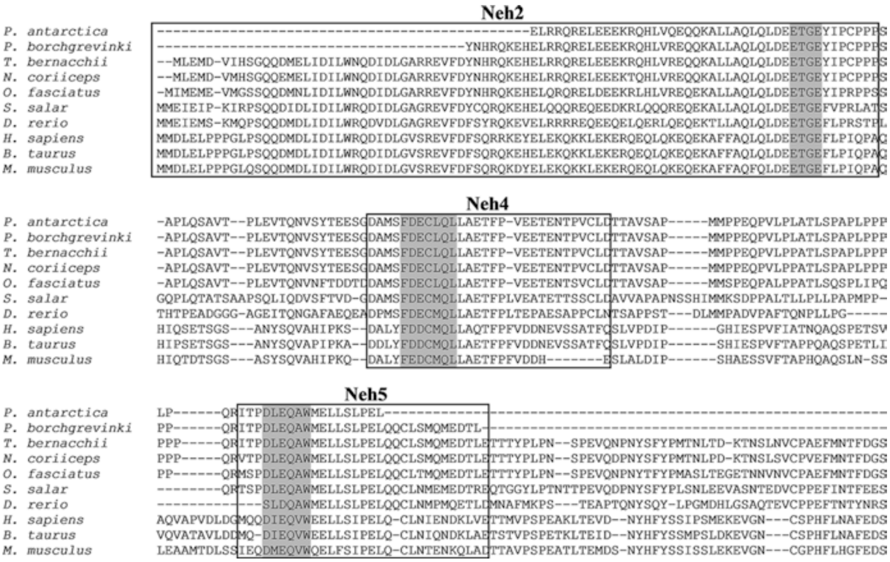

Fig. 4.4 Multiple alignment of the N-terminal portion of *P. antarctica* Nrf2 with the corresponding amino acid sequences from Antarctic fishes (*P. borchgrevinki, T. bernacchii* and *N. coriiceps*), other fish species (*O. fasciatus, S. salar*, *D. rerio*) and mammalian models (*H. sapiens, B. taurus, M. musculus*). The Neh domains of the N-terminal portion (Neh2, Neh4, Neh5) are shown in *boxes*. Conserved motifs are highlighted: ETGE for Keap1 binding in the Neh2 domain; the TRAM-binding motif in the Neh4 domain; the actin-related motif in the Neh5 domain

sensing and regulatory pathways similar to those of temperate animals, or different mechanisms evolved in extreme environmental conditions (Fulgentini et al. 2015).

4.2.2 *Transcriptional and Functional Modulation of Antioxidants in* P. antarctica

Besides analyses of nucleotide and protein sequences, evidences of adaptation of antioxidant defenses in polar organisms are also obtained from transcriptional studies, which are able to describe the adaptive capacity in response to environmental changes. Chen et al. (2008) have shown that three categories of genes involved in oxidative stress modulation are over-expressed in the nototheniid *Dissostichus mawsoni*. These include genes that act in ROS scavenging activities (phospholipid hydroperoxide GPx, peroxiredoxin-5, superoxide dismutase, haptoglobin); anti-apoptotic genes with known role in mitigating ROS-mediated apoptosis and cellular oxidative stress; genes involved in iron transport and storage, which control ROS formation catalysed by free Fe^{2+}. Transcriptomic analysis of *P. antarctica* (Shin et al. 2012) displayed 28 sequences (over a total of 8982 identified genes) related to antioxidant activity in the liver of adult fish and confirmed over-expression of the

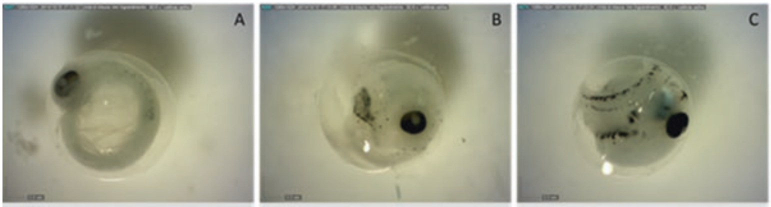

Fig. 4.5 Embryonated eggs of *Pleuragramma antarctica* sampled during the XXIX Italian Expedition within the platelet ice at Tethys Bay, Terra Nova Bay, in early November (**a**), middle November (**b**) and late November (**c**) 2013

Ub-proteasome pathway, involved in degradation of misfolded and damaged proteins as previously observed in *D. mawsoni*.

Transcriptional variations of *P. antarctica cat* and *gpx* during the final phases of embryo development in platelet ice have been investigated in *P. antarctica* sampled at Tethys Bay (Terra Nova Bay) in the Austral spring 2013 (XXIX Italian expedition; Fig. 4.5).

The real-time PCR analyses, performed with specific primers designed on *P. antarctica* sequences, show a gradual increase of *cat* and *gpx* transcription, with triple and double levels of mRNA, respectively, from the beginning to the end of November, when the eggs are near to the hatch (Fig. 4.6).

To better elucidate the complex relationship between modulation of antioxidant defenses in *P. antarctica* and platelet ice dynamics, variations of antioxidant parameters have been also measured at the functional, catalytic level in embryonated eggs sampled in the same area during two different Antarctic seasons, in 2003 and 2013.

Similarly to gene induction, the obtained results generally confirmed a marked and rapid increase of all antioxidants during the 3 weeks (from the beginning to the end of November), particularly for enzymatic activities of catalase, glutathione S-transferases, glutathione peroxidases and levels of total glutathione (Fig. 4.7; Regoli et al. 2005). The catalytic induction of these defenses supports their responsiveness toward natural and anthropogenic pro-oxidant stressors as previously shown in temperate and Antarctic species experiencing UV radiation, seasonality of feeding activities, presence of photosynthesizing symbionts, adaptation to diving and anaerobic metabolism, exposure to chemical oxidants and pollutants (Abele et al. 1998; Regoli et al. 1998, 2000b, 2002, 2005; Abele and Puntarulo 2004).

Comparing data obtained during the 2 years, samples of 2013 exhibited lower basal levels of catalase and an opposite temporal trend of glutathione reductase with a depletion (instead of induction) of activity (Fig.4.7; Table 4.4; Regoli et al. 2005). The lack of environmental data prevents the possibility to hypothesize the role of specific factors in influencing such biological defenses. However, it has been widely demonstrated, particularly in polluted organisms, that the complexity of antioxidant network makes variations difficult to predict: individual defenses can respond with different activation sequences and biphasic or temporary changes of antioxidants frequently occur toward natural or anthropogenic pro-oxidant challenge (Regoli et al. 2000b; Regoli and Giuliani 2014).

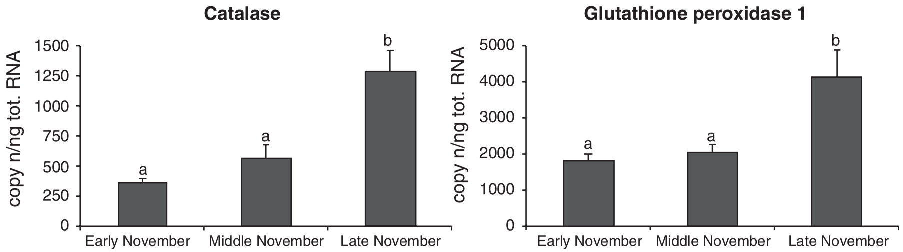

Fig. 4.6 mRNA levels of catalase and glutathione peroxidase in embryonated eggs of *P. antarctica* sampled within the platelet ice at Terra Nova Bay, in November 2013. *Different letters* indicate significant differences among weeks $p < 0.05$. Values are expressed as mean ± standard deviation

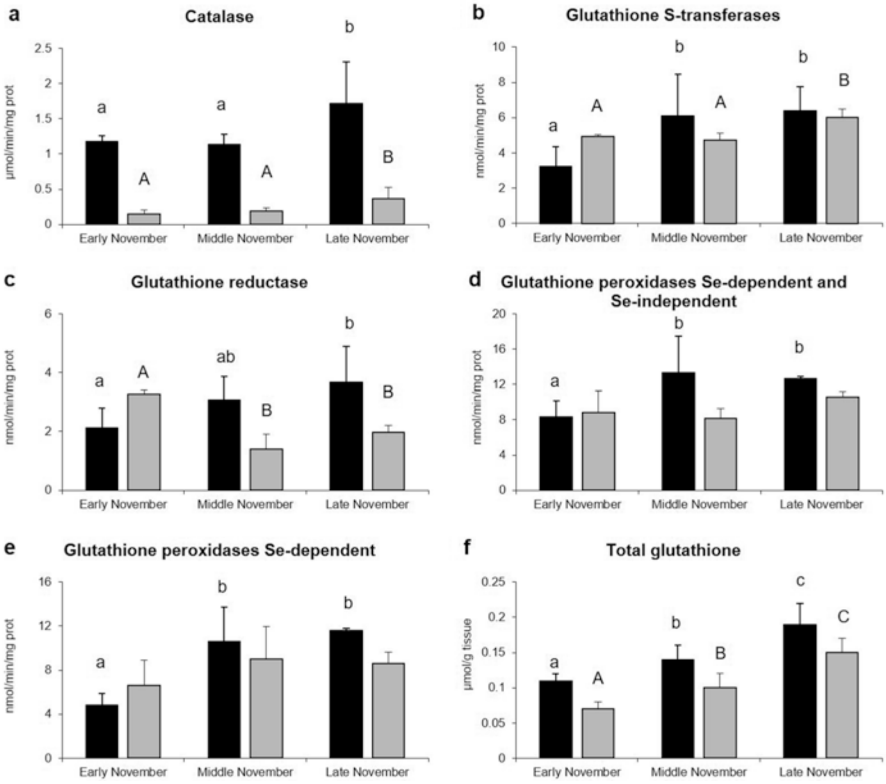

Fig. 4.7 Variation of antioxidant defenses measured in embryonated eggs of *P. antarctica*; *black* and *grey bars* indicate samples collected in 2003 and 2013, respectively. *Different letters* indicate significant differences among weeks in the same year $p < 0.05$. Values are expressed as mean ± standard deviation

The general induction of antioxidants did not prevent a certain unbalance between pro-oxidant forces and antioxidant defenses in *P. antarctica,* causing slight oxidative damages of biological molecules, such as the increase of lipid peroxidation, observed as content of malondialdehyde during the raise of pro-oxidant pressure in platelet ice (Fig. 4.8; Regoli et al. 2005). Similar results have been reported by other authors, with increased antioxidant defenses and lipid peroxidation in the Antarctic limpet exposed to hydrogen peroxide (Abele et al. 1998). Although individual antioxidants were sensitive in revealing a varied pro-oxidant challenge in *P. antarctica*, these changes did not significantly contribute to enhance the overall capability to neutralize different forms of oxyradicals (Fig. 4.8; Regoli et al. 2005). The apparent discrepancy between responses of individual antioxidants and TOSC values towards peroxyl and hydroxyl radicals might contribute to explain the increase of lipid peroxidation products, suggesting that other non-enzymatic scavengers, such as ascorbic acid and vitamin E (α-tocopherol), might significantly

Table 4.4 Antioxidants in *P. antarctica* and other fish species at embryonic stage

Species	Period/stage	Cat	GR	Se-dep GPxs	MDA	Tot glutathione (µmol/g tissue)	Ref
P. antarctica	Early Nov 2003	1.18 ± 0.08	2.12 ± 0.68	4.79 ± 1.1	3.65 ± 0.21	0.11 ± 0.01	
	Mid Nov 2003	1.13 ± 0.15	3.06 ± 0.81	10.6 ± 3.32	9.91 ± 6.23	0.14 ± 0.02	
	Late Nov 2003	1.72 ± 0.59	3.67 ± 1.23	11.57 ± 0.2	12.16 ± 5.48	0.19 ± 0.03	
P. antarctica	Early Nov 2013	0.15 ± 0.05	3.26 ± 0.16	6.59 ± 2.32	1.59 ± 0.43	0.07 ± 0.01	
	Mid Nov 2013	0.19 ± 0.05	1.41 ± 0.5	9.02 ± 2.96	1.72 ± 0.06	0.1 ± 0.02	
	Late Nov 2013	0.37 ± 0.16	1.98 ± 0.23	8.6 ± 1.02	1.42 ± 0.08	0.15 ± 0.02	
C. auratus	Hatching (168 hpf)	0.032 ± 0.003			40 ± 10[a]		[1]
H. molitrix	Hatching	0.2 ± 0.005		0.48 ± 0.1	0.2 ± 0.04[b]		[2]
G. morhua	Hatching gland	0.002 ± 0.3		2 ± 0.5		240 ± 2	[3]
D. rerio	Hatching					4900 ± 10[c]	[4]

C. auratus = *Carassius auratus*, *H. molitrix* = *Hypophthalmichthys molitrix*, *G. morhua* = *Gadus morhua*, *D. rerio* = *Danio rerio*, *Cat* = Catalase (µmol/min/mg prot), *GR* = Glutathione reduttasi (nmol/min/mg prot), *Se-dep GPxs* = Selenium Dependent Glutathione Peroxidases (nmol/min/mg prot), *MDA* = Malondialdehyde (nmol/g tissue)

[a] nmol/g prot

[b] nmol/mg prot

[c] µM

[1] Kong et al. (2013), [2] Liravi et al. (2014), [3] Skjærven et al. (2013), [4] Timme-Laragy et al. (2013)

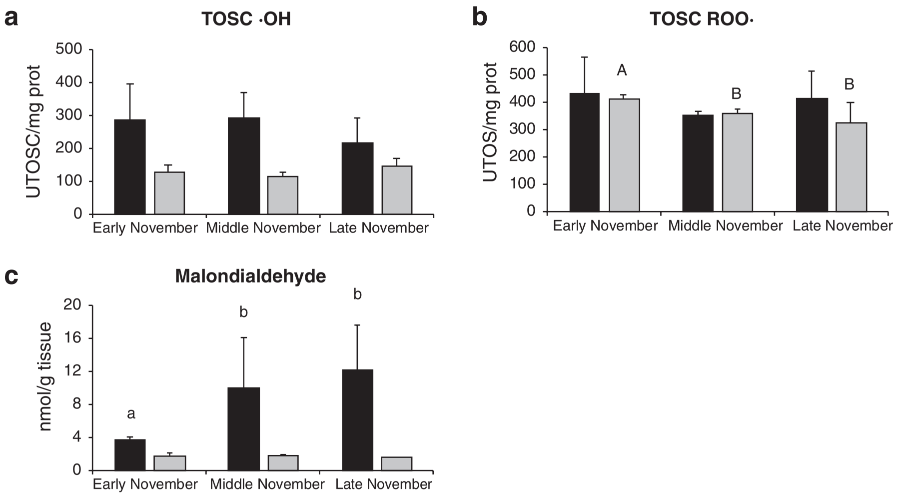

Fig. 4.8 Total Oxyradical Scavenging Capacity (**a**, **b**) and malondialdehyde content (**c**) measured in embryonated eggs of *P. antarctica*; *black* and *grey bars* indicate samples collected in 2003 and 2013, respectively. *Different letters* indicate significant differences among weeks in the same year p < 0.05. Values are expressed as mean ± standard deviation

contribute to balance the redox status of *P. antarctica* and onset of lipid peroxidation processes.

The marked increase of antioxidants observed at both transcriptional and functional level in *P. antarctica* embryos during their final development in platelet ice (Figs. 4.6, 4.7, 4.8; Table 4.4; Regoli et al. 2005) suggests a fundamental role of these defenses as a specific adaptive mechanism to the enhancement of pro-oxidant challenge due to both environmental and biological factors. The few data available for antioxidant levels in early developmental stages of fish (Table 4.4), generally confirm higher levels of these defenses in *P. antarctica*, further supporting the exposure of this species to a naturally high oxidative pressure.

Regoli et al. (2005) firstly associated the biochemical changes occurring in embryonated eggs before hatching to the elevated pro-oxidant conditions occurring in the lower layer of sea ice and ice platelets at the beginning of Austral spring. The capability to rapidly enhance the antioxidant efficiency is a fundamental and widely distributed strategy in those species which normally experience marked variations of environmental pro-oxidant challenge in their life cycle (Regoli et al. 2000b, 2004): in this respect, the photo-oxidation of DOM (Dissolved Organic Matter) and the increase of photosynthetically generated oxygen in the platelet zone represent an intriguing explanation for the need of rapid increase of antioxidant efficiency in *P. antarctica* embryos (Regoli et al. 2005). The elevated pro-oxidant conditions in the platelet ice-rich nursery of this fish, could support the hypothesis for a systematic selection of individuals with ample antioxidant protection or induction capability.

The hatching phase represents another critical challenge, since the silverfish larvae are released into an icy, oxidative microenvironment (Vacchi et al. 2004) becoming suddenly exposed to the cold oxygenated water and oxyradicals, once they escape from the chorion. In this respect, the anticipated increase of antioxidant defenses would act to protect the organisms from the potential risk of exposure to ROS, as typically observed in many hibernating species (Storey and Storey 2013). Moreover, the hatching is often associated with a peak of oxygen consumption by the organism, indicative of increased metabolic activities, associated with breaking and escaping from the protective chorion. Indeed, larvae of *P. antarctica* (1–3 days post-hatch) have a respiration rate of 11.60 ± 2.39 nmol O_2 h^{-1} ind^{-1}, almost double than embryonated eggs immediately before hatching (6.86 ± 1.87 nmol O_2 h^{-1} ind $^{-1}$) (Evans et al. 2012). Since ROS are normally produced as a product of the metabolism, an improved antioxidant system could also be required to deal with the increased cellular activity in the hatching and post-hatching periods. To our knowledge, only few studies evaluated the antioxidants fluctuations during embryonic stages in fishes. These trends are strictly related to the different species, and the comparison is made very difficult because of the difference in considered embryonic stage and in applied antioxidant detection methods. In order to provide a comparison among these data, a brief summary is shown, considering for all species data measured immediately before the hatching (Table 4.4).

The enhancement of antioxidant protection can represent an additional advantage also for the pelagic phase in highly irradiated surfaces after the ice melting; catalase can be inactivated in transparent animals exposed to UV-B radiation (Obermüller et al. 2005) and solar irradiance prevents the survival of some pelagic species in polar waters (Rautio and Korhola 2002) where reduced repair mechanisms further increase susceptibility to photochemical damage (Ross and Vincent 1998). The embryos of the Antarctic sea urchins, *Sterechinus neumayeri*, contained higher levels of both SOD and CAT, when compared with the developing eggs and larvae of other marine animals, and exposure to UV-B induced a small but significant increase of these antioxidants (Lister et al. 2010). Nevertheless, in organisms living in water column, the increase of antioxidant activities was not enough to completely protect embryos from oxidative damage to protein and lipids (Lister et al. 2010).

4.3 Concluding Remarks

The recent studies on antioxidant defenses of *P. antarctica* summarized in present chapter allowed a deeper insight on the adaptation to oxidative stress in Antarctic organisms. The sequences of two antioxidant enzymes (catalase and glutathione peroxidase) and of the transcription factor Nrf2 have been identified for the first time in *P. antarctica*. Catalase and glutathione peroxidase showed some features typical of cold-adapted enzymes. Both transcriptional and catalytic studies demonstrated the increase of antioxidant protection at the end of egg development, as adaptive responses to extreme environmental conditions, biological features and rapid changes of pro-oxidant pressure associated to platelet ice when the embryos hatch.

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Part II
Ecology and Life History

Chapter 5
Diet and Trophic Ecology of Adult Antarctic Silverfish (*Pleuragramma antarctica*)

Matthew H. Pinkerton

Abstract Antarctic silverfish, *Pleuragramma antarctica* Boulenger 1902 are the most abundant pelagic fish over the high-Antarctic shelf and one of the main ecosystem links in Southern Ocean shelf ecosystems, where they are a key prey item for fish marine mammals and birds. Consequently, information on the feeding ecology of silverfish forms an important component of research to understand the structure of Antarctic shelf ecosystems.

This chapter reviews what is known about the feeding of adult silverfish, what prey items make up the diet of adult silverfish and how the diet varies by fish size, geographic location and season. By "adult silverfish" we mean silverfish that are of a size where they are likely to have reached sexual maturity (greater than about 120 mm standard length).

Keywords Southern Ocean • Nototheniidae • Stable isotope • Trophic niche • Trophic level • Fatty acid • Stomach contents

5.1 Introduction

Antarctic silverfish (*Pleuragramma antarctica* Boulenger 1902) (hereafter "silverfish") are the most abundant pelagic fish over the high-Antarctic shelf (DeWitt 1970; Guglielmo et al. 1998). Silverfish are one of the main ecosystem links in Southern Ocean shelf ecosystems, where they are a key prey item for fish (La Mesa et al. 2004), marine mammals and birds (Takahashi and Nemoto 1984; Eastman 1985). Consequently, information on the feeding ecology of silverfish forms an important component of research to understand the structure of Antarctic shelf ecosystems.

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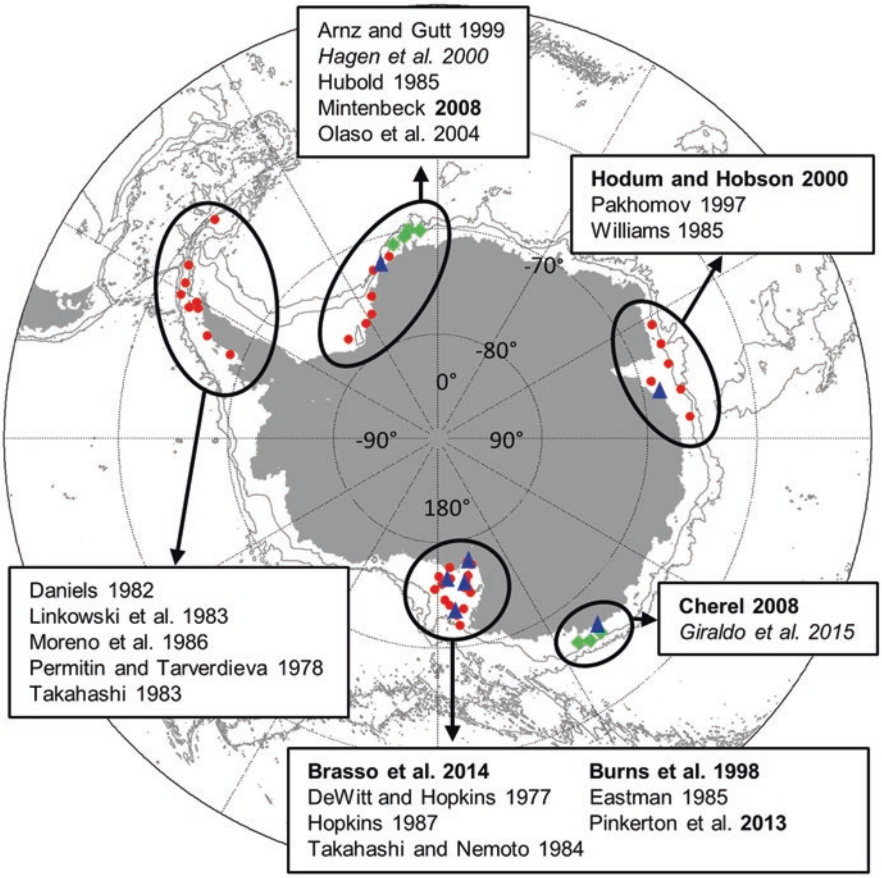

Fig. 5.1 Summary of diet studies of Antarctic silverfish in the Southern Ocean: (1) stomach contents analysis (*red circles, text in normal font*); (2) fatty acid/lipid analysis (*green diamonds, text in italic*); (3) stable isotope analysis (*blue triangles, text in bold*). Studies in normal font with the year in *bold* indicate both stomach contents and stable isotope analyses. Positions of sampling are approximate or indicative when not reported exactly

This chapter reviews what is known about the feeding of adult silverfish: what prey items make up the diet of adult silverfish and how does the diet vary by fish size, geographic location and season? By "adult silverfish" we mean silverfish that are of a size where they are likely to have reached sexual maturity. Based on data from the Weddell Sea and East Antarctica, the review by La Mesa and Eastman (2012) concluded that females mature for the first time at 130–160 mm standard length (SL), corresponding to 4–5 years of age, whereas males reach maturity at 120–180 mm SL, corresponding to 4–7 years of age. In this paper, we consider any fish greater than about 120 mm SL to be adults (Hubold 1984; Hubold and Tomo 1989; Mayzaud et al. 2011) but note that first spawning occurs about a year after reaching sexual maturity (Hubold and Tomo 1989; La Mesa and Eastman 2012).

Three main methods have been used to assess feeding by silverfish: (1) examination of stomach contents; (2) lipid-based methods; and (3) stable isotope methods (Fig. 5.1).

5.2 Diet Analyses of Adult Silverfish

5.2.1 Studies of Silverfish Stomach Contents

Most of the information on silverfish diet has come from the examination of the contents of silverfish stomachs (Table 5.1). Studies based on the analysis of stomach contents have shown a general predominance of pelagic and benthopelagic invertebrate prey, with euphausiids (*Euphausia superba* and *E. crystallorophias*) and copepods (especially the larger calanoid copepods) being especially important. Other invertebrates taken by adult silverfish include amphipods, gastropods (pteropods), ostracods, mysids, cumaceans, chaetognaths, polychaetes, siphonophores, jellyfish and salps (see Table 5.1) Piscivory by adult silverfish may be uncommon, but when it occurs, fish can make up a large proportion of the diet by weight (Eastman 1985; Pinkerton et al. 2013). Fish found to have been consumed include silverfish (cannibalism), icefish, myctophids and the barracudina *Notolepsis coatsi* (Eastman 1985; Williams 1985; Pakhomov 1997; Arntz and Gutt 1999; Olaso et al. 2004; Pinkerton et al. 2013).

5.2.2 Insights from Lipid Analysis

The composition of fatty acids can be used to investigate feeding relationships in marine ecosystems (e.g. Falk-Petersen et al. 2000). Some primary producers (e.g. diatoms) and zooplankton (calanoid copepods) have characteristic patterns of fatty acids which can be transferred to their predators with relatively little change (Dalsgaard et al. 2003) so that comparing "fatty acid trophic markers" in predators and potential prey can help to identify key predator-prey links in the food-web and exclude others. Of particular relevance to studying the diet of silverfish is the observation that the copepods *Calanoides acutus* and *Calanus propinquus* are the only known species of Antarctic zooplankton rich in the long-chain monounsaturated fatty acids ΣC20:1 and ΣC22:1 (Hagen et al. 2000). Elevated proportions of these two fatty acids were found in the tissue of adult silverfish in the Weddell and Lazarev Seas (Hagen et al. 2000) and in the Dumont d'Urville Sea in East Antarctica (Giraldo et al. 2015). This result implies feeding by silverfish on *C. acutus* and *C. propinquus* in these regions, though it is possible but unlikely that these species of copepod were consumed by an intermediate predator which was then consumed by silverfish.

Table 5.1 Stomach contents of adult *P. antarctica* from studies in different Antarctic areas and locations

Area Location	N	Stomach content	Ref
Antarctic Peninsula			
Ant. Peninsula and Scotia Sea	17	Predominantly euphausiids (69%V), cumaceans (10%V). Small amounts of polychaetes, amphipods, fish	[1]
South Shetland Islands	107	Only pelagic prey. Main: *Euphausia* spp. >80%W (mainly *E. superba*); secondary: amphipods (Hyperiidae), salps; minor: polychaetes, copepods, ostracods, fish	[2]
Bransfield Strait	106	Dominance of *M. gerlachei* (~60%W). Lesser: *C. acutus*, *E. superba*, *T. macrura*, ostracods, polychaetes, chaetognaths, amphipods and fish	[3]
South Orkney Islands	6	Euphausiids (*E. superba*, 76%O); amphipods (*T. gaudichaudii*, 11%O). Frequency of occurrence only.	[4]
South Shetland Islands	13	*E. superba* (100%)	[5]
Weddell Sea			
South Weddell Sea, East Weddell Sea	74*	Copepods (*M. gerlachei*; *C. acutus*; *C. propinquus*; *Euchaeta* spp.), gastropods, euphausiids (*E. crystallorophias*; *E. superba*), ostracods, mysids, isopods, polychaetes, siphonophores, jellyfish., one juvenile fish (channichthyid), fish scales of *P. antarctica*. Numbers of prey only.	[6]
Drescher Inlet	40	Main: copepods (*C. propinquus*, *M. gerlachei*, *R. gigas*); chaetognaths frequently ingested; minor: mysids, ostracods and hyperiid amphipods	[7] [8]
East Weddell Sea	108	Main: copepods (including *R. gigas*, *C. propinquus*) and euphausiids (including *E. superba*); minor: amphipods, ostracods, fish	[9]
East Antarctica			
Commonwealth Sea	306	*E. superba* (92%), *Pleuragramma antarctica* (6%), minor: *E. crystallorophias*, pteropods, amphipods	[10]
Prydz Bay	45*	Copepods (*C. acutus*, *M. gerlachei*, *E. antarctica*), fish (including *N. coatsi*, myctophid juvenile), euphausiids (*E. crystallorophias*, *E. superba*)	[11]
Ross Sea			
Ross Sea	32	Predominantly *E. superba* near Ross Sea slope and *E. crystallorophias* over mid-shelf and south Ross Sea. Copepods (*Euchaeta* spp., *C. acutus*, *M. gerlachei*), mysids (*A. ohlinii*)	[12]
McMurdo Sound	25	Mysids (65%O), copepods (47%O), larval and juvenile fish (22%O: *P. antarctica* 13%O and other fish unidentified). Frequency of occurrence only	[13]
McMurdo Sound	23	Copepods (*C. acutus*; *M. gerlachei*). Gastropods (*L. helicina*), furcilia (probably *E. crystallorophias*)	[14]

(continued)

Table 5.1 (continued)

Area Location	N	Stomach content	Ref
Ross Sea shelf and slope	136	Fish (myctophids; *P. antarctica*), euphausiids (*E. superba*, *E. crystallorophias*), with amphipods, mysids, copepods (*Paraeuchaeta* sp., *M. gerlachei*)	[15]
Western Ross Sea	35	Amphipods (mainly *T. gaudichaudii*), copepods, *E. crystallorophias*. Frequency of occurrence only	[16]

N = number of non-empty stomachs examined, *N** = all stomachs examined
References: [1] Daniels (1982), [2] Linkowski et al. (1983), [3] Moreno et al. (1986), [4] Permitin and Tarverdieva (1978), [5] Takahashi (1983), [6] Hubold (1985), [7] Mintenbeck (2008), [8] Arntz and Gutt (1999), [9] Olaso et al. (2004), [10] Pakhomov (1997), [11] Williams (1985), [12] DeWitt and Hopkins (1977), [13] Eastman (1985), [14] Hopkins (1987), [15] Pinkerton et al. (2013), [16] Takahashi and Nemoto (1984)

The common copepods *Rhincalanus gigas*, *Metridia gerlachei* and *Euchaeta antarctica* lack characteristic markers and hence analysis of fatty acids cannot be used to trace feeding on these copepods (Hagen et al. 2000). However, lipids found in different species of euphausiids do provide opportunities to investigate predation on them. *E. superba* produces mainly triglycerols, while *E. crystallorophias* and *Thysanoessa macrura* are especially rich in the fatty acid C18:1(n-9) (Bottino 1974; Hagen and Kattner 1998; Kattner and Hagen 1998). Higher levels of this lipid were found in silverfish sampled in the Weddell and Lazarev Seas consistent with feeding on *E. crystallorophias* and/or *T. macrura* (Hagen et al. 2000).

5.2.3 Stable Isotope Analyses

Physical, biological and chemical processes in ecosystems result in distinct isotopic signatures in the tissue of biota and these stable isotopic signatures can provide information on the structure of food-webs. Carbon isotopes are often used to identify carbon sources and fluxes within ecosystems (Fry and Sherr 1984; Peterson and Fry 1987), whilst nitrogen isotope ratios have been widely used in dietary studies, including for estimating trophic level and for considering long-term diet (DeNiro and Epstein 1981; Post 2002). The stable isotope signature in fish muscle depends on how fast the muscle is replaced and this has not been measured for silverfish. However, some studies have suggested that the temporal integration period for stable isotopes in silverfish is likely to be of the scale of weeks to months (Brasso et al. 2014).

Studies of the trophic position of adult silverfish using stable isotope analysis have been carried out in the Ross Sea, parts of East Antarctica and in the Weddell Sea (Table 5.2). In these studies, “baseline” δ^{15}N (of phytoplankton) was obtained from measurements in the same geographic region either from: (1) measurements of particulate organic matter (POM) in the water column (Mintenbeck 2008; Pinkerton

Table 5.2 Analyses of stable isotope values of adult *P. antarctica* from studies in different Antarctic areas and locations

Area	N	Isotope results			Ref
Location		δ^{15}N mean ± s.d.‰	δ^{13}C mean ± s.d.‰	Trophic level	
Weddell Sea					
Weddell Sea shelf and slope	10	9.2	−27.0	3.65	[1]
East Antarctica					
Pointe Géologie, Adélie Land	5	10.6 ± 0.3	−24.7 ± 0.4	4.0 ± 0.1[a]	[2]
Prydz Bay	13	10.7 ± 1.2	−23.9 ± 0.7	4.3 ± 0.4	[3]
Ross Sea					
Ross Sea shelf	31	10.7 ± 0.4	−25.4 ± 1.1	4.2 ± 0.1	[4]
McMurdo Sound	4[b]	10.9 ± 0.6[c]	−25.5 ± 0.4[d]	4.3 ± 0.2[e]	[5]
Ross Sea shelf and slope	86	10.6 ± 0.7	−24.9 ± 0.5	4.2 ± 0.2	[6]

The author is not aware of any stable isotope measurements of silverfish near the Antarctic Peninsula

Stable-isotope values are reported in the δ notation where δ^{13}C or δ^{15}N = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is ^{13}C/^{12}C or ^{15}N/^{14}N (Fry 2006)

N = number of samples, "*s.d.*" = standard deviation

References: [1] Mintenbeck (2008), [2] Cherel (2008), [3] Hodum and Hobson (2000), [4] Brasso et al. (2014), [5] Burns et al. (1998), [6] Pinkerton et al. (2013)

[a] Trophic level from δ^{15}N for *E. superba* assuming trophic level of 2.3 (Hodum and Hobson 2000) and trophic fractionation 3.2‰ per trophic step.

[b] Sample may include juveniles (51–259 mm given in Burns et al. 1998) but likely to be all adults from δ^{15}N values (based on Pinkerton et al. 2013, fig. 6a)

[c] Reported as "10.9 ± 9.2" assumed to be misprint and s.d. estimated from Burns et al. (1998), fig. 4

[d] Corrected for lipid effect, Burns et al. (1998), fig. 4

[e] Trophic level calculated using baseline of δ^{15}N = 0.4‰ and trophic fractionation 3.2‰ per trophic step as Pinkerton et al. (2013) for Ross Sea

et al. 2013; Brasso et al. 2014); or (2) from δ^{15}N of *E. superba* assuming a trophic level of 2.3 (Hodum and Hobson 2000). Nitrogen isotopes were assumed to enrich by ~3.2‰ per trophic level (Sweeting et al. 2007). Trophic levels of adult silverfish in the Ross Sea and East Antarctica estimated in this way are remarkably similar at 4.0–4.3, in contrast with DeWitt and Hopkins (1977) who predicted a low trophic level for silverfish in the Ross Sea on the basis of the dominance of euphausiids in the stomach contents. The trophic level of more than 4 seems unusually high for a small fish. For example, stable isotope analysis of five species of myctophids from north of the Ross Sea slope (*Electrona carlsbergi*, *E. antarctica*, *Gymnoscopelus nicholsi*, *G. opisthopterus*, *G. braueri*) suggested trophic levels of between 3.2 and 4.0 (Pinkerton, unpublished data).

The high trophic level of silverfish in the Ross Sea and East Antarctica measured by stable isotope studies (Burns et al. 1998; Hodum and Hobson 2000; Cherel 2008; Pinkerton et al. 2013) are consistent with substantial feeding on carnivorous rather than herbivorous or omnivorous prey, such as fish and carnivorous copepods. For "small" adult silverfish (152–178 mm SL) in the Ross Sea, Pinkerton et al. (2013) found that the $\delta^{15}N$ of silverfish muscle was consistent with the average diet from stomach contents analysis and the $\delta^{15}N$ values measured for the various prey items, assuming a trophic fractionation factor of 3.2‰ per trophic level (Sweeting et al. 2007). However, for "large" (>179 mm SL) adult silverfish in the Ross Sea, the stomach contents and trophic level of prey suggested an even higher trophic level of ~4.6 (Pinkerton et al. 2013).

Based on $\delta^{15}N$ measurements, the trophic level of adult silverfish in the Weddell Sea was estimated to be 3.6, consistent with feeding on omnivorous copepods (*C. propinquus*, *M. gerlachei*, *R. gigas*) and euphausiids, which together dominated the stomach contents (Mintenbeck 2008).

5.3 Discussion

5.3.1 Reliability of Diet Information from Analysis of Stomach Contents

Although useful information on the feeding of adult silverfish has been provided by the analysis of biochemical tracers (fatty acids and stable isotopes), by far the greatest insight has come from the examination of stomach contents. Stomach content analysis is probably still the best way to understand a predator's diet, but has several limitations (Hynes 1950; Hyslop 1980; Cortés 1997), as follows.

1. Stomach contents provide only a very short-term snapshot of diet. Given that sampling occurs almost exclusively in summer, estimates of the long-term diet of silverfish will be biased if feeding varies seasonally. This issue is discussed further below.
2. The proportions of prey in stomachs can be affected by variations in the digestion rates of different prey; hard items will be over-represented in stomach contents relative to soft-bodied prey because they remain for longer and are easier to identify. Bias due to differential digestion rates can be avoided by using only fresh (i.e. relatively undigested) material found in the stomachs.
3. Stomach contents can be affected by the timing of sampling compared to seasonal changes in foraging behavior.
4. Diel vertical migration patterns in adult silverfish have been found (e.g. Fuiman et al. 2002; Lancraft et al. 2004) but was not considered to have affected estimates of diet (DeWitt and Hopkins 1977; Moreno et al. 1986; Mintenbeck 2008).
5. There tends to be high variability in the quality of identification of prey items found in stomachs both within and between studies, and there is no consensus on how to handle unidentified prey in stomach contents analysis. Identification of prey does not seem to be a major problem with silverfish stomach analysis as

there are a relatively few main prey species, most of these are hard-bodied and are eaten whole.

6. Stomach contents are typically very variable between individuals so many stomachs must be analysed to give a reliable indication of diet in the population. One way to determine if the sample size is sufficient to describe diet is to plot the cumulative number of prey categories observed against the randomly pooled number of stomachs examined (Hurtubia 1973; Alonso et al. 2002). In the Ross Sea, Pinkerton et al. (2013) found that 121 non-empty stomachs were needed to sample 95% of prey diversity. About half the studies in Table 5.1 had this kind of sample size. However, the number of stomachs required depends on the prey diversity, and often a much smaller sample size than this would be adequate to describe the diet.
7. Some fish can feed in the net during capture and this can bias stomach contents. Net feeding was identified for samples caught at one station by DeWitt and Hopkins (1977) but does not seem to have been a common problem in studies of silverfish.
8. Cortés (1997) notes that ideally the energy value (or strictly, the metabolisable energy content) of prey should also be taken into account in evaluating dietary importance to a predator, but this is generally not done in feeding studies.
9. Finally, various different metrics have been used to estimate "diet" from stomach contents of silverfish. This final point is considered in more detail below.

Examination of stomach contents can be used to describe diet in terms of the number of different prey items, their frequency of occurrence, or by proportion of the total prey weight (%W) (Cortés 1997). Proportions of prey by weight (%W) is relevant to considering energy flow through food-webs or overall nutritional importance of a prey to a predator (Macdonald and Green 1983; Pinkerton et al. 2013), but many earlier studies on silverfish only reported frequency of occurrence of prey in stomachs (e.g. Permitin and Tarverdieva 1978; Takahashi and Nemoto 1984; Eastman 1985) or numbers of prey (Hubold 1985). It is not possible to obtain quantitative information on diet from these studies, especially when weights of different prey items vary substantially (e.g. fish versus invertebrate prey in Eastman 1985).

Composite metrics, such as the Index of Relative Importance (IRI; Pinkas et al. 1971), have also been used. While the IRI method is said to "cancel out biases in its individual components" (Bigg and Perez 1985), this has been contested (Macdonald and Green 1983; Hansson 1998; Cortés 1997). Estimating the confidence intervals (CIs) of the average proportions of various prey items by weight is recommended instead (Tirasin and Jorgensen 1999) as this method can detect the overall significance of any large and scarce prey items in the diet (wide CIs that may include zero). The only silverfish study to use %IRI was Pinkerton et al. (2013), which included data from 136 stomachs of adult silverfish collected from 21 stations over the Ross Sea shelf and upper continental slope (depths between 270 and 880 m) in February 2008. In this review, the measurements of the stomach contents of adult silverfish from Pinkerton et al. (2013) were re-analysed by bootstrapping the %W data (Fig. 5.2). A two-part bootstrap randomisation was used to preserve the struc-

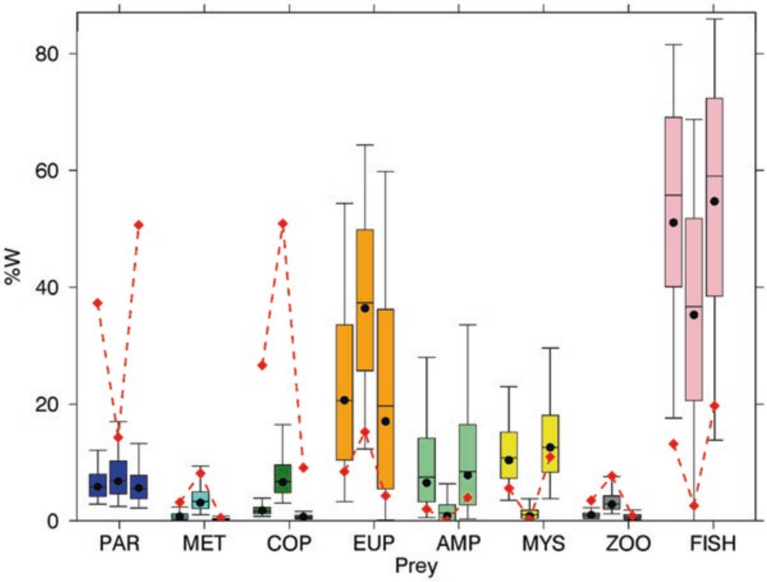

Fig. 5.2 Diet of Antarctic silverfish in the Ross Sea based on Pinkerton et al. (2013). The proportion of the total prey weight contributed by a given prey item is written %W. Colours show different prey items: PAR (*blue*) = *Paraeuchaeta* sp.; MET (*cyan*) = *M. gerlachei*; COP (*green*) = unidentified or other copepods; EUP (*orange*) = euphausids; AMP (*light green*) = amphipods; MYS (*yellow*) = mysids; ZOO (*grey*) = other zooplankton; FISH (*pink*) = fish. For each set of three bars: *left* = all adult silverfish (>151 mm standard length, SL; N = 136); *middle* = small adult silverfish (152–178 mm SL, N = 72); *right* = large adult silverfish (>179 mm SL; N = 64). The *black dots* are the mean proportions of prey by weight (%W) for all non-empty stomachs. The coloured bars show the bootstrap estimates of %W (*line* = bootstrap median; *boxes* are 25th–75th bias-corrected percentiles; *whiskers* are 5th–95th percentiles). The *red dots* and *red dashed lines* are the %IRI estimates of diet based on all data

ture of the data (Tirasin and Jorgensen 1999) as follows: (1) randomly select a station; (2) draw the same number of random samples from this station (with replacement) as actually collected in the survey; (3) select another random station and repeat until 136 samples had been selected; (4) estimate the diet metrics (%W and %IRI) from this set of samples; (5) repeat the process 1000 times; (6) calculate the median and percentiles (5th, 25th, 75th, 95th) from the 1000 bootstrap samples using the bias-corrected percentile method (Efron and Tibshirani 1993; Chernick 1999).

Figure 5.2 show that %IRI gives a different description of diet than that based on weight of prey. In the %IRI description (red dots and lines), the importance of *Paraeuchaeta* sp. and unidentified/other copepods is exaggerated compared to the weight-based approach, and the importance of euphausiids and fish in the diet of silverfish is under-represented. The large uncertainty on the contribution of euphausiids and fish to the diet of silverfish is shown by the large range of the coloured bars. This re-analysis shows that euphausiids and fish dominate the diet of silverfish in the Ross Sea in terms of energy flow, at least in the year and stations sampled. It is noted that these results are more consistent with stable isotope measurements than a diet description based on %IRI (Pinkerton et al. 2013).

5.3.2 *Biochemical Tracer Methods*

Analysis of fatty acids is a recent but increasingly-used approach to study the feeding ecology of a wide range of organisms, especially birds and mammals (Arts et al. 2009; Williams and Buck 2010). With respect to understanding diet, the technique has been used in two main ways. First, some fatty acids are characteristic of a particular prey or group of prey (e.g. feeding by silverfish on *C. acutus* and *C. propinquus* inferred from the presence of fatty acids ΣC20:1 and ΣC22:1; Hagen et al. 2000; Giraldo et al. 2015). Second, quantitative fatty acid signature analysis (QFASA) has been developed to estimate diet based on matching lipid signatures in predators and prey, after accounting for the predator's lipid metabolism (Iverson et al. 2004). Developed for mammals, this approach has rarely been applied to fish, partly because of variable and poorly characterised fatty acid metabolism in fish (Happel et al. 2016).

In contrast, stable isotope analysis has long been used to investigate feeding ecology (DeNiro and Epstein 1981; Fry and Sherr 1984) and its use is still increasing with the development of a range of stable isotope mixing models (Phillips et al. 2014). Inferring diet from knowledge of predator and prey stable isotope values works best when: (1) the range of prey items has been established (e.g. from examining stomach contents); (2) there are relatively few prey items; (3) the prey items have different trophic levels ($\delta^{15}N$) and/or sources ($\delta^{13}C$); (4) enough samples of predators and (all) prey have been analysed to account for intraspecific variability; (5) samples are from the same region; (6) trophic fractionation factors are known; (7) turnover times of predator tissue can be estimated (Fry 2006; Phillips et al. 2014). The depauperate nature of the pelagic, high-latitude Southern Ocean, and the large range in trophic level of prey items of silverfish mean that stable isotope methods have provided useful information on silverfish feeding when used in conjunction with analysis of stomach contents (Pinkerton et al. 2013). Further combination of detailed examination of stomach contents with one or more of these biochemical tracer methods is likely to provide new insights into silverfish feeding in the future.

5.3.3 *Insights into Feeding from Morphology*

Feeding morphology strongly influences the kind of prey a fish can consume (DeWitt and Hopkins 1977). Silverfish have slender gill rakers compared with some species of *Trematomus*, which DeWitt and Hopkins (1977) suggest implies a focus on smaller prey (Yasuda 1960). In terms of swimming ability, adult silverfish are sluggish (Johnston et al. 1988; Kunzmann 1990; Wöhrmann et al. 1997) which argues against the pursuit of mobile prey (such as fish), and suggests more of a lunging, opportunistic foraging behaviour on smaller, less mobile prey. The mouths of silverfish are directed upward, with the lower jaw protruding, and jaws are moderately protractile. This arrangement may has been taken as indicative of fish taking individual prey, as fish which strain large quantities of water for plankton typically do not have protrusible mouths (e.g. Clupeidae) (DeWitt and Hopkins 1977). This is consistent with silverfish having conical, curved and mostly small teeth, but with usually 3–4 larger teeth, including prominent canines (Gon and Heemstra 1990) which suggest that prey are seized and held individually (DeWitt and Hopkins 1977).

5.3.4 Variations in Diet with Location

In general, adult silverfish prey mainly on large copepods and euphausiids (Table 5.1). As a group, copepods are dominant most often, especially *C. acutus*, *C. propinquus*, *M. gerlachei*, *Paraeuchaeta* sp., and *R. gigas* (Moreno et al. 1986; Hubold 1985; Mintenbeck 2008; Olaso et al. 2004; Williams 1985; Hopkins 1987). In continental shelf waters away from the edge of the continental shelf, adult *E. crystallorophias* was often an important prey species whereas near the continental slope or over deeper water, *E. superba* frequently became the major part of the diet (DeWitt and Hopkins 1977; Daniels 1982). Indeed, *E. superba* was sometimes the only prey item found in stomachs (Takahashi 1983). Several other pelagic invertebrates frequently featured in the diet of adult silverfish, including amphipods (usually Hyperiidea, including *Themisto gaudichaudii*; Takahashi and Nemoto 1984) and mysids (e.g. *Antarctomysis ohlini*; DeWitt and Hopkins 1977; Eastman 1985). Several other groups of zooplankton are eaten only occasionally, including pelagic polychaetes (e.g. *Pelagobia longicirrata*), pteropods (*Limacina helicina*), ostracods (*Conchoecia* spp.), larvae of decapods, cumaceans, isopods, and chaetognaths (*Eukrohnia hamata*, *Sagitta* spp.) (DeWitt and Hopkins 1977; Daniels 1982; Linkowski et al. 1983; Hubold 1985; Moreno et al. 1986; Hopkins 1987; Olaso et al. 2004; Mintenbeck 2008).

The importance of piscivory in silverfish is still unclear, and seems to depend on location. Eastman (1985) and Williams (1985) gave the first reports of piscivory by silverfish – in McMurdo Sound and Prydz Bay respectively – with probably substantial (but unquantified in terms of %W) cannibalism also found by Eastman (1985). Since then, a number of other studies have confirmed that adult silverfish can be piscivorous (Pakhomov 1997; Arntz and Gutt 1999; Olaso et al. 2004; Pinkerton et al. 2013) which implies that this is not an anomalous response to an “extreme biotype” (Eastman 1985). Certainly, fish seem to be consumed less frequently than invertebrates, but, when consumed, fish prey may often dominate in terms of total weight of prey (e.g. Pinkerton et al. 2013). A bias against recognising the importance of piscivory will arise from using frequency of occurrence, number of prey items or composite indices like IRI to describe diet rather than %W (see Fig. 5.2). The high trophic level of silverfish in the Ross Sea and East Antarctica (Burns et al. 1998; Hodum and Hobson 2000; Cherel 2008; Pinkerton et al. 2013) suggests more feeding on fish prey than is suggested by stomach contents analyses to date.

5.3.5 Variations in Diet with Depth of Capture

The diet of silverfish ascertained from stomach contents does not seem to depend on the depth at which the silverfish were captured. In one study, when both benthic and pelagic methods were used, the main prey item was consistent (euphausiids), but the diversity of prey in stomachs was higher when silverfish were collected in the water column as a greater variety of copepods were consumed (DeWitt and Hopkins 1977). No studies find benthic prey having being consumed by adult silverfish, even when they were caught near to the seabed (Hubold 1985; Takahashi and Nemoto 1984; Takahashi and Iwami 1997; Pinkerton et al. 2013). Although some “pelagic” prey may also be present in the benthic boundary layer, it is likely that adult silverfish which were caught near the seabed move up into the water column to feed. Although this would disrupt the strict vertical separation of age/size classes as described by Hubold (1984, 1985) and Hubold and Ekau (1987), it would give silverfish access to higher abundances of many copepod species which tend to be most abundant in the upper water layers (50–200 m) in some areas (Mintenbeck 2008).

5.3.6 Variation in Diet with Fish Size

Information on variation in the diet of adult silverfish with body size is mixed. In one study, general linear model analysis of $\delta^{15}N$ of adult silverfish showed a small but significant increase in the average trophic level with fish size (Pinkerton et al. 2013) but no significant relationship between SL and $\delta^{15}N$ was found by Brasso et al. (2014). Stomach contents data from Pinkerton et al. (2013) showed that fish, amphipods and mysids contributed more to the diet of large silverfish (>178 mm SL), whereas euphausiids and copepods contributed more to the diet of smaller fish (152–177 mm SL; Fig. 5.2). In other studies (Williams 1985; Pakhomov 1997; Arntz and Gutt 1999), the proportion of fish prey found in silverfish stomachs increased with the size of silverfish, though the change was small.

Overall, it appears that fish size does not have a major effect on the diet of adult silverfish, except that fish prey are more likely to be taken by larger adult silverfish than smaller. Would we expect the diet of adult silverfish to vary with fish size from consideration of the size of individual prey items? Although there is substantial variability in the largest size of prey consumed by different species of fish, prey are usually smaller than about 30% of the predator size (Scharf et al. 2000; Shin and

Cury 2001). Scharf et al. (2000) show that the largest prey taken tend to be fish, and that predation on invertebrates by fish is not usually size-limited. A maximum prey-predator-prey size ratio of 0.3 would imply that a 100 mm SL silverfish could handle prey up to about 30 mm, whereas a 200 mm SL individual could predate prey smaller than 60 mm.

In the Ross Sea, adult silverfish were found to consume copepods with typical lengths of 1–10 mm (DeWitt and Hopkins 1977). Copepods in this size range include adult female *M. gerlachei* (~3.7 mm), adult *C. propinquus* (~5.0 mm) and adult female *Paraeuchaeta* sp. (~9.2 mm) (Hubold and Hagen 1997). Lengths of *E. crystallorophias* from silverfish stomachs had a mode of 20–25 mm, and *E. superba* had typical sizes 35–45 mm (DeWitt and Hopkins 1977). In the Commonwealth Sea, *E. superba* of 18–54 mm (modal size ~45 mm) were found in the stomachs of silverfish (Pakhomov 1997) but smaller *E. superba* (20–32 mm) were retrieved from silverfish caught in Croker Passage (Lancraft et al. 2004). Piscine prey of 46–76 mm SL (juvenile silverfish) have been found (Pakhomov 1997), and in McMurdo Sound, a 145 mm SL silverfish had a 45 mm SL silverfish in its stomach (Eastman 1985).

It seems therefore that whereas copepods and *E. crystallorophias* are well within the size range of prey that adult silverfish would be able to consume, fish and large adult *E. superba* may be close to the upper size of prey possible. These size considerations suggests little change in diet would be expected with size of adult silverfish, but would explain the substantial difference in diet between adults and juveniles that are seen in stomach contents (e.g. Pakhomov 1997), trophic level (e.g. Pinkerton et al. 2013), and niche width in $\delta^{13}C$-$\delta^{15}N$ space (Brasso et al. 2014).

5.3.7 Variations with Season

Almost all studies on silverfish diet have occurred during the austral summer (January–March) when ice conditions permit research vessels to sample at high latitudes and we have little information on feeding at other times. The high trophic level estimated from stable isotope data in the Ross Sea and over the East Antarctica shelf suggests feeding at a similar (or higher) trophic level year round to that in the summer. Year-round feeding would be consistent with high levels of triglycerol found in silverfish (Reinhardt and van Vleet 1986).

Whether the species consumed by silverfish outside summer are the same as found in stomachs collected in the summer is not known. It is possible that silverfish feed at the base of sea-ice outside summer similar to the behaviour observed by Daniels (1982) where silverfish fed on amphipods (*Nototropsis* sp.) under fast ice. This would be consistent with the results of a modelling study on the optimum ecological strategies of silverfish (Maes et al. 2006) which concluded that: "The dark, shallow waters under pack ice may offer a permanent food supply and probably provide a refuge against predators for all life history stages of *Pleuragramma*."

The type and abundance of copepods present for silverfish to feed on in the winter depends on the various life-strategies of the key prey species. Antarctic copepods have a long (1 or 2 year) life cycle which can include seasonal vertical migration, dormancy (diapause, quiescence) and the accumulation/use of energy reserves (Schnack-Schiel and Hagen 1995; Atkinson 1998; Schnack-Schiel 2001). Among the main prey of silverfish, *C. acutus* is the only species to include a true diapause at depth in the dark season; *C. propinquus*, *M. gerlachei*, and *Paraeuchaeta antarctica* are likely to remain active year round, using lipid reserves accumulated during the summer as well as on carnivorous feeding at low prey concentrations to remain active in the winter (Schnack-Schiel and Hagen 1995; Schnack-Schiel 2001). This potentially means these copepods are a year-round food resource for adult silverfish.

5.3.8 Selectivity Versus Opportunistic Predation

It remains unclear to what extent silverfish are selective – rather than simply opportunistic – in their feeding, as few studies have managed to measure the relative abundances of different prey concurrently with sampling of silverfish diet. Two features of the feeding of adult silverfish are obvious from the analyses of diet in the literature and are relevant to considering selective versus opportunistic feeding in adult silverfish. First, the major prey items consumed in different parts of the Southern Ocean are from a relatively small "menu": large calanoid copepods, euphausiids, intermittent and poorly quantified piscivory (including cannibalism), and, less frequently, amphipods and mysids. Hence, the scope for prey selection seems rather low. Second, the proportions of these main prey items in the diet of silverfish vary considerably across studies, from overwhelming dominance of copepods (Moreno et al. 1986), to dominance of *E. superba* (Pakhomov 1997) to substantial feeding of fish and a more diverse diet (Eastman 1985; Pinkerton et al. 2013). On a broad scale therefore, silverfish seem to be highly flexible in their feeding; any of a number of preferred prey items seem to be acceptable.

It is clear that the proportions of different prey available in the water column will vary substantially between areas. For example, amongst euphausiids, *E. superba* is generally more common in deeper water, and *E. crystallorophias* more common in shelf waters. Proportions of different prey will also vary between years. For example, at South Orkney Islands, the proportion of *E. superba* in the stomach of various notothens varied by more than 50%O between years (Permitin and Tarverdieva 1978). This interannual variation was probably in relation to availability of the prey species as three predators showed the same patterns of high and low consumption of *E. superba* between years (Permitin and Tarverdieva 1978). Hence, it is likely that much of the variation in diet in adult silverfish between studies results simply from a different mix of the potential prey items being present at the time of sampling.

That feeding is largely opportunistic is supported by some measurements of stomach contents and sampling of the zooplankton community. For example, Hopkins (1987) reports that, like the stomach contents, the pelagic zooplankton community was dominated by *C. acutus* and *L. helicina*; Moreno et al. (1986) found a dominance of *M. gerlachei* in silverfish stomachs and also in zooplankton net hauls. However, in contrast, sometimes *Paraeuchaeta* sp. seems to be selected for by silverfish. In some studies, the relatively high proportion by weight of this copepod in silverfish stomachs (DeWitt and Hopkins 1977; Pinkerton et al. 2013) is at odds with its low abundance in the mesozooplankton community generally (Mayzaud et al. 2002; Stevens et al. 2014). Pinkerton et al. (2013) report: "*M. gerlachei* was 2.3–20 times more numerically abundant than *Paraeuchaeta* sp. over the Ross Sea shelf and slope, but *Paraeuchaeta* sp. was three to four times more important as prey". Targeted feeding on some species over others seems to have ecological advantages: *C. acutus* and *M. gerlachei* have been found to have much higher total lipid content than other copepods (Reinhardt and van Vleet 1986). Confirmation of the degree to which adult silverfish are selective feeders hence awaits further research.

5.3.9 Final Comments

Feeding studies over the last 30+ years have given us a picture of the common prey taken by adult silverfish around over the Antarctic shelf and slope, though important questions remain: How important is piscivory? To what extent is feeding selective rather than purely opportunistic? What is the diet of silverfish outside the ice-free summer months? It is clear however, that silverfish are well adapted to make use of whatever pelagic prey is available in different parts of the Southern Ocean. By minimising their daily consumption rate and focussing on small prey throughout their adult life, silverfish have successfully established themselves as a keystone of high Antarctic ecosystems.

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Chapter 6
Trophic Ecology of Early Developmental Stages of Antarctic Silverfish

Eric Tavernier and Carolina Giraldo

Abstract The Antarctic pelagic ecosystem over the continental shelf is dominated by the Antarctic silverfish *Pleuragramma antarctica* (Nototheniidae) which represents up to 90% of the fish biomass. *P. antarctica* is the only notothenioid species to have an entire pelagic life cycle. This species is characterized by a particularly long larval stage that lasts over a year and a vertical distribution with larvae in the surface layer and the older individuals in deeper ones. The reproductive cycle of *P. antarctica* is closely linked to seasonal sea ice dynamics and early stages depend on the spatial and temporal match with zooplankton production. *P. antarctica* is planktivorous at all stages of development, larvae are omnivorous actively feeding on diatoms and small copepods such as *Oithona* and *Oncaea* spp., while juveniles and adults are strictly carnivorous and feed mainly on copepods and euphausiids. In the early years of its life-history Antarctic silverfish exhibit primarily a marked transition in terms of trophic ecology between larvae and juveniles. The food resource partitioning is relatively clear between larvae and older life stages with a negligible overlap. Although the shift in diet between juveniles and adults remains less obvious in several geographic locations, juveniles and adults seem to share a similar mid-trophic level around Antarctica.

Keywords Trophic ecology • Fish feeding • Antarctic fish larvae • Juveniles

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6.1 Introduction

Pleuragramma antarctica (Notothenioidei, Nototheniidae), also known as the Antarctic silverfish, has a circum-Antarctic distribution, is described as the dominant pelagic fish over the Antarctic continental shelf, and is well known as a planktivorous species and a keystone for its intermediate trophic level between zooplankton and a large range of top predators (reviewed in La Mesa and Eastman 2012). The contribution of *P. antarctica* to the Antarctic ecosystem functioning (e.g., as prey or predator) is generally taken into account as a whole without discriminating between the different developmental stages. The main object of this chapter is thus to review the trophic ecology of larval and juvenile stages of *P. antarctica* and to highlight some of our major knowledge gaps. Three age groups of *P. antarctica* were considered and defined according to data based on length frequency distributions for various areas of the Antarctic shelf (Hubold 1984; La Mesa et al. 2010; Koubbi et al. 2011). Specimens between 8 and 30 mm SL (Standard Length) were attributed to age 0 larvae, those of 30–50 mm and between 55 and 100 mm SL were two size classes of juvenile, respectively age 1 year and age 2 year (Fig. 6.1). Longer specimens, greater than 110 mm SL were considered as adults.

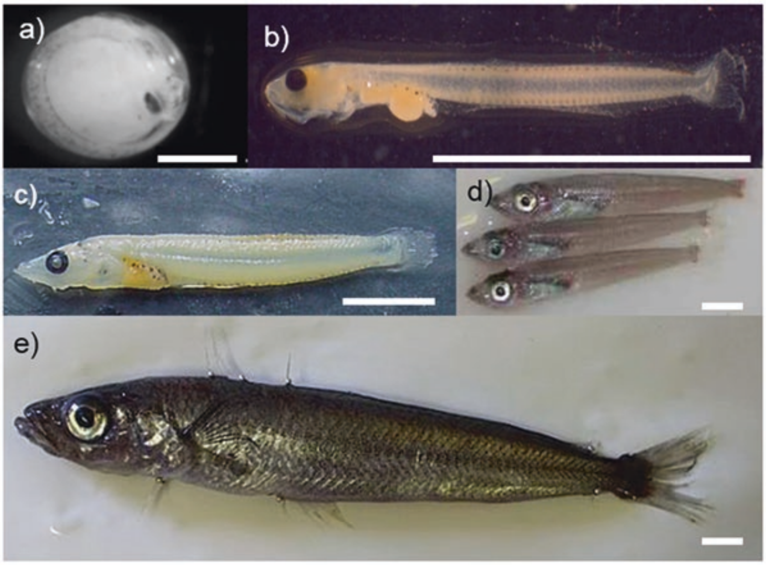

Fig. 6.1 *P. antarctica* developmental stages. (**a**) egg, (**b**) larva (**c**) juvenile 1+, (**d**) juveniles 2+, (**e**) adult. Bar scales for **a**, **b**, **c**, **d** = 1 cm; bar scale for **e** = 1 mm (Photo credits E. Tavernier and M. Vacchi)

6.2 Distribution and Mean Abundances

The spatial distribution pattern of different *P. antarctica* life stages is well described and seems to be consistent over the years and all around the continental shelf. Studies on the distribution and abundances of larvae and juveniles have been conducted since the "Discovery" and "Terra Nova" expeditions in the Ross Sea (Regan 1916), McMurdo Sound (DeWitt and Tyler 1960), Prydz Bay (Wohlschlag 1961), Weddell Sea (Hubold 1984; Keller 1983) and off the Antarctic Peninsula (Kellermann 1986). Relatively more recently, studies have been conducted on the East side from the Cosmonaut Sea (Van de Putte et al. 2010), Davis Sea (Hoddell et al. 2000) and the Dumont d'Urville Sea (Koubbi et al. 1997).

Larvae of the Antarctic silverfish constitute the great majority of ichthyoplankton in many locations around Antarctica representing from 90% to 98% of the ichthyoplankton biomass (Weddell Sea (Keller 1983), Ross Sea (Guglielmo et al. 1998; Granata et al. 2002), Dumont d'Urvile Sea (Koubbi et al. 1997)). Larval abundances reported during the summer varied depending on the location and the year, but ranged between 5.5 and 47.6 individuals per 10^3 m^3 in the Antarctic Peninsula (Kellermann 1986) up to 300–3700 individuals per 10^3 m^3 in the Weddell Sea (Hubold 1984; Keller 1983). Hubold (1984) and Koubbi et al. (2011) reported that mean abundances varied, from one year to the next, by a factor of 16–20 in age 0 larvae (Weddell Sea and Dumont d'Urville Sea, respectively) and could be as high as 33,560 individuals per 10^3 m^3. The factors influencing changes in larval abundances remain poorly understood, however, recent studies (Koubbi et al. 2011) suggest that variability could be greater linked to interannual variation of oceanographic features (e.g., ice-coverage) than to geographic spatial variation. Current data and observations support this hypothesis with abundance and size of a year class being strongly related to the percentage of ice coverage and the appearance and extension of coastal polynyas, delaying or preventing local onset of plankton production (Hubold 1984; La Mesa et al. 2010; Koubbi et al. 2011). Furthermore, high variability among samples (numerous samples without larvae) could be also partly explained by the fact that *P. antarctica* larvae live in dense swarms.

In contrast to larvae, juveniles represented lower abundances with also highly variability depending on the region and the year. Kellermann (1986) reported that juveniles formed 83–97% of the pelagic fish. Abundances ranged from 0.01 to 2 individuals per 10^3 m^3 (La Mesa et al. 2010) up to 1–125 individuals per 10^3 m^3 (Granata et al. 2002). Abundance data should be regarded with precaution when comparing different regions. Differences in abundance might come from different sampling gears (and efficiencies) used among studies. Larvae were indeed collected with multinet, Hamburg Plankton Net, RMT-1 (Rectangular Midwater Trawl-1) or double frame bongo net, towed in oblique hauls between the surface and 140 to 200 m among studies and with time of fishing ranging from one to three. As for most fish species, differences between larval and juvenile abundances might be explained by high rates of mortality at the larval stage, larval drift or migration that are still poorly understood in *P. antarctica*.

During ontogeny, larvae and juveniles are progressively transported from coastal areas to deeper and offshore waters, where they are segregated both horizontally and vertically. As reviewed by La Mesa and Eastman (2012), early larvae, mainly found close to hatching coastal areas, are transported and dispersed by local currents on the continental shelf up to the shelf break or the seaward limit of polynyas. During the summer period, the distribution pattern of early life stages is strongly influenced by geographical and oceanographical features such as inner shelf depressions, canyons, coastal gyres and katabatic winds. Larvae are accumulated in frontal systems where gyres and eddies favored retention as in northern and southern areas of Terra Nova Bay (Granata et al. 2002) or north of Terre Adélie (Koubbi et al. 2009). The only available study on otolith biochemistry (based on Sr/Ca ratios in *P. antarctica* indicated migration between onshore and offshore areas. Radtke et al. (1993) suggested that *P. antarctica* is exposed to colder water early in life for 2 years, gradually moving into warmer offshore waters as it grows older past 2–4 years. Although otolith biochemistry appears as a promising technique to resolve migration patterns in polar fish, the relationship between Sr/Ca ratios and water temperature remains poorly understood (Sturrock et al. 2015).

The characteristic vertical distribution of age/size classes of *P. antarctica* seems to be consistent among locations with individuals migrating into deeper waters as they mature. Larvae inhabit upper water layers, while older developmental stages occur in deeper depths (Hubold 1984, 1985; Kellermann 1986; Granata et al. 2002; Koubbi et al. 2011). Although found throughout the water column, age 0 larvae remain mostly near the surface (more than 50% caught on the upper 50 m layer) characterized by seasonally warmer and stratified water and thus providing a stable environment for plankton development and a significant advantage for growth (Hubold 1984; Guglielmo et al. 1998; Granata et al. 2002). Juveniles age 1 resemble in their vertical distribution to juveniles age 2, associated with cold “Ice Shelf Water” (−1.8 to −2.1 °C) and relatively shallow bottom depths from 200 to 500 m depth (Hubold 1984; La Mesa et al. 2010). Hubold (1985) stated that segregation of life stages could be viewed as a strategy to reduce intraspecific competition and cannibalism.

6.3 Nutritional Condition of Antarctic Silverfish Larvae

Unlike other notothenioid species, most of which spawn large eggs on the sea bottom, *P. antarctica* spawn pelagic eggs of small size (about 2 mm) that have been found floating in the platelet ice under the sea ice cover (Vacchi et al. 2004, 2012). In the first weeks after hatching, larvae show a relatively good feeding activity regardless of the location: 92.8% of stomach from larvae (8–17 mm) contained food in Western Ross Sea (Granata et al. 2009), feeding incidence among postlarvae (13–22 mm) caught at daylight and at night were 79.6% and 76.8% respectively in Bransfield Strait (Kellermann 1987), 30% of the larvae had empty guts when observed with a stereomicroscope but only 10.9% if observed with a scanning electron microscope in Dumont d'Urville Sea (Koubbi et al. 2007). To determine if the observed high feeding incidences translate into individuals in good condition, the nutritional status of *P. antarctica* larvae was studied using histological and biochemical approaches. Condition of the *P. antarctica* larvae (15–20 mm SL) investigated by histology of the digestive organs (Koubbi et al. 2007, Dumont d'Urville Sea) during the summer indicated that most larvae were in good condition or slightly starved. Some individuals were severely starved but none were at a point of no-return and the prey quality seemed to have an important impact on the condition of fish larvae. Generally, individuals feeding mainly on copepods (omnivorous diet) were in better condition than those feeding mainly on diatoms. However, despite their lower energy content compared to copepods, diatoms might provide a direct supply of some nutrients (e.g., amino-acids, essential fatty acids) and improve digestion (Vallet et al. 2011).

Lipid content and their dynamics can be linked to the condition and life strategies of the individuals. The Antarctic silverfish is characterized by a pronounced ontogenic lipid accumulation with increasing size (Wöhrmann et al. 1997; Mayzaud et al. 2011). During 4 years of sampling in Dumont d'Urville Sea (Giraldo et al. 2012; Tavernier et al. 2012) lipid content in larvae was low (mean value 2.1% wet weight with a dominance of structural polar lipids (87–94.6%) and low percentage of triglycerides (TAG, 2–7%) (Giraldo et al. 2012; Mayzaud et al. 2011). The total amount of lipids was linearly related to the quantity of structural polar lipids, suggesting that dietary energy could be mainly channeled towards fast growth rather than energy storage for potential starvation periods or overwintering. Taking advantage of the long monitoring of *P. antarctica* larvae in the Dumont d'Urville Sea nutritional condition of larvae was investigated using a lipid-based condition index from 2008 to 2011, in summer (Giraldo et al. 2012). TAG are the most responsive lipid class to changes in larvae feeding, as they can either be stored when the derived energy from exogenous feeding is higher than the metabolic demands, or preferentially catabolized during starvation (Fraser 1989; Håkanson 1989). Because the absolute TAG content depends on larval size, TAG was standardized using cholesterol (Chol) as a proxy of larval body mass and because Chol is not catabolized under starvation (Fraser 1989; Håkanson 1989). TAG/Chol ratios indicated large interannual differences in *P. antarctica* larvae but no significant spatial variability. Because *P. antarctica* larvae favor growth over lipid storage, low TAG/Chol values could be misinterpreted as individuals in poor condition. Only the combination of a low TAG/Chol ratio and low polar lipids content (open to be mobilized during starvation periods) index was able to better target individuals in poor condition with regard to growing specimens. Thus the combination of the two parameters showed significant differences in the physiological condition of larvae at particular stations displaying great inter-annual differences. The number of larvae estimated in poor condition ranged from 2% in 2009 to 33% in 2010 and could be related to the lowest copepod abundances in summer 2010. These results suggested that inter-annual variability in the condition of larvae was greater than the magnitude of spatial variability in the investigated area.

Taken together, the available information seem to indicate that *P. antarctica* larvae has good feeding activity in all the investigated areas during the critical period in summer when their development is likely to coincide spatially and temporally with plankton production (Cushing 1975). Given a long pelagic larval phase of over 1 year (Kellermann 1987), silverfish larvae have to survive also through the winter when the planktonic resources are low (Longhurst 1998). Hubold and Hagen (1997) reported no seasonal effect on lipid content at the end of winter for *P. antarctica* larvae in Weddell Sea, the good foraging activity observed in October/November being apparently preceded by a similar behavior during the winter period. Analysis of lipid class indicate that the mean strategy in *P. antarctica* larvae is the allocation of energy reserves primarily to somatic growth, which should increase survival (a) by increasing the opportunity to consume larger preys and more available food and (b) by decreasing the risk of predation by a continuous improvement of their evasion capacities. Indeed, beside starvation, predation upon larvae is considered as the most important source of mortality (Leggett and Deblois 1994). Although information concerning the predation upon silverfish larvae is rare, large larvae of icefish have been reported as specialized predators in the Ross Sea with silverfish larvae as their overwhelmingly dominant prey (La Mesa et al. 2011). By taking into account the previously described early traits of life, it could be argued that *P. antarctica* larvae may experience high mortality rates mostly linked to predation than to poor physiological conditions and feeding incidences.

Given the difficulties in obtain direct in vivo measures of larval growth, most of the available average growth rates of *P. antarctica* have been estimated using differences between mean lengths of co-occurring age groups during the austral summer. The larval growth rate in the Dumont d'Urville Sea (0.17 mm per day, Koubbi et al. 2011) was similar to the one indicated for *P. antarctica* larvae from the Western Ross Sea (0.15–0.21 mm per day, Granata et al. 2009) but slightly lower than rates (0.15–0.24 mm per day) reported in the Weddell Sea (Keller 1983; Hubold 1985) and the Antarctic Peninsula (0.32 mm per day, Kellermann 1986). These results indicate different larval growth rates among areas of the Southern Ocean as suggested by Radtke et al. (1993). Similar average growth rates of 0.07–0.08 mm per day for the first year were reported, between age groups 0 and 1, from different locations (Kellermann 1986; Guglielmo et al. 1998, Koubbi et al. 2011). Local and seasonal food supply and variations in prey sizes are likely to affect growth rate more substantially than changes in temperature as observed by Hubold and Tomo (1989). Differences between the growth rate estimated during summer and those calculated per year reflect a cyclic growth pattern, with higher growth rates during the peak of phytoplankton/zooplankton production (i.e. spring/summer) and a less important growth rate during the winter. This is in agreement with the data by Granata et al. (2009), indicating that *P. antarctica* young of the year grow slowly (about 0.02–0.03 mm per day) from March to November. In summary, although early life stages of Antarctic silverfish seem to feed year round, the species show distinct seasonal growth pattern depending primarily on variation in food supply and/or prey composition between summer (higher growth rates) and winter (lower growth rate).

6.4 Feeding Ecology

Considerable data from various sectors of the Southern Ocean support the notion that *P. antarctica* is a zooplanktophagous species in all phases of its life cycle. However given the seasonal constraints in collecting samples, data are fragmentary and mainly confined to the austral summer period. Most of the studies on feeding strategy and food preferences were carried out by the traditional analyses of stomach contents that provides precise identification of prey, as well as a good insight of abundance, frequency of occurrence of prey and prey's body size.

In the Antarctic Peninsula (Bransfield Strait) the diet of post-larvae (13 and 22 mm SL) collected in February consisted mainly of small copepods (*Oncaea* spp.), calanoid eggs and occasionally early stages of *Euphausia superba* and tintinnids while post-larvae (18–31 mm SL) collected in March consisted exclusively of copepods, as well as calanoid eggs (Kellermann 1987). Displaying an evident shift to larger prey with ontogeny, juveniles (33–60 mm SL) collected at the same period fed almost exclusively on copepods (mainly calanoids) and occasionally on larval stages of euphausiids (Kellermann 1987). In the Weddell Sea, Hubold and Ekau (1990) reported that calyptopis stages of ice krill and calanoid copepods represented the most frequent food of juveniles (40–60 mm SL). In the western Ross Sea, the most frequent prey of small larvae (8–17 mm SL) were calanoid eggs and pteropods (*Limacina helicina*) followed by small copepods and tintinnids while large larvae (14–29 mm SL) fed exclusively on copepods during spring in the same area (Granata et al. 2009). During their second summer, juveniles (36–50 mm SL) consumed mainly small copepods and less frequently ice krill calyptopes (Granata et al. 2009). Hopkins (1987) reported that in February, in the Ross Sea silverfish small larvae (mean 14 mm SL) fed exclusively on cyclopoids copepods whereas larger larvae (mean of 21 mm SL) exhibited a wider spectrum of prey, feeding on calanoids (mainly), secondarily on cyclopoids and occasionally on larval stages of euphausiids. The diet of second-year juveniles (mean of 39 mm SL) consisted exclusively of the same proportion of cyclopoids and calanoids (Hopkins 1987). A clear shift of food preference with ontogeny was described by Pakhomov et al. (1995) during summer in the Indian sector. The diet of larvae (13.5–21 mm SL) consisted exclusively of small cyclopoids (*Oncaea* and *Oithona* spp.) whereas juveniles (36–60 mm SL) fed preferentially on larger calanoids (mainly *Calanoides acutus*, *Calanus propinquus*, *Metridia gerlachei*) and less frequently cyclopoids and euphausiids. The proportion of young stages of euphausiids (mainly *E. superba*) was greatly increased in the diet of 3-year old juveniles, followed by large calanoids. Although a carnivorous diet seems to be the common feature among most of the studies, a different perspective emerges from studies using scanning electron microscopy for gut content analyses of larvae from Adelie Land (Koubbi et al. 2007; Vallet et al. 2011). This approach provided the first evidence that *P. antarctica* larvae also fed on phytoplankton (mainly diatoms *Fragilariopsis* spp.) during their first summer. The most frequent prey of small larvae (11–21 mm SL) were cyclopoid copepods, planktonic eggs and phytoplankton (Giraldo et al. 2011; Vallet et al. 2011). An evident shift in food preference was also observed in Dumont d'Urville Sea with juveniles (43–52 mm SL) feeding mainly on calanoids copepods, planktonic eggs and appendicularians (Giraldo et al. 2011).

In contrast to the summer months, there is very little information available on the feeding habits of young life stages during the rest of the year. During the first winter, thought as a critical period with low food supply, postlarvae seems to be able to feed sufficiently in the upper water layers in the Weddell Sea relying almost exclusively on cyclopoid copepods (Hubold and Hagen 1997). Furthermore, the food composition of larvae collected in spring in the Western Ross Sea indicated that they prey actively on sea ice associated copepods living underneath the sea ice (Granata et al. 2009). The diet consisted exclusively on both calanoids (*Paralabidocera antarctica* and *Stephos longipes*) and harpacticoids (*Harpacticus furcifer*) at various development stages. Hence, postlarvae could continue feeding throughout the year and seem to be well-adapted to feed on a wide spectrum of zooplanktonic prey depending on seasonal availability.

Stomach content analyses have been proved a valuable tool to evaluate detailed food sources and feeding strategy, however, results often represent only a snapshot of an organism's diet in time and space and also suffer from their inability to provide an integrated history of feeding. Moreover, stomach contents reflect what was ingested and not necessary what is really assimilated and might also underestimate the contribution of soft preys with a rapid digestion rate. To overcome these difficulties in identifying trophic relationships and diet, ecologists have turned to the use of indirect methods such as the use of stable isotopes and fatty acid trophic markers. Both techniques hold an advantage over gut content analysis because they reflect assimilated rather than potential carbon sources and can provide a longer integrated history of feeding strategies. Dietary fatty acids are incorporated in a relatively conservative manner into lipid reserves. The fatty acid composition (also refer to as "fatty acid signatures") of neutral lipids (triglycerides for *P. antarctica*) illustrate diet integrated over several weeks to months. Thus, combinations of fatty acids have been widely used as qualitative markers to trace predator-prey relationships in marine ecosystems (see for review Dalsgaard et al. 2003). Off Adelie Land, triglycerides from larval stages showed high levels of the fatty acids 16:0, 20:5n-3, 20:6n-3 together with elevated amounts of the 16:1n-7 and, to a lesser degree, of the 18:4n-3 fatty acids, usually associated with herbivorous feeding (Mayzaud et al. 2011; Tavernier et al. 2012). This fatty acid pattern was consistent with a contribution of phytoplankton and confirmed the underestimate of the part of primary producers in the diet of *P. antarctica* larvae during their first summer. Moreover the contribution of phytoplankton to the diet of fish larvae explains the lower $\delta^{15}N$ values for *P. antarctica* larvae when compared to strictly carnivorous species and corroborates the strong omnivory shown with fatty acid trophic markers. In agreement with fatty acids, stable isotope analysis (summer, Dumont d'Urville, Giraldo et al. 2011) showed a difference of one trophic level (based muscle $\delta^{15}N$ values) between *P. antarctica* larvae (6.7‰) and juveniles (9.7–10.0‰) with trophic levels ranging from 1.5 for herbivores to 3.2 for carnivorous species. *P. antarctica* larvae showed an intermediate trophic level of 2.6 similar to the one of *Euphausia crystallorophias*

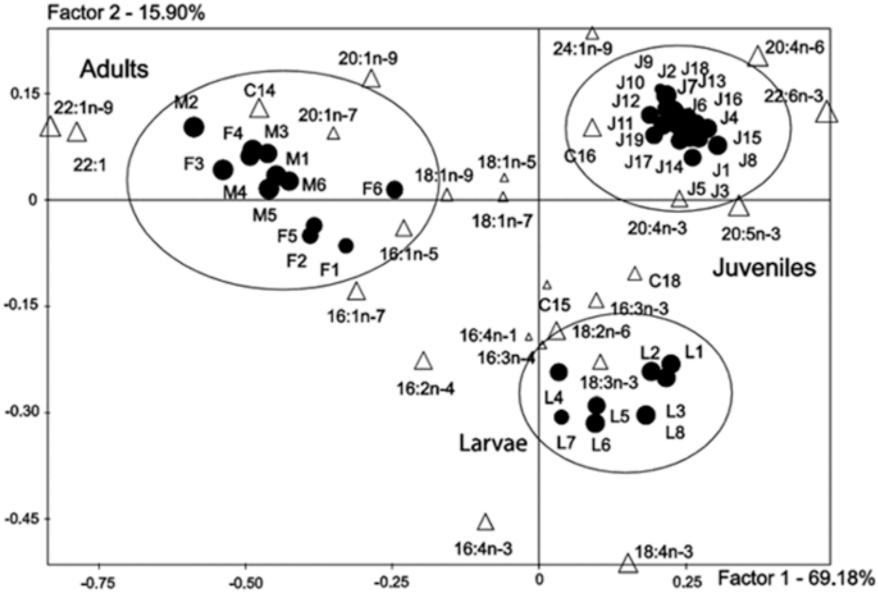

Fig. 6.2 Correspondence analysis on the fatty acids (FA) composition of the triacylglycerol lipid fraction in *P. antarctica* life stages. Muscle tissue was analyzed for juveniles and larvae. Muscle (M) and liver (F) were analyzed in adult specimens. Individuals (fish) are illustrated by *black circles* and FA by *triangles*. The size of the triangles is proportionate to the contribution of the FA to the factorial map. Analysis explains 85% of the variance and highlights different FA compositions indicating different diets depending on the developmental stage. Phytoplankton markers (C16-C18 PUFA) were present in larvae, while C20:1 and C22:1 (*Calanus* type copepods) were present in adult tissues. Although no clear FA marker was found in juvenile stages, the carnivory index C18:1n-9/C18:1n-7 significantly increased from 1.5 in larvae to 4.7 and 3.0 in juveniles and adults, respectively (Modified from Giraldo et al. 2015)

(2.4) known as a herbivorous/omnivorous species. Fatty acid trophic markers pattern from the same studies also confirmed a carnivorous dietary input and a negligible contribution of calanoid copepods in the diet of *P. antarctica* larvae, as indicating by the very low levels of 20:1 and 22:1 acids. The correspondence analysis, describing the triglyceride fatty acids composition of different developmental stages (larvae 9–30 mm SL, juveniles 42–69 mm SL and adults 160–176 mm SL, Giraldo et al. 2015) support ontogenetic diet changes (Fig. 6.2). All three age classes are well segregated confirming a shift from a phytophagous and omnivorous diet for larvae to a carnivorous diet for juveniles. Among juveniles stages, studies have shown a gradual and increasing shift from a copepod dominant diet in 1 year juveniles, to an increasing contribution of euphausiids in the diet of older juveniles (Mayzaud et al. 2011). Using orthogonal projections to latent structures-discriminant analysis (OPLS-DA), a multivariate method predicting the association predator-main potential prey using total fatty acids as predictive variable, Giraldo et al. (2015) showed that the dominant preys of juveniles were euphausiids and to minor extent copepods, while all adults fed exclusively on large non-herbivorous copepods (*C. acutus*, *Paraeuchaeta antarctica* and *C. propinquus*). Difference between juveniles and adults were surprising as it was expected to find larger prey for older stages. Poor contribution of copepods in the diet of juveniles might be explained by a greater availability of euphausiid larvae over the period before the catch. This study indicates also slight or no effect of spatial variability in dietary patterns and suggests that diet composition is relatively constant along the Dumont d'Urville Sea.

Taken together studies show that during the first 3 years of its life span, *P. antarctica* feed during all seasons and appear as generalist predator well-adapted to feed on a wide spectrum of zooplankton (mainly copepods). Major prey species reported for each life stage are usually similar among areas and studies, and main differences on the relative abundance of prey seem to reflect local prey availability. Diet of larvae during their first summer was dominated by small prey (consumable prey size is strongly limited by mouth width in early life stages), mainly cyclopoid copepods (*Oncaea* and *Oithona* spp.), occasionally planktonic eggs (mainly calanoid eggs), early life stages of euphausiids and phytoplankton. From the second summer, food preferences of juveniles indicated an evident shift with ontogeny as individuals switched to large copepods (*C. propinquus*, *M. gerlachei*, *C. acutus* and *Rhincalanus gigas)* and larval stages of euphausiids, decreasing the consumption of small cyclopoids. Although the prey consumed by young life stages of Antarctic silverfish outside summer is not well documented, they could potentially feed year-round consuming available prey species, especially copepods.

P. antarctica appears as a zooplanktophagous throughout its life, with sub-adults and adults also feeding primarily on copepods and euphausiids (La Mesa and Eastman 2012). If food resource partitioning is relatively clear between larvae and older life stages (feeding on different size fractions of zooplankton with negligible overlap), the dietary shift between juveniles and small adults remains less obvious. Stable isotopes indicate no differences on $\delta^{15}N$ values between juveniles and adults, suggesting that both developmental stages share the similar mid-trophic level position all around Antarctica (see hereafter). While copepods could be a dominant prey year round, young developmental stages of *P. antarctica* can be considered as generalist feeders rather than specialist feeders, as their diet consist of a relatively high number of food items. Consequently, this dietary plasticity should favour a high tolerance in fluctuating environmental conditions, switch between several prey species if necessary. Compared with the generalist consumer behavior of silverfish, larvae and juveniles of the family Channichthyidae appear as trophic specialists, with a narrow food spectrum that is exclusively composed of early developmental stages of krill and fish (Pakhomov et al. 1995) which might result in higher tropic vulnerability. However, to clearly evaluate the feeding strategies of early life stages of *P. antarctica* and determine if individuals exert food preferences, a direct comparison with prey availability and abundances is needed. Off the Antarctic Peninsula, at low food density conditions, larger prey species were selectively chosen, but when food density was high, size-selective feeding mode was distinctly less pronounced for larvae and *P. antarctica* juveniles (Kellermann 1986, 1987).

Moreover, prey forming large aggregations and patchily distributed in the water column or under the sea ice are negatively selected by larvae and juveniles in the Western Ross Sea (Granata et al. 2009). Accordingly, Vallet et al. (2011) showed that prey selection was apparently food-density dependent for phytoplankton, with an inverse relationship between food abundance and selection feeding of larvae in the Dumont d'Urville Sea. As most diatom taxa presented a low food-specific abundance and high occurrence. Larvae seem to select positively some diatom species, such as *Coscinodiscus* spp. and *Thalassiothrix antarctica*, that presented lower concentration in the water column compared to *Fragilariopsis* spp., (strongly negatively selected). Nevertheless, it remains unclear to what extent early life-history stages of *P. antarctica* show a truly selective feeding strategy rather than an opportunistic behavior. Determining if larvae and juveniles can be selective or partially selective feeders, and if they can adapt their feeding strategy depending on seasonal prey availability or quality and their implications to withstand environmental changes definitely requires further investigation.

6.5 Early Stages of *P. antarctica* Within the Trophic-Web

The Antarctic silverfish is commonly known as a keystone within the pelagic ecosystem of the Antarctic continental shelf, functioning as a major link between the lower trophic positions and top predators including fish, penguins, flying seabirds and marine mammals (reviewed by La Mesa and Eastman 2012). Isotopic measures of δ^{15}N, in samples collected in the Dumont d'Urville Sea during CEAMARC (Collaborative East Antarctic Marine Census) showed a positive relationship between *P. antarctica* length (SL, mm) and δ^{15}N (Fig. 6.3).

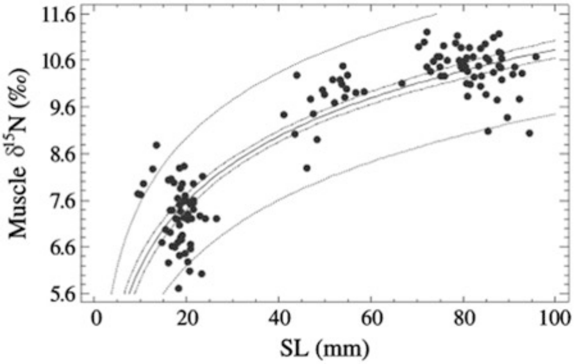

Fig. 6.3 Logarithm regression model between δ^{15}N and standard length (SL, mm). The plot shows the least squares regression line and 95% prediction limits for new observations. The following equation is issue from the regression; $\delta^{15}N = 2.89 + 1.5*Log(SL(mm))$, $R^2 = 0.70$, ANOVA F = 357, p-value <0.0001) (Modified from Giraldo et al. 2011)

These results (Giraldo et al. 2011; Cherel et al. 2011) highlighted that (i) larval stages were significantly segregated from older stages with a difference of ~2.5‰ in $\delta^{15}N$ values, reflecting one trophic level of difference between early developmental stages, and (ii) mean $\delta^{15}N$ values did not differ between juveniles and adults. The contribution of phytoplankton to the diet of silverfish larvae explains the lower value when compared to juveniles or other strictly carnivorous species. Values of $\delta^{15}N$ for juveniles (2–3 years old) and adults (10.4‰ ± 0.4 and 10.2‰ ± 0.7, respectively) are consistent among studies regardless of the location (reviewed by Pinkerton et al. 2013). Taken together, the above studies suggest that juveniles and adults of Antarctic silverfish share a similar mid-trophic level around the Antarctic continental shelf. Furthermore, $\delta^{13}C$ values used as an indicator of the origin of carbon sources, confirm an ontogenetic variation in the vertical and horizontal foraging habitat of *P. antarctica* moving progressively from shallow depths to deeper and more offshore waters. Brasso et al. (2014) reported higher variances in $\delta^{13}C$ linked to size classes suggesting a dietary niche expansion with ontogeny relying on a progressively larger and more variable foraging habitat range. *P. antarctica* may, as a general rule, continue to feed upon small and lower trophic level prey items throughout life span, notwithstanding leading to ontogenetic increases in niche breadth rather than trophic level. Nevertheless there is a marked dietary overlap between juveniles and adults, which could be understated by their spatial and vertical segregation preventing intraspecific competition rather than interspecific competition.

The isotopic signatures of Antarctic silverfish, their main potential prey, competitor and predator species have been primarily investigated in three areas including the Ross Sea (Pinkerton et al. 2013), the Weddell Sea (Mintenbeck 2008) and the Dumont d'Urville Sea (Cherel et al. 2011; Giraldo et al. 2011). As summarized in Fig. 6.4, the trophic position of life stages of *P. antarctica* among zooplankton species, that have been documented as part of their diet, is characterized by a continuum with all stages occupying the tertiary consumers' level. Zooplankton $\delta^{15}N$ values were in close agreement with the diet composition previously documented in this chapter. Trophic level of potential prey ranged from 1.5 for herbivores such as *R. gigas* to 3.2 for carnivorous species such as *Paraeuchaeta antarctica* while *P. antarctica* larvae showed an intermediate trophic level of 2.6. The isotopic signature of the two dominant euphausiids species and calanoid copepods (*C. propinquus*, *C. acutus* and to a lesser extent *M. gerlachei*) reflects an herbivorous/omnivorous diet and the rather similar trophic level (1.3–2.3) of these prey could explain why silverfish juveniles and adults share the same trophic level.

Carnivorous zooplankton (e.g., hyperiid amphipod *Themisto gaudichaudii*, Chaetognaths and numerous jellyfish) encompassed intermediate trophic levels between those of larvae and juveniles of *P. antarctica*, suggesting that these species prey upon same trophic-level organisms and could be potential competitors to early life stages of *P. antarctica*. Interestingly, the large and carnivorous copepod *Paraeuchaeta antarctica* shared the same trophic level as silverfish juveniles in different localities (Mintenbeck 2008; Giraldo et al. 2011; Pinkerton et al. 2013). *Paraeuchaeta antarctica* is a carnivorous species feeding mostly on copepods which

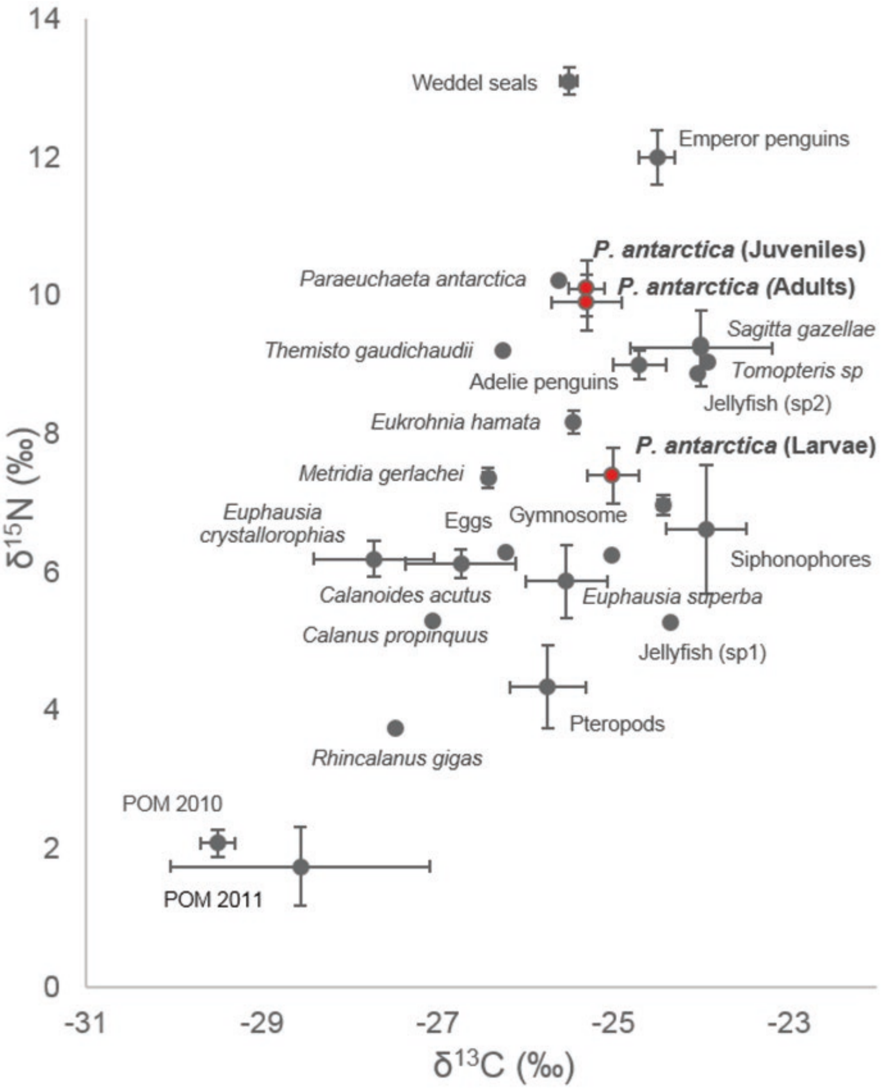

Fig. 6.4 Food web representation of *P. antarctica* (*red*) and its prey and predators in Dumont d'Urville Sea during summer based on stable isotopes analysis ($\delta^{13}C$, $\delta^{15}N$) (Modified from Giraldo et al. 2011 and Cherel et al. 2011)

explain why it has the same trophic levels as juveniles and adult silverfish. With the exception of channichthyids larvae, most of notothenioid larvae presented similar trophic levels as the silverfish larvae (Cherel et al. 2011).

Along with the ice krill *E. crystallorophias*, the intermediate trophic position of *P. antarctica* in relation to other fish species and other predators has been reported in numerous regions of Antarctica and is mainly based on dietary studies of organisms as well as the distribution of prey and predators (Smith et al. 2014). Since most predators preferentially forage on specific size classes, we can question what is the real contribution of young stages of *P. antarctica* in the Antarctic food web. Considering their high abundances (in times and locations), young life stages of silverfish are preyed upon by a large range of predators, particularly where the ice krill is absent or at reduced biomass (e.g., higher latitudes). *P. antarctica* is then believed to dominate energy transfer within the water column. This species showed a pronounced ontogenic lipid accumulation with increasing size from 12% to 47% of dry weight in larvae and adult respectively (Friedrich and Hagen 1994; Mayzaud et al. 2011). With ontogeny, *P. antarctica* increases its energetic value as prey compared to euphausiids and copepods that have moderate energy contents (Mintenbeck 2008). With only a few exceptions, all sizes of channichthyids (*Chionodraco hamatus*, *C. myersi*, *Dacodraco hunteri*) feed exclusively on fish and mostly on early life stages of *P. antarctica* (Hubold 1985, 1990; Eastman 1999; La Mesa et al. 2004). Accordingly, larvae and postlarvae of *P. antarctica* were the most frequent and abundant prey in the diet of larvae of *C. hamatus* and *D. hunteri* in the Ross Sea (La Mesa et al. 2011; La Mesa and Eastman 2012) and of *C. myersi* larvae in the Indian sector (Pakhomov et al. 1995). Some other piscivorous notothenioids (e.g., *Dissostichus mawsoni*, *Gymnodraco acuticeps*) feed mostly on early life stages of silverfish (Eastman 1985). Postlarval and juvenile *P. antarctica* were also described as a small contribution to the diet of cryopelagic fish (*Pagothenia borchgrevinki* and *Trematomus newnesi*) in the Ross Sea (La Mesa et al. 2000). Moreover, Eastman (1985) reported that in McMurdo Sound, adults of *P. antarctica* could have a cannibalistic behavior preying on its own larvae (13% frequency of occurrence). Juveniles and larger silverfish are in turn fed upon a large range of predators living and foraging on the shelf such as penguins (including Adélie penguin *Pygoscelis adeliae*, emperor penguin *Aptenodytes forsteri*), marine mammals (such as Weddell seals *Leptonychotes weddellii*, minke whales *Balaenoptera bonaerensis* and killer whales *Orcinus orca*) and flying seabirds (South polar skuas *Stercorarius maccormicki,* snow petrels *Pagodroma nivea* and Antarctic petrels *Thalassoica antarctica*) (Smith et al. 2014). While penguins fed mostly on euphausiids for themselves, Cherel et al. (2011) suggested that they enhanced the capture of *P. antarctica* and of channichthyid juveniles for their chicks.

By its high biomass and abundance and its intermediate trophic level, the Antarctic silverfish (including all phases of its life cycle) can be considered as a keystone species in coastal and neritic zones much like *E. superba* (Antarctic krill) is for waters beyond the continental shelf. Along with *E. crystallorophias* (ice krill), the silverfish is one of the few dominating pelagic species at an intermediate trophic level that is a crucial link between the highly diverse lower trophic levels (assemblage of phytoplankton and zooplankton species) and top predators (e.g. predatory fish, seabirds and sea mammals). Consequently, Koubbi et al. (2011) suggested that the Antarctic silverfish could play the same role as do other small plankton-feeding pelagic species such as clupeids in other productive oceanic zones (e.g. upwelling regions) in a termed "wasp-waist" ecosystem (Cury et al. 2000).

6.6 Conclusive Remarks

Substantial features for the early life traits of Antarctic silverfish emerge from the literature such as the high trophic plasticity and the keystone role as intermediate trophic species over the continental shelf, as summarized in Fig. 6.5. In the early years of its life-history Antarctic silverfish exhibit primarily a marked transition in terms of trophic ecology between age 0 larvae and age 1 juveniles. The food resource partitioning is relatively clear between silverfish larvae and older life stages feeding

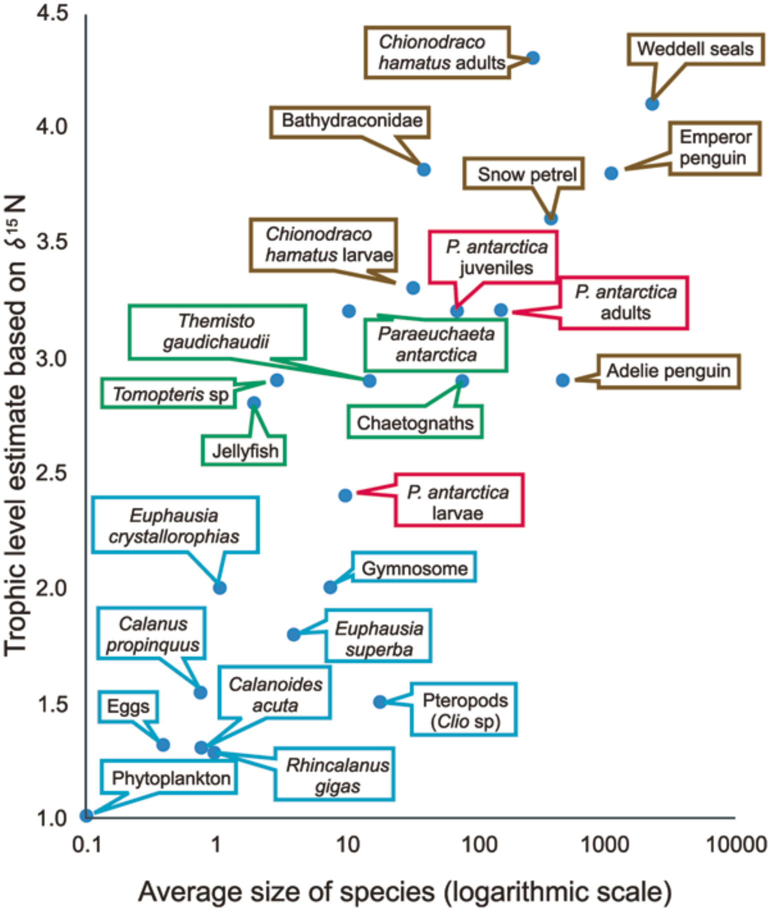

Fig. 6.5 Main trophic relationship of early developmental stages of *P. antarctica*. Potential prey, predators and competitors are in *blue*, *brown* and *green*, respectively. The y-axis correspond to the average range of trophic level values reported from different localities (Hodum and Hobson, 2000; Mintenbeck 2008; Cherel et al. 2011; Giraldo et al. 2011; Giraldo 2012; Pinkerton et al. 2013). The x-axis correspond to the average size of each category of silverfish life stages and potential prey, predators and competitors

on different size fractions of zooplankton with negligible overlap. Although the shift in diet between juveniles and adults remains less obvious, juveniles and adults seem to share a similar mid-trophic level around Antarctica. This review underlines that it is relevant to use different methods simultaneously, since each method provides a level of resolution that cannot easily be achieved by the other one. Although *P. antarctica* is one of the most studied Antarctic fish, specific areas of knowledge are sometimes based on a limited number of studies (e.g., overwintering, spawning areas) or indirect assumptions (e.g., recruitment success) and need hence further investigation.

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Chapter 7
The Role of Lipids in the Life History of the Antarctic Silverfish *Pleuragramma antarctica*

Wilhelm Hagen and Gerhard Kattner

Abstract A unique characteristic of the pelagic Antarctic silverfish *Pleuragramma antarctica* is the massive accumulation and storage of lipids in special oil sacs. The enormous lipid deposition beyond 50% of body dry mass functions primarily as buoyancy aid compensating for the missing swim bladder in these fishes, although the depot lipids could also serve as energy reserves. The lipid signature clearly reflects the life cycle of *P. antarctica*. Trophic marker fatty acids of the early larval and post-larval stages reveal feeding preferences on phyto- and zooplankton, mainly copepods, which these stages utilize for rapid somatic growth without special lipid storage. The juvenile stages tend to feed on calanoid copepods, while the adults shift to krill (*Euphausia superba, E. crystallorophias*) as major food items. The findings from fatty acid trophic markers are in accordance with gut content analyses. Juveniles to adults exhibit a pronounced lipid deposition, namely triacylglycerols, in the oil sacs. These triacylglycerols are composed of unmodified dietary fatty acids, but may also partially be synthesized *de novo*. This substantial lipid accumulation not only represents a key adaptation of *P. antarctica* to life in the pelagic realm. It is also of major importance as high-quality and high-energy food for other marine vertebrates such as seabirds and seals and ultimately ensures an efficient energy flow through the lipid-based high-Antarctic food web.

Keywords Nototheniidae • Lipid deposition • Life cycle

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7.1 Introduction

The Antarctic fish fauna is unique, due to the overwhelming dominance of one perciform group, the Notothenioidei, with 97% of the species endemic to the Southern Ocean (Andriashev 1987). These species originate from a benthic ancestor without a swim bladder (Clarke and Johnston 1996; Eastman 2005), which may explain that only very few species permanently invaded the pelagic zone, a niche with plenty of lipid-rich food, e.g. copepods and krill, largely unoccupied by fishes (Eastman 2005). The Antarctic silverfish *Pleuragramma antarctica* is the key species among these pelagic fishes of the Antarctic ichthyofauna (Koubbi et al. 2009; La Mesa and Eastman 2012). The biomass of this circum-Antarctic shoaling species is estimated with 500,000 t (1 t km^{-2}) in the Weddell Sea (Hubold 1992). The complete life cycle of the Antarctic silverfish has first been intensely studied by Hubold and colleagues (e.g. Hubold 1984, 1992, 2009) in the Atlantic sector of the Southern Ocean, especially the Weddell Sea. The ontogenetic development of *P. antarctica* relies strongly on lipids from the energy-rich eggs and yolk-sac larvae via post-larval and juvenile developmental stages to the adults (Wöhrmann et al. 1997). The incorporation of large amounts of triacylglycerols in the lipid sacs (Fig. 7.1), which are fully developed in the juveniles and adults, is an exclusive key adaptation of this pelagic species to counter negative buoyancy (Eastman and De Vries 1982; Friedrich and Hagen 1994; Hagen et al. 2000). Although not very flexible with regard to buoyancy regulation, these low-density lipid compounds compensate for the lacking up-thrust of a swim bladder and allow these sluggish fish to maintain their position in the water column without additional swimming effort. However, these lipid deposits may also be utilized for energetic requirements (Eastman and de Vries 1989).

For the first time, ripe eggs of *P. antarctica* were detected in the stomachs of benthos-feeding fish (*Trematomus* spp.) at 450 m depth in the Weddell Sea in

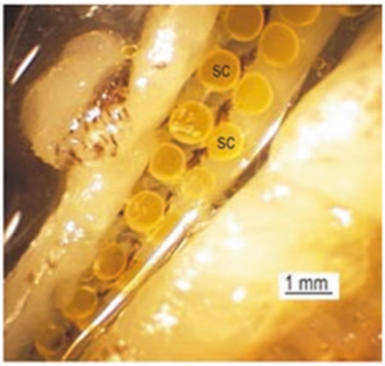


Fig. 7.1 *Pleuragramma antarctica*. Double row of subcutaneous lips sacs (sc) from a mid-ventral location in the trunk of a 90 mm length formalin-preserved specimen. Melanophores between sacs are in the mid-ventral line. Sacs are 0.7–0.9 mm in diameter (Figure and caption from La Mesa and Eastman (2012) (with kind permission of John Wiley and Sons Inc))

October 1986 (Hubold 1992, 2009). The embryonated eggs of *P. antarctica* have a diameter of about 2.0–2.5 mm and contain large amounts of lipid-rich yolk, which explains their initial positive buoyancy, but before hatching the eggs start to sink, as lipids are catabolized and converted to proteins (Evans et al. 2012). Lipids are also crucial for growth of the early larvae, which at least partially rely on the yolk sac for energy. Yolk-sac larvae of 8–10 mm length can survive more than 3 weeks of starvation (Hubold 1992), although if food is available in the field they may feed soon after hatching, as the mouth is already well developed (Bottaro et al. 2009). Hatching has never been observed in the Weddell Sea, but apparently it commences in early spring (November), with very small larvae also occurring in January and February. First yolk-sac larvae (9 mm mean length) were only collected (by Multinet) at depths below 500 m in mid-November. Within a few days very high concentrations of larvae occurred in the productive surface layer (<50 m depth) and in spite of near-freezing seawater temperatures the larvae showed surprisingly high growth rates comparable to boreal herring larvae (Hubold 1992). This early developmental phase of *P. antarctica* in the Weddell Sea appears to deviate from that described for the Antarctic silverfish in the Ross Sea, a phenomenon that may be due to different hydrographic conditions, but clearly requires further investigations. In Terra Nova Bay, Ross Sea, developing eggs and freshly hatched larvae of *P. antarctica* occur in very high concentrations near the surface among the platelet ice, directly under the congelation ice (Vacchi et al. 2004, 2012a), although the early larvae showed negative phototaxis and positive gravitaxis (Evans et al. 2012).

Larvae and post-larvae feed mainly on small cyclopoid copepods and early juveniles switch to calanoid copepods at a standard length of >60 mm (Hubold and Hagen 1997). During this developmental phase they start to store lipids via ingested food and/or *de novo* synthesis. In the juvenile stages enormous lipid depots, namely triacylglycerols, are accumulated. In the ice-covered regions the older stages of *P. antarctica* shift from copepods to the dominant neritic “ice krill”, *Euphausia crystallorophias*. Part of the *P. antarctica* population is transported by currents from the southern Weddell Sea towards the eastern side of the Antarctic Peninsula, where the fish mainly feed on the Antarctic krill, *E. superba* (Kellermann 1987). Pronounced lipid storage is a typical characteristic of the adults, with lipids reaching average levels of 50% of body dry mass (DM), but they may vary from 30% to 60%DM (Friedrich and Hagen 1994). With increasing age the adults descend to greater depths and return to the southern Weddell Sea shelf areas. The circle of this life cycle closes, when the adult *P. antarctica* migrate to their spawning grounds over the northeastern shelf areas of the high-Antarctic Weddell Sea (Hubold 1992).

Although *P. antarctica* is a key component of the high-Antarctic food web and an important and very lipid-rich prey for marine mammals and birds, e.g. toothed whales, seals and emperor penguins, there are few detailed reports on its lipid and fatty acid compositions as a unique characteristic of the Antarctic silverfish. We will summarize the lipid data and elucidate the role of these high-energy/low-density compounds in the life history of *P. antarctica* by tackling questions such as these: Is the strong lipid increase in the older stages based on *de novo* biosynthesis or is it accumulated from the diet? Is the lipid in the oil sacs rather inert or does it show an intense turnover, perhaps depending on a seasonally varying food supply? Is the huge amount of lipids necessary to maintain neutral buoyancy or is it also utilized as energy reserve? Has the sluggish mode of life of *P. antarctica* any influence on the lipid storage and demand?

7.2 Larval Stages: Rapid Growth and Low Lipid Levels

Pleuragramma larvae can be defined – besides their size – by their rather low total lipid content dominated by phospholipids. This group comprises larvae between 10 and 19 mm body length, with the largest specimens overlapping with the post-larval phase. They show rapid growth rates of 0.15–0.21 mm per day, which are similar to Atlantic herring larvae (Hubold 1985). Body dry mass extends over a very wide range from 0.3 to 2.8 mg per specimen. This also holds true for the total lipid amount, which increases accordingly from 0.06 mg in the youngest to 0.4 mg in the oldest larvae. The relative lipid levels (in % body dry mass, %DM) are rather low. Smallest larvae (ca. 10 mm) with yolk sac exhibit slightly elevated lipid levels with 19%DM, which decrease with further development to <14%DM in 15–16 mm specimens utilizing their yolk lipids. In larvae of 19 mm size lipid levels start to increase again to 23%DM (calculated from % of total lipid (%TL) in wet mass) (Table 7.1 and references therein). The respective lipid compositions of larval stages are characterized by phospholipids (ca. 70–80%TL) as the dominant component of biomembranes. These early stages contain only low but variable amounts of storage lipid, namely triacylglycerols (ca. 7–18%TL, Table 7.1). The lipid composition of the larvae is strongly determined by yolk lipids, which are essential to fuel initial somatic growth. During early larval development of the Atlantic herring for instance, these lipids are dominated by phospholipids, mainly phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Tocher et al. 1985). These lipid classes also prevail in the larvae of *P. antarctica* with PC and PE accounting for 34% and 20%TL, respectively (Hagen 1988), while Tavernier et al. (2012) reported 40% PC and 13% PE.

The fatty acid compositions of the larvae (Table 7.2) consist mainly of three principal components, 22:6(n-3), 20:5(n-3) and 16:0, which make up about 24%, 17% and 18% of total fatty acids (%TFA), respectively. This composition reflects the typical fatty acid pattern of marine biomembrane lipids. Other important but less abundant fatty acids (usually between 5 and 10%TFA) are 18:1(n-9), 18:1(n-7) and 16:1(n-7). The fatty acid compositions of the two major lipid classes, phospholipids and triacylglycerols, were analysed separately by Mayzaud et al. (2011) and Tavernier et al. (2012) for the larval size range of 17–19 mm.

Differences between these lipid classes mainly concern the fatty acids 22:6(n-3) and 16:0, which show twice as high percentages in the phospholipids than in the triacylglycerols (Table 7.3). In addition, 16:1(n-7), 18:4(n-3) and 18:1(n-9) were clearly higher in the triacylglycerols as compared to the phospholipids. Typically, the fatty acids of triacylglycerols reflect dietary preferences, whereas phospholipids

Table 7.1 *Pleuragramma antarctica*. Lipid composition according to stage/age and season

Stage/Age	Season (n)	BL (mm)	DM (mg)	TL (mg)	TL (%DM)	PL (%TL)	TAG (%TL)	CHOL (%TL)
L	Spring (8)	10.0 ± 0.0	0.30 ± 0.05	0.06 ± 0.01	18.9 ± 1.8	73.4 ± 2.2	17.8 ± 3.6	8.4 ± 1.6
L	Summer (15)	15.5 ± 0.3	1.70 ± 0.33	0.23 ± 0.04	13.6 ± 0.7	66.7 ± 7.1	14.2 ± 3.7	10.6 ± 1.6
L	Summer[a] (6)	17.2			13.6	68.4	12.5	11.9
L	Summer[b] (6)	19.0	2.82[c]	0.40	23.1[c]	83.0 ± 6.1	7.2 ± 3.0	4.1 ± 0.8
PL	Autumn (3)	31.5 ± 0.6	13.0 ± 2.0	2.8 ± 0.4	21.7 ± 0.6	42.1 ± 3.4	48.3 ± 3.5	6.7 ± 0.5
PL	Spring (17)	39.9 ± 6.1	28.0 ± 14.0	5.8 ± 3.9	19.4 ± 3.5	55.8 ± 12.5	33.2 ± 10.6	7.3 ± 1.9
PL	Summer (7)	44.1 ± 5.9	49.8 ± 22.2	13.9 ± 7.5	26.8 ± 6.2	39.3 ± 6.7	47.4 ± 18.1	6.4 ± 3.6
J1	Summer[b] (3)	54.0		35.4	37.2[c]	34.3 ± 1.6	60.8 ± 1.6	4.5 ± 0.3
J2	Spring (11)	67.4 ± 8.7	329 ± 187	130 ± 83	38.0 ± 3.9	25.6 ± 5.8	70.0 ± 6.2	3.2 ± 0.9
J2	Summer (3)	73.3 ± 10.4	474 ± 184	171 ± 60	36.5 ± 3.6	21.3 ± 2.2	74.8 ± 1.5	2.6 ± 0.6
J2+	Summer[b] (6)	82.0		170	44.1[c]	21.6 ± 3.7	72.4 ± 4.0	4.1 ± 0.9
J3	Spring (8)	92.9 ± 3.9	1197 ± 274	566 ± 181	46.5 ± 5.7	16.9 ± 2.8	78.5 ± 4.1	1.8 ± 1.9
J3	Summer (4)	97.5 ± 6.5	1052 ± 248	327 ± 132	30.2 ± 5.0	22.5 ± 4.0	66.6 ± 5.7	2.8 ± 1.8
A4	Summer[d](4)	120.0 ± 22.0	2100 ± 1800	792	37.7 ± 9.7	17.3 ± 5.4	80.4 ± 6.0	1.5 ± 0.8
A4+	Summer[e] (17)	158.2 ± 17.4			45.2 ± 8.2			

General data, body length (*BL*), body dry mass (*DM*), total lipid mass (*TL*), phospholipids (*PL*), triacylglycerols (*TAG*), cholesterol (*CHOL* (values are reported as means ± standard deviation)

L larvae, *PL* post-larvae, *J* juveniles, *A* adults, *n* number of analyses

[a] Tavernier et al. (2012), means calculated of 6 separate data sets

[b] Mayzaud et al. (2011)

[c] Authors' unpublished data, calculated via wet mass

[d] Hagen et al. (2000)

[e] Friedrich and Hagen (1994)

Larval samples consisted of 50–100 pooled specimens

Table 7.2 *Pleuragramma antarctica*. Fatty acid compositions of total lipids (means only in mass %); <0.05% or not detected; for n refer to Table 7.1

Season	Spring	Summer	Summer[a]	Autumn	Spring	Summer	Spring	Summer	Spring	Summer	Summer[b]
Stage	Larvae			Post-larvae			Juv. 2	Juveniles 2–3			Adults 4
Length (mm)	10.0	15.5	17.2	31.5	39.9	44.1	67.4	73.3	92.9	97.5	120.0
14:0	3.1	3.3	2.7	4.4	3.2	8.2	5.7	7.9	5.8	8.4	14.0
16:0	17.8	19.5	16.9	19.0	17.2	14.0	13.0	13.0	12.6	14.2	15.8
16:1(n-7)	5.1	4.8	5.3	4.5	5.2	6.0	7.2	9.2	8.0	10.6	9.5
16:2(n-4)	0.6	1.1	0.1	0.7	0.7	0.7	0.7	0.9	0.5	0.1	1.1
16:3(n-4)	–	–	0.9	–	0.2	0.2	0.1	0.3	0.1	0.4	0.2
18:0	2.2	2.7	2.6	1.3	2.4	1.4	1.1	0.9	1.0	1.1	1.0
18:1(n-9)	13.7	9.8	5.6	13.7	13.9	16.8	12.2	11.3	11.0	20.4	24.6
18:1(n-7)	4.7	6.7	5.8	2.7	4.1	3.6	3.0	3.2	2.8	6.1	6.3
18:2(n-6)	1.1	1.8	1.2	1.0	1.1	1.0	1.3	1.2	1.3	1.5	1.4
18:4(n-3)	0.5	1.2	1.4	2.6	0.8	1.1	1.5	1.6	2.1	1.5	1.8
20:1(n-9)	3.1	0.7	–	0.2	3.9	9.7	10.9	17.3	9.3	7.5	5.7
20:1(n-7)	0.1	–	–	–	0.1	0.3	0.5	0.9	0.6	0.7	0.4
20:4(n-6)	0.8	0.7	0.4	–	0.7	0.4	0.2	0.1	0.1	0.4	0.3
20:5(n-3)	14.9	17.3	19.5	13.1	13.5	11.9	7.1	8.4	6.0	7.4	4.8
22:1(n-11)	2.9	–	–	1.0	2.7	3.1	10.7	7.9	12.2	3.3	4.5
22:1(n-9)	1.9	–	–	1.3	2.1	1.5	7.7	4.1	10.2	2.5	3.0
22:5(n-3)	1.1	1.1	0.5	1.0	1.1	0.9	0.6	0.7	0.5	0.3	0.2
22:6(n-3)	20.4	25.5	28.1	22.7	19.3	13.2	8.5	5.8	5.7	6.6	3.8

[a] Tavernier et al. (2012): means calculated of 6 separate data sets

[b] Hagen et al. (2000)

Table 7.3 *Pleuragramma antarctica*. Fatty acid compositions of major lipid classes (mass%)

Lipid class	Triacylglycerols					Phospholipids				
Stage	[a]Larvae	[b]Larvae	[b]Juv. 1	[b]Juv. 2+	[c]Adults 4	[a]Larvae	[b]Larvae	[b]Juv. 1	[b]Juv. 2+	[c]Adults 4
Length (mm)	17.2	19.0	54.0	82.0	120.0	17.2	19.0	54.0	82.0	120.0
14:0	2.8	1.2	8.3	9.6	17.0	2.3	0.9	1.3	0.9	2.3
16:0	12.8	11.0	10.2	11.8	17.3	22.1	20.5	21.9	19.7	16.4
16:1(n-7)	6.8	8.1	8.6	10.5	10.1	2.8	2.9	2.2	2.0	2.8
16:2(n-4)	0.2	0.2	0.5	0.5	1.0	0.8	–	–	–	0.8
16:3(n-4)	0.4	–	0.1	0.1	0.2	–	0.4	0.3	0.3	–
18:0	4.1	5.2	0.7	0.7	0.9	3.5	3.9	2.3	2.8	3.3
18:1(n-9)	11.2	8.8	9.3	12.5	26.7	7.3	6.2	6.7	8.5	13.6
18:1(n-7)	7.7	6.6	4.0	5.2	7.7	7.2	7.2	5.3	6.4	8.9
18:2(n-6)	2.4	2.7	1.5	1.4	1.5	1.2	2.0	1.1	1.1	1.2
18:4(n-3)	3.6	7.5	1.6	1.6	1.6	0.7	1.8	0.6	0.5	–
20:1(n-9)	1.0	1.8	18.4	14.4	3.5	0.6	0.7	2.9	3.3	1.3
20:1(n-7)	0.4	0.3	2.8	2.3	0.3	–	0.1	0.3	0.4	–
20:4(n-6)	–	0.2	0.2	0.1	0.2	0.7	0.6	0.9	1.2	2.0
20:5(n-3)	17.9	14.7	7.3	3.4	5.2	15.5	16.9	20.9	17.9	18.1
22:1(n-11)	–	0.9	7.7	7.9	2.1	–	–	–	–	–
22:1(n-9)	0.3	1.0	4.1	5.7	1.7	–	0.2	0.2	0.4	–
22:5(n-3)	0.6	0.4	0.4	0.1	0.2	0.5	0.1	0.1	0.1	0.6
22:6(n-3)	12.2	11.6	4.3	1.0	1.4	24.3	25.2	23.8	25.6	27.6

[a] Tavernier et al. (2012): means calculated of 6 separate data sets
[b] Mayzaud et al. (2011): means ($n = 2$–6)
[c] Hagen et al. (2000)

have a more conservative and homogeneous composition less influenced by dietary interactions (Dalsgaard et al. 2003). A trophic signature is partially reflected in the fatty acid compositions of the larvae, although it is unknown to what extent the fatty acid compositions of eggs and yolk influence the overall composition. The fatty acids 16:1(n-7) and 18:4(n-3) in the larvae may originate from feeding on diatoms and flagellates, respectively. However, it cannot be excluded that these algae were ingested by e.g. herbivorous copepods and that this prey together with the phytoplankton markers was incorporated by *P. antarctica*. Gut content analyses by Koubbi et al. (2007) showed that the larvae (15–30 mm) from Dumont d'Urville Sea, East Antarctica, are omnivorous, feeding mainly on diatoms and copepods. Vallet et al. (2011) and Tavernier et al. (2012) reported that about 70% of the larvae feed on a mixture of phytoplankton and zooplankton, while the rest ingests exclusively phytoplankton, mainly diatoms. In contrast, diatoms represented only a negligible food item in the guts of larvae from the southern Weddell Sea (von Dorrien 1989; Hubold and Hagen 1997). In both Antarctic regions the ingestion of diatoms by the larvae is reflected by the 16:1(n-7) marker fatty acid, although the signal is not as high as expected from the gut content analyses. In addition, polyunsaturated fatty acids with 16 carbon atoms, another typical diatom marker, are almost missing. These findings do not suggest an intense incorporation of diatom fatty acids by *P. antarctica*. Vice versa, fragile flagellates are often damaged and thus very difficult to detect in the guts of the larvae. This may explain why higher portions of the 18:4(n-3) flagellate marker are not corroborated by gut content analyses. It should also be kept in mind that gut contents provide only a snapshot impression of the ingested food items, whereas fatty acid trophic markers integrate dietary signals over several weeks (Graeve et al. 1994). This emphasizes an advantage of trophic marker fatty acid studies over conventional gut content analyses (which may provide higher taxonomic resolution). On the other hand, the rapid growth of the *P. antarctica* larvae may limit the applicability of trophic marker fatty acids, due to their immediate conversion and intense utilization for growth and energetic requirements.

7.3 Post-Larval Stages: Slower Growth and Initial Lipid Accumulation

With the development from larvae to post-larvae (ca. 30–50 mm length) there is a clear increase in total lipid levels, indicating the onset of lipid accumulation in special subcutaneous and intermuscular depots, the oil sacs typical of *Pleuragramma* (Eastman and De Vries 1989). Dry mass and lipid mass increase clearly with length to 50 mg DM and 14 mg TL, respectively (Table 7.1). This results in a near doubling of the lipid content from about 19%DM in the smaller to 30%DM in the larger post-larvae. This initial lipid increase in the post-larvae is clearly due to an accumulation of triacylglycerols (Table 7.1). These compounds comprise the only neutral lipid component stored by *P. antarctica* and reach about 40% of total lipids in these post-larvae.

Apparently, during this critical phase of early development the post-larvae need to invest energy into somatic growth, but also channel already substantial amounts of energy, namely triacylglycerols, towards lipid deposition. It is a matter of conjecture, whether this lipid storage is primarily functioning as buoyancy adaptation, since there are no density data available for the post-larval phase. This lipid deposition may explain that – in contrast to the rapid growth of the larvae – growth of post-larvae slows down drastically to 0.06–0.08 mm per day (La Mesa and Eastman 2012). These post-larvae use the ingested energy partially for a distinct lipid deposition, which is quite unique among early developmental stages. It is in contrast to other species, e.g. herring or hake post-larvae, which suffer from high predation pressure and tend to rapidly outgrow this critical early developmental phase (Grote et al. 2012).

In accordance with the increasing triacylglycerol portions, the fatty acid compositions of the post-larvae reflect more clearly their dietary preferences. Unfortunately, for the post-larval stages only fatty acid data of total lipids are available, but not of triacylglycerols, which would provide stronger dietary signals. This signal is indicated by higher concentrations of long-chain monounsaturated fatty acids, in particular 20:1(n-9), in the most advanced post-larvae. Other principal fatty acids are similar to those of the larval stages. The 20:1(n-9) fatty acid supports the intense ingestion of calanid copepods, namely the older lipid-rich copepodite stages of *Calanoides acutus*. This trophic marker comprises about a quarter of total fatty acids in *C. acutus* (Kattner et al. 1994). It is the only Antarctic copepod species characterized by high amounts of wax esters with long-chain monounsaturated fatty acids and alcohols, both moieties dominated by 20:1(n-9). These wax esters make up 90% of total lipids in this copepod species. The other principal prey items are cyclopoid copepods of the genus *Oncaea* (Hubold and Hagen 1997). They are similarly rich in wax esters as *C. acutus*, but its fatty acids are strongly dominated by 18:1(n-9) (33–79%TFA), while 14:0 and 16:0 prevail in the fatty alcohol moieties (Kattner et al. 2003). This is partially reflected in the most advanced post-larvae. *P. antarctica* hardly contains any wax esters. The trace amounts detected may still originate from undigested food in the guts, although according to Giraldo et al. (2013) the gut content has no significant influence on the lipid analyses. Thus, wax esters have to be cleaved into fatty acids and alcohols, probably in the liver. Both moieties may be used for metabolic demands and for the production of triacylglycerols, with fatty alcohols following conversion into fatty acids. Alternatively, the alcohols may not be absorbed and egested unutilized, making no use of this high-energy compound. However, Sargent et al. (1979) suggested that fatty alcohols are efficiently assimilated and converted to fatty acids, usually as triacylglycerol moieties, by marine fish, e.g. Atlantic herring. The suggested feeding preferences of *P. antarctica* based on its fatty acid markers is supported by conventional gut content analysis, since the wax ester-rich older *C. acutus* have been reported as the dominant prey item in *P. antarctica* specimens <50 mm comprising more than 40% of prey biomass (Hubold and Hagen 1997). Small cyclopoid copepods of the genus *Oncaea* were the second most important prey and accounted for 25% of the ingested biomass and 60% in terms of prey abundance.

7.4 Juveniles and Adults: Slow Growth and Pronounced Lipid Storage

The strong lipid accumulation already noted in the post-larvae continues exponentially in the juveniles, which comprise several age classes. First-year juveniles had a total lipid mass of about 35 mg and a lipid content of 37% DM (calculated from wet mass by Mayzaud et al. 2011). The exponential increase in dry and lipid mass from the younger to the older juveniles (size range 54–98 mm) is shown in Figs. 7.2 and 7.3, reaching about 1.2 and 0.6 g, respectively (Table 7.1). Second and third-year juveniles exhibit slightly higher mean lipid levels between 30% and 47%DM. Adult *P. antarctica* (size range 120–190 mm) reach highest mean lipid levels around 47%DM with a maximum of 58%DM (Friedrich and Hagen 1994). These total lipids are clearly dominated by the storage lipid triacylglycerol, increasing from 60%TL in first-year juveniles to 80%TL in the adults (Fig. 7.4). The remaining lipid compounds comprise primarily phospholipids, but also low levels of cholesterol (Table 7.1).

The fatty acid compositions reveal a change in trophic preferences from larvae to juveniles and adults. As already indicated by the older post-larvae, portions of 20:1(n-9) reached up to 17%TFA in juveniles, but decreased again towards the adults to 6%TFA. A similar trend occurred for 22:1(n-11) and 22:1(n-9) with maxima

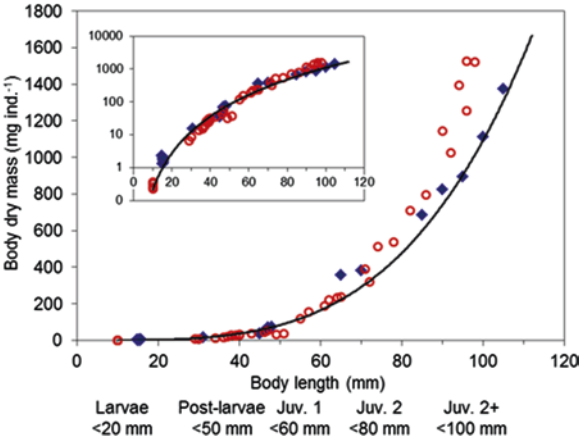
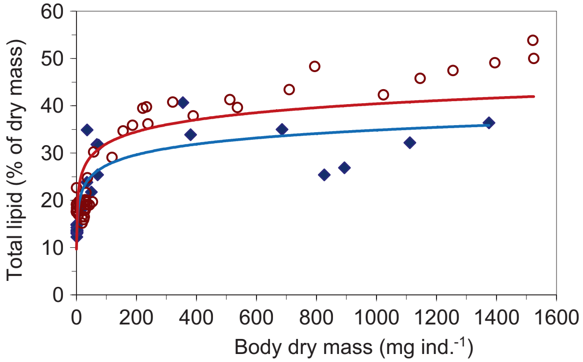


Fig. 7.2 *Pleuragramma antarctica*. Growth curve includes major lipid accumulation. Body dry mass in dependence of body length for larvae to juvenile age 2+. The *insert* shows the data in a semi-logarithmic scale. Spring (*red dots*), summer (*blue diamonds*); *Juv.* juveniles

Fig. 7.3 *Pleuragramma antarctica*. Lipid content versus body dry mass for larvae to juvenile age 2+. Logarithmic trendlines are presented. Spring (*red dots/line*), summer (*blue diamonds/line*)



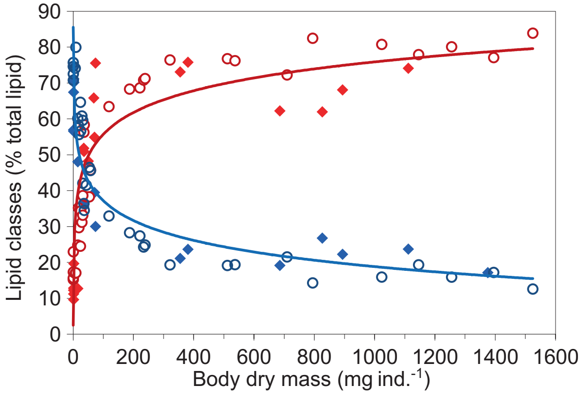


Fig. 7.4 *Pleuragramma antarctica*. Changes in lipid classes during growth. Logarithmic trendlines are shown. Triacylglycerols (*red symbols/line*), phospholipids (*blue symbols/line*), *dots*: data from spring, *diamonds*: data from summer

of 12% and 10%, respectively, in juveniles decreasing to about 3–4%TFA in the adults (Table 7.2). These 22:1 isomers suggest an increasing importance of the larger calanoid copepod, *Calanus propinquus*, in the diet of juvenile *P. antarctica*. *C. propinquus* deposits triacylglycerols instead of wax esters and it is the only dominant Antarctic copepod, which biosynthesizes very high amounts of the fatty acid 22:1(n-9) with up to 26%TFA (Kattner et al. 1994). This intriguing shift from *C. acutus* to *C. propinquus* by larger *P. antarctica* (60–100 mm) is clearly supported by gut content analyses (Hubold and Hagen 1997). The 22:1(n-11) isomer shows even higher percentages in *C. propinquus*, but it is also typical of *Calanoides acutus*, as discussed above. Wax esters may contribute 90% of the total lipids in *C. acutus*, hence the percentage of the 22:1(n-11) alcohol is comparable to that of the corresponding fatty acid in *C. propinquus*. Although the 22:1(n-11) trophic marker is a powerful tool to identify these two dominant calanid copepods as food items, it is limited with regard to the differentiation between these species. Apart from their dominance, it is probably also energetically advantageous for *P. antarctica* to feed on these two calanid species, since long-chain moieties have a higher energy content than shorter ones (Albers et al. 1996). The preference for *C. propinquus* as prey suggests that it is easier for *P. antarctica* to utilize the large amounts of triacylglycerols in comparison to wax esters.

The decreasing importance of the long-chain monounsaturated fatty acids with increasing size of *P. antarctica* may be compensated by higher lipid deposits. It also emphasizes the change in dietary preferences of the older specimens supported by the corresponding change in fatty acid compositions. 18:1(n-9) is by far the dominant fatty acid in the adults (25%TFA), followed by similar amounts of 16:0 and 14:0 (ca. 15%TFA each, Table 7.2). These fatty acids are not as specific trophic indicators as the long-chain monounsaturates, since they are rather common end-products of the fatty acid biosynthesis. The fatty acid 18:1(n-9) usually originates from the elongation and desaturation of 14:0 and 16:0 dietary precursors, but may also derive from *de novo* biosynthesis. This may reflect a considerable biosynthetic production of lipids in adult *P. antarctica*. Despite its limitation as a trophic marker, 18:1(n-9) can provide dietary information, as it is a major fatty acid component of the ice krill *Euphausia crystallorophias* (Bottino 1975, Kattner and Hagen 1998), especially in juveniles and adults with up to 75% in the wax ester fraction, which may comprise 50% of total lipids. In addition, the 14:0 fatty alcohol, the predominant wax ester moiety in ice krill (75% of total alcohols), may be converted to the corresponding 14:0 fatty acid by *P. antarctica*. Hence, these fatty acid components provide supportive evidence that older *P. antarctica* rely on ice krill as major prey item. In addition to calanid copepods, various authors report the importance of euphausiids, especially ice krill in high-Antarctic waters, in the diet of *P. antarctica* (Hubold 1985; Hubold and Ekau 1990). In more northerly regions of the Southern Ocean, e.g. off the Antarctic Peninsula, older silverfish specimens shift to the Antarctic krill (La Mesa and Eastman 2012 and references therein). However, the lipid and fatty acid compositions of *E. superba* do not exhibit specific trophic markers (Hagen et al. 2001) and thus do not provide evidence for the ingestion of this krill species. It should be noted that Hubold (1991) reported a strong seasonal shift for juvenile *P. antarctica* from a krill-based diet in summer to a copepod-dominated diet in late winter. This diet was mainly composed of deep-living copepods (Spinocalanidae, etc.), which the juveniles apparently encountered at depth. The fatty acid composition of these copepods is not known, however, due to their carnivorous feeding mode, they are very unlikely to biosynthesize long-chain monounsaturated fatty acids typical of herbivorous calanids.

Other relevant food items include the calanoid copepods *Rhincalanus gigas* and *Euchaeta* spp. (Hubold and Hagen 1997). These species also accumulate large amounts of wax esters and have similar fatty acid and alcohol compositions as the ice krill, although 18:1(n-9) is less dominant in the omnivorous *R. gigas* than in carnivorous *Euchaeta* spp. (Kattner et al. 1994; Hagen et al. 1995; Albers et al. 1996). Hence, these lipid components provide no distinguishing dietary resolution. Nevertheless, the high percentage of 16:1(n-7) in those copepod species (up to 25%TFA in *Euchaeta*) seems to be reflected in older *Pleuragramma* specimens (ca. 10%TFA). This high percentage is intriguing, since 16:1(n-7) is a typical marker of diatoms (Graeve et al. 1994), but it may be incorporated unmodified via the ingestion of these copepods and retained by *P. antarctica*.

An overview of the fatty acid data of the various *P. antarctica* stages from larvae to adults sorted by Principal Component Analysis (PCA) is given in Fig. 7.5, which highlights the differences and similarities. The first two principal components explain 86% of the variance. The first axis (PC1) discriminates mainly between developmental stages with the early larvae arranged towards the left hand side and the juveniles and adults towards the right hand side. Post-larval data are not as tightly sorted due to their higher variability and comprise a larger area, partially overlapping with larvae and juveniles. PC1 is negatively correlated with the fatty acids 22:6(n-3), 20:5(n-3), 16:0, 18:1(n-7) and 18:0 (in decreasing order of explanatory power) typical of larval stages, especially from summer. The positive values of PC1 are associated with the isomers 22:1(n-11) and (n-9), the isomers 20:1(n-9) and (n-7) as well as 14:0 and 16:1(n-7) characteristic of the juveniles. Within the stages the data are also discriminated according to season, but this influence is less pronounced. The few data of the adults and some juveniles, both from summer, are

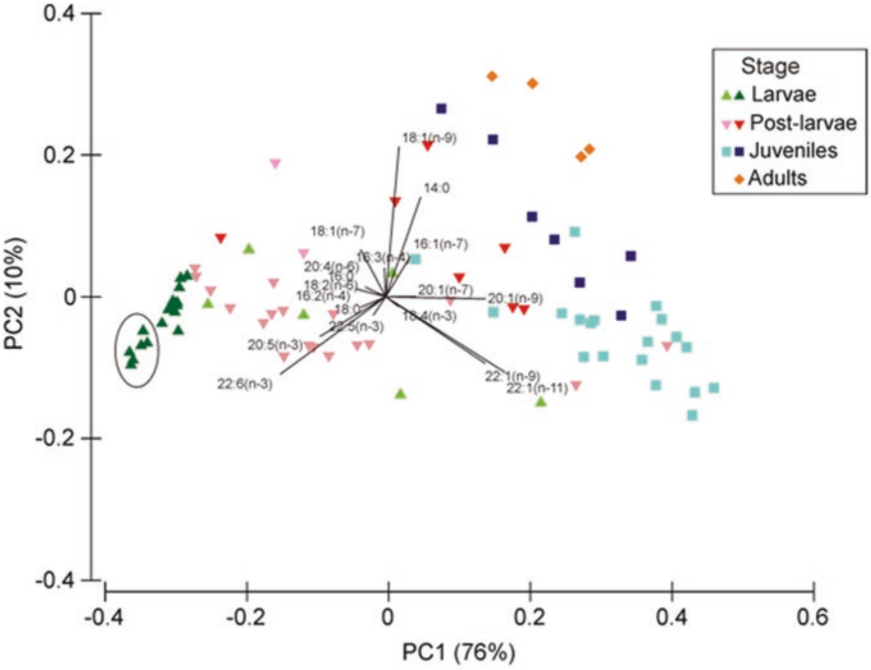


Fig. 7.5 *Pleuragramma antarctica*. Principal Component Analysis (PCA) of larvae to adults, sorted based on their fatty acid compositions. Light colours: spring data, dark colours: summer data, data in circle from Tavernier et al. (2012). PCA was conducted based on the composition of the most abundant fatty acids using the Primer v6 software. Prior to PCA, proportions of the fatty acids were normalized with arcsine-square-root transformation to correct deficiencies in normality and homogeneity of variance

separated from the younger stages and mostly represented by positive correlations with 18:1(n-9), 14:0, 18:1(n-7), 16:1(n-7), 16:3(n-4), and negative values of 22:6(n-3), 22:1(n-11, n-9) and 20:5(n-3) (PC2). The figure emphasizes the close correlation of the ontogenetic development and lipid accumulation, reflected by the changing fatty acid compositions as one of the most important features of *P. antarctica*.

7.5 Importance of Lipids as Buoyancy Aid and Energy Reserve

Two key aspects are discussed with regard to the function of lipids in *P. antarctica*, buoyancy and energetics (Eastman 1988). The Antarctic silverfish is one of the very few fully pelagic Antarctic fish species, which originates from a bottom-dwelling notothenioid ancestor without a swim bladder (La Mesa and Eastman 2012). Hence, *P. antarctica* has no efficient buoyancy aid to regulate its density in the water column, an obvious disadvantage for a pelagic life style. However, due to different adaptive mechanisms, the species was able to strongly reduce its density and thus achieved almost neutral buoyancy in seawater (Eastman 1985). *P. antarctica* shows various adaptations to increase its sinking resistance and to reduce its density, e.g. enlargement of pectoral and pelvic fins, reduced ossification and calcification of the skeleton, replacement of bones by cartilage, small otoliths, and a persistent notochord. The species is also retaining larval features, which delays the formation of scales (Albertson et al. 2010; La Mesa and Eastman 2012).

The other prominent feature of *P. antarctica* contributing to neutral buoyancy are the intermuscular and subcutaneous oil sacs, which apparently serve to compensate for the lack of a swim bladder. Eastman and de Vries (1989) suggested that the lipid sacs are primarily used as buoyancy aid and doubted their utilization as energy reserves, due to the limited cell membrane surface area. To fulfil this buoyancy function, it would be much more effective for *P. antarctica* to store these deposits as wax esters (specific gravity at 5 °C: 0.90 g cm^3) instead of triacylglycerols (specific gravity at 5 °C: 0.96 g cm^3), because wax esters provide one third more up-thrust than triacylglycerols (Lee and Patton 1989). Many marine copepod species follow this strategy of wax ester deposition and they are able to biosynthesize enormous amounts of these lipids. Ingesting wax ester-rich copepods could enable *P. antarctica* to transfer and incorporate huge amounts of these low-density lipids in their oil sacs. However, *P. antarctica* and apparently the whole group of Notothenioidei do not accumulate wax esters (Phleger et al. 1999b; Hagen et al. 2000; Mayzaud et al. 2011), which also indicates their inability to biosynthesize these lipids. (The high wax ester contents in flesh and lipid sacs of adult *P. antarctica* as well as the very high hydrocarbon levels in the larvae reported by Reinhardt and Van Vleet (1986) must be erroneous results.) During digestion wax esters are cleaved into fatty acids and fatty alcohols, the latter are obviously converted into the corresponding fatty acids. This is a common biochemical pathway known of many triacylglycerol-storing fish species, e.g. the well-investigated Atlantic herring (Sargent et al. 1979), and apparently it is also used by *P. antarctica.* In contrast, Antarctic myctophids (Phleger et al. 1999a) and many other marine fish species, e.g. capelin, as well as meso- and bathypelagic fish, e.g. deep-sea cod, biosynthesize wax esters, as already reviewed by Nevenzel (1970).

The fat reserves in the lipid sacs of *P. antarctica* would be quite useful during periods of food shortage, e.g. in winter, or in times of higher energy demand such as gonad maturation and egg formation (vitellogenesis). The few seasonal data from early spring and from summer show no clear differences in total lipid contents between seasons for the juveniles (Hubold and Hagen 1997). Surprisingly, lipid levels of these juveniles were lower in summer than in early spring (Fig. 7.3), but the few data do not allow a sound explanation. It is a matter of conjecture, if this difference indicates poorer feeding conditions in summer than in spring. The development of *P. antarctica* demands maximum amounts of lipid during the formation of the oil sacs, which requires plenty of (lipid-rich) food. Once they have reached adulthood and filled their oil sacs, there is less need for further energy investment to maintain the buoyancy function of the sacs. This is in accordance with the rather sluggish and thus energy-saving mode of life suggested for *P. antarctica* (Zimmermann and Hubold 1998). If lipids were crucial for neutral buoyancy, utilization of the lipid sacs by *P. antarctica* would result in negative buoyancy. The species would be forced to increase its swimming activity to maintain its position in the water column, further depleting its lipid depots, a vicious circle. Accordingly, a model applied by Maes et al. (2006) suggests maximum fitness of *P. antarctica*, if the oil sacs function as metabolically inert buoyancy aids and are not utilized as energy stores. However, the variability of lipid levels in *P. antarctica*, which range from 30% to almost 60%DM in the adults (Friedrich and Hagen 1994) indicates that lipids are not only maintained for optimum buoyancy, but may suggest utilization, depending on the available food supply. We know from feeding experiments of copepods with labelled phytoplankton that the turnover in their oil sacs is considerable and lipids are exchanged within 2–3 weeks during good feeding conditions (Graeve et al. 2005). Unfortunately, information about the fatty acid composition of adult *P. antarctica* during ageing is still missing.

The pronounced lipid accumulation of *P. antarctica* represents not only an impressive adaptive mechanism to occupy the productive pelagic realm, this species also represents a crucial component within the Antarctic food web. Trophodynamics in the Southern Ocean are largely based on the efficient transfer of high-energy and high-quality (rich in (n-3) fatty acids also known as omega-3 fatty acids) lipids from one trophic level to the next, from extremely lipid-rich calanoid copepods and euphausiids via oily *P. antarctica* to the top predators, warm-blooded vertebrates with a high-performance metabolism. This fragile and well-balanced Antarctic system is quite vulnerable to climate change and may result in the displacement of *P. antarctica* populations for instance in the Antarctic Peninsula region, which already shows dramatic changes due to warming (Mintenbeck et al. 2011). Investigations in the Ross Sea emphasize the close association of *Pleuragramma*'s life cycle, especially spawning and larval development, with the cryopelagic habitat and sea-ice cover (La Mesa and Eastman 2012; Vacchi et al. 2012b). The reduction and eventually disappearance of *P. antarctica* stocks in more northerly Antarctic regions may result in a dramatic shift from a lipid-based to a less energy-rich food web with drastic effects for the whole community. In this respect, the Antarctic silverfish may prove to be a keystone predator, an essential component of the Antarctic Ocean.

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Chapter 8

Energetics of the Antarctic Silverfish, *Pleuragramma antarctica*, from the Western Antarctic Peninsula

Eloy Martinez and Joseph J. Torres

Abstract The nototheniid *Pleuragramma antarctica*, commonly known as the Antarctic silverfish, dominates the pelagic fish biomass in most regions of coastal Antarctica. In this chapter, we provide shipboard oxygen consumption and nitrogen excretion rates obtained from *P. antarctica* collected along the Western Antarctic Peninsula and, combining those data with results from previous studies, develop an age-dependent energy budget for the species. Routine oxygen consumption of *P. antarctica* fell in the midrange of values for notothenioids, with a mean of 0.057 ± 0.012 ml O_2 g^{-1} h^{-1} (χ ± 95% CI). *P. antarctica* showed a mean ammonia-nitrogen excretion rate of 0.194 ± 0.042 µmol NH4-N g^{-1} h^{-1} (χ ± 95% CI). Based on current data, ingestion rates estimated in previous studies were sufficient to cover the metabolic requirements over the year classes 0–10. Metabolism stood out as the highest energy cost to the fish over the age intervals considered, initially commanding 89%, gradually declining to 67% of the annual energy costs as the fish aged from 0 to 10 years. Overall, the budget presented in the chapter shows good agreement between ingested and combusted energy, and supports the contention of a low-energy lifestyle for *P. antarctica*, but it also resembles that of other pelagic species in the high percentage of assimilated energy devoted to metabolism. It differs from more temperate coastal pelagic fishes in its large investment in reproduction and its pattern of slow steady growth throughout a relatively long lifespan.

Keywords Fish metabolism • Energy budget • Antarctic fishes

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8.1 Introduction

8.1.1 Pelagic Lifestyle

Few Antarctic fishes have evolved to exploit the pelagic realm. Though a number of notothenioids display benthopelagic habits, or exploit the water column in early life, only one species, the Antarctic silverfish *Pleuragramma antarctica* Boulenger, 1902, retains a pelagic lifestyle through all its life stages (DeWitt 1970; DeVries and Eastman 1978; Eastman and DeVries 1982; Williams 1985; Hubold and Ekau 1987; Donnelly et al. 2004).

Notothenioids, including *P. antarctica*, do not possess a swim bladder. Near-neutral buoyancy in *P. antarctica* is achieved with reduced bone density, a characteristic trait of notothenioids (Eastman et al. 2014; Voskoboinikova et al. 2017), high water content (Reisenbichler 1993), and additional hydrostatic lift provided by a high lipid content (Hagen et al. 2000; Hagen and Kattner 2017). Lipids contributing to buoyancy are composed of triacylglycerols at various levels of saturation and are stored subcutaneously in lipid sacs surrounded by adipocytes (Eastman and DeVries 1989). Depending on the energetic state of the individual, the lipids providing buoyancy may also serve as energy fuel (DeVries and Eastman 1978; Eastman and DeVries 1989; Hagen and Kattner 2017).

P. antarctica dominates the pelagic environment in most regions of coastal Antarctica (Hubold 1984; Donnelly et al. 2004), comprising as much as 90% of the local ichthyofauna (Hubold and Ekau 1987). Comparable biomass dominance is usually only observed in highly productive areas such as the La Plata Estuary in Argentina, where early stages of the anchovy *Engraulis anchoita* range from 60–90% of total biomass (Hubold 1982). The dominance of *P. antarctica* in both numbers and biomass, its circumpolar distribution, and the lack of other pelagic fishes able to tolerate near-freezing waters (Cullins et al. 2011), combine to make *P. antarctica* a keystone species in the Antarctic coastal food web (DeWitt and Hopkins 1977; Hubold 1985; Kellermann 1987).

P. antarctica exploits a variety of habitats in the shelf waters of Antarctica during the course of its life. Floating eggs and early larval stages are associated with seasonal pack ice which provides structure, refuge, and a food source for early stages (Vacchi et al. 2004; Vallet et al. 2011; Guidetti et al. 2015). Post-larval and juvenile stages are found mainly in the upper 100 m of Antarctic shelf waters (Hubold 1984). Adults are found throughout the midwater at depths ranging from 0 to 900 m (Gerasimchuk 1986; Fuiman et al. 2002; Robison 2003). Larger individuals usually reside at greater depth, a size-dependent depth distribution that may reduce cannibalistic feeding on larvae and juveniles by adults (Hubold 1985, 1992; Hubold and Hagen 1997). A similar size-dependent segregation by depth is commonly observed in mesopelagic fishes (Marshall 1971).

Within its circumpolar distribution, *P. antarctica* is restricted to shelf waters. Further offshore, oceanic teleosts such as myctophids predominate (DeWitt 1970; Donnelly and Torres 2008). Though they are similar to *P. antarctica* in size, depth profiles and feeding habits (Lancraft et al. 2004), myctophids lack the antifreezes required to survive in the frigid ice-shelf waters that typify most of the coastal Antarctic (Cullins et al. 2011). An exception to the clear-cut separation between oceanic and coastal faunal assemblages is found on the Western Antarctic Peninsula (WAP), where *P. antarctica* co-occurs with the myctophids *Electrona antarctica, Gymnoscopelus braueri, and G. nicholsi*, due to the presence of warm (2 °C) circumpolar deep water at mid-depths.

8.1.2 Vertical Migration

P. antarctica exhibits a range of different foraging strategies and daily behavior patterns. They have been observed feeding inshore in large schools (Daniels 1982; Daniels and Lipps 1982), as well as performing diel vertical migrations of 600 m and more in the deeper inshore troughs and fjords (Reisenbichler 1993; Robison 2003; Lancraft et al. 2004). Its migratory pattern allows *P. antarctica* to avoid visual predators such as penguins, seals and flighted-seabirds during the day and to capitalize on abundant prey such as *Euphausia superba* nearer the surface at night (Hopkins and Torres 1988; Robison 2003; Lancraft et al. 2004). Undoubtedly, vertical migration also incurs an energy expenditure as a result of the directed swimming to and from near-surface waters.

8.1.3 Feeding Habits

Larval *P. antarctica*, though primarily carnivorous, are capable of omnivory (Kellermann 1987; Vallet et al. 2011; Koubbi et al. 2007). The prey list of larval stages and juveniles includes diatoms, euphausiid eggs and larvae, copepods, pteropods, and tintinnids (DeWitt and Hopkins 1977; Kellermann 1987; Vallet et al. 2011; Tavernier and Giraldo 2017). Details of prey selection, if present at these stages, are not clear due to regional variations in the distribution and abundance of specific prey types.

Studies on the feeding habits of juvenile and adult *P. antarctica* suggest a diet mainly consisting of copepods, amphipods and euphausiids. Species and relative abundance of prey classes vary according to region, age class and season (DeWitt and Hopkins 1977; Moreno et al. 1986; Reisenbichler 1993). Overall, the feeding strategy of *P. antarctica* is that of a food-generalist throughout its life (Hubold and Hagen 1997). A generalist approach ensures energy acquisition throughout the year for a planktivore in a highly seasonal planktonic community. For recent reviews on diet and trophic ecology of Antarctic silverfish see Pinkerton (2017), and Tavernier and Giraldo (2017).

8.1.4 Growth Pattern

P. antarctica is a slow-growing species, reaching 45 g in 10–11 years (specimen collected in this study, based on growth curves in Reisenbichler 1993, Hubold and Tomo 1989, respectively). Growth rates of *P. antarctica* have been addressed in multiple studies. Region-dependent growth curves are subtly different, but all exhibit a low growth rate (Hubold and Tomo 1989; Radtke et al. 1993; Reisenbichler 1993; La Mesa and Eastman 2012). Reproductive maturity occurs at 13–16 cm (Hubold 1985; Duhamel et al. 1993, Faleyeva and Gerasimchuk 1990) or about 7–9 years of age (Hubold and Tomo 1989; Kock and Kellermann 1991). The observed slow growth and late reproductive maturity of *Pleuragramma* contrast with the life history strategies of temperate analogues such as the sardines and anchovies, which are often shorter-lived and may reproduce as early as the second year of life (e.g. *Sardinops caerulea*, Lasker 1970).

8.2 What Is an Energy Budget?

The energy budget of a species is a summary of how its food energy is utilized. It is grounded in the laws of thermodynamics, which, simply put, states that the “energy ingested by a fish (I) must turn up in one form or another through metabolism (M), growth (G), or excretion (E), where I = M + G + E” (Brett and Groves 1979). The amount of energy devoted to each category reflects a species’ life history strategy as well as its current life stanza. Thus, active pelagic species such as tuna and sardines have a higher percentage of their ingested energy devoted to metabolism than sluggish species such as sargassum fish. Likewise, younger, quickly growing fish would be expected to devote a larger percentage of their energy intake to growth than older fish already at their maximum size. Comparisons between species are instructive in deducing aspects of species’ life history that may be difficult to obtain otherwise, such as activity level.

It is important to recognize that each element of the energy budget represents a rate process that must be measured directly, either on the living fish (metabolism as oxygen consumption rate and nitrogen excretion as ammonium excretion rate), derived from compositional analyses of gut contents collected on a daily basis (ingestion rate or daily ration), or analyses of growth rate using the measured mass of individuals in conjunction with the ages recorded in their earbones (otoliths). The energy budget reported below combines new data for oxygen consumption and ammonium excretion with literature values for ingestion, growth and reproduction. It is a first cut at examining *Pleuragramma*’s energy usage.

8.2.1 Budget Components

The basic energy budget equation is usually stated as I = M + G + E, with its elements subdivided as needed to accommodate data sets within each component. For example, growth can be divided into somatic, or organismal growth, and reproductive growth, which refers to the increase in mass of the gonads as they mature prior to spawning. In this chapter the new (oxygen consumption and ammonium excretion) data will be presented first followed by a discussion of the literature-derived (ingestion, growth and reproduction) data used to assemble the budget, the budget itself and comparisons with similar species. Note that oxygen consumption, respiration, and metabolism are used interchangeably throughout the chapter.

8.3 Oxygen Consumption and Ammonium Excretion

8.3.1 Notes on Sampling and Methods

P. antarctica specimens were captured during multiple research cruises to the WAP in 2001, 2002 and 2010. Fishes were collected using a 10 m^2 multiple opening and closing net, with an environmental sensing system (MOCNESS) during two Global Ocean Ecosystem Dynamics (GLOBEC)-sponsored cruises aboard the R/V *Laurence M. Gould* and the R/V *Nathaniel B. Palmer* in the austral autumns and winters of 2001 and 2002. Additional specimens were collected during the austral autumn of 2010 aboard the R/V *Nathaniel B. Palmer*, using a 10 m^2 MOCNESS or a Tucker trawl. Immediately after collection, specimens were transferred to large flow-through tanks filled with 0 °C seawater. All specimens were placed in respiration chambers within 4 h of collection.

Oxygen consumption rates were determined using the methods of Torres and Somero (1988b). Specimens selected for measurements were in excellent condition, but had recently been captured in a midwater trawl. Rates should therefore be considered as estimates of routine metabolism; they are not comparable to rates determined on animals that can be maintained for long periods of time in the laboratory.

Ammonium (NH_4) excreted by each specimen during the respirometric trial was quantified by following the methods described in Jones (1991) as modified by Masserini and Fanning (2000). Briefly, frozen water samples (20 ml) collected before and after each respiratory run were thawed, diluted with deionized water and analyzed for ammonium with a Technicon® Auto Analyzer II. An ammonium-permeable membrane separated the water sample from a continuous flow of o-phthaldialdehyde reagent. The reagent binds to the permeating ammonium, forming a fluorescent product later quantified by the autoanalyzer. Ammonium concentrations in the respirometric chamber prior to each run were subtracted from the ammonium present in the final sample. Corrected ammonium concentrations were

Table 8.1 Oxygen consumption, nitrogen excretion, and O:N (atomic ratio) of *Pleuragramma antarctica* in three different mass ranges

Mass (g) χ ± 95% CI (n) range	VO_2 (ml O_2 g^{-1} h^{-1}) χ ± 95% CI (n) range	Nitrogen excretion (μmol-N g^{-1} h^{-1}) χ ± 95% CI (n) range	O:N χ ± 95% CI (n) range
1.608 ± 0.327 (6) 1.024–2.187	0.058 ± 0.011 (6) 0.04–0.08	0.218 ± 0.033 (6) 0.161–0.277	23.352 ± 3.514 (6) 17.656–30.858
16.800 ± 2.856 (3) 14.1–19.1	0.059 ± 0.043 (3) 0.022–0.097	0.144 ± 0.073 (3) 0.096–0.218	34.108 ± 13.858(3) 20.042–42.397
36.5 ± 9.510 (3) 28.7–45.4	0.051 ± 0.030 (3) 0.023–0.076	0.194 ± 0.144 (3) 0.057–0.309	26.657 ± 9.392 (3) 21.826–36.241
14.129 ± 8.729 (12) 1.024–45.4	0.057 ± 0.012 (12) 0.022–0.097	0.194 ± 0.042 (12) 0.057–0.309	26.967 ± 4.695 (12) 17.655–42.397

Bottom line is overall mean for data set

divided by the total run time to acquire an estimate of ammonium excretion per individual per hour.

8.3.2 Metabolism

Mean oxygen consumption of *P. antarctica* for the entire data set was 0.057 ± 0.012 ml O2 g^{-1} h^{-1} (χ ± 95% CI) (Table 8.1). VO_2 scaled with mass according to the equation $y = aX^b$, where y = oxygen consumption (μl O_2 $individual^{-1}$ h^{-1}) and X = mass in mg (Fig. 8.1), with a slope or "b-value" of 0.932, indicating a nearly direct scaling with mass.

The fish sizes shown in Fig. 8.1 include standard lengths from 60 to 174 mm and masses from 1 to 45 g, roughly corresponding to year classes of 2–10 years (Reisenbichler 1993). Experimental subjects reflected the size structure of the silverfish population during the time of sampling (Parker et al. 2015). Smaller fish dominated the 2001–2002 cruises while larger fish were more abundant in 2010.

Routine oxygen consumption of *P. antarctica* fell in the midrange of values for notothenids normalized to a mass of 10 g (low: 0.037 ml O_2 g^{-1} h^{-1} for *Trematomous hansoni*; high: 0.175 ml O_2 g^{-1} h^{-1} for *T. bernacchii*; Table 8.2) and likewise for the (non-nototheniid) notothenioids in general (0.036–0.061 ml O_2 g^{-1} h^{-1} for *Harpagifer georgianus* and *Gymnodraco acuticeps* respectively, Table 8.2). Interestingly, *P. antarctica* respiration was quite similar to that of *Pagothenia borchgrevinki* (0.050–0.069 ml O_2 g^{-1} h^{-1}). Mesopelagic fishes, normally excluded from coastal regions by the very cold (−2 °C) Antarctic shelf waters (DeWitt 1970; Donnelly and Torres 2008) and a lack of endogenous antifreezes, are well represented on the WAP shelf, largely due to the presence of warm (2 °C) circumpolar deep water at mid-depths (Klinck et al. 2004). *P. antarctica* and the myctophid *E. antarctica* exhibit a similar vertical migration pattern where they co-occur on the WAP shelf, and have a similar diet (Lancraft et al. 2004). The metabolic rate of

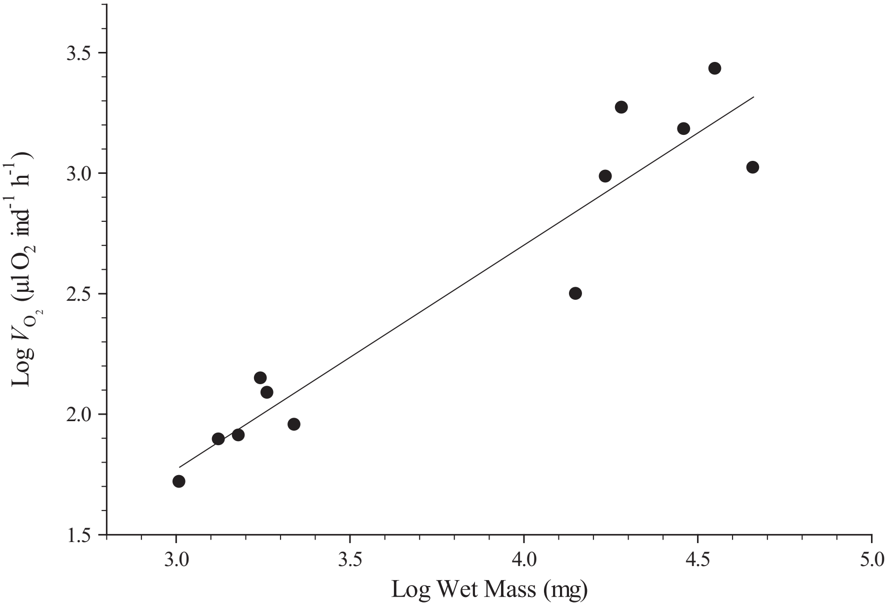

Fig. 8.1 Whole-individual respiration rate as a function of wet mass in *Pleuragramma antarctica* from the Western Antarctic Peninsula. Data were fitted to the equation Log y = −1.0208 + 0.9306 Log x; where y is the whole individual respiration rate and x is wet mass ($r^2 = 0.91$). Oxygen-consumption rates were determined following the methods described by Torres and Somero (1988b)

Electrona is slightly less (0.039 vs 0.050 ml O_2 g^{-1} h^{-1}; Table 8.2) than that of *P. antarctica* but its foraging strategy and vertical profile are quite similar. The deeper living myctophids, *G. braueri* and *G. opisthopterus*, are more similar to *P. antarctica* in size. They also vertically migrate, but remain at greater depth in their daily vertical excursions (Torres and Somero 1988a; Lancraft et al. 1989). The two deeper-living myctophids and the bathylagid, *Bathylagus antarcticus,* exhibited metabolic rates less than half those of *Electrona* and *Pleuragramma*.

A comparison of *P. antarctica* respiration with temperature- and mass-corrected rates of coastal pelagic species from other regions revealed similar rates to those of the two anchovies (Table 8.2), but about half that of the California sardine, a species it more closely resembles in size. Overall, its routine respiration rate, like that of its benthic relatives, suggests a capability for modest activity but not a lifestyle that involves continuous swimming (cf. La Mesa and Eastman 2012). The ratio of the maximum rate recorded in each run to the routine rate (see methods) produced an overall mean of 2.25 ± 0.59 (χ ± 95% CI) for the data set, suggesting a factorial scope of at least 3 for the ratio between minimum and maximum rates. Forster et al. (1987) reported a range of 3.94 to 5.68 for *P. borchgrevinki,* which is believed to alternate periods of activity with periods of rest in the near-surface platelet ice.

Table 8.2 Mass specific oxygen consumption rates in Antarctic pelagic and coastal species at intermediate weight and a standardized 10 g individual

Refs.	Species (Family)	Habits and depth distribution	IW (g)	OCR at 0.5 °C (μl O_2 mg wet weight^{-1} h^{-1})		Slope (b)	Intercept (a)
				IW V_{O_2}	10-g V_{O_2}		
[1]	*Pleuragramma antarctica* (Nototheniidae)	Pelagic planktivore	22.5	0.048	0.050	0.932	0.059
[2]	*Bathylagus antarcticus* (Bathylagidae)	Deep pelagic planktivore	25	0.015	0.016	0.889	0.021
	Electrona antarctica (Myctophidae)	Pelagic planktivore	5	0.040	0.039	0.946	0.044
	Gymnoscopelus braueri (Myctophidae)	Pelagic planktivore	–	–	0.026	1.029	0.024
	Gymnoscopelus opisthopterus (Myctophidae)	Pelagic planktivore	20	0.021	0.022	0.942	0.025
[3]	*Pagothenia borchgrevinki* (Nototheniidae)	Cryopelagic active	50	0.040	0.050	0.825	0.075
[4]	*Chaenocephalus aceratus* (Channichthyidae)	Hemoglobin-free ice fish	1048	0.019	0.061	0.750	0.108
	Pseudochaenichtys georgianus (Channichthyidae)	Benthic, 70–200 m	36	0.028	0.039	0.750	0.069
	Parachaenichthys charcoti (Bathydraconidae)	Ice fish, benthic 120–150 m	557	0.017	0.047	0.750	0.083
[5]	*Chaenocephalus aceratus* (Channichthyidae)	Benthic 90–160 m	~1000	0.016	0.051	0.750	0.090
	Notothenia gibberifrons (Nototheniidae)	Benthic, 90–160 m	470	0.016	0.042	0.750	0.074
	Notothenia neglecta (Nototheniidae)	Shallow water benthic	1000	0.020	0.063	0.750	0.112
[6]	*Harpagifer georgianus* (Harpagiferidae)	Nearshore benthic-sedentary	4	0.040	0.036	0.670	0.076
	Notothenia angustifrons (Nototheniidae)	Nearshore bentho-demersal	20	0.067	0.088	0.861	0.121
	Notothenia nudifrons (Nototheniidae)	Nearshore bentho-demersal	15	0.049	0.061	0.888	0.079
	Notothenia rossii (Nototheniidae)	Off-shore demerso-pelagic	350	0.023	0.108	0.616	0.262

(continued)

Table 8.2 (continued)

Refs.	Species (Family)	Habits and depth distribution	IW (g)	OCR at 0.5 °C (µl O_2 mg wet weight^{-1} h^{-1})		Slope (b)	Intercept (a)
				IW V_{O_2}	10-g V_{O_2}		
	Trematomus hansoni (Nototheniidae)	Nearshore and off-shore bentho-demersal	45	0.019	0.037	0.693	0.075
[7]	*Notothenia neglecta* (Nototheniidae)		1000	0.037	0.143	0.785	0.234
	Notothenia rossii (Nototheniidae)		500	0.027	0.046	0.958	0.051
[8, 9]	*Pagothenia borchgrevinki* (Nototheniidae)		108	0.038	0.069	0.750	0.123
	Gymnodraco acuticeps (Bathydraconidae)		74	0.037	0.061	0.750	0.108
	Rhigophila dearborni (Zoarcidae)		32	0.012	0.016	0.750	0.029
[10, 11, 12]	*Trematomus bernacchii* (Nototheniidae)		148	0.064	0.175	0.752	0.309
	Trematomus hansoni (Nototheniidae)		30	0.072	0.092	0.845	0.132
	Trematomus loennbergii (Nototheniidae)		106	0.051	0.043	0.997	0.043
	Rhigophila dearborni (Zoarcidae)		30	0.018	0.026	0.965	0.028
[13]	*Sardinops caerulea* (Clupeidae)	Pelagic filter feeder	85	0.075	0.128	0.75	0.228
[14]	*Engraulis mordax* (Engraulidae)	Pelagic filter feeder	9	0.055	0.053	0.750	0.095
[15]	*Engraulis capensis* (Engraulidae)	Pelagic filter feeder	6	0.067	0.059	0.750	0.105

Slopes (b) and intercepts (a) correspond to the equation $y = aX^b$ relating whole individual oxygen consumption rate (y; ml O_2 individual^{-1} h^{-1}) and the total wet body weight (x; g). Values for b and a came from the reference cited except in Hemmingsen and Douglas (1970) and Holeton (1970), where b = 0.75 was arbitrarily assigned for purposes of calculation. Temperature conversion to 0.5 °C was necessary only in the case of Morris and North (1984), whose rates were determined at 3 °C and in James et al. (1989), whose rates were determined at 16.2 °C; a Q_{10} of 2.0 was used. Information on Antarctic fish habits and depth distribution from Targett (1981) and Morris and North (1984). For information on additional species see Macdonald et al. (1987). *OCR* Oxygen Consumption Rate, *IW* Intermediate Weight. References: [1] Present study; [2] Torres and Somero (1988a); [3] Forster et al. (1987); [4] Hemmingsen and Douglas (1970); [5] Holeton (1970); [6] Morris and North (1984); [7] Ralph and Everson (1968); [8] Wells (1987); [9] Steffensen (2005); [10] Wohlschlag (1960); [11] Wohlschlag (1963); [12] Wohlschlag (1964); [13] Lasker (1970); [14] Childress and Somero (1979); [15] James et al. (1989)

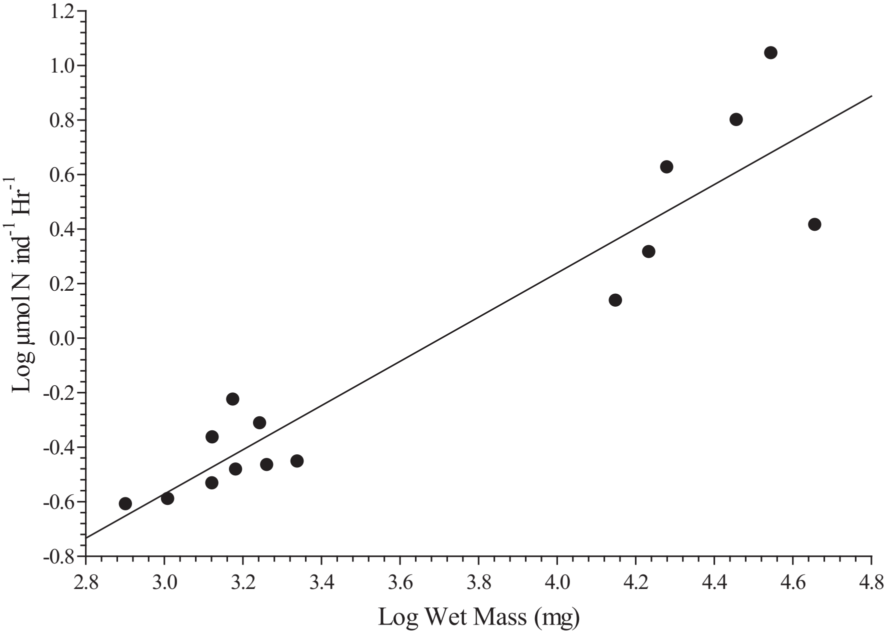

Fig. 8.2 Whole-individual nitrogen excretion rate as a function of individual wet mass of *Pleuragramma antarctica* from the Western Antarctic Peninsula. Data were fitted to the equation Log y = −3.004 + 0.8105 Log x; where y is the whole individual nitrogen excretion rate and x is wet mass ($r^2 = 0.89$)

8.3.3 Nitrogen Excretion

P. antarctica showed an ammonium-nitrogen excretion rate ranging from 0.057 to 0.309 µmol NH4-N g^{-1} h^{-1} (Table 8.1) and an overall mean of 0.194 ± 0.042 µmol NH4-N g^{-1} h^{-1} ($\chi \pm 95\%$ CI). Nitrogen excretion scaled with mass according to the equation $y = aX^b$, where y represents ammonium-nitrogen excretion rate (µmol NH4-N individual^{-1} h^{-1}) and X represents mass in mg (Fig. 8.2). The intermediate slope or “b-value” of 0.808 indicated a more pronounced decrease in nitrogen excretion rate with increasing mass than was observed with oxygen consumption rate, and is in the mid-range of b-values reported for other species (Boyce 1999).

An index that is useful for determining the biological substrate being oxidized is the O:N ratio, the ratio of the moles of oxygen consumed to the moles of ammonia N excreted. If all amino acids resulting from protein catabolism are deaminated with all N excreted as NH_4, and all C-skeletons go to CO_2 and H_2O, then the theoretical O:N minimum is 9.3; generally for carbohydrates 20–30; lipids >30. Since metabolizable carbohydrates are only present in minute quantities outside of the algal community (cf. Donnelly et al. 1994; Torres et al. 1994), the metabolic substrates encountered by carnivorous pelagic species such as *Pleuragramma* will

Table 8.3 Summary of ammonium-Nitrogen excretion rates for *Pleuragramma antarctica* and other marine fishes with disparate life histories

Family	Species	Habit location	AE	WMR	Refs.
Nothotheniidae	*Pleuragramma antarctica*	Pelagic zooplanktivore Antarctic	0.194	1–45	[1]
Harpagiferidae	*Harpagifer antarcticus*	Benthic Antarctic	0.066	6–36	[2]
Blenniidae	*Blennius pholis*	Benthic intertidal North Atlantic	0.045	5	[3]
Clupeidae	*Brevoortia tyrannis*	Pelagic planktivore North Atlantic	0.046	302	[4]
Engraulidae	*Engraulis capensis*	Pelagic planktivore South Africa coastal	0.187	200–600	[5]
	Engraulis mordax	Pelagic planktivore California coastal	0.17	9	[6]
Cottidae	*Taurulus bubalis*	Intertidal North Atlantic	0.207	27	[7]
Labridae	*Crenilabrus melops*	Intertidal North Atlantic	0.156	109	[7]
Salmonidae	*Oncorhynchus nerka*	Ne pacific	0.191	29	[8]
	Oncorhynchus mykiss	Temperate fresh water	0.094	129	[8]

AE Ammonium Excretion (μmol g wet mass^{-1} h^{-1}), *WMR* Wet Mass Range (g). References: [1] present work (Table 8.1); [2] Boyce (1999); [3] Sayer and Davenport (1987); [4] Durbin and Durbin (1981); [5] James et al. (1989); [6] McCarthy and Whitledge (1972); [7] Sayer and Davenport (1987); [8] Brett and Groves (1979)

be a mix of protein and lipid, with the O:N value indicating which, if either, predominates.

The O:N ratio (NH_4-N) ranged from 17.66 to 42.40 with a mean of 26.97 (Table 8.1) suggesting a prey spectrum including some items with high lipid levels, such as *Euphausia crystallorophias* for the larger fish, and lipid-rich copepods such as *Calanoides acutus* and *Calanus propinquus* for the smaller specimens (Torres et al. 1994; Donnelly et al. 1994). Crystal krill and the two calanoids are well established as important in the diets of silverfish at the sizes addressed in the present study (La Mesa and Eastman 2012). Since all rates were obtained in the austral autumn, overwintering lipid deposition would have been well underway in the copepods.

Few data on nitrogen excretion in Antarctic fishes are available for comparison. The benthic plunderfish, *Harpagifer antarcticus,* exhibits a rate about a third that of *P. antarctica* (Table 8.3), which is fairly typical of comparisons between benthic and pelagic species (Handy and Poxton 1993; Brett and Groves 1979). *H. antarcticus* exhibits a low VO_2 as well (Table 8.2), suggesting a sedentary life-style. Temperature-corrected values from other species are the only other available comparisons, but they are informative. The two anchovies showed rates very similar to that of the silverfish (Table 8.3), but another pelagic species, the Atlantic menhaden (*Brevoortia tyrannus*), was about 25% of that in *P. antarctica* (Durbin and Durbin 1981). Techniques employed for measurement in the two anchovies were most similar to that used in the present study, with determinations made on fishes in individual chambers. Menhaden determinations were taken on a small school of fish in a controlled laboratory setting, a near-ideal situation. For all species but *P. antarctica* in Table 8.3, experimental subjects were starved for a minimum of 36 h, and most for 6 days or more. Overall, silverfish NH4 excretion most closely resembled that of moderately active species of similar size.

8.4 Energy Budget Components

A simple energy budget that uses a combination of the best, and in many cases the only, information available for each budget category is presented in Tables 8.4 and 8.5. Table 8.4 shows data presented in kcal; Table 8.5 shows the budget broken down in terms of percent of assimilated energy. For a species that must be manipulated only in the field, usually on board ship due to its limited longevity after capture, the information is reasonably good. In Table 8.4 data are presented in kcal for ease of comparison with previous literature. To convert to kJ multiply by 4.19.

Table 8.4 Energy budget for *Pleuragramma antarctica*

A	SL	WM	GE	I	Met	Gr	NH_4 exc	Tot N exc	RE (kcal year^{-1}) GSI 15	GSI 25	GSI 45	O:N
1	46.73	0.27	0.09	2.65	1.88	0.09	0.16	0.20				24.43
2	63.62	1.28	0.58	7.61	5.04	0.49	0.37	0.46				24.63
3	79.91	3.18	1.85	15.77	9.95	1.27	0.67	0.83				25.01
4	95.60	6.07	4.31	27.42	16.65	2.46	1.05	1.30				25.59
5	110.69	10.01	8.41	42.78	25.20	4.10	1.50	1.87				26.38
6	125.18	15.07	14.62	62.03	35.63	6.21	2.03	2.52				27.39
7	139.07	21.30	23.43	85.35	47.98	8.81	2.63	3.26	3.51	5.86	10.54	28.64
8	152.36	28.74	35.35	112.87	62.25	11.92	3.29	4.08	5.30	8.84	15.91	30.12
9	165.05	37.44	50.92	144.73	78.49	15.56	4.03	4.99	7.64	12.73	22.91	31.86
10	177.14	47.42	70.66	181.05	96.70	19.75	4.82	5.98	10.60	17.67	31.80	33.86

Values for caloric growth and ingested energy from equations in Reisenbichler (1993). Metabolism, excretion, and O:N calculated from data acquired in the present study. Reproductive energy was estimated using gonadosomatic indices (GSIs) of 15%, 25%, and 45% (Kock and Kellermann 1991) and age-specific caloric densities (kcal g wm^{-1}) from Reisenbichler (1993). Reproductive energy values assumed that the caloric densities for the gonads were equivalent to that of whole *P. antarctica*.

A Age (years), *SL* Standard Length (mm), *WM* Wet Mass (g), *GE* Gross Energy (kcal ind^{-1}), *I* Ingestion (kcal year^{-1}), *Met* Metabolism (kcal year^{-1}), *Gr* Growth (kcal year^{-1}), *NH^4 exc* NH_4 excretion (kcal year^{-1}), *Tot N exc* Total N excretion (kcal year^{-1}), *RE* Reproductive Energy

Table 8.5 Energy budget for *Pleuragramma antarctica*

					RE		
A	M	Gr	NH_4 exc	Total N exc	GSI 15	GSI 25	GSI 45
1.00	88.98	4.09	7.51	9.31			
2.00	82.82	8.05	6.13	7.60			
3.00	78.81	10.06	5.33	6.60			
4.00	75.90	11.23	4.79	5.94			
5.00	73.64	11.98	4.40	5.45			
6.00	71.80	12.51	4.09	5.08			
7.00	70.26	12.90	3.85	4.77	5.15	8.58	15.44
8.00	68.94	13.20	3.65	4.52	5.87	9.79	17.62
9.00	67.78	13.44	3.48	4.31	6.60	10.99	19.79
10.00	66.76	13.63	3.33	4.13	7.32	12.20	21.95

Caloric values from Table 8.4 expressed as percent of total annual assimilated energy

8.4.1 Ingestion (I)

Data for ingestion in calories come from Reisenbichler (1993), whose values for ingestion (1.22 ± 0.38% wet mass: χ ± SD for ages 4–9) agree well with those of Olaso et al. (2004) (1.03% over the same age interval), although Reisenbichler (1993) provided caloric values for the stomach contents. Based on the results of both studies, the gastric evacuation rate was assumed to be once per day for purposes of generating the value for ingestion. Using the general equation of Brett and Groves (1979; see below) assimilated energy was assumed to be 80% of the ingested energy listed in Table 8.5.

8.4.2 Somatic Growth (G)

Caloric growth for WAP silverfish was obtained from Reisenbichler (1993) whose growth curve is nearly superimposable on that of Hubold and Tomo (1989), but who also provided equations for calculating yearly caloric (kcal g WM^{-1}) values for ages 1–10. As in Hubold and Tomo (1989), Reisenbichler's ages were determined using the annular rings of otoliths (sagittae). Landmark ages, such as onset of reproduction at age 7, were the same as those reported in Hubold and Tomo (1989) and Kock and Kellermann (1991).

8.4.3 Reproductive Investment

In the absence of data on reproductive output, energy used in reproduction was estimated using gonadosomatic index (GSI: mass of the gonad as a percent of total mass) values of 15%, 25%, and 45% (Kock and Kellermann 1991); caloric values for the gonad were calculated based on the caloric value of whole-fish tissue for that age interval. Values of 15% to 40% have been recorded for other nototheniids (Kock 1992), and what data are available suggest that *P. antarctica* is a particularly fecund species (La Mesa and Eastman 2012; La Mesa et al. 2014).

8.4.4 Metabolism (M)

Oxygen consumption rates were collected as previously described in *8.4.1*. Yearly values were estimated for the age interval preceding the listed age, e.g. for age 2, between ages of 1 and 2. Mid-year values of mass were used to compute the metabolism and excretion numbers for the age interval. Oxygen consumption rates were converted to kcal g WM^{-1} day^{-1} using an oxycalorific equivalent of 4.63 kcal l^{-1} O_2, assuming mixed metabolic substrates (Brett and Groves 1979).

8.4.5 Excretion (E)

Ammonium excretion rates were collected as previously described in *8.4.1*. As with metabolism, yearly values were generated for the age interval preceding the listed age, e.g. for age 2, between ages of 1 and 2. Mid-year values of mass were used to compute the excretion numbers for the age interval. In studies where nitrogen excretion values are reported (e.g. Durbin and Durbin 1981; James et al. 1989), values are usually given for excreted ammonium, or total ammonium nitrogen (TAN), and, if measured, dissolved organic nitrogen (DON). For marine fishes, TAN is 70 to 80% of the total nitrogen excreted, and the remainder is mainly urea and a differing suite of other nitrogenous compounds including creatine, creatinine and tri-methyl amine oxide (McCarthy and Whitledge 1972; Durbin and Durbin 1981). The values for *P. antarctica* in Table 8.4 used our data for TAN only, using the caloric conversion for the combustion of ammonia (5.94 kcal g^{-1} ammonia) obtained from Elliot and Davison (1975). Durbin and Durbin (1981) give a ratio for DON to TAN of 0.437, i.e. DON-N / TAN-N = 0.437, or 69.6% TAN to 30.4% DON by mass. If it is assumed that the TAN is 70% of the total excreted N, their equation provides a useful way of arriving at a total N if DON data are unavailable. Further, if the remaining 30% is assumed to be solely composed of urea (cf. James et al. 1989) a caloric value for a hypothetical total N excretion can be estimated. Working within these assumptions and using a caloric conversion of 2.57 kcal g^{-1} urea, an estimated value for total N excreted is reported in Table 8.5 both in terms of kcal year^{-1} and % of assimilated energy. O:N uses only the TAN data for the ratio. If hypothetical total N were used, the ratio would be adjusted downward by 26%.

8.5 Energy Budgets: *Pleuragramma* and Other Coastal Pelagic Fishes

Brett and Groves (1979) reviewed the literature on energy budgets in fish up until the time of publication, and, based on data available from 15 studies, presented a general energy budget for young, fast-growing carnivorous fishes:

$$100\,\mathrm{I} = (44 \pm 7)\,\mathrm{M} + (29 \pm 6)\,\mathrm{G} + (27 \pm 3)\,\mathrm{E}\,(\textit{all}\ \text{values}\ \chi \pm 95\%\,CI)$$

This equation provides an excellent standard of comparison for fishes in general and carnivorous fishes in particular. From the total calories ingested (I) 27% of those calories are lost through excretory processes (E) including energy lost as feces (20%) and as excreted nitrogen (7%). Growth (G) included only somatic growth; no reproductive losses were noted. Metabolism (M) was derived from respirometric analysis and, as above, converted to kcal g WM^{-1} day^{-1} using an oxycalorific equivalent of 4.63 kcal l^{-1} O_2. The spectrum of life-styles covered did not include any pelagic species, but did include salmonids, a variety of freshwater species including pike and perch, and the cod *Gadus morhua*. Most species used were moderately active ambush predators. Metabolism ranged from 36 to 76.5%; growth ranged from 3 to 74% and excretion from 1.3 to 49.4%. Most of the values for each budget category were within 10% of the figures in the equation above.

A few characteristics stand out in *Pleuragramma*'s acquisition and use of energy (Tables 8.4 and 8.5). First, the ingestion rate is fairly low relative to other species. Results from two studies, Reisenbichler (1993) and Olaso et al. (2004), reported nearly identical values for ingestion as a percent of wet mass (1.2% and 1.0% respectively). Field-based estimates of the daily ration for a variety of oceanic fishes were in the range of 2–6% of wet body mass (Gartner et al. 1997), with fishes of comparable size to *P. antarctica* in the range of 2–4%. Most of these were from subtropical temperatures. Laboratory based measurements of daily ration are available for brown trout (Elliott 1975a, b) in closely controlled conditions. Elliot reported a maintenance ration level for brown trout of 1.4% of wet mass per day at a fish mass of 50 g and a mid-range temperature for the species of 10 °C. A maintenance ration provides enough energy for survival, but not enough for growth. The optimum ration for growth (maximum growth per unit ration) was 2.4% of wet mass per day, and the maximum ration was 2.8%. Experiments were conducted with the amphipod, *Gammarus pulex,* as food. Similar experiments were conducted on sockeye salmon (Brett et al. 1969) using a variety of prepared feeds as well as marine zooplankton (frozen *Calanus plumchrus).* The maintenance ration for the highest calorie diet was about 0.6% of wet mass per day, and the maximum ration was 2.1%

of wet mass per day for a 50 g fish at 15 °C. The maximum ration of the commercial feed produced a daily growth rate of 3% of body mass per day. In contrast, the maintenance ration for the marine zooplankton diet was 1.8% of wet mass and the maximum ration was 4.2% per day. Corresponding daily growth for maximum ration using the copepods was only 1% per day, a big drop from the 3% observed with the commercial feed.

Despite the fact that the daily ration for *P. antarctica* was in the low end of the continuum for oceanic fishes, ingestion was sufficient to cover the metabolic requirements over the age intervals considered here (Tables 8.4 and 8.5). Metabolism stands out as the highest energy cost to the fish, commanding between 89% and 67% of the annual energy costs as the fish aged from 0 to 10 years. Though clearly quite high, the California sardine, *Sardinops caerulea,* showed a similar high cost of respiration, only in a reverse pattern with age. Metabolism consumed 81.5–97.9% of the total energy used by the fish between the year classes of 0 and 6 (Lasker 1970). The main difference between the two species was in the pattern of growth and its share of the energy budget.

The sardine grows most rapidly in its first 4 years of life, reaching 78% of the species' maximum length (280 mm; Butler et al. 1996) and exhibiting a rapidly declining investment in assimilated energy from years 1 to 4 (18.5–3.0%; Lasker 1970) as growth in length and mass levels off at the asymptotic size. In contrast, though the silverfish shows large annual increases in length from years 2–5 (36% declining to 16%, Table 8.5) and similarly in mass (373% declining to 65%), it only reaches 44% of its maximum length (maximum about 250 mm; La Mesa and Eastman 2012) by age 5 and it shows the opposite pattern of energy devoted to growth. Investment in growth increases annually from 8 to 12% of assimilated energy and remains within 2% of that level for the entire age interval considered here (Table 8.5). Low-cost growth is achieved with a low caloric density in the first 4 years and a steady low energy of investment and low rate of growth until death (Hubold and Tomo 1989; Reisenbichler 1993).

Sexual maturity occurs in *P. antarctica* at a length of 13–16 cm (La Mesa and Eastman 2012) corresponding to an age of 7–10 years in Table 8.5 (Reisenbichler 1993; Hubold and Tomo 1989). The literature provides good agreement on size at maturity, less so on age at maturity, which is the result of discrepancies in size at age. The majority of growth curves agree well with the size at age in Table 8.5, which come from Reisenbichler (1993). Data from Hubold and Tomo (1989) are essentially identical and like Reisenbichler (1993), were generated for fishes from the WAP. Sutton and Horn (2011), using fishes from Ross Sea, put the 13–16 cm size range at ages of 6–10 years, whereas Gerasimchuk (1992) puts the size at maturity at ages of 4–5 years for females and 4–7 years for males. All studies on reproductive output agree on the time of spawning as July–August (e.g. Kock and Kellermann 1991; Ghigliotti et al. 2017), time of hatch at October–November (Hubold and Tomo 1989; Vacchi et al. 2004) and a very high fecundity (Gerasimchuk 1987; La Mesa et al. 2014). Gerasimchuk reported 4315–17,774 eggs per female for silverfish captured in the Mawson Sea, scaling directly with fish mass (124.9 ± 4.4 eggs g^{-1}; $\chi \pm$ SD) (Gerasimchuk 1987). More recently, fecundity of silverfish captured on the WAP shelf was reported by La Mesa et al. (2014) to be between 3000 and 12,000 eggs per female and between 80 and 190 eggs g^{-1}, also scaling directly with fish size.

Hypothetical values for reproductive investment are presented in Tables 8.4 and 8.5 for ages between 7 and 10 years, using GSI values proposed for silverfish by Kock and Kellermann (1991) and assuming that increases in mass due to gonad development are equivalent in energy density to that of the whole fish (likely an underestimate). Values for reproductive investment as a percentage of assimilated calories range from 5.2% at a GSI of 15 at age 7, to 22% for a GSI of 45 at age 10. Enough latitude exists within the budget to accommodate a GSI of about 30 at ages 7 and older, supporting a case for high fecundity in *P. antarctica*, even with the many assumptions used to generate the numbers.

GSI for the California sardine was assumed to be 10 in the energetics study of Lasker (1970), representing an energetic investment of between 0.7% and 1.2% of assimilated calories between the ages of 1 and 6. Depending on latitude, young sardines are capable of reproduction in their first or second year of life (Butler et al. 1996), a very different reproductive strategy than that exhibited by *P. antarctica*. A GSI of 10 is also typical of the gadoids (Kock 1992).

Nitrogen excretion, whether as computed total N or measured NH4, is a continuous low level energy loss originating from protein breakdown and tissue maintenance. In a laboratory setting, N excretion can be monitored with precision, and it spikes for a few hours after feeding before returning to a fairly consistent endogenous, or maintenance level (Durbin and Durbin 1981). The excretion rates reported here most closely resemble the endogenous values, as any feeding in the recently captured fish would have taken place hours before data acquisition. N excretion when combined with VO_2 as the O:N ratio is also useful in producing insights on composition of ingested prey, since it reflects their protein-lipid composition. For *P. antarctica*, the O:N ratio produces the fairly unremarkable conclusion of a diet high in protein and lipid, typical of the chemical composition of known prey such as *E. crystallorophias*.

Overall, the budget in Tables 8.4 and 8.5 shows good agreement between ingested and combusted energy despite the fact that the data come from a variety of sources. It supports the contention of a low budget lifestyle for *P. antarctica*, but it also resembles that of other pelagic species in the high percentage of assimilated energy devoted to metabolism, particularly when compared to the general energy budget of Brett and Groves (1979). It differs from more temperate coastal pelagics in its large investment in reproduction, its slow pattern of steady growth throughout its life, and its fairly long life.

8.6 Concluding Remarks

Pleuragramma's lifestyle most closely resembles that of a mesopelagic vertically migrating fish, with a few caveats. First, the fact that it spawns *en masse* under the fast ice in Terra Nova Bay (Vacchi et al. 2004) points to a shoaling and schooling component to its behavioral repertoire that is never observed in other oceanic migrators. Schooling behavior involving thousands of individuals was observed first-hand by Daniels (1982) while diving under fast-ice on the WAP shelf, and was believed to be associated with feeding on biota associated with the underside of the ice. Whether schooling is the norm and vertical migration only occurs at low population densities is difficult to resolve with the data available. Certainly *P. antarctica* is capable of purposeful horizontal movement; it has been directly observed (Daniels 1982) and must be inferred from the data of Vacchi et al. (2004, 2012). In addition, the presence of benthic fauna in its gut reported by Daniels (1982) suggests occasional interaction with the shallow benthos on the WAP shelf.

Mesopelagic vertical migrators, which include myctophids and *P. antarctica* (at least part of the time), are found pan-globally (Robison 2003). They typically spend their daytime hours at depths from 400 to 800 m and swim to near-surface waters at night. Submersible observations indicate that migrating fishes exhibit a greatly reduced activity level ("lethargic behavior") at depth (Barham 1971), reminiscent of that reported by Fuiman et al. (2002) and La Mesa and Eastman (2012) for *P. antarctica* using critter-cams and ROVs. A diel rhythmicity in activity level greatly reduces energy expenditure, particularly for near-neutrally buoyant species that are able to reduce swimming activity to very low levels when not actively hunting or migrating. Vertical migration to depths of 400 m or greater effectively reduces their predator spectrum for part of the day to deep-diving species such as emperor penguins, seals, and toothfish (Williams 1995; Ainley and DeMaster 1990; Robison 2003). *Pleuragramma*'s center of distribution during the day is below 500 m in waters of sufficient depth (e.g. Croker Passage, Lancraft et al. 2004) and between 300 and 500 m at normal shelf depths (Parker et al. 2015).

A few characteristics of *P. antarctica* that are considered unusual for both nototheniids and the Antarctic marine ecosystem are a result of its pelagic lifestyle, and are mirrored in other mesopelagic species. High water (80%; Reisenbichler 1993) and high lipid (48% dry weight; Reinhardt and Van Vleet 1986) levels in their tissues confer near-neutral buoyancy (Near et al. 2009). *E. antarctica*, a vertically-migrating myctophid that co-occurs with *P. antarctica* in the WAP system and has a similar daily vertical excursion, exhibits a lipid level ranging from 57 to 73% of dry weight (Reinhardt and Van Vleet 1986), though with the lower water level of 66%. In the cold temperate waters of the California borderland, the tissue composition of vertically-migrating myctophids ranged from 66.1 to 80.4% water (72.9 ± 5.7; $\chi \pm$ SD) and 10.0 to 58.1% lipid (38.7 ± 17.7; $\chi \pm$ SD) as a function of dry weight (Childress and Nygaard 1973): in the same range as that of silverfish. The quest for neutral buoyancy is a universal one, and the mechanisms employed are similar from system to system. None of the myctophids considered here has a functional swimbladder as an adult, so their buoyancy is a function of their compositional attributes.

The co-occurrence of *P. antarctica* and *E. antarctica* on the WAP shelf, their similar migratory habit and diet (Lancraft et al. 2004), and their similar compositional attributes begs for a comparison between the two species. From a life history perspective, they are quite different. *E. antarctica* is believed to live for about 4 years reproducing at the end of its life, though the nature of the reproductive event, i.e., repetitive spawning over the productive season or one *big bang* is unknown (Greely et al. 1999). If it is similar to myctophids from warmer climates, it would be multiple spawnings (Gartner 1993). A simple energy budget for year 3 of its life suggests a rough breakdown of 56% of assimilated energy for metabolism, 34% for growth, and 10% for nitrogen excretion, a much higher investment in somatic growth than that observed for *P. antarctica* at any time in its first 10 years of life. Faster growth, a low reproductive age, a broadcast spawning reproductive strategy and high lipid accumulation for energy security during winter make *E. antarctica* a successful species on the WAP shelf. However, its lack of antifreeze (Cullins et al. 2011) excludes it from all low temperature shelf systems, that is, all but that of the WAP, which coincidentally is experiencing the most rapid warming of any marine ecosystem (Vaughan et al. 2003).

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Chapter 9
Reproductive Strategies of the Antarctic Silverfish: Known Knowns, Known Unknowns and Unknown Unknowns

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Abstract Reproduction is a key step of a species life history and includes a suite of strategies and tactics enacted to allow the maximization of reproductively active offspring in relation to available energy and parental life expectancy. Gender system, oogenesis pattern, maturation schedule, shifts in habitat utilization, spawning seasonality, mating behavior, and fertilization pattern are among the traits involved in the process of species adaptive optimization of reproduction.

In this frame, here we review the reproductive traits of the Antarctic silverfish (*Pleuragramma antarctica*) based on the available macroscopic and histological data. Then, we will step forward by focusing on two aspects of this species' reproduction: (i) skipped spawning; (ii) potential location of reproduction sites along the Antarctic coasts.

Keywords Antarctic fish • Ovarian maturation • Atresia • Skip spawning • Nursery area

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9.1 Introduction

It is widely accepted that reproduction encompasses a whole suite of strategies which enable the species to maximize its reproductive output in relation to the available energy and parental care (Murua and Saborido-Rey 2003). The optimization of these two components is achieved through an adaptive process fine tuning reproductive traits such as the gender system, oogenesis pattern, maturation schedule, shifts in habitat utilization, spawning seasonality, mating behaviour, and fertilization pattern. A thorough understanding of the reproductive characteristics of a species is paramount not only for understanding its biology and ecology, but also for management purposes. Here we focus on the reproductive features of the Antarctic notothenioid *Pleuragramma antarctica* (Antarctic silverfish), a dominant fish in high-Antarctic shelf and slope waters (Duhamel et al. 2014) where the species plays a similar key role in the food web as does krill in the Seasonal Pack-ice Zone (SPZ).

The evolution and adaptation of Antarctic notothenioids is closely linked to the opening of the Drake Passage and the subsequent cooling and other changes that occurred (Eastman 1993). The next 20 million years provided ample time to shape many aspects of their biology and ecology, including reproduction.

Antarctic notothenioids share a number of reproductive traits such as low fecundity, large eggs (size spanning between 2 and 5 mm), prolonged gametogenesis, and group-synchronous oocyte maturation (e.g. Kock and Kellermann 1991; Shandikov and Faleeva 1992; Calvo et al. 1999; Kock 2005; La Mesa et al. 2003, 2007, 2008; Parker and Grimes 2010). Despite the fact that they share common features of their reproduction on the one hand, notothenioids exhibit a high diversity in reproductive strategies. For example, most Antarctic notothenioids lay demersal eggs (e.g. Kock and Kellermann 1991; Kock and Everson 1997; van der Molen and Matallanas 2004; Kock 2005) that, in a number of species, are deposited in nests and guarded (e.g. Kock et al. 2006; Jones and Near 2012; Ferrando et al. 2014). Others lay pelagic eggs, which drift in the upper part of the water column up to several months (e.g. Yukhov 1982; Kock and Kellermann 1991).

Here, we review the reproductive traits of the Antarctic silverfish. We focus on two aspects of their reproductive strategy: (i) skipping annual spawning when unfavourable conditions prevail; (ii) identifying locations where the species are known to spawn and/or potentially spawn along the Antarctic shelves and slope.

9.2 Reproductive Traits of Antarctic Silverfish

In his monography of Antarctic fish, Andriashev (1965) compiled what was known on the Antarctic silverfish until the mid-1960s. Reproduction was one of the biological characteristics of which little beyond that spawning occurred apparently in austral winter and larvae were first recorded from October–December was known (Regan 1916; DeWitt and Tyler 1960).

The Soviet Union started commercial fishing in the Southern Ocean in the late 1960s. Soviet scouting and commercial fishing vessels were fishing close to the Antarctic continent from the second half of the 1970s. Aggregations of the Antarctic silverfish were discovered in the northern Weddell Sea, in the Prydz Bay and Ross Sea in the late 1970s by FRV *Mys Yunony* (Shust 1998). Comparatively little was published in the accessible Soviet scientific literature at that time. These exploratory fishing cruises confirmed that the species was apparently one of the most abundant fish species in the high-Antarctic. In particular, the abundance and biomass of this fish in the north-western part of the Weddell Sea was considered as very high. In 1985, the relative biomass of *P. antarctica,* even in the less productive central area of the Weddell Sea, was 300 kg/km^2 (Shust 1998). Some data on catches of *P. antarctica* by Soviet trawlers during exploratory fishing reported in CCAMLR documents can be found in Koubbi et al. (2017).

The species is drawn to greater depths than other plankton-feeders, and adult *P. antarctica* are generally found at depths beyond 300 m (Sosinski and Skora 1979; Shust et al. 1984; Ekau 1990).

German Antarctic expeditions, conducted between 1979 and 1981 to the Weddell Sea down to the Filchner Shelf Ice, found that Antarctic silverfish larvae made up to 95% of the plankton biomass obtained from net catches. Of particular interest was the occurrence of larvae between 8 and 12.5 mm standard length in January–February, which reinforced Andriashev's assumption of a winter spawning of the species (Hubold 1984). The presence of silverfish eggs in the stomach of the benthic nototheniid *Trematomus pennellii* (Ekau et al. 1987) first suggested that eggs may be deposited on the bottom (Kellermann 1986). However, the very thin membrane of eggs, and significant yolk deposits, made pelagic eggs more likely. The finding of early embryonated eggs under the fast ice in September (Ghigliotti et al. 2015), and their location in the upper part of the platelet ice, close to the solid ice, as found later in the season (Guidetti et al. 2015), confirmed that eggs were positively buoyant, and floating in their latest stage of development before hatching.

Unfavorable weather and ice conditions during the winter period have thus far limited year-round sampling of adult individuals which could provide an unambiguous determination of when and where Antarctic silverfish spawn. For the time being histological methods are considered to be the only techniques which could provide further insight into the process and cycle of gonad maturation.

A time series of 10 year of data collected during austral summer (December–April) to East Antarctica (Cosmonauts, Commonwealth and Mawson Seas) allowed Faleeva and Gerasimchuk (1987, 1990) to refine information on length and age at sexual maturity, which had previously been overestimated due to the limitation of macroscopic observations. Furthermore, their investigations provided evidence that in early autumn the Antarctic silverfish gonads are still far from pre-spawning condition, supporting the notion of a winter or even winter/spring spawning (June to September).

The presence of sometimes large numbers of oocytes in regression state some 2–3 months prior to spawning suggests that a proportion of Antarctic silverfish females does not spawn annually and that the process of gonad maturation can be

Table 9.1 Length and age at sexual maturity as reported in the scientific literature

Region	Sex	SL (cm)	Age (years)	Refs.
East Antarctica Cosmonauts Sea	f	14–16	5–6	[1]
	m	13–15	4–5	
East Antarctica Commonwealth Sea	f	14–15	5–6	[1]
	m	13–15	4–5	
East Antarctica Mawson Sea	f	15–16	5–6	[1]
	m	13–15	4–5	
Weddell Sea	f/m	15.8		[2]

interrupted in case of unfavorable environmental conditions, thus saving energy for survival (Faleeva and Gerasimchuk 1990). Later studies extending the geographic range of such investigation by including the Antarctic Peninsula (La Mesa et al. 2015a) and Ross Sea (Ghigliotti et al. 2017) confirmed the presence of readsorbing follicles sporadically present or massively occurring in the ovaries.

9.2.1 *Macroscopical Observations*

The Antarctic silverfish is a gonochoristic dimorphic species. Males are characterized by on average smaller size, and pelvic fin which are longer than in females (Gerasimchuk 1987a) (please note that the spelling might change according to the Russian or Ukrainian transliteration as Gerasimchuk or Herasymchuk, respectively).

Differences between sexes have also been detected in the length at which specimens reach sexual maturity (Table 9.1) that is smaller in males and larger in females. [1] Gerasimchuk (1992); [2] Duhamel et al. 1993.

In the early austral summer, the gonadosomatic index (GSI) of sexually mature individuals, a widely used proxy of gonad development that expresses gonad weight as a percentage of total body weight, is low (ranging from 1 to 2% in both sexes). The GSI is slowly increasing towards the end of summer but in early autumn it is still well below 15–30%, usually found in females of many nototheniids and channichthyids immediately prior to spawning (Kock and Kellermann 1991; Duhamel et al. 1993) (Table 9.2 and references therein).

In low-Antarctic nototheniids and channichthyids, there appears to be a trend in the same species of increasing egg size and decreasing relative fecundity towards higher latitudes (Kock and Kellermann 1991), i.e. the same species generally produces more eggs at South Georgia than in the South Shetland Islands. Antarctic silverfish do not appear to follow that trend (Hubold 1992). Although not as high as in some Patagonian and low-Antarctic nototheniid species (e.g. Brickle et al. 2006), relative fecundity in Antarctic silverfish is fairly high (Table 9.3), and coupled with a relatively small egg diameter of about 2 mm.

Table 9.2 Range and variability in gonad maturity coefficient (GSI) of Antarctic silverfish specimens at maturity over the stage III, from various Antarctic areas, during the Southern Hemisphere summer and early autumn

Month	Region	Sex	n	GSI range	GSI mean ± σ	Refs.
January	East Antarctica	f	143	1.00–4.48	1.47 ± 0.46	[1]
		m	44	1.00–3.56	1.36 ± 0.45	[1]
February	East Antarctica	f	247	1.03–4.98	1.78 ± 0.51	[1]
		m	160	1.08–8.06	2.11 ± 0.94	[1]
	Weddell Sea	f	67	1.3–3.6	2.20 ± 0.49	[2]
		m	53	1.0–4.9	2.40 ± 0.81	[2]
	Ross Sea	f	25	0.97–3.43	2.20 ± 0.51	[4]
		m	16	1.29–4.50	2.23 ± 0.77	[4]
March	East Antarctica	f	400	1.07–5.00	2.64 ± 0.78	[1]
		m	291	1.00–9.27	4.77 ± 1.57	[1]
April	Antarctic Peninsula Charcot Island	f	41	1.48–3.46	2.25 ± 0.06	[3]
		m	15	1.09–4.00	2.87 ± 0.20	[3]
	Antarctic Peninsula Marguerite Bay	f	46	1.54–4.35	2.62 ± 0.08	[3]
	Antarctic Peninsula Joinville Island	f	3	1.29–5.25	3.27 ± 1.98	[3]

n sample size, *GSI* GonadoSomatic Index. References: [1] Faleeva and Gerasimchuk (1990); [2] Duhamel et al. (1993); [3] La Mesa et al. (2015b); [4] Ghigliotti et al. (2017)

9.2.2 Histological Data

Progress and timing of gonad maturation occurs in the three investigated areas, East Antarctica (Faleeva and Gerasimchuk 1990), Antarctic Peninsula (La Mesa et al. 2015b), and Ross Sea Region (Ghigliotti et al. 2017) at a similar time of the year. Within the 4 months of the austral summer, males pass through two stages: a resting stage is observed early in the season with inactive, sometimes dividing, spermatogonia at the periphery of the seminal gland, residual spermatozoids, and epithelium desquamation in proximal part of the testicular tubule. Active spermatogenesis then occurs the further summer progresses. It is characterized by intensive spermatogonial proliferation and emergence of primary spermatocytes. Limiting investigations to visual observation of testicles often resulted in over-estimation of maturity stage given the comparatively large size of the testis. Testis has often been found to occupy the rear 2/3 or the entire abdominal cavity and to be turbid white and yellowish. Nevertheless, spermatogenesis completion and emergence of spermatozoids had never been observed.

As notothenioids in general (see Shandikov and Faleeva 1992), females of Antarctic silverfish close to spawning are characterized by having group synchronous ovaries which consists of a batch of eggs maturing and ovulate in the current season, and a second batch of previtellogenic much smaller oocytes representing the next season’s spawn. Oocytes in the primary growth stage are homogeneous in size. A large nucleus occupies most of the cell surrounded by a thin strongly basophilic layer of cytoplasm (Fig. 9.1a). They are regularly occurring along with a cohort of

Table 9.3 Absolute and relative fecundity (calculated in relation to weight) in developing females of the Antarctic silverfish collected in various Antarctic regions

Region	n	size range (cm)	OD (μm)	F_{abs} range	F_{abs} mean ± σ	F_{rel} range	F_{rel} mean ± σ	Refs.
EA (MS)	30	15.4–25.6	450	4315–17,774	7499 ± 2868	67.7–156.8	124.9 ± 4.4	[1]
AP (CI)	12	n.a.	400	2953–11,613	6818 ± 1968	78–193	136 ± 39	[2]
AP (MB)	12	n.a.	400	3637–11,492	6700 ± 1934	108–189	157 ± 45	[2]
AP (JI)	2	n.a.	400	10,043–10,174	10,109 ± 7148	119–171	145 ± 103	[2]
RS	25	13–19	460	2360–6700	4939 ± 1289	65.8–188.4	111.3 ± 27.8	[3]

n sample size, *OD* maturing oocyte minimum diameter, *EA* East Antarctica, *MS* Mawson Sea, *AP* Antarctic Peninsula, *CI* Charcot Island, *MB* Marguerite Bay, *JI* Joinville Island, *RS* Ross Sea, *n.a.* not available. References: [1] Gerasimchuk (1987b); [2] La Mesa et al. (2015b); [3] Ghigliotti et al. (2017)

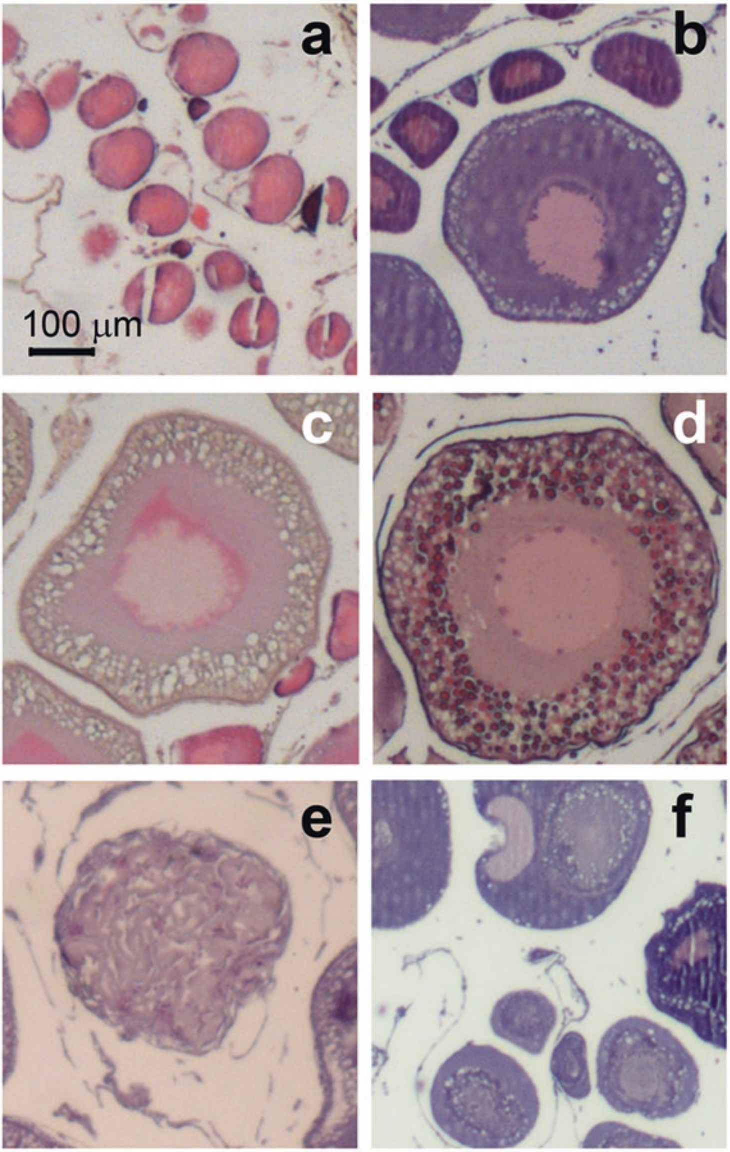

Fig. 9.1 Antarctic silverfish ovary. Cross sections of regular and reabsorbing follicles, hematoxylin-eosin stained: primary growth oocytes (**a**), initial alveolus oocyte (**b**), cortical alveolus stage oocyte (**c**), vitellogenic oocyte (**d**), post-ovulatory follicle (**e**), alpha atretic follicles (**f**). Scale bar = 100 μm

oocytes in various stages of trophoplasmatic growth. Three distinct stages of vitellogenic cells have been observed: initial alveolus (IA), cortical alveolus (CA), and vitellogenic (Vtg). The cells are round with a thin acidophilic layer at the periphery of the cytoplasm (the future zona radiata) beneath the follicle. The nucleus is spherical in the IA cells (Fig. 9.1b), and becomes irregularly shaped, with niches along its wall, in the CA (Fig. 9.1c) and Vtg (Fig. 9.1d) cells. A large number of basophilic nucleoli are accumulated at the periphery of the nucleus; small grainy basophilic, not marginated, nucleoli are sometimes interspersed in the nucleoplasm or circularly displaced around the central area of the nucleus. Vesicles start to be accumulated at the periphery of the cytoplasm, in a few rows (IA), many rows (CA) or multiple rows occupying almost the entire cytoplasm (Vtg). In the vitellogenic oocytes, yolk granules start to be deposited from the periphery of the oocyte toward the centrally located nucleus.

In January and February post ovulatory follicles (POFs), reaching 1600–1800 μm in size, were occasionally observed in the ovaries (Faleeva and Gerasimchuk 1990; Ghigliotti et al. 2017). They are clearly recognizable as having completely homogenized yolk, reduced lumen, convoluted shape and deep folded membrane (Fig. 9.1e).

The presence of developing follicles undergoing resorption (atretic follicles) is reported from the majority of females exhibiting maturing gonads from East Antarctica (Faleeva and Gerasimchuk 1990), a few females from the Antarctic Peninsula (La Mesa et al. 2015a), and a large number of maturing females from the Ross Sea Region (Ghigliotti et al. 2017). In most cases, the atretic process is reported to be in its initial phase (alpha atresia): oocytes shrinking in size, distorted follicles, disorganization of the cytoplasm in the oocyte, fragmentation of the chorion with cytoplasm flowing out, deformation and sometimes dissolution of the nucleus are among the most commonly described features (Fig. 9.1f).

9.3 Skipped Spawning: A Reproductive Strategy in the Antarctic Silverfish?

Field observations to be conducted year-round close to the Antarctic continent are still extremely difficult, dangerous, and cost-expensive. In particular, winter investigations suffer from these constraints. The limitations in mind, the available information nevertheless allows to develop a hypothesis with respect to the reproductive strategy which is probably followed by Antarctic silverfish.

Spawning is likely to occur in austral winter and early spring (July to end of August, beginning of September). This hypothesis is supported by the fact that both male and female gonads are developing but still far from spawning in late summer. The presence of newly hatched larvae in November (e.g. Hubold 1984; Vacchi et al. 2012) provides additional support for winter spawning of the Antarctic silverfish. The presence of large numbers of atretic follicles, also known as mass ovarian atresia, in the months leading up to spawning suggest that Antarctic silverfish are able to skip the process of gonad maturation when unfavorable living conditions are encountered. This may be interpreted as a tactic in order not to jeopardize survival of the fish.

A number of observations in recent years suggest that in other Antarctic fish species, such as mackerel icefish at South Georgia, 10–20% of the fish do not spawn each year. In years when feeding conditions were poor such as in those years when few krill was transported to South Georgia, up to 60% of mackerel icefish are able to interrupt the maturation process of the ovaries in order to save energy to sustain their living (Kock and Everson 2003). Skip spawning has been hypothesized for a number of Patagonian, Sub-Antarctic, and Antarctic species, Patagonian toothfish *Dissostichus eleginoides* (Arana 2009), the Antarctic toothfish *D. mawsoni* (Parker and Grimes 2010), the blackfin icefish *Chaenocephalus aceratus* (Vanella et al. 2005), the mackerel icefish *Champsocephalus gunnari* (Kock and Kellermann 1991; Kock and Everson 2003), and the pike icefish *C. esox* (Calvo et al. 1999).

Over evolutionary time, skipped spawning occasionally enacted when unfavorable environmental and physiological conditions prevail, can be interpreted as a manifestation of phenotypic plasticity in a species, in a wider adaptive sense. In other words, the capability to skip spawning, demonstrated at individual level, can be viewed as a facet of the overall specific reproductive strategy influencing the reproductive potential of fish populations and thus enhancing the species fitness (Jorgensen et al. 2006; Rideout and Tomkiewicz 2011; Shaw and Levin 2011).

The Antarctic silverfish is suggested to perform energy costly spawning migrations from open waters to ice-laden coastal waters where they were born or where environmental conditions are similar to those experienced at the larval stage following a homing-behaviour (Koubbi et al. 2011). In such a scenario, the capability to down-regulate the oocytes development through follicular atresia would reduce individual reproductive investment during breeding migration, at annual scale, but would result in a long-term adaptive advantage for the populations (Ghigliotti et al. 2017).

9.4 Reproduction Areas: Evidences and Hypotheses

There is sufficient evidence now to support the hypothesis that Antarctic silverfish reproduction occurs in coastal areas along major continental ice shelves (e.g. Faleeva and Gerasimchuk 1990; Eastman 1993; Hubold 1990; Kellermann 1987; La Mesa et al. 2010; Moline et al. 2008; Vacchi et al. 2012). However, running ripe females and males have never been sampled. Nevertheless, information is available on known and potential nursery areas of early larvae. They can serve as a proxy for the location of spawning grounds. In its wider sense, the term “nursery area” is used to define those regions or habitat where larvae, post-larvae and early juveniles of a given species occur and reside in significant amounts, with a substantial potential to contribute to the renewal of the adult fish population (Beck et al. 2001). Given the diversity in the life histories and developing times of the various species, a nursery area in its wide definition does not necessarily coincide with the place where reproduction occurs (Harden-Jones 1968). Being Antarctic fish characterized by a protracted larval development, in order to limit vagueness we will use the term “nursery area” in its strict sense, considering nursery grounds only areas where larvae up to 25 mm SL (0+ age class) have been recorded. Records of larger larvae, post-larvae, and juveniles, although important for reconstructing the life history of the Antarctic silverfish, have been excluded from our analysis because of the high spatial dispersal of these stages (La Mesa et al. 2010).

Early stages of silverfish, namely embryonated eggs or 0+ larvae (up to 25 mm SL), have been recorded in various locations around the Antarctic continent. In order to attempt a rough quantitative comparison among localities, the abundance of early life stages of Antarctic silverfish available in the scientific literature are ranked into four categories (see Table 9.4 and pertaining legend). By coupling the presence

Table 9.4 *Pleuragramma antarctica,* hatching and nursery areas along the Antarctic coasts

Region locality	Typology	Life stage (SL)	Period	Abundance	References
Ross Sea					
Terra Nova Bay	HA NG	Eggs & larvae (mean 9.3 mm)	Sep., Nov., early Dec.	++++	Vacchi et al. (2004), (2012), Guidetti et al. (2015), and Ghigliotti et al. (2015)
Terra Nova Bay (North side)	NG	Larvae 0+ (mean 13.9 mm)	Jan., Feb.	++++	Guglielmo et al. (1998) and Granata et al. (2000)
McMurdo Sound	LNG	Larvae 0+ (18–25 mm)	Oct., Nov.	+++	Outram (2000)
McMurdo Sound	ID	Larvae 0+ (mean 8.9 mm)	Nov. – Jan.	++	DeWitt and Tyler (1960)
McMurdo Sound	ID	Larvae 0+ (mean 14 mm)	18–21 Feb.	++	Hopkins (1987)
Ross Island	ID	Larvae 0+ (6–7 mm – 15–19 mm)	14 Dec. – 21 Feb.	++	Regan (1916)
Bay of Whales	LNG	Larvae 0+ (mean 10 mm)	mid Dec. – Mid Jan.	+	Biggs (1982)
Bay of Whales	LNG	Larvae 0+ (mean 9.8 mm)	Mar.	+	Caccavo et al. (2015), Brooks and Goetz (2014)
D'Urville Sea					
Pierre Lejay Bay	NG	Larvae 0+ (mean 17.3 mm)	Jan. – early Feb.	++++	Koubbi et al. (1997)
MGT & Commonwealth Bay	NG	Larvae 0+ (mean 13.8 mm)	Jan.	++++	Koubbi et al. (2011)
Davis Sea					
Gauss Station (Wilhelm II coast)	LNG	Larvae 0+ (20 mm)[a]	22 Nov. – 01 Dec.	++++	Pappenheim (1912)

(continued)

Table 9.4 (continued)

Region locality	Typology	Life stage (SL)	Period	Abundance	References
Cosmonauts and Cooperation Seas					
Prydz Bay	NG	Larvae 0+ (mean 15 mm)	Jan. – Feb.	++++	Williams (1991)
Cosmonauts Sea and Prydz Bay	?	Larvae 0+ (18 mm)	Jan. – Mar.	+	Van de Putte et al. (2010)
Weddell Sea					
Vestkapp (Eastern Weddell Sea)	LHA	Hatched larvae	mid Nov.	unrep.	Hubold (1990)
FIS (Vahsel and Gould Bays)	NG	Larvae 0+ (8–25 mm)	end Jan. – early Mar.	++++	Keller (1983) and Hubold (1984)
R-LIS (Camp Norway – Halley Bay)	NG	Larvae 0+ (8–16 mm)	Jan.	++++	Hubold (1985) and White and Piatkowski (1993)
Antarctic Peninsula					
Tip AP-North West Weddell Sea	LHA		December	unrep.	Kellermann (1986) and La Mesa et al. (2015b)
S-W AP-North Bellinghausen Sea	LHA			unrep.	Kellermann (1986)
Bransfield Straits	LNG	Larvae 0+ (means 15.9–20.2 mm)	Jan. – Feb.	++	Kellermann (1986) and Sinque et al. (1986)
Bransfield Straits	LNG	Larvae 0+ (17.7 to 26.7 mm)	26 Feb. – 3 Mar.	++	Jones et al. (2014) and La Mesa et al. (2015b)
Bransfield Strait-Marguerite Bay	LNG	Larvae 0+ (15.0–19.8 mm)[a]	early Jan. – mid Feb.	+++	Ross et al. (2014)
Gerlache Strait	LNG	Larvae 0+ (16.8–23.4 mm)	19 Jan. – 15 Feb.	++	Morales-Nin et al. (1995)
Marguerite Bay	LNG	Larvae 0+ (9–13 mm)	Dec.	++	Outram (2000)

Localities are grouped by region. Acronyms have occasionally been used to make the table more easily readable: *MGT* Mertz Glacier Tongue, *FIS* Filchner Ice Shelf, *R-LIS* Reeser-Larsen Ice Shelves, *AP* Antarctic Peninsula, *HA* Hatching Area, *LHA* Likely Hatching Area, *NG* Nursery Ground, *LNG* Likely Nursery Ground, *ID* Insufficient Data to draw any hypothesis, *SL* Standard Length. Abundance of Antarctic silverfish specimens have been expressed as follows: + = sporadic occurrence (<10), ++ = moderate occurrence (10–100), +++ = high occurrence (101–1000), ++++ very high occurrence (>1000); unrep = abundance not reported in the paper

[a] Size range referred to the Total Length (TL) not Standard Length

of the various Antarctic silverfish early stages and their abundance, each of the locations has been assigned to one of the following typology: (i) Hatching Area (HA, area where hatching eggs have been recorded), (ii) Likely Hatching Area (LHA, area in which eggs have never been found but just hatched larvae have been found abundant), (iii) Nursery Grounds (NG, area where early larval stages are occurring in large or very large numbers) and (iv) Likely Nursery Grounds (LNG, where silverfish larvae up to 25 mm SL were recorded in limited amount).

Terra Nova Bay (TNB), in the Ross Sea, is the only area from where embryonated eggs and newly hatched larvae have been recorded. In TNB, Antarctic silverfish embryonated eggs have been found floating in large quantities among platelet ice under the sea-ice in September (Ghigliotti et al. 2015). Mass hatching has directly been observed in November (Vacchi et al. 2004, 2012; Guidetti et al. 2015). In the same areas, early larvae (7–9 mm SL) have been recorded in abundance in the following summer months (Guglielmo et al. 1998; Granata et al. 2000). This makes TNB both a hatching area (HA) and a nursery ground (NG) and strongly supports the notion that mass spawning occurs in the northern part of the Ross Sea. The finding, during several annual surveys, of adult silverfish remains trapped in the sea-ice in the TNB nursery area is a further strong indication for spawning in the northern Ross Sea (Vacchi et al. 2012). Alternative hypotheses are that eggs are spawned close to the sea-ice and remain in a challenging environment for all the embryonic development period, or dispatched in deeper ice-free water and then rise in the water column later on owing to their buoyancy and local hydrodynamic flows (Vacchi et al. 2012).

Although lower in numbers, larvae had been collected on several occasions in southern parts of the Ross Sea. *P. antarctica* early larvae were collected at Ross Island during the “Discovery” and “Terra Nova” Expeditions (Regan 1916). Early larvae were collected in McMurdo Sound during spring and summer months (DeWitt and Tyler 1960; Hopkins 1987; Outram 2000). Recently, several newly-hatched larvae, which were genetically assigned to *Pleuragramma,* were sampled in mid-March in the Bay of Whales, eastern Ross Sea (Brooks and Goetz 2014; Caccavo et al. 2015). This finding, together with the occurrence of a number of 10 mm long larvae, previously reported by Biggs (1982) in the same area, strongly suggests the potential role of the Bay of Whales in the eastern Ross Sea as a nursery ground for this species.

In the D’Urville Sea region, annual surveys carried out from 1996 to 2010 (Koubbi et al. 1997, 2009, 2011) provided data to identify a second nursery ground for *Pleuragramma*. The Terre Adélie coastal area (Pierre Lejay Bay) and seabed depression adjacent to the Mertz Glacier Tongue and Commonwealth Bay hosted *P. antarctica* larvae in large numbers with mean size of 17.3 and 13.8 mm SL, respectively. At Pierre Lejay Bay a dense aggregation of Antarctic silverfish larvae, extending about 1.8 by 1.5 nautical miles, was noticed. Such an aggregation was made of at least 17/m^3 individuals, while densities in the remainder of the study area were on average lower than one individual/m^3 (Koubbi et al. 1997).

In other regions of the Indian Sector, an interesting record on the mass occurrence of early-stage *Pleuragramma* in Wilhelm II coast (Davis Sea) came from the Heroic Age of the Antarctic Expeditions around the turn to the Twentieth century. From 22 November to 1 December 1902 more than one thousand *Pleuragramma* larvae of about 20 mm TL were taken by fine-meshed tow-nets at the German Gauss Station (Pappenheim 1912). These large density suggest the presence of another nursery ground in the Davis Sea.

A survey carried 1991 in the Prydz Bay region (Cooperation Sea) identified another nursery ground. In 93 near-surface tows (30–50 m depth) of 30 min duration with the International Young Gadoid Pelagic Trawl (IYGPT, *P. antarctica* constituted the most important part of the catch. Up to 9100 individuals/30 min haul occurred in the central part of the bay. Most of the fishes were age class I juveniles of 45 mm SL but a noticeable part of the catch was also represented by *Pleuragramma* larvae of 15 mm SL (Williams 1991). A recent extensive survey in the pelagic zone of the Cosmonauts Sea and Prydz Bay (Van de Putte et al. 2010) confirmed the occurrence of Antarctic silverfish fingerlings of age class 2+ (54–95 mm SL) and larvae of age class 0+ in coastal waters of Prydz Bay, although in low numbers.

The Weddell Sea region has been studied in some detail for the presence and abundance of ichthyonekton since 1979/1980 (Hempel et al. 1983; Kellermann 1986; Hubold and Ekau 1987; Ekau et al. 1987; Piatkowski 1987; Boysen-Ennen and Piatkowski 1988; Hubold 1990; Piatkowski et al. 1990; White and Piatkowski 1993). Larval *P. antarctica* typically predominated shelf waters of the southern and southeastern Weddell Sea (Keller 1983; Hubold 1985, 1990) representing some 90% of the fish larvae in summer (Hubold 1990; Piatkowski et al. 1990). *P.antarctica* larvae ranging 8–16 mm SL in size, were aggregating in the upper 25–50 m of the water column over the continental slope and innershelf depressions located in the southern Weddell Sea off the Filchner Ice Shelf and in the south eastern part of Weddell Sea in January–February (Hubold 1984, 1985). The density of larvae peaked at 3000–4000 individuals/1000 m^3 (Keller 1983) and supports the hypothesis that *P. antarctica* hatched near Vestkapp by mid-November (Hubold 1990), making this zone a likely hatching area (LHA) for the species.

A large number of ichthyoplankton surveys were performed in the western Antarctic Peninsula (wAP) since 1975/1976 (Kellermann 1986; Kellermann and Schadwinkel 1991, Kellermann and Kock 1988; Loeb 1991; Morales-Nin et al. 1995; Donnelly and Torres 2008; Jones et al. 2014; Ross et al. 2014; Parker et al. 2015; Mintenbeck and Torres 2017). Typically, the number of fish larvae up to 25 mm SL was low, ranging from zero to about one hundred individuals in the catches (higher numbers of more developed postlarvae and juveniles were routinely collected).

At least, three sites of wAP (Bransfield Strait, Gerlache Strait, and Marguerite Bay) can be considered as LNGs. In these wAP areas, the larval density is generally 10 times less than in the Weddell Sea. A substantial decline has occurred in the northernmost location of larvae after 1999–2000 (Ross et al. 2014). Despite the occurrence of substantial numbers of larvae, it remains unclear so far if Antarctic silverfish spawn successfully in the wAP region. Recruitment of *Pleuragramma* to the waters of wAP shelf has been attributed to larval dispersal from spawning occurring in the western Weddell Sea (Kellermann 1986; La Mesa et al. 2015b) and the Bellinghausen Sea, southwest of the wAP (Kellermann and Schadwinkel 1991). A LHA could be located in the northwestern Weddell Sea. Larvae originating from this area could be transported from the Weddell Sea via the Weddell gyre/Antarctic Coastal Current as it flows through the Antarctic Sound and around the tip of the

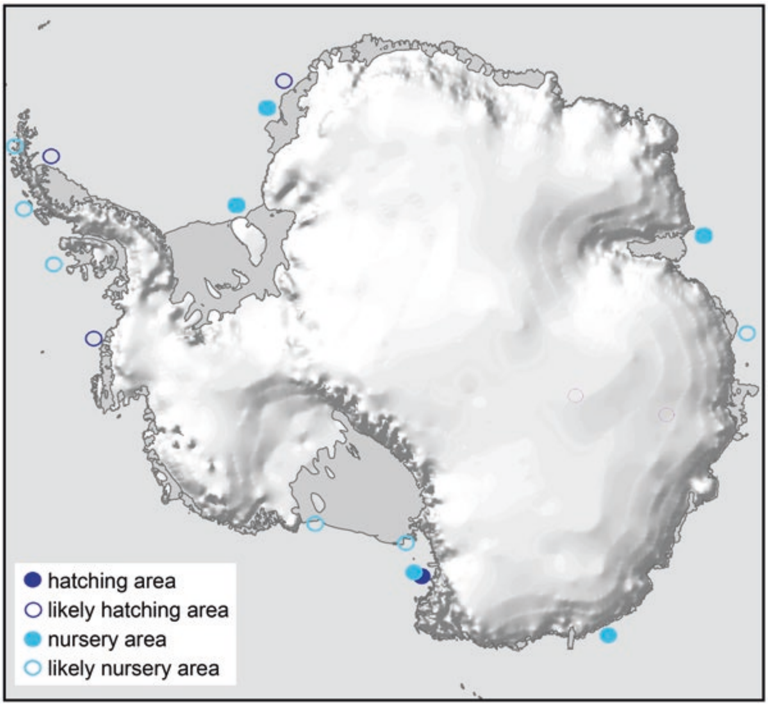

Fig. 9.2 *Pleuragramma antarctica* actual hatching areas, likely hatching areas, nursery areas and likely nursery areas are mapped

Antarctic Peninsula into the eastern Bransfield Strait (La Mesa et al. 2015b). Larvae originating from the other LHAs in the Bellinghausen Sea south of the Peninsula could be drifted northeast by the Antarctic Circumpolar Current merging with the general northeasterly flow at the shelf break along the Peninsula (Mintenbeck and Torres 2017).

The resulting distribution of hatching areas, likely hatching areas, nursery areas and likely nursery areas along the coasts of Antarctica is shown in Fig. 9.2.

Hubold (1984) related the retention of developing eggs and young stages in the Weddell Sea to the presence of polynyas that are known to act as hydrographic engines, and that are widespread along the Antarctic coast (e.g. Kern 2009). However, no evidences are available to date that might confirm or reject this interesting hypothesis.

9.5 Conclusive Considerations

Scientific knowledge on the reproduction cycle of the Antarctic silverfish has improved in the past 30 years, with most of the information originating from the Ross Sea. In Fig. 9.3 the year-round events characterizing the Antarctic silverfish reproduction in the Ross Sea are shown along a timeline.

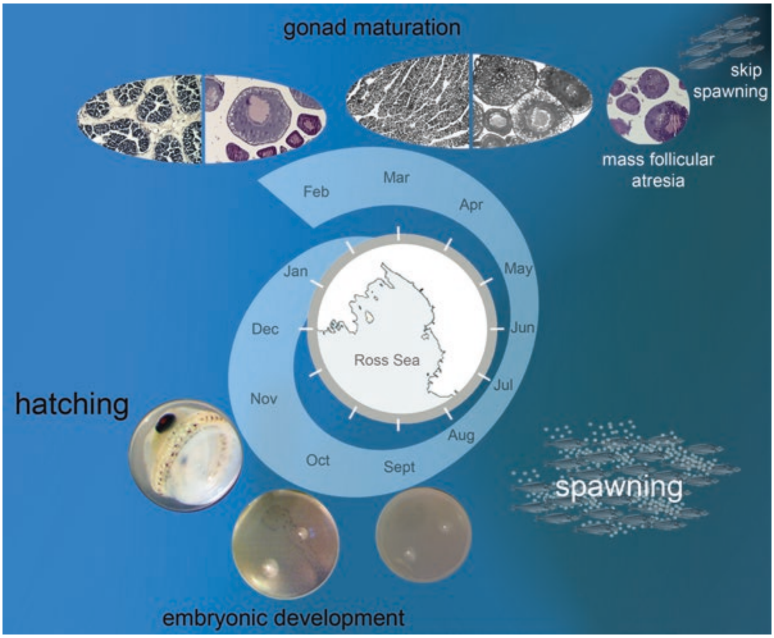

Fig. 9.3 Diagrammatic representation of the year-round events related to the Antarctic silverfish reproduction in the Ross Sea summarizing what reported in current literature. Pictures correspond to actual data, drawings stand for events whose timing or happening is indirectly deduced

Besides illustrating actual information, Fig. 9.3 also highlights areas where knowledge are still lacking. Indeed, the time of spawning is still approximate, but it is likely to occur from July to September. Moreover, the occurrence of small larvae as late as February–March may suggest that spawning is extending over a comparatively long period of several months. Eggs are pelagic but if they are released in surface waters or close to the sea-floor from where they may rise later is still unknown. Logistic constraints have historically limited year-round field activity, however future scenarios in this regard are encouraging.

For instance, newly established permanent Antarctic Stations are expected to provide new opportunities for Antarctic silverfish winter sampling, such as the new Korean Jang Bogo Station in the Ross Sea, in close vicinity of the Silverfish Bay – Cape Washington hatching area, that already offered the possibility to extend backwards to September the sampling of embryonated eggs (Ghigliotti et al. 2015).

In addition, advances in the logistic capabilities of research vessels, by allowing to operate year-round in ice-covered waters, could further improve both a geographical and temporal extension of researches focusing on the Antarctic silverfish life cycle and reproduction.

New tools for non-invasive, spatial and temporal extensive surveys have been developed in recent years improving researches by visual and acoustic methods

(Azzali et al. 2010; O'Driscoll et al. 2011; Fox 2015; Guidetti et al. 2015). The definition of Antarctic silverfish adults acoustic target strength (Azzali et al. 2010), and the improvement of acoustic technology in general (reviewed in O'Driscoll et al. 2017), provides new possibilities for recording silverfish presence, abundance and movements over a wide time frame.

On the whole, the availability of new infrastructure for all-year-round research in the Southern Ocean, progress in remote sensing and image processing technology offer the possibility to collect data in areas and periods previously unattainable, laying the bases to step forward in the knowledge of the Antarctic silverfish reproductive strategies.

Acknowledgments This work is also intended as a tribute to the memory of Tatiana I. Faleeva who greatly contributed to the knowledge on the reproductive biology of the Antarctic silverfish.

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Chapter 10

Population Structure and Life History Connectivity of Antarctic Silverfish (*Pleuragramma antarctica*) in the Southern Ocean Ecosystem

Julian Ashford, Lorenzo Zane, Joseph J. Torres, Mario La Mesa, and Alexander R. Simms

Abstract Antarctic silverfish (*Pleuragramma antarctica*) are the most important pelagic forage fish on the Antarctic continental shelf. They have an exclusively pelagic life history, including cryopelagic eggs and early larvae. The discovery of extensive distributions of eggs and larvae under fast-ice inside Terra Nova Bay, and the revelation that the aggregates were stable between years, suggested dispersal over the continental shelf by older larvae and juveniles and a return as adults to spawning areas, consistent with observations from the Antarctic Peninsula of large-scale adult movement inshore. This life history hypothesis holds promise for understanding population structure in silverfish found around the Antarctic continental shelf. However, the hypothesis is challenged by inconsistencies concerning the

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distribution of spawning and feeding areas, a low-energy life strategy, and lack of phylogenetic diversification.

We review recent and past population and genetic studies in the context of published literature on the physical environment, including hydrography and a recent geological reconstruction of Antarctic Ice Sheet deglaciation. We suggest physical-biological interactions between glacial trough systems, circulation, and life history processes in shaping distributions of silverfish along and across the shelf. We discuss how these processes may result in a richly diverse population structure around the Antarctic and over time, and show how the synthesis can help account for (1) variability in spawning and feeding areas over succeeding glacial cycles, (2) connectivity without invoking active migration on large spatial scales, and (3) persistence over successive glacial cycles without further phylogenetic diversification. Based on this synthesis, we make predictions concerning population structuring and genetic signatures of paleo-climatic events, and suggest multi-disciplinary approaches to test these in future research.

Keywords Population dynamics • Southern Ocean • Antarctic shelf • Antarctic fish

10.1 Introduction

Antarctic silverfish (*Pleuragramma antarctica*) are an important forage species in the marine ecosystem of the Southern Ocean, with a circumpolar range along the Antarctic continental shelf that extends to the South Orkney Islands (e.g. La Mesa and Eastman 2012; Duhamel et al. 2014) (Fig. 10.1). Unusually for a notothenioid, they have an exclusively pelagic life history, including a cryopelagic egg stage. The discovery of extensive distributions of eggs and larvae during November under fast-ice inside Terra Nova Bay in the Ross Sea (Vacchi et al. 2004) has stimulated interest in the processes structuring their populations and ecology. Recent sampling indicated that the aggregations were stable between years, restricted to embayments either side of the Campbell Glacier tongue, and associated with concentrations of platelet ice under the ice canopy (Vacchi et al. 2012; Guidetti et al. 2015). Distributions of young stages in the upper water column during December–January indicated dispersal over the continental shelf facilitated by the general circulation of the Ross Sea (La Mesa et al. 2010). Return as adults to spawning areas presumably completes the life cycle, consistent with observations from the Antarctic Peninsula (e.g. Daniels and Lipps 1982) of large-scale adult movement inshore.

This emerging life history hypothesis holds general promise for helping understand the population structure, as well as the distribution and abundance of silverfish around the Antarctic. Newly hatched larvae have been found in the south-eastern Weddell Sea (Hubold 1984), around the northern tip of the Antarctic Peninsula (Kellermann 1986; La Mesa et al. 2015), in Marguerite Bay (Donnelly and Torres 2008), and recently in the Bay of Whales in the eastern Ross Sea (Brooks and Goetz 2014). Growing evidence of population heterogeneity is consistent with life histories

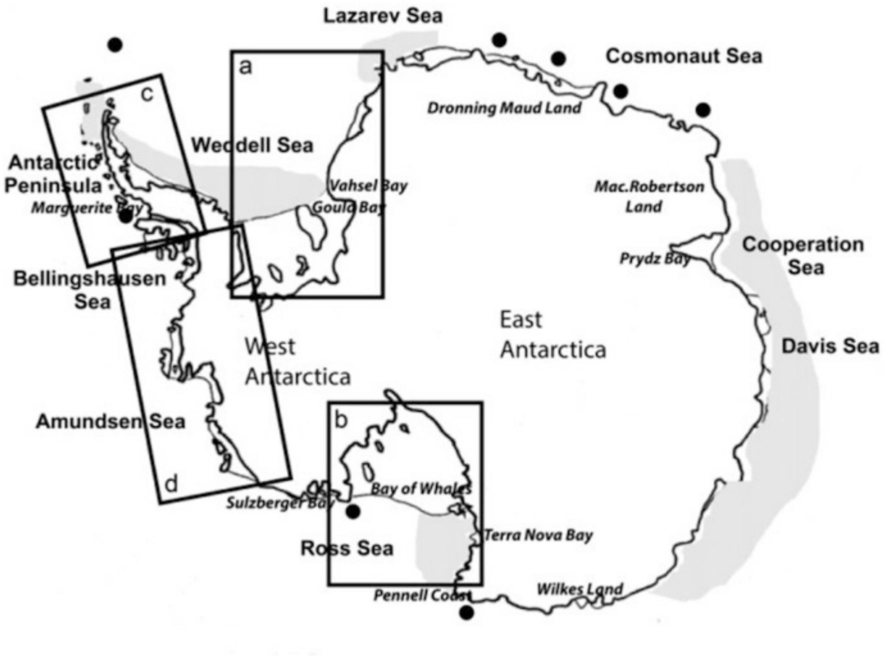


Fig. 10.1 Distribution of silverfish along the Antarctic continental shelf (Reproduced from La Mesa and Eastman 2012). *Black dots* indicate single record. *Frames* a, b, c, d enclose areas of interest whose details are shown in Figs. 10.2, 10.3, 10.4, and 10.5, respectively

linked to disparate spawning areas. Small but significant genetic differences detected both spatially and temporally (Zane et al. 2006) have been corroborated in a recent study (Agostini et al. 2015) that discounted panmixia, or random mixing, and indicated genetic structuring along the Antarctic Peninsula. Further evidence from differences in otolith chemistry (Ferguson 2012), reproductive measures (La Mesa et al. 2014), and distributions of faunal assemblages (Parker et al. 2015) also indicated spatial structuring.

However, several inconsistencies challenge the hypothesis in its present form. Firstly, La Mesa and Eastman (2012) pointed out that successive cycles of expansion and contraction of the Antarctic ice sheet (e.g. Anderson 1999) mean that current spawning areas, and feeding areas over the continental shelf, were covered over large periods during the evolutionary ecology of silverfish. Thus, a recent molecular phylogenetic study estimated that the Antarctic clade of notothenioids arose at approximately 22.4 Ma (million years before present, where present is defined as AD 1950) and Pleuragrammatinae, the neutrally buoyant clade to which silverfish belong, formed approximately 17 Ma (Near et al. 2012). The Pleuragrammatinae are characterized by only six species, including two members of the genus *Gvozdarus* (Eschmeyer et al. 2016) that are poorly known and not characterized at the molecular level. The latest split between the toothfish *Dissostichus mawsoni* and *D. eleginoides* occurred approximately 7.3 Ma, but earlier differentiation between *P.antarctica* and *Aethotaxis mitopteryx* dated at approximately 15 Ma (Near et al. 2012). By comparison, the Last Glacial Maximum (LGM) occurred at approximately 20 ka (kiloyears before present).

Secondly, the few species that are pelagic contrast with the spectacular diversification that occurred during late Miocene cooling (11.6–5.3 Ma) among benthic and bentho-pelagic Antarctic notothenioids. More than 100 species are currently recognized (Eastman 2005). Increased ice scouring, local extinction of the near-shore benthic fauna, survival of refugee populations in suitable areas, allopatric divergence and subsequent colonization of open niches have been invoked as the factors that allowed recurrent diversification of benthic and bentho-pelagic notothenioids (Near et al. 2012), as well as other Antarctic benthic taxa surviving the last glaciations (Thatje et al. 2008; Convey et al. 2009). In the Pleuragrammatinae, diversification is thought to have filled the niche space over the Antarctic shelf (Eastman 2005) consistent with a process of ecological speciation driven by divergent natural selection between environments (e.g. Schluter 2009). However, subsequent stability in the neutrally buoyant clade is harder to reconcile with mounting evidence of heterogeneity in shelf waters, both around the Antarctic and over time. The survival of only a few, old, highly divergent pelagic species suggests that lineage diversity was restricted either by increasing extinction rates or by reduced opportunities for speciation (Bortolotto et al. 2011). This raises the question: on an evolutionary time scale, how did silverfish adjust to environmental heterogeneity in a way that allowed populations to persist but without further phylogenetic diversification?

Thirdly, La Mesa and Eastman (2012) also pointed out that, in pelagic notothenioids, older fish do not maintain themselves in the water column by continuous swimming. Diversification from a benthic ancestry was achieved in large part through accumulation of lipids and paedomorphic adaptations, in which adult stages retain juvenile or larval features. Paradoxically in silverfish, this resulted in a pelagic species with an inactive, energy-efficient strategy that relies on extensive lipid sequestration in large sacs composed of adipocytes (Eastman and DeVries 1989). The resulting neutral buoyancy facilitates a sedentary lifestyle in which fish hang in the water column to feed (La Mesa and Eastman 2012). Questions immediately arise concerning silverfish movement: given the habitat constraint imposed on connectivity by their cryopelagic egg stage, how are the observed circumpolar distributions maintained? Secondly, how do adults return to spawning areas in order to close their life history? In this review, we start to resolve these issues by considering data from recent and past population studies in the context of glacial features and ice-sheet extent around the Antarctic continental shelf, as well as the shelf circulation and hydrography. We integrate the biological data with published studies on the physical environment, including a recent geological reconstruction of Antarctic Ice Sheet deglaciation. This synthesis showed that the ice sheet grounding line was near the shelf edge around much of the continent during the LGM, but the spatial pattern of deglaciation was highly variable (The RAISED Consortium et al. 2014). We suggest further development of the life history hypothesis that can account for variability in spawning and feeding areas, connectivity that does not rely on active swimming on large spatial scales, and persistence over successive glacial cycles without further phylogenetic diversification. In doing so, we take some initial steps towards an ecosystem perspective, with predictions concerning population structuring and genetic signatures of paleo-climatic events that can be tested in future research. Finally, we briefly consider some implications for other components of the ecosystem, including issues concerning fishing and climate variability.

10.2 Precedents

10.2.1 Prior Studies Suggest Early Life History Associated with Glacial Features on the Shelf

Kellermann (1986) noted that prior studies recorded silverfish early stages adjacent to ice shelves, which could be regarded as potential spawning areas. In particular, Hubold (1984) examined the spatial distribution of different age groups off ice shelves in the south-eastern Weddell Sea (Fig. 10.2). He found larvae <12.5 mm SL confined near the surface and associated with troughs off the Filchner Ice Shelf and Camp Norway. Higher post-larval concentrations (age 0+) coincided with troughs on the shelf as well, especially along the Filchner Ice Shelf in Gould Bay and Vahsel Bay, and also off the Brunt and Riiser-Larsen Ice Shelves. Distributions tended to be deeper with age. He noted the strong katabatic wind field and speculated that recruitment was influenced by yearly ice conditions associated with a polynya appearing along the coast during summer (Hubold 1984).

Extending out from under the Filchner Ice Shelf, Filchner Trough is the largest of a series in the south-eastern Weddell Sea that include the Ronne and Hughes Troughs (Fig. 10.2). Glacial reconstructions show that the grounding line, which delineates the limit where the ice sheet rested on the continental shelf, was near that of the present-day since 5 ka (Hillenbrand et al. 2012, 2014). However at 25 ka, it was located at the shelf break or on the outer shelf; off the Brunt and Quar Ice shelves, it had already retreated from the shelf edge (Hillenbrand et al. 2014). Ice streams extended along Filchner Trough to the outer continental shelf, sourced from both West and East Antarctica (Elverhoi 1981; Bentley and Anderson 1998; Larter et al. 2012; Hillenbrand et al. 2014). Available age constraints have not confirmed that the trough was filled with grounded ice during the LGM rather than a prior time period (Stolldorf et al. 2012; Hillenbrand et al. 2014), and the grounding line may have been confined to the inner shelf along the Ronne Trough, the Filchner Trough, and the continental margin farther east. Nevertheless, ice shelves are thought to have extended across these regions. Retreat was asynchronous: parts of the East Antarctic Ice Sheet (EAIS) retreated earlier than the West Antarctic Ice Sheet (WAIS) and the outer shelf off the Filchner Trough and farther east was free of grounded ice by between 15 ka and 10 ka (Stolldorf et al. 2012; Hillenbrand et al. 2014).

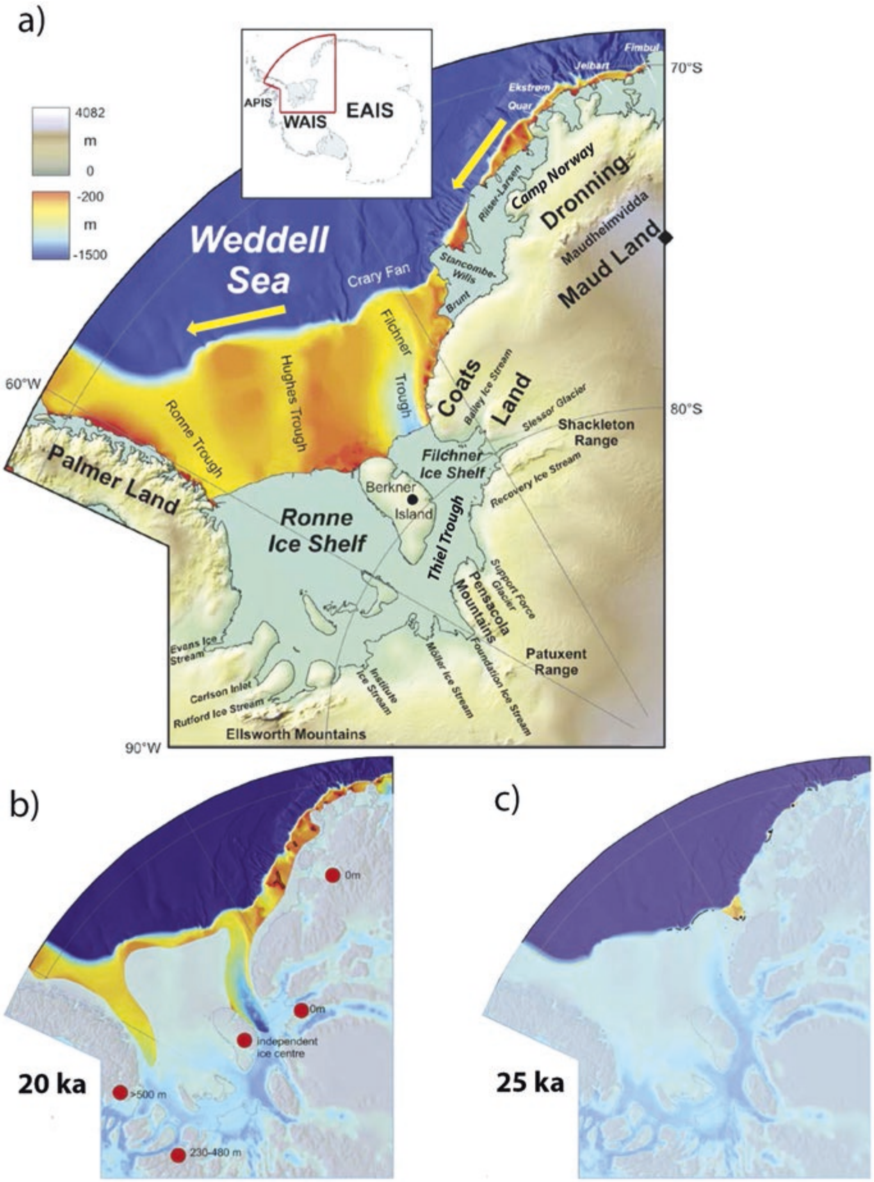


Fig. 10.2 South and southeastern Weddell Sea (Reproduced from Hillenbrand et al. 2014, Figs. 1, 8, and 12). (**a**) Shelf bathymetry and ice-sheet surface elevations (m above sea level). *APIS* Antarctic Peninsula Ice Sheet, *EAIS* East Antarctic Ice Sheet, *WAIS* West Antarctic Ice Sheet. *Yellow arrows* show direction of southern limb of Weddell Gyre. (**b** and **c**) Glacial reconstructions under alternative interpretations of available datasets (details in Hillenbrand et al. 2014): at 20 ka under Scenario A (**b**), at 25 ka under Scenario B (**c**)

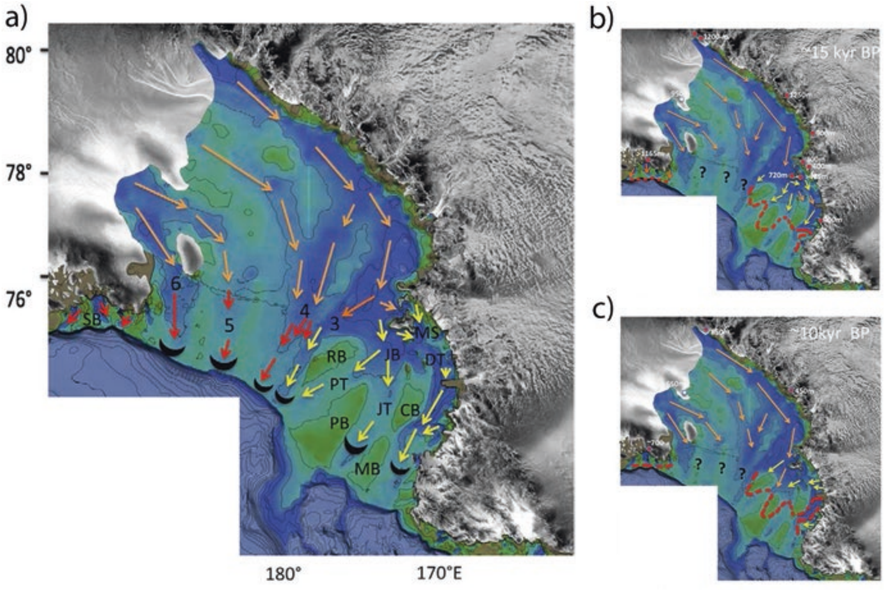


Fig. 10.3 Ross Sea (Reproduced from Anderson et al. 2014). (**a**) Bathymetry and paleo-drainage for maximum ice sheet advance showing ice draining East (*yellow arrows*) and West Antarctica (*red arrows*), with boundary at ca. 180° longitude. *Orange arrows* indicate inferred drainage in area beneath Ross Ice Shelf. – *Dark moon shapes* show grounding zone wedges; *MS* McMurdo Sound, *DT* Drygalski Trough, *CB* Crary Bank, *JB* JOIDES Basin, *JT* Joides Trough, *MB* Mawson Bank, *PB* Pennell Bank, *PT* Pennell Trough, *RB* Ross Bank, *SB* Sulzberger Bay. Trough numbering 3–6 using system by Mosola and Anderson (2006). *Insets*, also reproduced from Anderson et al. (2014), show inferred ice sheet elevations and grounding line (*dashed red lines*) in the western Ross Sea at (**b**) 15 ka and (**c**) 10 ka

In Terra Nova Bay, the David and Reeves Glaciers drain into the Drygalski Trough from the Transantarctic Mountains, directly offshore of the embayments where eggs and larvae were found in Silverfish Bay and the Gerlache Inlet. The Drygalski Trough is the largest of a series of cross-shelf troughs in the Ross Sea (Fig. 10.3). The region is also characterized by strong katabatic winds and a summer polynya, linked to considerable inter-annual variability in production that has been implicated in silverfish life history (La Mesa et al. 2010). However, an ice sheet occupied the Ross Sea embayment during the LGM, and it did not become largely ice-free until 7 ka (Licht et al. 1996; Hall et al. 2004; Anderson et al. 2014). Sourced from West and East Antarctica, the confluence was situated between Trough 3 and Trough 4 (numbering system used by Mosola and Anderson 2006) at approximately 180° longitude. The Drygalski Trough was occupied by ice streams draining from the EAIS, and the ice sheet was grounded north of Coulman Island (Anderson et al. 2014). Prior to 34 ka, an earlier ice sheet advanced to the edge of the continental shelf (Mosola and Anderson 2006). After the LGM, retreat followed a complex pattern with episodes of back-stepping and stability (Shipp et al. 1999; Domack et al. 1999; McKay et al. 2008). The grounding line reached the Drygalski Ice Tongue by 11 ka (Licht et al. 1996; Domack et al. 1999) but, outside of the trough, ice remained north of Terra Nova Bay until 8.2 ka (Baroni and Hall 2004) and did not retreat to the vicinity of Ross Island until ca 7.8 ka (Licht et al. 1996; Anderson 1999; McKay et al. 2008; Hall et al. 2013; Anderson et al. 2014) although more recent work suggests earlier deglaciation (McKay et al. 2016). By contrast, available age constraints suggest earlier retreat of the WAIS in the eastern Ross Sea where recently hatched larvae were found in the Bay of Whales (Brooks and Goetz 2014). The grounding line may have already been inshore of the shelf margin by the LGM, possibly forming a deep embayment (Licht et al. 1996; Bart and Cone 2012; Anderson et al. 2014).

Along the western Antarctic Peninsula (AP), the largest glacial feature on the shelf is Marguerite Trough, which extends from inside Marguerite Bay to the shelf break (Fig. 10.4, H). Donnelly and Torres (2008) caught 0+ age silverfish in Marguerite Bay during surveys in 2001 and 2002; large abundances of larvae and juveniles were found around the coastal fjords in the northern bay and inside George VI Sound. Nevertheless, the AP Ice Sheet (APIS) was grounded to the outer shelf edge during the LGM (Fig. 10.4) and contained ice streams that drained along cross-shelf bathymetric troughs (Pudsey et al. 1994; Evans et al. 2005; Heroy and Anderson 2005, 2007; O'Cofaigh et al. 2014). Off Marguerite Bay, the grounding line was located on the outer shelf and retreat was well underway by 14 ka (Kilfeather et al. 2011). Radiocarbon dating from glacial marine sediment indicated that retreat was initially slow between 13.5 and 12.6 ka across the outer to middle shelf but was rapid across the inner shelf around 9.6 ka (Bentley et al. 2011; Kilfeather et al. 2011). The larger inner fjords such as Neny Fjord were ice free by 9 ka (Allen et al. 2010) and the smaller fjords were ice free by around 6 ka (Simkins et al. 2013).

Farther to the north-east, Kellerman (1986) recorded silverfish post-larvae and juveniles in the Bransfield Strait and along the northern AP shelf during a series of surveys between 1976 and 1982. He hypothesized that larvae reach the Bransfield Strait by advection in water flowing from the north-western Weddell Sea around Joinville Island (e.g. Thompson et al. 2009). More recently, La Mesa et al. (2015) coupled otolith micro-increment data from larvae caught off Joinville Island with particle simulations from a circulation model, to predict where the larvae were spawned. They estimated two main areas of origin, one on the inner shelf off the Larsen A ice shelf connecting to the second at the shelf-break, both corresponding to the vicinity of the Larsen Inlet Trough (Fig. 10.4, L). The glacial reconstruction for the region (Evans et al. 2005; O'Cofaigh et al. 2014) shows ice streams that occupy the trough extending out to a maximum grounding position running along the shelf break (Fig. 10.4).

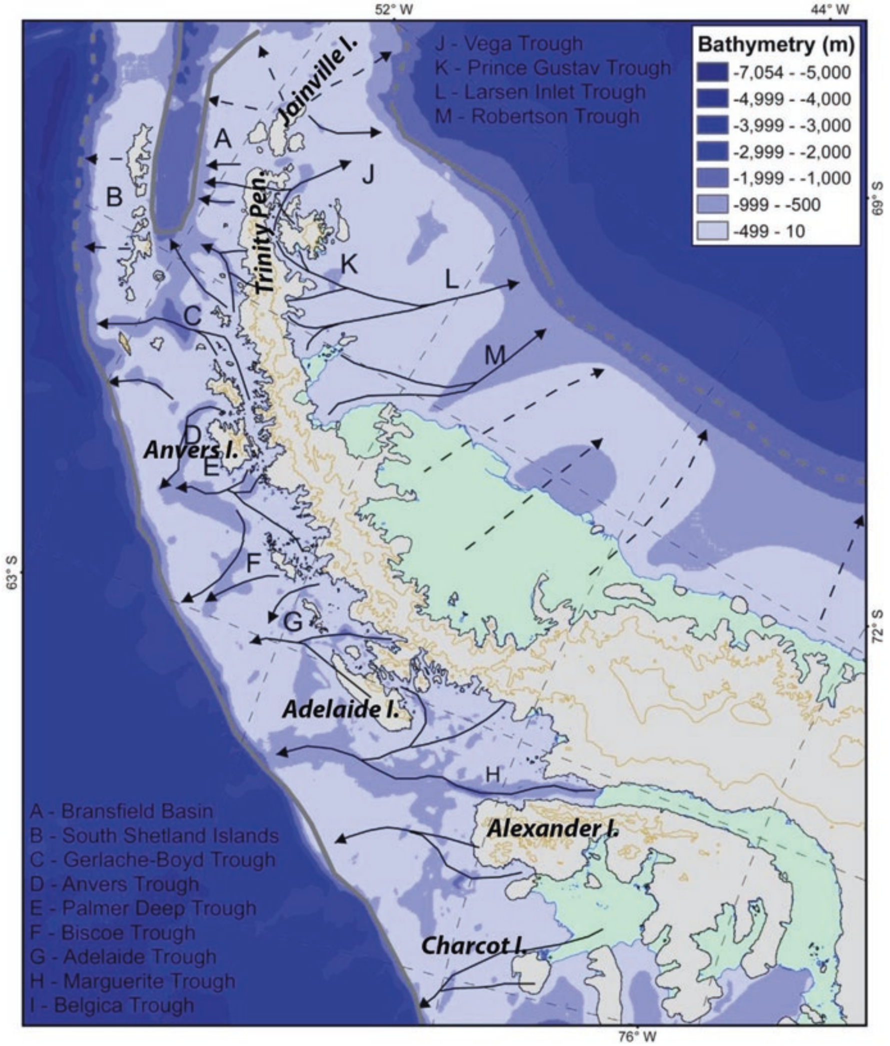


Fig. 10.4 Antarctic Peninsula (Reproduced from O'Cofaigh et al. 2014), showing reconstruction of ice streams and grounding line at LGM, with strong relationship to cross-shelf bathymetric troughs. *Arrowed lines* show ice-streams; *heavy grey line* shows LGM grounding line (*inferred where dashed*); modern ice shelves are shown in *light green*. *H* shows the Marguerite Trough system extending along Alexander Island from the ice shelf in George VI Sound (See also Fig. 10.9)

10.2.2 Geological Studies Document Widespread Distribution of Glacial Trough Systems Across the Shelf

Similar large trough systems occur across the shelf around the Antarctic and suggest present-day availability of silverfish habitat. In East Antarctica, the glacial trough in Lützow-Holm Bay reaches to the continental slope off Dronning Maud Land; Iceberg Alley and Nielsen Basin similarly dissect the shelf off Mac.Robertson Land (Mackintosh et al. 2014). Prydz Bay contains several large troughs, and glacial flow into the Amery Ice Shelf accounts for a substantial part of the drainage from the EAIS (Anderson 1999). Off Wilkes Land, the Mertz, Ninnis, Adelie and Clarie Troughs all originate at the mouths of ice streams draining the EAIS, and connect to the shelf edge (Mackintosh et al. 2014). Large trough systems are also found along the Pennell Coast (Anderson 1999), associated with ice flow from the northern edge of the Transantarctic Mountains and the EAIS.

Further east in Sulzberger Bay, drainage is from the WAIS. Similarly, the Dotson-Getz, Pine Island and Abbott Troughs in the Amundsen Sea, and the Belgica and Latady Troughs in the Bellingshausen Sea (Fig. 10.5), were all occupied by major ice streams that drained the WAIS and flowed across the continental shelf (Larter et al. 2014). North of Marguerite Bay, the AP is transected by systems that drain from the APIS. They include the Adelaide (Fig. 10.4, G), Biscoe and Anvers Troughs (Fig. 10.4, F and D respectively), and the Palmer Deep (Fig. 10.4, E), which may have been eroded by ice flowing in from the Gerlache Strait (Heroy and Anderson 2005). In the north-western Weddell Sea, the Prince Gustav Trough (Fig.10.4, K) is located to the north of the Larsen Inlet Trough, and the Robertson Trough (Fig. 10.4, M) is to the south (O'Cofaigh et al. 2014). By contrast, cross-shelf trough systems are relatively few in the southern Weddell Sea, because ice streams from the WAIS are directed eastward along the Thiel Trough into the Filchner Ice Shelf (Anderson 1999; Hillenbrand et al. 2014).

However, such habitat has varied considerably over time. Regional reconstructions show that, like the Weddell Sea and Ross Sea, ice extended to the edge of the shelf along the continental margin off much of East Antarctica including Wilkes Land (Mackintosh et al. 2014). Off West Antarctica, it extended to the shelf edge off Sulzberger Bay (Anderson et al. 2014) and along the Amundsen and Bellingshausen Seas (Larter et al. 2014), as well as the western and eastern AP (O'Cofaigh et al. 2014). Nevertheless, at 20 ka, the grounding line did not reach the shelf edge everywhere, and the local LGM and subsequent retreat were not synchronous (Anderson et al. 2002, The RAISED Consortium et al. 2014). The ice sheet retreat from Prydz Bay occurred by ca 13.5 ka (Domack et al. 1998) whereas, in Sulzberger Bay, most of the deglaciation is thought to have occurred in the last 11 ka (Anderson et al. 2014). In the Amundsen and Bellingshausen Seas, retreat varied considerably (Hillenbrand et al. 2010, 2013; Kirshner et al. 2012). It was well underway before 29 ka in the Belgica Trough (Hillenbrand et al. 2010), but not until 20 ka in the other major troughs (Larter et al. 2014). The grounding line retreated rapidly in the

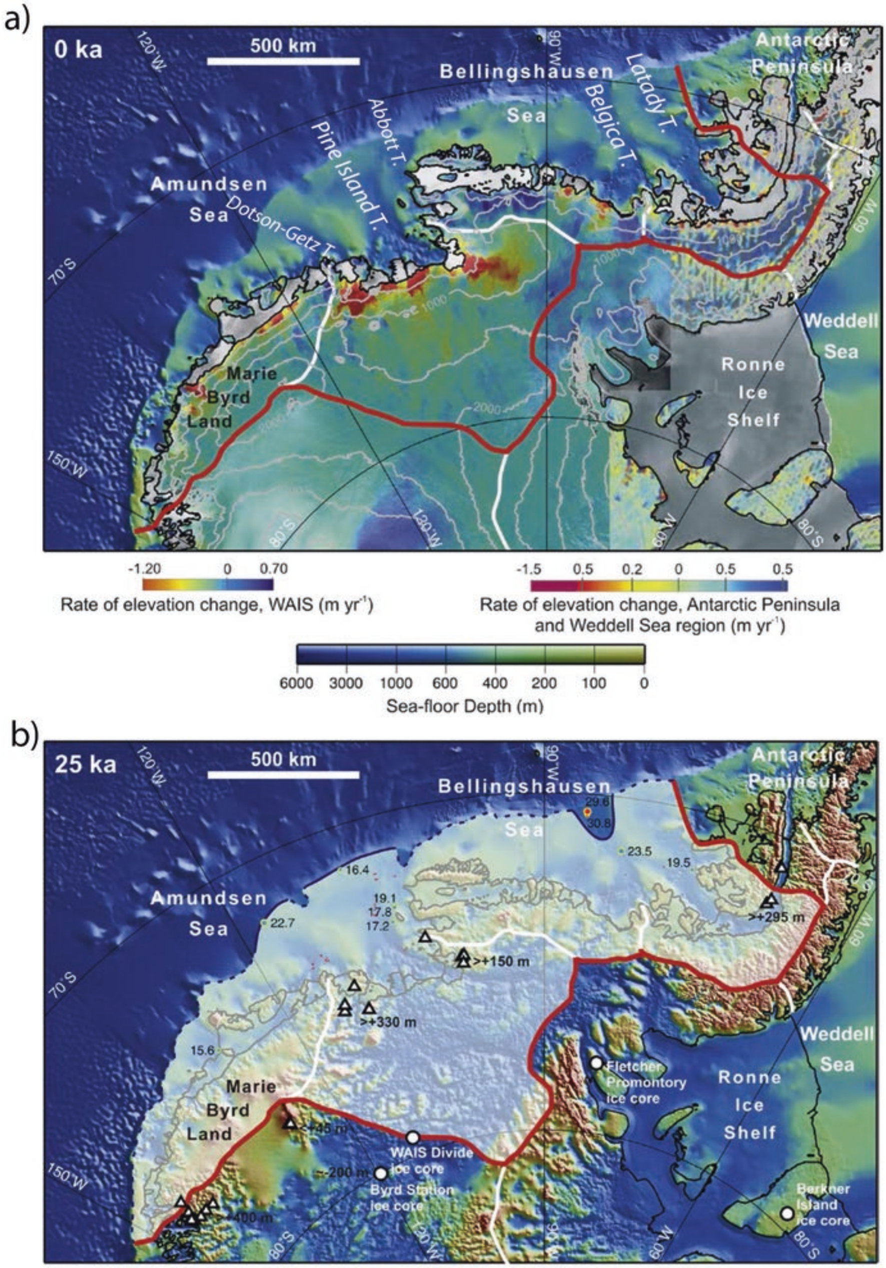


Fig. 10.5 Amundsen and Bellingshausen Seas (Reproduced from Larter et al. 2014). (**a**) Modern ice sheet configuration, showing surface elevation, ice sheets, and shelf bathymetry, (**b**) glacial reconstruction at 25 ka, showing extent of ice sheet as semi-transparent *white fill* and margin as *dark blue line* (*dashed* where less certain). *Thick white lines* mark major ice divides

Amundsen Sea to near modern limits by 10 ka (Lowe and Anderson 2002; Smith et al. 2011). By contrast in tributaries feeding into the Belgica Trough, it had reached the inner shelf in Eltanin Bay by 15 ka, but was very limited along the Ronne Entrance where it was still retreating at 5 ka (Larter et al. 2014). Initial retreat along the AP progressed southward over time; it also varied considerably between nearby troughs (O'Cofaigh et al. 2014). Retreat was earlier along the eastern AP than the western AP, and underway by 18 ka. From sampling in the Prince Gustav Channel, deglaciation and the transition to an ice shelf occurred prior to 11–12 ka (Evans et al. 2005). Grounded ice is likely to have been near its present configuration by 10 ka. However, fringing ice shelves remained more extended, with major retreat episodes suggested for the Larsen-A Ice Shelf between 3.8 ka and 1.4 ka (O'Cofaigh et al. 2014).

Recent consensus attributes thinning in modern ice shelves and retreat of grounded ice in ice streams to basal melting. Increasing inflow of warm Circumpolar Deep Water (CDW) along troughs, combined with topographic effects on ice flow and warm water inflow, may help explain the differences in retreat history between troughs (Larter et al. 2014). Topographic effects include bottlenecks and confluences in the configuration of ice streams, and submarine reverse slopes. These slopes are inclined towards the continent as a result of isostatic depression caused by the weight of the ice sheet that creates deep basins along the inner shelf (Larter et al. 2014).

10.2.3 Previous Studies Suggest Life History Associated with Ocean Circulation

Ocean circulation plays an important role in structuring populations across a range of Southern Ocean species (e.g. Loeb et al. 1993). The Antarctic Circumpolar Current (ACC) has been implicated in the transport of young Antarctic krill (*Euphausia superba*), entrained in fronts, from the western AP to South Georgia (e.g. Hofmann et al. 1998; Fach and Klinck 2006). According to simulations of the large-scale circulation, transport pathways connect widely separated regions of the Southern Ocean (Thorpe et al. 2007), and empirical studies suggest population structuring in *D. eleginoides*, a member of the neutrally buoyant clade of notothenioids, that is linked to the position of fronts and the direction of water flow (e.g. Shaw et al. 2004; Ashford et al. 2006, 2008). The Ross Gyre in the Southeast Pacific Basin (SPB) has also been implicated in the life history of *D. mawsoni* (Hanchet et al. 2008; Ashford et al. 2012). Matschiner et al. (2009) further pointed out that long larval and early juvenile pelagic stages, and connectivity generated by oceanographic currents, may promote gene flow around Antarctica in many notothenioid species. Damerau et al. (2012) found evidence of eastward gene flow in seven species from Elephant Island and the South Shetland Islands towards the South Orkney Islands. In the icefish *Chaenocephalus aceratus,* westward gene flow has been found as well (Papetti et al. 2009, 2012), and may be linked to an active post-larval stage (Ashford et al. 2010b). These results suggest that a complex interplay between oceanographic currents and life history features can act together to determine population structure, distribution and abundance across a range of notothenioid species.

Like La Mesa et al. (2010), earlier authors studying silverfish have linked dispersal from spawning habitat to regional circulation over the continental shelf. In the southeastern Weddell Sea, Hubold (1984) implicated surface Ekman transport induced by the katabatic wind field in transporting age 0+ post-larvae distributed over troughs to the offshore side of the coastal polynya and as far as the outer shelf edge. Moreover, he observed that post-larvae were largely absent over the Weddell Sea basin, and suggested that fish might be concentrated by the slope front, as well as locally by eddies. Kellermann (1996) proposed that, as well as connectivity from the north-western Weddell Sea (Kellermann 1986), extensive distributions of juvenile and adult silverfish found along the western AP shelf (e.g. Daniels and Lipps 1982) were the result of advection along the southern ACC from a source in the Bellingshausen Sea. He suggested advective pathways from the Bellingshausen to the shelf off the Biscoe and Palmer Archipelagos, and also inshore south of Anvers Island through the Gerlache Strait into the Bransfield Strait.

Genetic studies have also considered the potential effect of hydrography on silverfish connectivity. Zane et al. (2006) examined whether a pelagic life history and exposure to major circum-Antarctic currents resulted in weak population structure around the Antarctic. Genetic samples were taken in different years in the southeastern Weddell Sea off Halley Bay, in Terra Nova Bay and in the northern Bransfield Strait off Elephant Island and the South Shetland Islands. The results suggested considerable gene flow, but significant differences were found between years at Halley Bay and in a single comparison between Halley Bay and Terra Nova Bay. Similarly, Agostini et al. (2015) found evidence that supported geographic genetic structuring among silverfish sampled off the Larsen Ice Shelf, around Joinville Island, and along the southwestern AP, but also confirmed the weakness of genetic differentiation. They found genetic homogeneity between samples collected off the south-western AP at Marguerite Bay and Charcot Island and a weak net gene flow southward from the northern Peninsula. They suggested that these results, and considerable variability found between years, could be explained by dispersal from spawning areas into deeper water that exposes fish differentially to circulation features over the shelf. The resulting interplay between transport and retention determines spatial distributions along the AP.

Parallel studies found evidence corroborating connectivity along the south-western AP. Using the same fish as Agostini et al. (2015), Ferguson (2012) found differences in the trace element chemistry laid down in the otolith nuclei of fish sampled from the south-western AP and off Joinville Island in Antarctic Sound; however, there were no differences between fish from Marguerite Bay and off Charcot Island, consistent with a single population. Examining this further, La Mesa et al. (2015) found no structuring between Marguerite Bay and Charcot Island using reproductive metrics. Finally, Parker et al. (2015) found oceanic assemblages of invertebrates and fish mixing inshore over the western AP shelf, and silverfish strongly associated with neritic assemblages constrained along the northern and south-western coasts. They noted the contrast with prior distributions of silverfish, and suggested that a strengthening oceanic regime was consistent with increasing intrusion of CDW, notably along the Marguerite Trough (e.g. Dinniman and Klinck 2004). Building on the conclusions of Agostini et al. (2015), they hypothesized that, restricted inshore, dispersing silverfish are no longer exposed to circulation features over the outer shelf of the western AP, and this fundamentally alters their subsequent connectivity and distribution.

Taken together, these studies implicated physical-biological interactions between glacial trough systems, circulation and life history processes in shaping population structure and connectivity in silverfish, and hence gene flow and genetic differentiation. Moreover, compared to studies in other species, they highlighted the present-day importance of westward connectivity via the shelf circulation, and suggested diminishing influence of eastward transport in the ACC along the western AP slope. Because circulation is frequently constrained by topography, these interactions also suggest important associations between habitat and connectivity, ultimately structured by geological processes over successive glacial cycles.

10.3 Topography, Circulation and Connectivity Around the Antarctic Continental Shelf

10.3.1 Circumpolar Flow in the ACC

Frequently invoked as the major current shaping biological dispersal in the Antarctic, eastward flow in the ACC is concentrated in high-speed jets associated with fronts (e.g. Orsi et al. 1995, Fig. 10.6). The southern of these is the Southern ACC Front (SACCF), associated with shoaling of CDW towards the surface. The southern boundary of the ACC is located over the continental slope along the eastern Amundsen and Bellingshausen Seas (Orsi et al. 1995); off the western AP, flow along the slope is associated with the SACCF (Savidge and Amft 2009). The resulting eastward transport provides a potential pathway that can facilitate along-shelf movement of silverfish, consistent with Kellermann (1996). Connectivity with the shelf is facilitated by Upper CDW transported in the ACC that floods the shelf along the Peninsula. The intruding water mixes with intermediate Winter Water (WW) to form a deep layer of Modified CDW (e.g. Smith et al. 1999).

Downstream of the AP, the ACC southern boundary passes to the north of the South Orkney Islands (Fig. 10.7), where silverfish are found over the continental shelf (e.g. Jones and Kock 2009). However, further east, it moves far offshore, passing out into the southern Atlantic Ocean through a trough north of the South Sandwich Islands. Similarly, the SACCF is steered offshore by the Northwest Georgia Rise after looping around the eastern shelf of South Georgia.

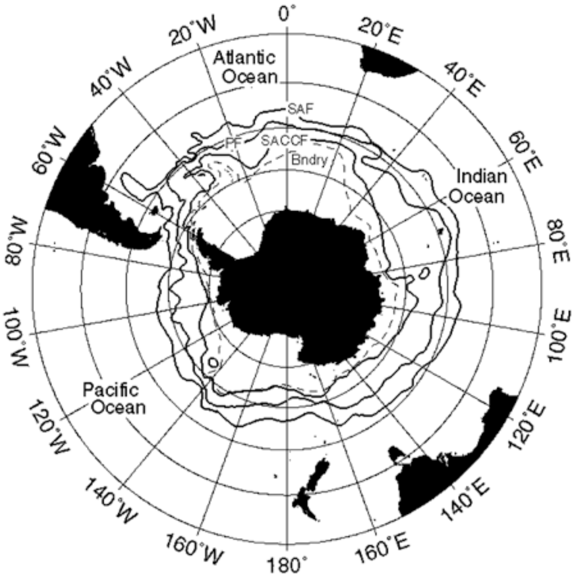


Fig. 10.6 The Southern Ocean (Reproduced from Ashford et al. 2008), showing the mean position of the main fronts in the Antarctic Circumpolar Current (ACC) (Orsi et al. 1995) in relation to West and East Antarctica. *SAF* Subantarctic Front, *PF* Polar Front, *SACCF* Southern ACC Front, *Bndry* southern boundary of the ACC

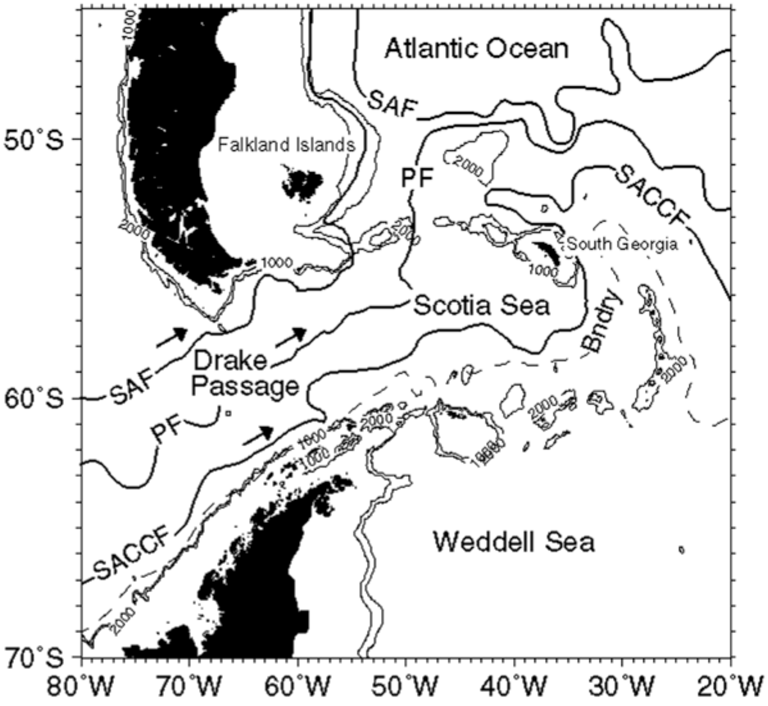


Fig. 10.7 The Drake Passage, Scotia Sea and southern Atlantic Ocean reproduced from Ashford et al. (2008), showing the frontal systems of the ACC (Orsi et al. 1995) in relation to the western Antarctic Peninsula and southern Scotia Ridge. Abbreviations as in Fig. 10.6

Silverfish have been found only rarely off South Georgia and the South Sandwich Islands (e.g. Trunov 2001). In contrast to the concentrations of krill, their sporadic occurrence argues that they are not entrained in the same ACC transport pathways in this region.

Continuing eastward, the ACC flows around the Weddell Gyre and southward, again bringing CDW close to the continental shelf along East Antarctica (Orsi et al. 1995). In the western SPB, the southern boundary follows the bathymetry along the Pacific-Antarctic Ridge: rapid shoaling of lower CDW marks the northward limit of the Ross Gyre, and it forms a warm, salty layer that reaches south to the continental slope. Further east, the ACC extends southward again, creating a bifurcation in the Amundsen Sea between eastward transport along the continental slope towards the AP, and westward transport along the eastern Ross Sea in the southern limb of the Ross Gyre. Thus, as a result of the large-scale circulation, these pathways can potentially transport fish larvae around the Southern Ocean. However, connectivity to habitats along the Antarctic continental shelf depends on the proximity of the southern boundary to the slope, its interaction with the shelf circulation, and the spatial availability of fish over their life history.

10.3.2 Along-Shelf Structuring

The southward extension of the ACC east of the Ross Gyre forces its eastward flow along the continental slope from the Amundsen Sea to the north-western AP. In sharp contrast, the flow of recirculating water along the southern limb of the Weddell Gyre is westward (e.g. Orsi et al. 1993; Loeb et al. 1993). In the western Weddell Basin, water flows northward (Fig. 10.8): in the Weddell Front off the continental slope; in the Antarctic Slope Front (ASF) along the slope; and in the Antarctic Coastal Current (CC) over the shelf (Thompson et al. 2009). Water transported in the Weddell Front flows around the Powell Basin to reach the South Orkney Islands, where Warm Deep Water (WDW) from the Weddell Sea is found over the southern shelf (Gordon et al. 2001; Heywood et al. 2004). The CC and ASF are steered by the topography to flow northwest around the tip of the Peninsula, forming transport pathways for along-shelf recruitment of silverfish from the Weddell Sea to larval aggregations in the Bransfield Gyre (Kellermann 1986; La Mesa et al. 2015). The two fronts merge at the mouth of the Bransfield Strait and a branch flows northeastward eventually into the southern ACC, while another forms a pathway that continues southwestward inshore along the Trinity Peninsula.

However, recent hydrographic evidence does not support extension of this advective pathway further southward through the Gerlache Strait, as suggested by Kellermann (1986). Similarly, the evidence does not support a strong advection pathway over the shelf south of Anvers Island and northward into the Bransfield Strait, as hypothesized by Kellermann (1996). Instead, westward flow off the northern AP forms the southwestward limb of the cyclonic circulation in the Bransfield

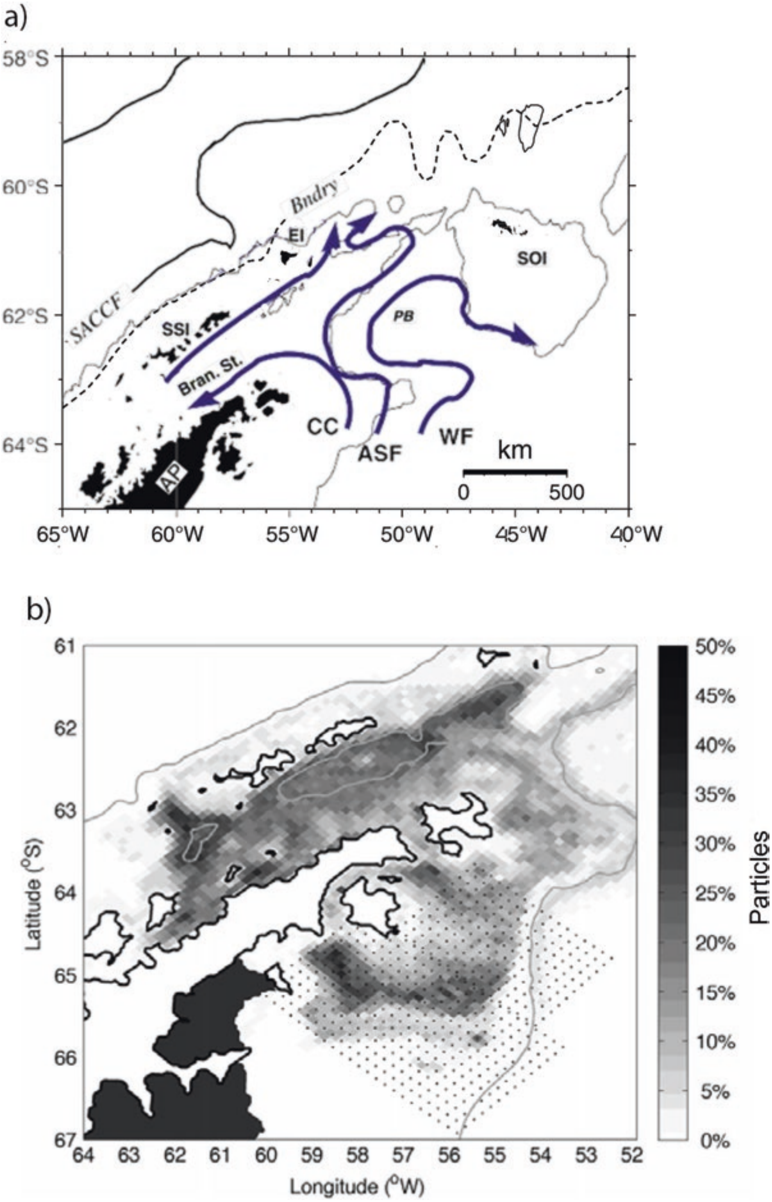


Fig. 10.8 The Western Weddell Sea and Bransfield Strait (**a**) Showing dominant currents, reproduced from Ashford et al. (2010b) and drawn originally from Thompson et al. (2009) (**b**) Distribution of simulated particles released along the eastern Antarctic Peninsula (Reproduced from La Mesa et al. 2015). Particles (%) indicate preferred transport pathways. *SACCF* Southern ACC Front, *Bndry* ACC southern boundary, *CC* Coastal Current, *ASF* Antarctic Slope Front, *WF* Weddell Front, *SSI* South Shetland Islands, *EI* Elephant Island, *SOI* South Orkney Islands, *Bran. St.* Bransfield Strait

Strait (Savidge and Amft 2009), and sharp changes in water properties (e.g. Smith et al. 1999) suggest a strong discontinuity that accounts for population structuring between silverfish found along the northern and southwestern AP (Ferguson 2012; Agostini et al. 2015; Parker et al. 2015). Minimal mixing between waters in the Gerlache Strait and south of Anvers Island, and a relatively shallow sill in the Neumayer Channel, create a barrier that prevents large-scale southward connectivity as fish age and move deeper during their migration. Nevertheless, mixing occurs between the water masses, and may account for the low levels of gene flow measured (Agostini et al. 2015). A strong western boundary current in the Bransfield Strait brings water from the Weddell Sea past the South Shetland Islands, potentially accounting for catches of silverfish in research trawls over the continental shelf (e.g. Jones et al. 2001).

Off the southwestern AP, Hofmann and Klinck (1998) described nearshore southward flow as part of a shelf-scale gyre, with circulation at the shelf-break dominated by the ACC. An independent southward circulation along the inner shelf has been described by Moffat et al. (2008). Strongly seasonal, the Antarctic Peninsula Coastal Current (APCC) is typical of coastal buoyant plumes with freshwater inputs largely from precipitation over the ocean and meltwater run-off from land (Moffat et al. 2008). A freshwater tongue in which the signature of WW is weak or absent extends laterally offshore to approximately 20 km, where a salinity front separates it from shelf waters. The velocity field shows a strong current co-located with the salinity front, extending to 150 m depth with surface velocities of 0.15–0.40 m s^{-1}. In winter, the formation of sea-ice and reduced horizontal gradients of seawater properties are associated with a shut-down of the APCC. Velocities rarely exceed 0.05 m s^{-1} and flow reverses episodically (Moffat et al. 2008).

The current creates a seasonal transport pathway along the inner shelf. The APCC reappears after the ice starts to retreat in late October, coinciding with spawning and hatching of silverfish. Based on drifter tracks (Beardsley et al. 2004), flow southward along Adelaide Island is thought to enter Marguerite Bay and exit around Alexander Island (Fig. 10.9). This generates a transport pathway that connects spawning in Marguerite Bay to areas where fish were caught over trough systems near Charcot Island. Based on evidence from otolith chemistry (Ferguson 2012), the fish are from the same population and movement occurs in the first year after hatching. The shallower distribution of young fish is consistent with the location of the APCC velocity front in the water column, ensuring exposure to westward transport. Moffat et al. (2008) pointed out that the front creates a favorable environment for biological production, suggesting that westward transport and foraging opportunities are correlated.

Indeed, coastal buoyancy forcing may be a general feature of silverfish population structure, connecting spawning areas to shelf distributions downstream. Dispersal along the APCC from spawning areas in Marguerite Bay is congruent with that predicted by La Mesa et al. (2015) for the northern Peninsula in which the CC connects spawning in the vicinity of the Larsen Inlet Trough to silverfish distributions around the Bransfield Strait. Moreover, APCC flow southward past Adelaide Island is thought to originate in the vicinity of Anvers Island (Moffat et al. 2008) and

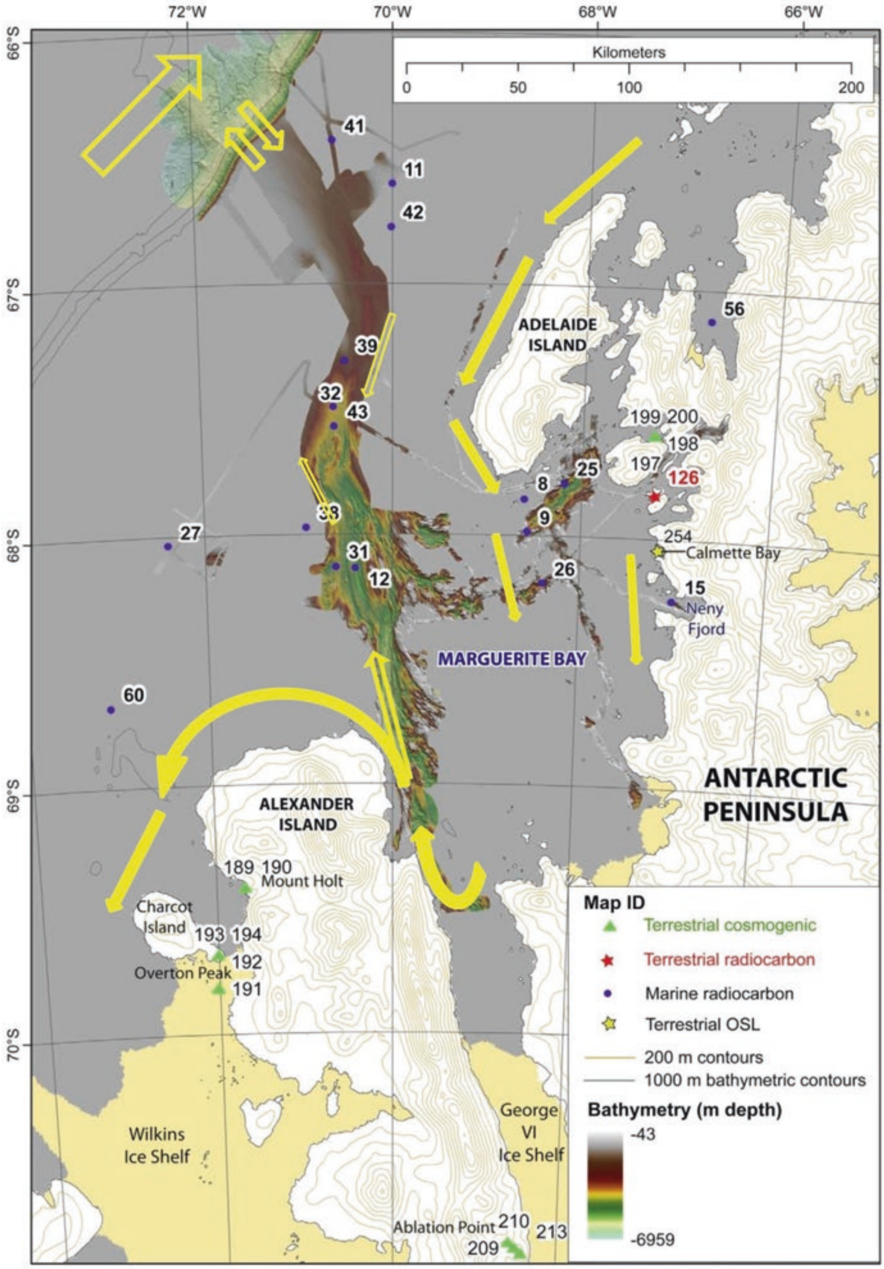


Fig. 10.9 Marguerite Trough showing bathymetry and location of marine radiocarbon samples (Reproduced from O'Cofaigh et al. 2014), with schematic of the shelf circulation. Net trough inflow and outflow (*transparent arrows at mouth*) based on Dinniman and Klinck (2004); location of the APCC (*filled arrows*) is drawn from Moffat et al. (2008). *Large arrow* indicates direction of flow over the shelf slope along the southern boundary of the ACC

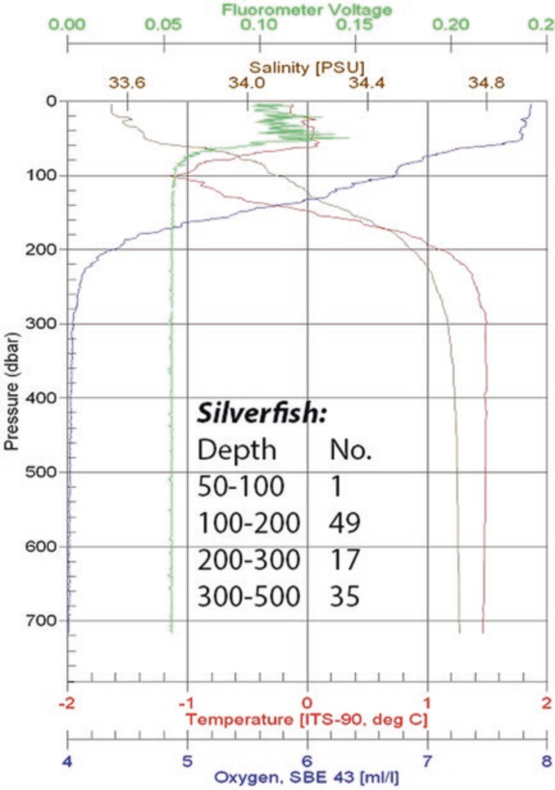
a similar system may have operated historically along the mid-Peninsula. Thus, Parker et al. (2015) argued that a locally reproducing population would account for historic concentrations of silverfish south of Anvers Island, spawning under fast ice close to one of the trough systems that transect the AP at this latitude. Flow along the APCC that entrained young fish foraging in the surface layer would have connected spawning areas to distributions over the mid-Peninsula shelf. In conclusion, recent studies around the AP suggest that silverfish distribution and population structure may be closely related to the location and transport of coastal buoyancy plumes, and hence precipitation and meltwater run-off from ice-sheets along the shelf.

10.3.3 Cross-Shelf Structuring

If along-shelf flow from spawning habitat can help explain circumpolar distributions of silverfish over the shelf, how then do sufficient numbers of adults return to spawning areas to maintain individual populations? Consistent with Parker et al.'s (2015) argument for localized reproduction along the western AP, cross-shelf flow following the bathymetry along troughs may provide a recirculating mechanism. Inertia forces water onto or off the shelf where the shelf-break curves, notably off the Marguerite Trough and into the Biscoe and Anvers Troughs (Dinniman and Klinck 2004). The deeper, nutrient-rich Upper CDW driven upward by the shallowing bathymetry connects via the on-shelf flow to cross-shelf circulation. Off Marguerite Bay (Fig.10.9), cross-shelf flows at depth (Dinniman and Klinck 2004) contrast with broad jets in the upper layer that extend to a depth of 200 m (Savidge and Amft 2009). These follow the northeastern flank of the trough system inshore and the southwestern flank in a seaward direction at velocities of ca. 0.05 m s^{-1}. In the Marguerite Trough, adult fish distribute throughout the water column below 100 m, and notably around the interface where CDW and WW mix between 100 and 200 m (Fig. 10.10, Ferguson 2012). Water following bathymetric contours along the trough sides potentially facilitates connectivity between productive areas associated with mixing CDW, and spawning areas near the head of the trough. Other retention features may contribute. Piñones et al. (2011) found in simulations that cyclonic circulation in Laubeuf Fjord retained particles in deeper water, associated with a productivity hotspot. Moreover, the mean circulation field suggested retention of younger stages in a cyclonic eddy formed in the surface layer of Marguerite Bay, where offshore flow meandered to join inflowing water along the northeastern side of the trough (Piñones et al. 2011).

Interactions between flows across and along shelf may be important to connectivity between populations as well. Because intrusions of CDW are episodic and vary in intensity, the frequency and extent of their penetration are likely to influence the distance that silverfish disperse along the trough. Agostini et al. (2015) hypothesized that dispersal from spawning areas into deeper water exposes fish to circulation over the shelf, and the resulting interplay between transport and retention

Fig. 10.10 Distribution of silverfish with depth and water properties in Marguerite Trough (latitude 068° 09.11, longitude 069° 59.63), April 2010. Volume of water sampled was approximately proportional to depth range



determines population structure and distribution. Variability in the frequency and penetration of CDW along the Marguerite Trough may regulate silverfish exposure to flow eastward along the shelf break, and hence connectivity with trough systems further to the northeast. Congruent with Kellermann's (1996) advective pathway along the southern ACC to south of Anvers Island, such a mechanism could help account for the historic observations of silverfish concentrations by Daniels (1982), with fish potentially sourced from troughs located upstream along the shelf as far as the bifurcation in the Amundsen Sea. Conversely, a strengthening oceanic regime that constrains silverfish distributions away from the slope (Parker et al. 2015), also shifts the population away from eastward connectivity along the ACC. Nevertheless, westward connectivity remains viable along the APCC. Water flowing along the shelf inshore becomes entrained in the trough circulation, providing a pathway for migration and gene flow between populations.

In the western Ross Sea (Fig. 10.11), similar considerations apply but with important differences. Inflow from the shelf-break follows the bathymetry along the west side of the series of banks oriented north-south on the shelf, with outflow on the eastern side (Dinniman et al. 2003). Lower CDW modified by mixing with AASW floods onto the shelf at the shelf-break in the vicinity of troughs separating the banks

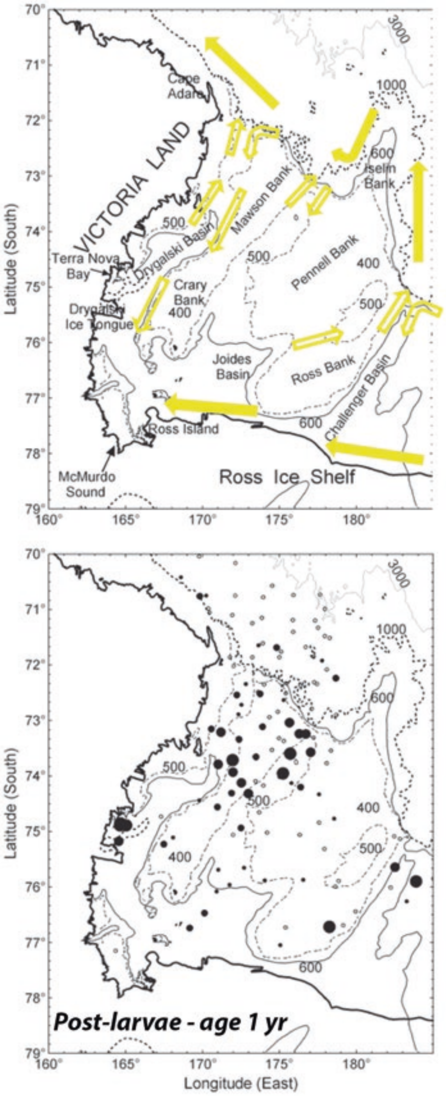


Fig. 10.11 Distributions of young silverfish in the upper water column of the western Ross Sea (Reproduced from La Mesa et al. 2010), in relation to the shelf circulation. *Upper left panel* shows schematic of westward flow along the Ross Ice Shelf and in the Antarctic Slope Current (*filled arrows*), and cross-shelf flow in inflows and outflows along troughs (*transparent arrows*). Other panels show standardized abundances of silverfish at pre-flexion, post-larval and juvenile stages in net tows that sampled to a maximum depth of between 130 and 300 m

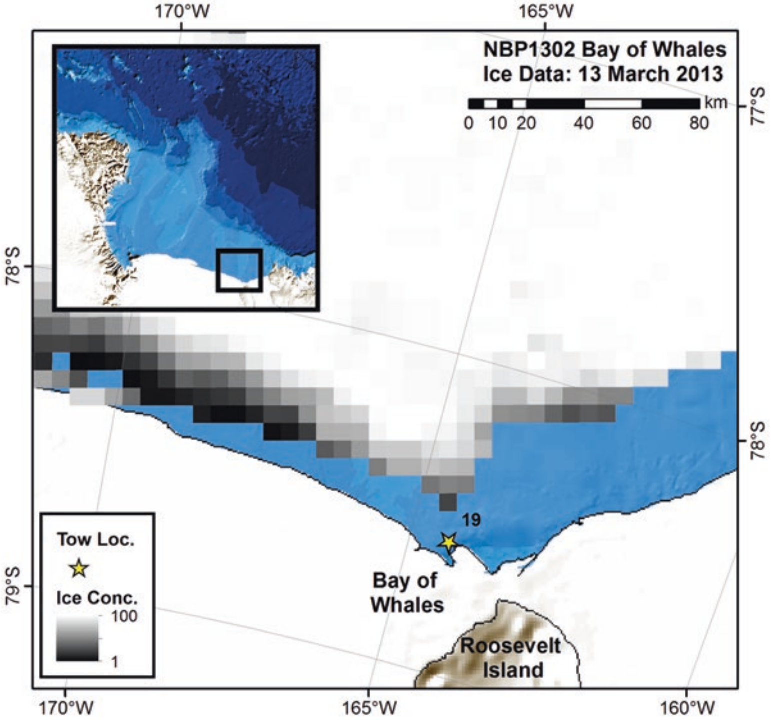


Fig. 10.12 Bay of Whales, showing ice concentration and site sampled by Brooks and Goetz (2014)

(e.g. Stover 2006; Orsi and Wiederwohl 2009). It meets equatorward flow of dense, cold High Salinity Shelf Water (HSSW), mixing to form Modified Shelf Water (Gordon et al. 2009). Consistent with La Mesa et al. (2010), young silverfish dispersing into the Ross Sea polynya from Terra Nova Bay encounter shelf outflow that follows the western side of the Drygalski Trough, facilitating movement to productive areas associated with inflowing nutrient-rich modified CDW. As fish age, they move deeper, away from surface waters. However, rather than returning fish to the spawning areas near the head of the trough, the inflow attenuates along the Crary Bank and into the Drygalski Basin, from where shelf outflow can facilitate movement back past Terra Nova Bay. Another difference from Marguerite Trough is that flow along the slope is westward in the Antarctic Slope Current (Dinniman et al. 2003; Locarnini 1994; Rickard et al. 2010). As a result, although silverfish can disperse in counter-clockwise flow around the Mawson and Crary Banks, eastward connectivity is severely constrained no matter how far fish disperse out over the shelf.

Similarly, in the eastern Ross Sea, cross-shelf circulation along bathymetric contours can facilitate movement between spawning in the Bay of Whales (Fig. 10.12) and feeding areas along the eastern troughs. Westward transport occurs along the Antarctic Slope Front, and a current that flows along the front of the Ross Ice Shelf. However, any eastward connectivity is severely constrained by the circulation.

10.4 Developing the Life History Hypothesis

10.4.1 Variability in the Structure of Marine Fish Populations

The previous section illustrates how physical-biological interactions can help resolve issues over connectivity that challenge the life history hypothesis. The present-day structure and hydrography of major trough systems provide a series of mechanisms that can help explain how an inactive, energy-efficient species moves between pelagic habitats occupied over its life history, and eventually returns to spatially constrained spawning areas necessary for a cryopelagic egg stage. Even before invoking behavior, predictable transport pathways along the shelf suggest how circumpolar distributions of silverfish can be maintained.

Nevertheless, a life cycle structured by hydrography implies considerable leakage. Biology can play a mitigating role through reinforcing behavior as well as physiological adaptation. For instance, as indicated by Moffat et al. (2008), fronts with rapid velocity shear are often zones of high productivity; fine-scale movement relative to feeding opportunities can facilitate entrainment in transport pathways supporting life history connectivity. Moreover, silverfish are unusually fecund for a notothenioid species, which renders them resilient under changing environmental conditions (La Mesa et al. 2014). It can also be seen as an adaptation to offset advective losses over their life history. Observations recorded by Daniels and Lipps (1982) among others suggest shoaling and aggregating behavior in adults as well, and active shoreward movement at spawning time. Active swimming that reinforces transport inshore would serve to optimize energy costs and reduce advective mortality.

The link between population structure and physical barriers has been widely recognized. Frontal systems, depth, or changes in gradients in water properties can obstruct directed movement and create spatial separation (e.g. Kingsford 1993; Loeb et al. 1993; Shaw et al. 2004). As a result, environmental variability can lead to changes in abundance through variation in mortality rate or self-recruitment, with their extent delineated by population boundaries. Yet ocean circulation can produce more complex population structure. Retention features can structure population diversity (Iles and Sinclair 1982), and currents that connect disparate areas can create opportunities for succeeding stages during the life history (e.g. Harden Jones 1968). But population membership can only be maintained if these trajectories eventually allow return to spawning areas (Sinclair 1988; Sinclair and Iles 1989), either by actively migrating adult stages (e.g. Cushing 1981) or facilitated by transport. Alternatively, fish can be entrained in trajectories that do not allow closure, becoming either non-breeding vagrants (Sinclair 1988) in habitats where abundance is determined by immigration and local mortality, or migrants if they eventually join another population to spawn. In the latter case, source populations with high recruitment relative to mortality can maintain sink populations in which self-recruitment is insufficient to offset mortality (Pulliam 1988; Polachek 1990). Interruptions to net migration can lead to extinctions of sink populations, even without local changes in births and deaths. As a result, variation in the spatial and temporal delivery of life stages between habitats can impact abundance and distribution over large areas.

Physical-biological interactions like these affect genetic differentiation and gene flow. High fecundity and early mortality can enhance the effect of genetic drift (David et al. 1997) in situations where many individuals fail to contribute to recruitment, and stochasticity associated with reproduction influences the proportion of individuals that contribute to the next generation (Hedgecock 1994). Several factors, including local oceanographic features such as troughs and upwelling nutrient-rich areas that exert temporary spatial constraints on individuals, can influence the success of parents and therefore the genetic composition in the pool of recruits (Aglieri et al. 2014). Among the consequences of variable reproductive success are a reduction in the effective genetic population size compared to census size, and a pattern of temporal genetic patchiness (unpatterned genetic heterogeneity) among local populations on a small spatial scale (Pujolar et al. 2009). Notably, both these effects have been observed in silverfish. Agostini et al. (2015) estimated the effective population size of silverfish collected off the western AP to be about 6500–11,000 individuals. They also found increasing differentiation over time between the southwestern and northern AP, and differences among length classes at Joinville Island. Zane et al. (2006) found a similar result for the Weddell Sea, with samples collected in 1989 differing significantly from samples collected in 1991.

Physical and population processes can interact with gene flow in complex ways. Populations exposed to divergent selection pressures can develop local adaptations even in the presence of high levels of gene flow (Canino et al. 2005; Hemmer-Hansen et al. 2007). Genetic differences at loci under selection often correlate with environmental variables (e.g. Milano et al. 2014), suggesting that there is a cost in terms of mortality associated with significant gene flow between locally adapted populations (Zane 2007). Similarly, the genetic-environment association (GEA) hypothesis (Bierne et al. 2011) predicts pre- or post-zygotic genetic incompatibilities that are independent from adaptation to habitats, and can determine a genetic load during immigration and successful reproduction. The GEA hypothesis has been recently invoked to explain growing evidence from genome-wide scans of genetic markers that show a much higher level of differentiation than expected under conditions of strong gene flow (Bierne et al. 2011). When incompatible genetic backgrounds come into contact, endogenous barriers negatively affect the fitness of immigrant offspring, increasing mortality and preventing the flow of certain combinations of alleles (Milano et al. 2014).

Moreover, range changes through expansion and contraction (e.g. MacCall 1990) can alter the spread and retention rate of mutations under selection pressure. They can also generate contact zones between populations that are adapted to different selective pressures or are genetically incompatible. In these cases, connections between divergent local populations can result in stable metapopulations in which genetic diversity is preserved at the cost of increased local mortality and extinction rates. Conversely, gene flow through immigration can homogenize differences between populations over the long term. The level of migrants needed to achieve homogenization is as low as one migrant per generation at equilibrium in an

Infinite Island Model (Waples and Gaggiotti 2006; see also Agostini et al. 2015 for silverfish in the AP). The evidence of weak but significant genetic differentiation in many Antarctic fish implies an ongoing exchange that is nevertheless insufficient to counteract genetic drift: in these situations, small increases in the level of connectivity can rapidly cause the differences to disappear, strongly impacting genetic structure.

However, rather than greater connectivity, recent observations in the AP suggest an increasing pattern of isolation for silverfish (Parker et al. 2015). Thus, in a stepping stone-type scenario, genetic differentiation between previously homogeneous adjacent populations seems reasonable, especially if temperature-related reductions in larval-stage duration (O'Connor et al. 2007) alter the complex spatial mechanisms of recruitment and connectivity between locations (Patarnello et al. 2011). Nevertheless, the accumulation of genetic differences through drift is extremely slow in populations of thousands of individuals. Ecological populations are generally nested within larger genetic units (Lowe and Allendorf 2010), since the amount of migration needed to maintain genetic homogeneity is far smaller than the lower bound required to render them ecologically independent (Bortolotto et al. 2011). As a result, inferences of genetic homogeneity should not be interpreted as implying the existence of a single ecological unit for management purposes. But conversely, heterogeneity can have complex causes: as well as discrete populations with independent recruitment and mortality, temporal genetic patchiness or mixing between two or more populations in different proportions can generate spatial variation in allele frequencies. Local populations that are connected, for instance in a metapopulation, also imply vital rates that are not independent, in which changes in connectivity can fundamentally influence their genetic structure and diversity.

10.4.2 Silverfish Population Structure: Variability Around the Antarctic

General theoretical considerations like these, taken with empirical evidence of interaction between physical forcing and life history processes, argue strongly for a richly diverse population structure in silverfish around the Antarctic. So far, there has been no comprehensive study at different spatial scales and over time. However, the series of studies from the AP indicate separation between Marguerite Bay and the northern Peninsula, with coherent feeding and spawning areas centered around the Marguerite Trough system. Similarities with fish found off Charcot Island can be explained by advection of young stages in the APCC along the coast of Alexander Island, but the lack of a return pathway suggests the Charcot fish are non-breeding vagrants. Lack of differentiation in the otolith trace element chemistry (Ferguson 2012) argues against mixing with a population reproducing locally off Alexander Island. Similarly, lack of any return pathway to the northern Weddell Sea suggests that fish reaching the Bransfield Strait and the shelf off the South Shetland Islands are vagrants. Low levels of gene flow are consistent with some southward emigration via hydrographic mixing in the Gerlache Strait. Emigration into the Bellingshausen and Amundsen Seas may also occur from the southwestern population by advection along the APCC. However, it remains unclear how far the coastal plume extends. It also remains unclear whether the trough systems downstream support suitable spawning areas that might account for distributions of silverfish caught off Marie Byrd Land and in the eastern Ross Sea (Donnelly et al. 2004).

In the northern Weddell Sea, the simulation study by La Mesa et al. (2015) predicted that larvae advected around Joinville Island largely originated in the Larsen Inlet Trough, suggesting a source population with a coherent life history. Nevertheless, Agostini et al. (2015) also found temporal instability in genetic frequencies. They hypothesized that physical-biological interactions during dispersal over the shelf may shape the genetic patterns observed, generating variability and hence genetic patchiness. However, the study also noted that the instability could reflect mixing with another unsampled population. Under this hypothesis, prevailing flow and the presence of several trough systems along the shelf imply advection from two or more source populations, themselves related through migration via flow along the coast and shelf slope. However, it has yet to be documented whether populations occupy the Prince Gustav, Robertson and Jason Troughs; or whether fish in Antarctic Sound represent a separate system with outflow into the southern Bransfield Strait. Although current evidence cannot discount genetic instability due to pulses of immigration from unsampled populations, the differences were weak and the hypothesis requires further testing.

Distributions of silverfish in the southeastern Weddell Sea gave a clearer indication of unidirectional movement between coherent populations linked to trough systems along the shelf. Hubold (1984) observed that warmer water reaches the shelf edge at depths of 500 m, and the troughs contained warm water at depth. He also noted a large cyclonic gyre over Filchner Trough. He hypothesized its involvement in the distribution of silverfish larvae. Thus, the gyre and an eddy system off Camp Norway represent retention areas for young fish following local hatching; dispersed over the shelf by cyclonic water movement and the katabatic wind field, they collect along the slope front accounting for concentrations found 30 km from shore. Moreover, consistent with westward connectivity, Hubold (1984) found post-larvae distributed over troughs along the shelf from Camp Norway to the Filchner Ice Shelf, as well as smaller numbers of 1 + yr. and adult fish. While the survey did not extend west of Filchner Trough, the relative paucity of trough systems along the southern Weddell Sea argue that any fish dispersing further westward may reach neighbouring populations only rarely, and mostly become vagrants instead.

By contrast, fish around the South Orkney Islands appear isolated. Although water transported in the Weddell Front flows around the Powell Basin to reach the South Orkney Islands, the obstacles to entrainment of silverfish in the current off the continental shelf and the distances involved make connectivity doubtful from either the northwestern or southeastern Weddell Sea. Evidence from otolith chemistry indicated structuring between fish caught in Antarctic Sound and over the South Orkney shelf (Ferguson 2012). By comparison, there was no differentiation along the shelf south of Coronation and Laurie Islands, suggesting that silverfish around the South Orkney Islands represent a separate and currently discrete population.

In the Ross Sea, the Antarctic Slope Current and the current along the ice shelf suggest pathways for westward advection from the eastern trough systems. However, Zane et al. (2006) found no genetic instabilities in the western Ross Sea. Moreover, homogeneity in the otolith nucleus chemistry of fish caught along the Drygalski Trough, Crary and Ross Banks (Ferguson 2012) corroborated a lack of mixing from the Bay of Whales and further east. The lack of connectivity suggested that either distances were too large relative to flow velocity; or alternatively, that fish in the Bay of Whales were spawned late in the season and seasonal flow along the ice shelf was limiting. Further west, outflow from the mouth of the Drygalski Trough and westward along the shelf may promote connectivity with extensive silverfish distributions found along East Antarctica (La Mesa and Eastman 2012). However, distances are large and, away from the surface, much of the outflow at the Drygalski mouth contributes to gravity flows that cascade down the slope (Gordon et al. 2009). Instead, recent evidence points to a single, discrete population in the western Ross Sea, centered on spawning in Terra Nova Bay. Several return pathways may be involved, facilitated by cross-shelf flow along bathymetric contours. Adult silverfish caught in trawls in the Drygalski Basin, acoustic backscatter indicating others in concentrations along the Drygalski and Joides Troughs, and comparatively large densities of juveniles along the Joides Trough (O'Driscoll et al. 2011), suggest trajectories along the Drygalski Trough, around the Mawson and Crary Banks, and around the Drygalski Basin.

10.4.3 Silverfish Population Structure: Variability over Time

The interaction between physical and life history processes also provides insight into how silverfish populations may have persisted over successive glacial cycles without giving rise to further phylogenetic diversification. Although current spawning areas and most of the continental shelf were covered during large periods, system shifts along troughs during ice sheet retreat and subsequent advance may have shaped complex and dynamically changing populations. Newly hatched fish in the Bay of Whales (Brooks and Goetz 2014) (Fig. 10.12) and in Gould Bay and Vahsel Bay (Hubold 1984) demonstrate that indentations along ice-sheets can provide the conditions necessary for spawning. As evident in Terra Nova Bay, active ice streams can help maintain ice distributions during ice-sheet retreat, creating embayments on either side with a source of meltwater and protection from near-shore advection. The relationships between CDW intruding into the deep basins inshore and the retreat of ice streams and break-up of ice-shelves along the AP (e.g. Dinniman et al. 2012; Larter et al. 2014) suggests that, as silverfish spawning areas retreated across the shelf, productive feeding areas increasingly advanced up troughs, mitigating variability in the distance between silverfish feeding and spawning grounds.

As ice sheets have retreated close to the coast, however, populations have become less exposed to circulation features associated with the outer shelf. Movement along the shelf-break and slope is reduced, and interaction with buoyancy-forced features inshore dominates connectivity instead. Increasingly, structure and processes at the head of the trough are important to the persistence of spawning areas: glacial dynamics, including the size and variability of ice flow and meltwater, and the configuration of the shoreline influence the predictable formation of fast ice. Along the western AP, such a mechanism is consistent with Parker et al.'s (2015) hypothesis that the recent disappearance of silverfish was due to extinction of a population south of Anvers Island as a result of regional warming. Recent failure of recruitment at Marguerite Bay suggests a similar extinction in process. Parker et al. (2015) also suggested a spatial connection between the two populations. Reductions in eastward connectivity could have contributed to the decline off Anvers Island if persistence depended on northeastward immigration from Marguerite Bay in a source-sink relationship.

The contrast with strong continuing recruitment in the Ross Sea implies variation at regional scales. Regions like the western AP that are strongly influenced by warming (e.g. Stammerjohn et al. 2008) show a higher rate of extinction related to the timing and intensity of succeeding cycles. Populations in regions like the Ross Sea where present-day warming trends lag, or that occupy deeply indented systems with continuing strong ice-streams, tend to survive as sources for westward connectivity. Moreover, the glacial reconstructions show regional variation in the timing of deglaciation that suggests differential changes in habitat availability, population structure, and connectivity during ice sheet retreat. Thus, the rapid retreat of the APIS across the inner shelf of Marguerite Bay around 9.6 ka (Bentley et al. 2011) meant that the deep inshore basins along the western AP were available before those in the Ross Sea. Retreat of the EAIS from Prydz Bay by ca 13.5 ka (Domack et al. 1998), and of the WAIS by 10 ka in the Amundsen Sea (Lowe and Anderson 2002), indicate much earlier availability than in Sulzberger Bay, where retreat occurred mostly in the last 11 ka (Anderson et al. 2014). In Belgica Trough, continuing retreat along the Ronne Entrance at 5 ka (Hillenbrand et al. 2010; Larter et al. 2014) suggests changes in habitat and circulation, with ongoing effects on silverfish abundance and life history connectivity.

Conversely, as ice sheets advance, it seems reasonable that many of these dynamics reverse. Changes in meltwater input would reduce buoyancy-driven westward connectivity during summer, just as currently during winter. With decreasing intrusion of warm water along troughs, feeding areas of surviving populations would trend outwards across the shelf, exposing fish to circulation features that provide new opportunities for connectivity and colonization. Consistent with the hypothesis of Parker et al. (2015), eastward connectivity could promote re-colonization of the Anvers and Biscoe Troughs as spawning areas became viable again after the warming phase ends. By subsidizing births, immigration from Marguerite Trough would stabilize any newly colonized population south of Anvers Island. Alternatively, consistent with Kellermann's (1996) hypothesis, connectivity eastward along the ACC could contribute vagrants from troughs in the Bellingshausen or Amundsen Seas to south of Anvers Island, accounting for historic distributions even without local self-recruitment. Similar considerations apply in the Weddell Sea. Katabatic activity further out over the slope and increasing exposure to circulation features offshore could promote westward connectivity to troughs downstream along the ASF, and colonization of the South Orkney Shelf along the Weddell Front.

At the LGM, however, the geological reconstructions demonstrate that the ice grounding line reached the shelf-break along large areas of the AP, Weddell and Ross Seas, as well as the Bellingshausen and Amundsen Seas and East Antarctica (Anderson 1999; O'Cofaigh et al. 2014; Hillenbrand et al. 2014; Larter et al. 2014; Anderson et al. 2014; Mackintosh et al. 2014; other references therein). An ice shelf is thought to have covered the Bransfield Strait, and the South Shetland Ice Cap extended onto the outer continental shelf (Simms et al. 2011). The resulting exposure to ice conditions and oceanic water at trough mouths implies a new set of extinction threats, with changes in the structuring of fast ice and the areas of productivity available, as well as the distances between them. Nevertheless, the sedimentary evidence indicates that the grounding line at the LGM may have been strongly indented inshore from the mouths of the Filchner and Drygalski Troughs (Hillenbrand et al. 2014; Anderson et al. 2014). In these systems, residual cross-shelf exchange may have allowed populations to persist, while others went to extinction. Re-colonization could then resume as the ice-sheet retreated back across the shelf once more.

Genetic data provide some corroborating evidence. Based on mitochondrial DNA sequencing and analysis of mismatch distribution, silverfish experienced a population expansion in the past (Zane et al. 2006). However, the timing needs clarification. The starting of the expansion was originally dated to about 120 ka, during the last (Eemian) inter-glacial period. This was revised to about 200 ka, corresponding to the beginning of the previous glacial period, after incorporation of new fossil and geological evidence in the molecular clock calibration (Janko et al. 2007). A concordant pattern of population expansion has been reported for Antarctic krill (Zane et al. 1998; Goodall-Copestake et al. 2010), suggesting a similar effect of paleo-climate on Antarctic pelagic species. However, for both silverfish and krill, the estimate of the date at which the expansion began suffers from lack of reliable calibration of the molecular clock and the width of the confidence interval associated with the point estimate. Thus, the original hypothesis that the expansion could be linked to a range expansion due to equator-ward migration of Antarctic planktonic ecosystems during cold periods (Charles et al. 1991) should be taken with extreme caution. In addition, both silverfish and krill studies were based on a single mitochondrial DNA marker, and the use of nuclear markers in krill provided discordant and much more recent estimates (Bortolotto et al. 2011). Finally, the approaches used for the estimation of the expansion are based on simplified approaches that assume one single population size change (mismatch distribution used in Zane et al. 1998, 2006) or limit the overall number of population size changes (skyride plots used in Goodall-Copestake et al. 2010). Nevertheless, the study of historical demography in Antarctic species, and in silverfish in particular, is still in its infancy and has great potential. It would benefit from the availability of a robust time frame for differentiation of notothenioids (Near et al. 2012); use of multiple mitochondrial and molecular markers; and application of new powerful approaches, such as those based on Approximate Bayesian Computation (Beaumont 2010) that allow comparison of complex scenarios of population changes (Marino et al. 2013).

Moreover, populations may have persisted during the LGM in areas other than the Filchner and Drygalski Troughs. Although deglaciation is not well constrained for much of the East Antarctic, available data suggest that the shelf remained ice free in Lützow-Holm Bay and along Prydz Channel (Mackintosh et al. 2014). In the Weddell Sea, the ice off Ronne Trough may have been strongly indented as well, providing conditions for a population to persist (Hillenbrand et al. 2014). Conversely, conditions in the Filchner and Drygalski Troughs may not have allowed persistence over time scales prior to the LGM. The grounding line appears to have reached the outer shelf in both systems during earlier glacial cycles (Hillenbrand et al. 2014; Anderson et al. 2014), whereas sampling along the continental slope indicated that glacial ice has not been grounded off Prydz Channel since the mid-Pleistocene (Mackintosh et al. 2014). This evidence suggests that population structure varied between glacial periods, with episodes of increased extinction rates, and re-colonization between troughs that enabled silverfish to persist over succeeding glacial cycles. Moreover, gene flow facilitated by the rapid bursts of connectivity involved may have made harder the evolution of reproductive isolation by divergent selection (Schluter 2009), hence limiting the opportunities for ecologically-based speciation.

10.5 Hypothesis Predictions and Testing

The preceding sections describe how the life history hypothesis can be developed conceptually by integrating past and recent population results with evidence from a range of studies examining physical components of the Southern Ocean ecosystem. Nevertheless, the resulting synthesis needs testing analytically using population and circulation models, and directly in the field. Advances in scientific theory become increasingly convincing as the possible outcomes of empirical testing become more specific and narrowly defined (Popper 1959), facilitating the use of powerful and highly targeted sampling and experimental designs. Particularly useful when examining physical-biological interactions, in this section we discuss several predictions concerning population structure that can be tested in further research.

Building on the results from La Mesa et al. (2015), the synthesis predicts a coherent population currently occupying the Larsen Inlet Trough, with spawning areas under fast ice near its head. Based on the distributions of young fish found by Hubold (1984), the same applies for the Filchner Trough and that off Camp Norway in the southeast Weddell Sea. Trough features associated with coherent silverfish populations appear to include the following: size; extension to the shelf edge; large active ice streams at their head, frequently associated with strong katabatic winds and a coastal polynya; and upwelling of warmer, nutrient-rich water over the shelf at their mouth. The occurrence of these features in trough systems in the Bellingshausen and Amundsen Seas, in particular the Belgica Trough, suggests suitable habitat to support silverfish populations.

The ACC also brings CDW close to the continent along East Antarctica (Orsi et al. 1995) where extensive distributions of silverfish are found over the shelf (La Mesa and Eastman 2012). Off Wilkes Land, large numbers of silverfish have been found south of the ASF, principally inshore along the western side of the Ninnis Trough and over the eastern Adélie Bank (Moteki et al. 2011). Post et al. (2011) described characteristic features: a deep inner basin adjacent to the Mertz Glacier Tongue, and oceanography dominated by the Mertz Polynya sustained by the katabatic wind field over Buchanan Bay. HSSW is formed there and modified CDW upwelling onto the shelf raises salinity and provides heat that contributes to sea-ice melting in the polynya. Large numbers of silverfish were also found associated with the Adélie Trough further west and out over the shelf (Moteki et al. 2011). This suggests spawning grounds under fast ice in the coastal bays along the George V Basin. Fish over the Adélie Trough may be the result of local reproduction or advection from the Ninnis system. In addition, concentrations in the Mawson, Davis, Cooperation, Cosmonaut and Lazarev Seas highlighted by La Mesa and Eastman (2012) suggest that at least some of the large cross-shelf troughs found west of Wilkes Land contain suitable habitat and maintain coherent populations that supply the shelf offshore and downstream. Although the topography is not as well described elsewhere off East Antarctica, systems in Lützow-Holm Bay, Iceberg Alley, Nielsen Basin and Prydz Bay appear promising candidates.

Spatial predictions like these facilitate the use of sampling frames in surveys that are highly targeted to cover the expected areas and timing of life history events (La Mesa et al. 2015). The hypothesis also predicts strong physical-biological relationships at each stage of the life history that are amenable to empirical measurement. Considered in the form of a life table, biological variables like adult fecundity rate (e.g. La Mesa et al. 2014) and the number of spawners are likely to be an important influence on egg abundance. So is the amount of habitat availability, indicating an expected relationship with ice extent and variables involved in platelet formation. Similarly, the katabatic wind field appears important in transport reaching productive larval habitat like polynyas so, as well as egg predation, local wind direction, velocity, and frequency can be expected to have strong relationships with larval abundance. Katabatic wind variables are also likely to influence the availability of larval habitat through their effect on polynya structure and extent, including upwelling of warmer water and hence temperature and salinity. Moreover, the hypothesis highlights how the retention of young fish in the population is likely to be influenced by interactions between offshore flow along the trough and buoyancy-forcing associated with advection losses along the inner shelf. As a result, strong relationships can be expected between juvenile abundance and variables describing trough flow such as the velocity, volume, and distance to intruding CDW, as well as inverse associations with rates of precipitation and meltwater run-off. For adults, the relative contributions of active swimming and transport needs further examination, but the velocity and volume of shelf outflow and flow along the slope are likely to influence losses by advection. A relationship between inflow along the trough and adult return to spawning areas can also be expected under the hypothesis.

Such physical-biological interactions suggest the geological features that are likely to be important predictors of population variability between troughs. Momentum-driven upwelling at points of curvature along the slope argues for the importance of formations like the Crary Fan off the mouth of the Filchner Trough, and the Belgica Trough Mouth Fan. The configuration of individual troughs, including the location of reverse slopes and glacial features like moraines, mega-scale glacial lineations and grounding zone wedges, are likely to be important in shaping eddies, meanders and areas of upwelling in the trough circulation. Areas where wind-driven mixing brings oxygen and iron rich meltwater to interface with upwelled deeper water are likely to be particularly productive. In turn, the influence of geological features on retention and transport, and the distribution of productive feeding areas, imply relationships with population vital rates and abundance, as well as connectivity.

These physical-biological relationships also lead to predictions under the life history hypothesis concerning the potential effect of glacial cycles on both genetic diversity and structuring. Thus, repeated cooling cycles can be expected to result in cyclic fluctuations of local population size, consistent with evidence of historic dietary shifts in predators (Emslie et al. 1998; Emslie and McDaniel 2002). If populations during the warmer period are more constrained along the coast and less exposed to the outer shelf circulation, increased fragmentation and reduced habitat availability may have resulted in decreases in population size. These reductions would have been followed by expansion during the cooling periods, when distributions shifted outward over the shelf, resulting not only in increased habitat availability (Zane et al. 2006), but also in increased mixing between previously isolated populations. Overall, these population size fluctuations might be expected to reduce genetic diversity. However, because estimates of effective population size are still in the order of thousands of individuals (Agostini et al. 2015), the reductions in population size may not have resulted in substantial genetic losses, making these episodes more difficult to detect. Even so, genetic analyses have already rejected the hypothesis of population size equilibrium and detected a signal of population expansion (Zane et al. 2006), and new analytical approaches show promise for detecting multiple fluctuations in population size and testing their correlation with glacial cycles (Beaumont 2010).

Predictions can be made for genetic structuring on recent time scales as well. Connectivity in both westward and eastward directions along the AP implies that, contrary to other Antarctic fish (Matschiner et al. 2009), unidirectional gene flow is not expected for silverfish in this region. The recent study by Agostini et al. (2015) supports this prediction, but the result was based on an indirect estimate of genetic connectivity, and further tests with direct approaches based on paternity or kinship analysis (Schunter et al. 2014) would be appropriate. Additionally, the relationship between connectivity and circulation implies potential for population differentiation from a stepping stone-type scenario. However, this may have been further complicated by episodic long distance dispersal events, especially along the shelf slope, and circum-antarctic gene flow that fluctuated over succeeding glacial cycles. As a result, differentiation may not be strongly related to distance. Future studies should focus on neighbouring populations, and the hydrodynamic and ecological features governing their separation and connectivity. Thus, they should be designed with sufficient power to resolve differentiation by using a large range of genetic markers and large sample sizes, incorporating a multidisciplinary approach that includes other fisheries population techniques. Because of the temporal heterogeneity demonstrated in silverfish (Zane et al. 2006; Agostini et al. 2015), experimental designs should also include temporal replicates to discriminate geographic differences from those over time, with sampling frames that exclude as far as possible mixtures of fish between populations.

Multi-disciplinary approaches to testing are particularly useful, incorporating markers that can cross-reference and complement the genetic information (e.g. Begg et al. 1999). As demonstrated by La Mesa et al. (2015), the extent of distributions downstream can be predicted through simulations using circulation models. For the related *D. mawsoni*, Ashford et al. (2012) complemented simulations with empirical data from age distributions and otolith chemistry, using genetic analyses by Kuhn and Gaffney (2008) to establish an external comparison. Where silverfish distributions are associated with troughs lacking features necessary to support a population, similarities in trace element chemistry in the nucleus can be used to test for vagrancy from the parent population. The reproductive study by La Mesa et al. (2014) usefully complemented approaches using otolith chemistry (Ferguson 2012), genetics (Agostini et al. 2015) and faunal assemblages (Parker et al. 2015) along the AP. Reproductive studies can also help identify spatial differences in fecundity (e.g. Riginella et al. 2015), and variation in gonad development indicative of vagrancy. By contrast, when advection is predicted to reach a trough occupied by a neighbouring population, mixing can be detected as a bimodal distribution in the nucleus chemistry, and reproductive studies used to help confirm that immigrants contribute to spawning. On the other hand, in situations where simulations predict that advection falls short, population separation can be tested by differentiation in the nucleus chemistry characterized by underlying unimodal distributions. Finally, spatial age distributions can effectively examine dispersal effects and demographic structuring related to life history movement.

10.6 Conclusion: Silverfish Population Structure in the Southern Ocean Ecosystem

In this chapter, we suggest further development of an emerging life history hypothesis that can enhance our understanding of population structure in silverfish. The synthesis helps resolve inconsistencies highlighted by previous authors concerning cyclical variability in spawning and feeding areas, connectivity without active large-scale migration, and persistence over successive glacial cycles without phylogenetic diversification. The hypothesis needs rigorous testing and further development, but it suggests broad potential implications for silverfish population structure and life history connectivity in the context of the Southern Ocean ecosystem.

Population considerations are important at an ecosystem level because of the role of silverfish as a forage species. For example, their disappearance from the diets of Adélie penguins nesting near Anvers Island may have been driven by reduced connectivity from Marguerite Bay along the shelf slope, as the distribution of neritic assemblages became increasingly constrained inshore (Parker et al. 2015). In the Ross Sea, silverfish form a large component in the diet of the toothfish *D. mawsoni* (Eastman 1985; La Mesa et al. 2004), which is fished commercially and follows life history movement to spawning grounds in the western Southeast Pacific Basin (Hanchet et al. 2008). Ontogenetic changes to neutral buoyancy may facilitate dispersal via trough outflows into the Ross Gyre (Ashford et al. 2012) and, as in silverfish, result from the accumulation of lipids during feeding. How the life history trajectories of the two species intersect in the southwestern Ross Sea, and along troughs connecting to the shelf edge, appears fundamental to the life cycle of *D. mawsoni*, and their entrainment in flows to spawning grounds off the continental shelf.

Population structure and life history connectivity are a critical step to predicting the abundance and distribution of silverfish, and their availability to higher components of the ecosystem. A life cycle can be considered as a continuity solution, linking areas within a physical system that imposes spatial constraints (Sinclair and Iles 1989). Such a solution demands at least one trajectory that returns adults to the areas where they were spawned. For silverfish that remain members in this way, births and deaths directly govern abundance and population persistence. By contrast, vagrants have no effect on whether a population persists or not, because they no longer contribute to spawning. Their abundance depends on supply and local mortality. In populations where immigration subsidizes recruitment (Pulliam 1988), both local births and supply are important. Estimates of effective population size (Agostini et al. 2015) suggest that a relatively small proportion of adult silverfish contribute to the next generation. Vagrancy is likely to be high. As a result, distinguishing the life history trajectories used by members from those of vagrants, and fish moving between populations, is an important step toward understanding the spatial dynamics that sustain higher predator assemblages.

Temporal variability in silverfish abundance may also have widespread implications for the marine ecosystem. Losses of silverfish in the rapidly changing system along the western AP have caused increasing concern among scientists. We argue that silverfish have persisted through successive glacial cycles and variation in population structure and size may form part of their, so far successful, response. Historical shifts in the diets of Adélie penguins near Anvers and Adelaide Islands (Emslie et al. 1998; Emslie and McDaniel 2002) indicate associations between episodes of cooling and silverfish availability to higher trophic levels. As a result, silverfish distribution and abundance can usefully be examined in relation to present-day glacial and hydrographic conditions. Changes in abundance compared to historical baselines can be helpful in gauging spatial responses to climate variability and warming trends. However, this synthesis suggests that anthropogenic effects should be considered in the context of their potential for disrupting normal population responses to succeeding phases of glacial cycles, for example by accelerating the rate of warming or intensifying the peaks and troughs. In this way, understanding the complex physical-biological interactions that determine life history connectivity can help resolve population structure in silverfish, and its relationship with variability in the Southern Ocean ecosystem.

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Part III
Challenges and Conservation Perspectives

Chapter 11
Acoustic Methods of Monitoring Antarctic Silverfish Distribution and Abundance

Richard L. O'Driscoll, Iole Leonori, Andrea De Felice, and Gavin J. Macaulay

Abstract Acoustic methods have the ability to detect and quantify distribution and abundance of Antarctic silverfish (*Pleuragramma antarctica*) across a range of spatial and temporal scales. The main advantage of acoustic surveys over traditional net-based sampling methods for silverfish is that the larger adult fish are unlikely to avoid the echosounder. Acoustic surveys also allow relatively wide coverage over the whole water column in a short time period because data are collected while the vessel is steaming at 8–10 knots. The key uncertainties, as with most acoustic surveys, are target identification and target strength. These uncertainties are compounded because silverfish do not have a gas-filled swim-bladder and so are a relatively weak acoustic target. Use of multi-frequency acoustic data helps discriminate silverfish from krill and other associated species, and broadband acoustics has considerable potential in this regard. Acoustic target strength has been derived from scattering models and *in situ* and *ex situ* measurements. Adult silverfish exhibit different scattering properties to post-larvae and juveniles. In the Ross Sea, adult silverfish are distributed widely over the shelf and tend to form layers at 100–400 m depth. Juvenile silverfish of 50–80 mm standard length occur shallower and were observed as a weak layer centred at about 80 m depth.

Keywords Acoustics • Target strength • Geographical distribution • Forage fish • Ross Sea • Antarctica

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11.1 Introduction

Despite their importance in the Antarctic ecosystem, relatively little is known about the spatial distribution and abundance of silverfish, and this has been identified as a research priority for ecosystem modelling in the Ross Sea (Pinkerton et al. 2010). Fisheries acoustics methods (Simmonds and McLennan 2005) provide a potential tool to study silverfish at biologically relevant spatial and temporal scales.

11.1.1 Fisheries Acoustics

Underwater acoustics is based on transmitting sound waves into water and measuring what is reflected back (acoustic backscatter). Organisms in the water column such as fish and zooplankton scatter sound that is detected by the echosounder or sonar and the amount of backscatter can be used to estimate abundance. While simple in concept, fisheries acoustics relies upon many technical details, with key principles described briefly here.

Acoustic systems have varying physical forms that depend on their usage. For example, echosounder transducers are usually mounted on the hull of vessels, but may be towed behind a vessel, lowered to be closer to the fish of interest, or affixed to autonomous underwater vehicles. Echosounders can also be mounted on the seafloor or on fixed platforms to collect data from a single location over extended periods. Regardless of the platform or method of deployment, acoustic equipment needs to be carefully calibrated (Demer 2015) to yield data which can be compared between systems, and to estimate abundance.

While acoustics can indicate the presence of an organism, it doesn't directly measure the species. Target identification often requires the use of other sampling techniques, such as nets and cameras. However, with experience, and using aspects like the shape and acoustic properties of the aggregation, its behaviour, depth, location, time of day, time of year, etc., it may be possible to reliably deduce the species of schools without actually having to catch them (Fernandes 2009).

Another important parameter that is needed to interpret acoustic data is target strength (TS). This is a measure of how well an organism, such as fish, reflects sound. The TS is usually expressed as the logarithm of the ratio of the reflected sound intensity at 1 m from the target divided by the incident intensity: $TS = 10*\log_{10}(I_r/I_i)$. In fisheries acoustics, the reflected sound intensity is always less than the incident sound intensity, so the logarithm of the ratio is always less than 1, and hence TS values are always negative. TS values that are more negative indicate weaker reflections: for example, a large strongly reflecting fish can have a TS of −30 dB, while a small krill can have a TS of −80 dB. Sound is reflected from changes in acoustic impedance – which is the product of sound speed through the material and its density. Many fish have a swim-bladder which contains gas used to regulate their buoyancy – this generates a strong acoustic echo due to the relatively low acoustic impedance of the gas. Some fish don't have a swim-bladder and acoustic echoes from them result from much smaller impedance changes and hence have lower TS. These differences in reflectivity between species can be used to distinguish them, but can also make some species very hard to see – something that has very low contrast with water, such a jellyfish, are difficult to detect (Brierley et al. 2004).

The acoustic frequency also affects the TS and this can be used to help identify the species. As a general guide, lower acoustic frequencies reflect well from large organisms and also from small fish with gas-filled swim-bladders where the sound can cause the swim-bladder to resonate. Inversely, small organisms and those without a gas-filled swim-bladder reflect poorly at low frequencies, but better at high frequencies. Most fisheries applications tend to use frequencies in the range of 12–200 kHz. Lower frequencies travel further in water, but have a lower resolution, require larger and heavier transducers, and more powerful transmitters. In addition, as the frequency increases, the sound is absorbed by water more rapidly, so the higher frequencies cannot propagate as far, but the equipment is lighter, more portable, and cheaper. For example, in seawater, it is possible to see large fish at distances of 2000 m with an 18 kHz signal, while at 200 kHz the range reduces to about 300 m. Recent advances in acoustic technology use broadband echosounders which transmit a continuous range of frequencies rather than discrete well-separated frequencies, and these broadband systems may further improve the ability to discriminate species through their much finer frequency resolution.

11.1.2 Acoustics and Antarctic Silverfish

Although regular acoustic surveys have been conducted to estimate abundance of krill in Antarctic waters (e.g., Watkins et al. 2004), there was little acoustic work before 2008 on Antarctic silverfish. Ackley et al. (2003) noted that net tows suggested that "individual targets" in the coastal shelf region of the Ross Sea may have been juvenile silverfish, but no further details are given. O'Driscoll et al. (2011) described the first attempt to estimate silverfish abundance using acoustics, based on data collected during New Zealand's International Polar Year Census of Antarctic Marine Life (NZ IPY-CAML) voyage on R.V. "Tangaroa" to the Ross Sea region in February-March 2008. O'Driscoll et al. (2011) presented multifrequency echograms showing aggregations of silverfish, verified by targeted trawling, and use acoustic data to describe the spatial and vertical distribution and to estimate abundance of juvenile and adult silverfish in the Ross Sea. O'Driscoll et al. (2011) also used anatomically detailed scattering models based on computed tomography (CT) scans of frozen specimens (Fig. 11.1) to estimate acoustic TS for silverfish at 12, 18, 38, 70, and 120 kHz.

Azzali et al. (2010) carried out nine *in situ* acoustic-trawl experiments targeted at post-larvae and juveniles of Antarctic silverfish (total length 13.3–68.9 mm) in the Ross Sea during the 1997–1998, 2000 and 2004 Italian cruises on R/V "Italica" and

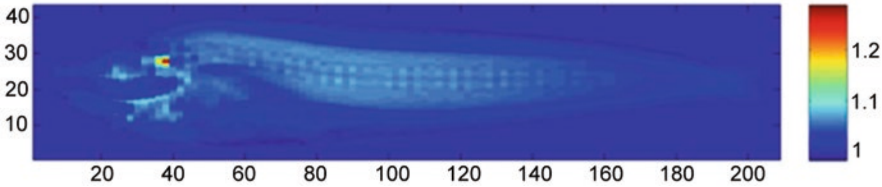


Fig. 11.1 Relative acoustic impedance of a 203 mm SL silverfish presented as a lateral mean through the fish. The pixel-to-pixel change in impedance indicates the scattering strength. The only strong acoustic reflectors are the otoliths (the *red pixels* in the head)

measured TS of preserved (frozen and defrosted) silverfish of total length 110–202.5 mm *ex situ* (Ancona Bay, Adriatic Sea) at 38, 120 and 200 kHz. Azzali et al. (2010) also used a general TS modelling approach based on the measured morphological characteristics of the silverfish (obtained by X-rays). Scattering from the fish body and backbone were estimated from generalised theoretical scattering models where the fish body was represented as an ellipsoid and the backbone as a cylinder with constant acoustic impedance. Model results could predict the TS measured both *ex situ* and *in situ* with a mean error less than 1.5 dB, although the differences between the predicted and measured TS at some fish lengths were high.

Both models suggest that adult silverfish exhibit different scattering properties to post-larvae and juveniles (Azzali et al. 2010; O'Driscoll et al. 2011).

In this paper we summarise available information on estimating abundance and distribution of silverfish, based mainly on the results of O'Driscoll et al. (2011) and Azzali et al. (2010). We discuss the advantages and limitations of acoustic methods in relation to other sampling methods, and describe recent advances in acoustic technology and research initiatives that may address some of the limitations.

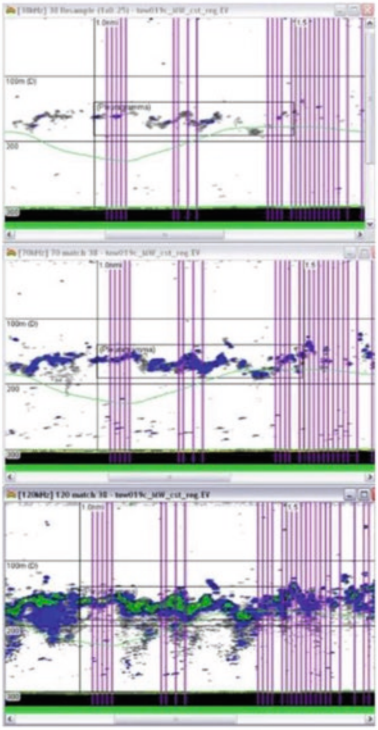
11.2 Antarctic Silverfish Acoustic Characteristics and Identification

Identification of Antarctic silverfish from acoustic recordings was based on linking catches in midwater trawls with acoustic characteristics on the NZ IPY-CAML voyage (O'Driscoll et al. 2011). On this 2008 voyage acoustic data were collected using four Simrad EK60 echosounders with hull-mounted transducers: 12 kHz (2 kW, single beam), 38 kHz (2 kW, split beam), 70 kHz (1 kW, split beam), and 120 kHz (500 W, single beam). The 38, 70, and 120 kHz echosounders were calibrated in the Ross Sea on 12 February 2008. It was not possible to calibrate the 12 kHz echosounder so results were not reported for this frequency. Acoustic data were collected from the sea surface to the seabed or to a maximum depth of 1000 m. However the effective ranges of the 70 kHz and 120 kHz echosounders were only about 750 m and 300 m respectively. Transmitted pulse length on all frequencies was 1.024 ms with a minimum of 1.5 seconds between transmits on the shelf with longer transmit intervals in deeper water. Targeted midwater trawls for acoustic target identification were carried out using a fine-mesh midwater trawl. The trawl had a circular mouth opening of about 12 m diameter, a codend mesh of 10 mm, and was rated to a maximum depth of 1200 m. It is similar to the IYGPT (International Young Gadoid Pelagic Trawl), which was recommended by CAML for sampling pelagic fish layers. During tows for acoustic target identification, the midwater trawl was targeted at the aggregation of interest and towed for 20–30 min at 3–4 knots. A temperature-depth logger was attached to the midwater trawl on each deployment and this provided an accurate time/depth profile for each tow.

A total of 10 targeted midwater tows were carried out in the shelf region. Catches in four of these tows were dominated (87–99% by weight) by adult and juvenile silverfish (50–200 mm standard length, SL). Example echograms from two of these trawls are shown in Figs. 11.2 and 11.3. Adult silverfish were caught from layers at 150–450 m depth, but were sometimes present close to the bottom and were also frequently caught in demersal trawls shallower than 500 m on the shelf (O'Driscoll et al. 2011). A weak layer at about 80 m depth (Fig. 11.4) was found to be associated with 'juvenile' silverfish of 40–80 mm SL. Although, the tow shown in Fig. 11.4 caught 83% icefish by weight, this represented only three individual adult *Neopagetopsis ionah* (weights 334–466 g) and these are not thought to have contributed significantly to the acoustic signal observed. Acoustic backscatter of silverfish schools increased with increasing frequency (Fig. 11.5), which is characteristic of species without an air-filled swim-bladder (Simmonds and MacLennan 2005).

The main uncertainty in target identification noted by O'Driscoll et al. (2011) was separating silverfish from ice krill (Fig. 11.6), which were the only other major source of pelagic backscatter on the Ross Sea shelf. Silverfish could not be simply discriminated from krill using multifrequency acoustics as both groups had a similar frequency response, with backscatter increasing with increasing frequency (Fig. 11.6). However, there were differences in school structure and density between krill and silverfish that allowed subjective classification. Target identification trawling suggested that separation of adult silverfish and krill was quite reliable. This was aided by apparent differences in depth distribution, with adult silverfish tending to occur at 150–450 m depth (e.g., Figs. 11.2 and 11.3), while most krill schools were in the upper 100 m (e.g., Fig. 11.6). Separation between juvenile silverfish and krill was less certain, as both had a similar vertical distribution and tended to coexist. Separation was based entirely on density, with krill tending to form discrete schools, while juvenile silverfish occurred as a diffuse layer (e.g., Fig. 11.5). Acoustic densities and target strength of juvenile silverfish were so low that they were, at times, similar to the acoustic noise level for the 38 kHz echosounder.

Fig. 11.2 Echograms at (*top* to *bottom*) 38, 70, and 120 kHz showing aggregation of adult silverfish at about 150 m depth on the Ross Sea shelf on 10 February 2008. *Green line* shows tow path of a midwater trawl which caught 204 kg of silverfish (94% of total catch by weight). *Pink vertical lines* show data rejected due to aeration



11.3 Spatial and Vertical Distribution of Silverfish from Acoustics

During the NZ IPY-CAML voyage, both adult and juvenile silverfish occurred widely over the western Ross Sea shelf from 72° to 77° S and 167° 30′ E to 180° over bottom depths from 200 to 500 m (Fig. 11.7). The highest acoustic densities of adults were observed at about 73° 30′ S and 178° 30′ E, southwest of the Iselin Bank. Juvenile silverfish appeared to be more abundant in the east, but the survey coverage was insufficient to provide good information on spatial distribution.

Juvenile silverfish and krill observed on the NZ IPY-CAML tended to occur in the upper 100 m. Adult silverfish were concentrated from 150 to 450 m depth. O'Driscoll et al. (2011) found no evidence for diurnal migration of silverfish. However, this survey was done at a time with limited periods of darkness. O'Driscoll et al. (2011) reported a clear relationship between silverfish size and depth, with larger silverfish caught deeper. Size distribution appeared to be related to depth rather than proximity to the seabed, as midwater and demersal trawls at similar depths caught similar fish sizes.

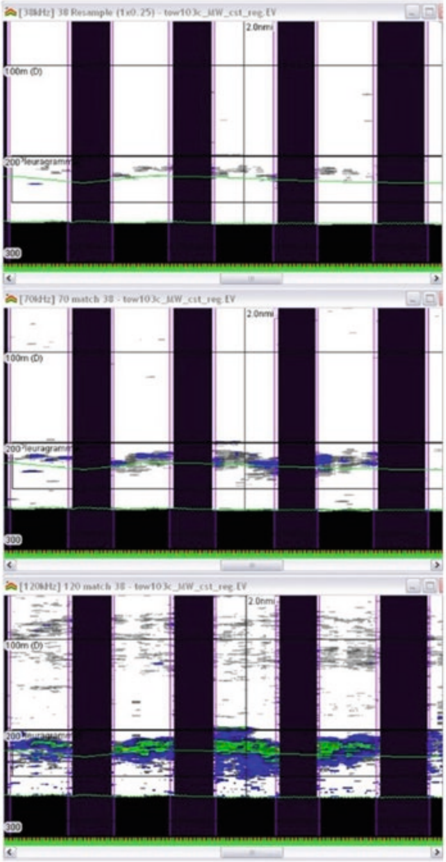


Fig. 11.3 Echograms at (*top* to *bottom*) 38, 70, and 120 kHz showing aggregation of adult silverfish at about 225 m depth on the Ross Sea shelf on 18 February 2008. *Green line* shows tow path of a midwater trawl which caught 220 kg of silverfish (99% of total catch by weight). *Black bands* show data rejected due to electrical noise

11.4 Acoustic Target Strength

Antarctic silverfish do not have a swim-bladder and are poor reflectors of sound. Silverfish have few dense structures and this is readily observed in CT scans (see Fig. 11.1). Most of the skeleton consists of cartilage (density 1060–1180 kg m^{-3}), with only a small amount of higher density bone (density 1300–2000 kg m^{-3}). The vertebrae consist of a thin collar of bone surrounding the large unconstricted notochord, a persistent larval feature. Buoyancy is provided by triglyceride lipids (density 930 kg m^{-3}), which are mostly stored in large sacs (Eastman and DeVries 1989).

The average ('base case') silverfish relative density contrast derived by O'Driscoll et al. (2011) from CT scans of seven silverfish (67–203 mm SL) was 0.974 (standard deviation of 0.018), suggesting that the fish were all less dense than seawater. It is biologically unlikely that a species *in situ* would be positively buoyant, and O'Driscoll et al. (2011) suggested that low CT density measurements may be an artefact of freezing. Other published measurements of silverfish buoyancy give

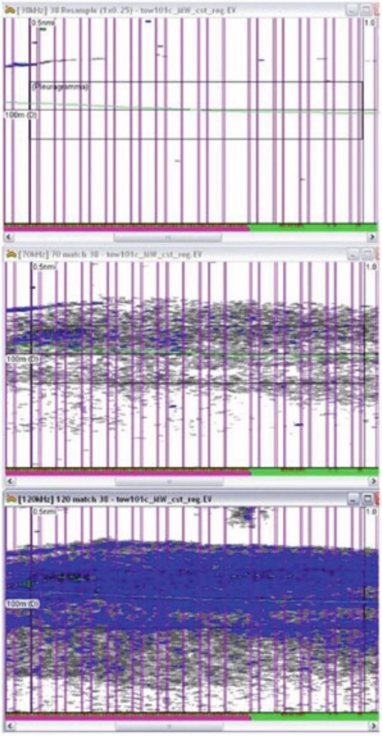


Fig. 11.4 Echograms at (*top* to *bottom*) 38, 70, and 120 kHz showing layer of juvenile silverfish at about 80 m depth on the Ross Sea shelf on 18 February 2008. *Green line* shows tow path of a midwater trawl which caught 0.2 kg of silverfish (10% of total catch by weight). *Pink vertical lines* show data rejected due to aeration

values that range from neutrally to negatively buoyant (Chu and Wiebe 2005; Near et al. 2009). In acoustic scattering model simulations, increasing the relative density generally increased the TS (O'Driscoll et al. 2011). The increase in TS was large for the two smallest fish, often exceeding 20 dB, but much less for larger fish and higher frequencies.

The outcome of the target strength modelling presented by O'Driscoll et al. (2011) was two TS equations: one suitable for specimens less than 11 cm standard length (SL) and the other for the individuals larger than 11 cm SL. For 38 kHz these relationships were:

$$TS = 146.8 \log\left(SL_{cm}\right) - 216 \, SL \le 11 \, cm \tag{11.1}$$

$$TS = 18.7 \log\left(SL_{cm}\right) - 82.1 \quad SL > 11 \, cm \tag{11.2}$$

This two-part relationship was unusual, having an extremely high slope for fish less than 11 cm.

As already reported above, Azzali et al. (2010) attempted a TS estimation of silverfish on the base of two *ex situ* experiments and other *in situ* analyses. *Ex situ* experiments allowed the calculation of TS for adult individuals with total length

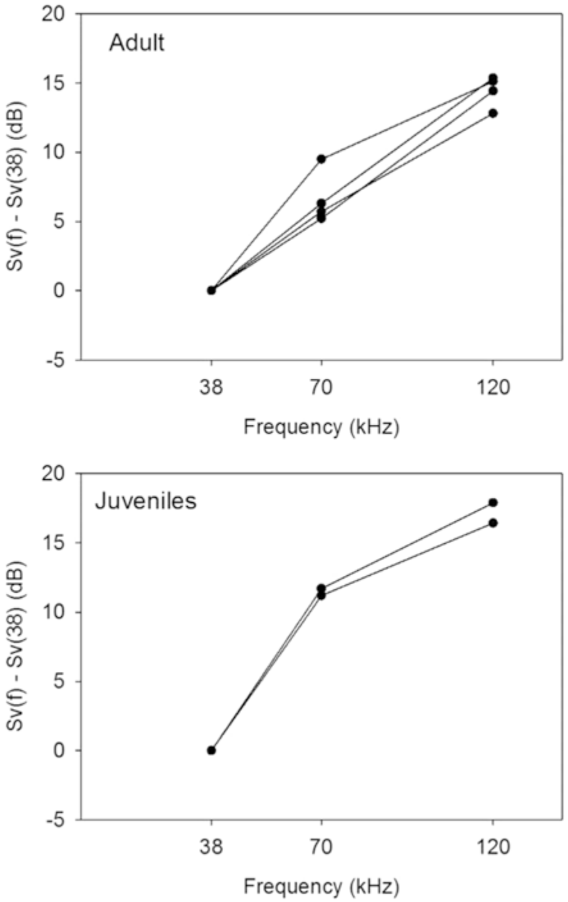


Fig. 11.5 Frequency response from aggregations of adult (*upper panel*) and juvenile (*lower panel*) Antarctic silverfish. Response is expressed as a dB-difference relative to 38 kHz

(TL) between 110 and 202.5 mm. The TS relationships at 38, 120 and 200 kHz were then:

$$TS_{38}\left(\text{adult}\right) = 36.83\log\left(TL_{cm}\right) - 103.62\,\mathrm{R}^2 = 0.75 \tag{11.3}$$

$$TS_{120}\left(\text{adult}\right) = 34.75\log\left(TL_{cm}\right) - 84.20\,\mathrm{R}^2 = 0.73 \tag{11.4}$$

$$TS_{200}\left(\text{adult}\right) = 26.71\log\left(TL_{cm}\right) - 73.74\,\mathrm{R}^2 = 0.78 \tag{11.5}$$

In situ TS data came from 9 hauls of 131 analysed from three research cruises in the western Ross Sea in 1997–1998, 2000 and 2004. The 9 hauls were selected for similar size and species composition (80% or more by number of *P. antarctica*). TS equations estimated for a TL range of 13.3–68.9 mm (i.e., juvenile silverfish) were:

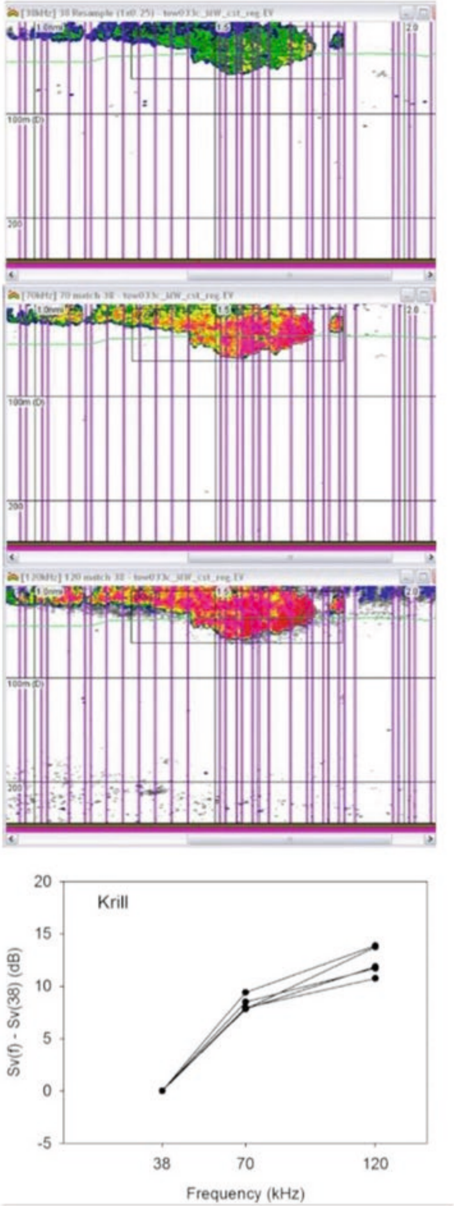
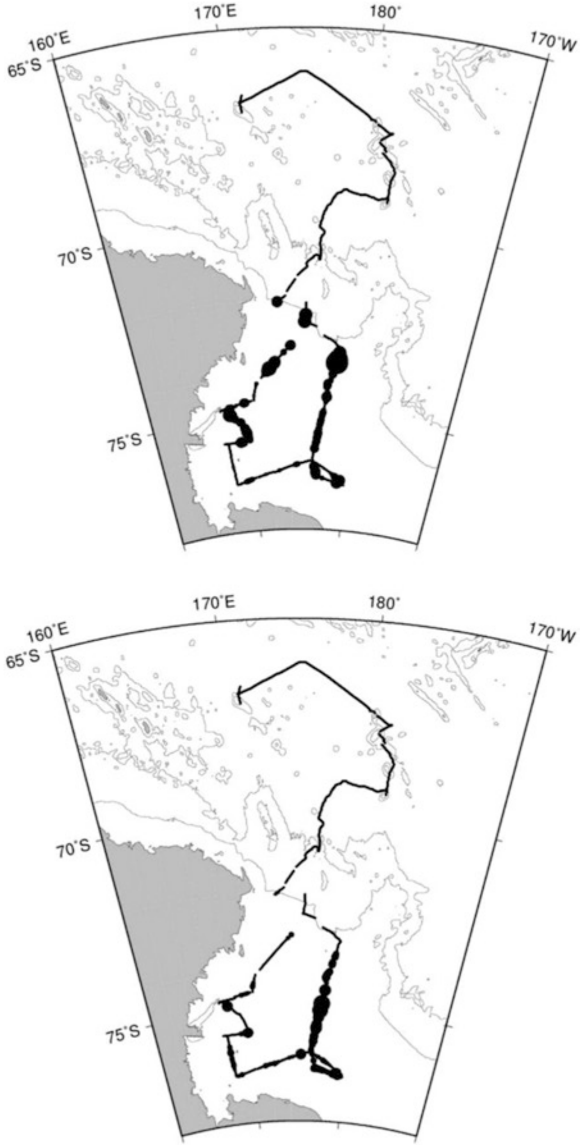


Fig. 11.6 Echograms at (*top* to *bottom*) 38, 70, and 120 kHz showing ice krill at about 50 m depth on the Ross Sea shelf on 12 February 2008. *Green line* shows tow path of a midwater trawl which caught 2.3 kg of krill (55% of total catch by weight). *Pink vertical lines* show data rejected due to aeration. *Lower panel* shows frequency response from aggregations of krill. Response is expressed as a dB-difference relative to 38 kHz

Fig. 11.7 Spatial distribution of acoustic backscatter from adult (*upper panel*) and juvenile (*lower panel*) silverfish on the NZ IPY-CAML voyage in Feb-Mar 2008. *Circle areas* are proportional to acoustic density scaled to the maximum backscatter (0.5 m² km⁻²)



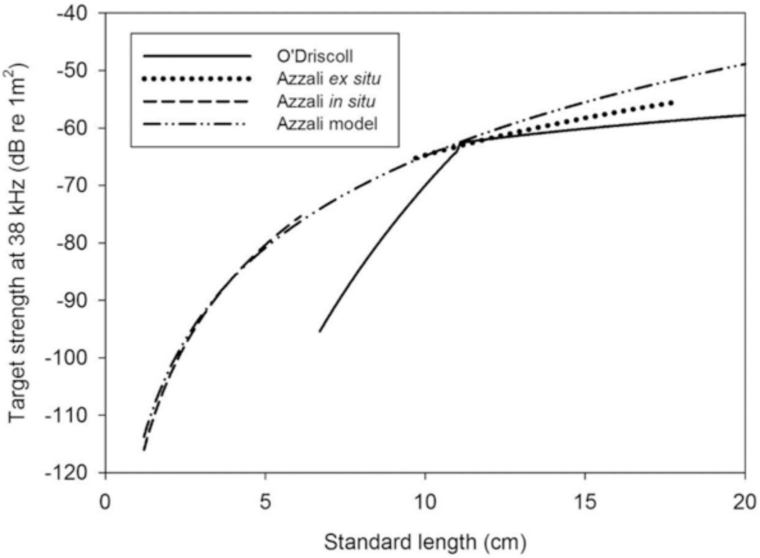


Fig. 11.8 Comparison of target strength to standard length (TS–SL) relationships for Antarctic silverfish at 38 kHz: O'Driscoll estimates from model scattering simulations based on CT scans (Eqs. 11.1 and 11.2), Azzali *ex situ* estimates derived from experiments on dead adult silverfish in Ancona Bay (Eq. 11.3), Azzali *in situ* estimates derived from fishing experiments on post-larvae and juvenile silverfish carried out in the Ross Sea (Eq. 11.6) and Azzali model estimates from generalized theoretical models based on morphological characteristics from X-rays (Eq. 11.9). Where original equations were based on total length (TL) (Eqs. 11.3, 11.6 and 11.9), these were converted to standard length based on the empirical relationship TL = 0.88 SL (Azzali et al. 2010). Relationships are only plotted across silverfish length ranges on which these were derived

$$TS_{38}\left(juv.\right) = 57.58\log\left(TL_{cm}\right) - 123.77\,\mathrm{R}^2 = 0.99 \tag{11.6}$$

$$TS_{120}\left(juv.\right) = 51.94\log\left(TL_{cm}\right) - 105.92\,\mathrm{R}^2 = 0.98 \tag{11.7}$$

$$TS_{200}\left(juv.\right) = 46.61\log\left(TL_{cm}\right) - 95.29\,\mathrm{R}^2 = 0.97 \tag{11.8}$$

Equations based on the theoretical model in Azzali et al. (2010) were in good agreement with both *in situ* and *ex situ* data (Fig. 11.8):

$$TS_{38} = 53.1\log\left(TL_{cm}\right) - 120.9\,\mathrm{R}^2 = 0.97 \tag{11.9}$$

$$TS_{120} = 48.3\log\left(TL_{cm}\right) - 100.65\,\mathrm{R}^2 = 0.99 \tag{11.10}$$

$$TS_{200} = 41.1\log\left(TL_{cm}\right) - 89.8\,\mathrm{R}^2 = 0.97 \tag{11.11}$$

Comparing the TS-L relationships from Azzali et al. (2010) and O'Driscoll et al. (2011) (Fig. 11.8), there were similar estimated TS at 38 kHz for individuals around

11 cm SL, while there was poor agreement for juveniles and larger adults at the same frequency (see Fig. 11.8). The difference in reflectivity between adult and juvenile silverfish is likely caused by the fact that soft tissue is dominant in juveniles, while hard tissues (backbone) prevail in adults, as the bones are well-developed in adults only.

11.5 Abundance Estimates

Using their base-case TS model, O'Driscoll et al. (2011) estimated that the biomass of adult silverfish in the Ross Sea was 206,000 t, with 95% confidence intervals (CI) from non-parametric bootstrapping of transect data of 107,000–292,000 t. The biomass of juvenile silverfish was highly variable depending on the choice of TS.

The biomass estimates of O'Driscoll et al. (2011) were based on data from the 38-kHz echosounder only. However, 70 kHz would have been a preferred frequency for abundance estimation, as this frequency had the best signal-to-noise ratio across the range of depths sampled. Unfortunately on the NZ IPY-CAML voyage in 2008, the 70-kHz echosounder on R.V. "Tangaroa" was susceptible to weather-induced bubbles. The 70 kHz transducer on this vessel has since been moved to improve its performance in poor weather. The effective range of the 120-kHz echosounder on R.V. "Tangaroa" meant that this frequency was not suitable for abundance estimation for silverfish aggregations deeper than about 300 m.

O'Driscoll et al. (2011) compared acoustic estimates of silverfish density with those estimated from trawling. Wingspread swept-area density estimates of silverfish from demersal trawls on the NZ IPY-CAML survey were 0.003–0.285 g m^{-2}. The acoustic estimates for the equivalent strata based were 0.08–1.24 g m^{-2}. In another study, Donnelly et al. (2004) present density estimates of 0.025–0.684 g m^{-2} for adult silverfish sampled by MOCNESS and Tucker trawls from the eastern Ross Sea. Therefore, acoustic and trawl estimates of adult silverfish were of a similar magnitude. However, acoustic estimates might be expected to be higher because, unlike a bottom trawl, acoustics samples the whole water column and there are no issues with net avoidance. Estimated silverfish biomass from a balanced trophic model of the Ross Sea (Pinkerton et al. 2010) was about 410,000 t. The acoustic biomass estimates of O'Driscoll et al. (2011) for adult silverfish are about half of this, but, depending on the choice of TS, inclusion of juvenile biomass could lead to much higher acoustic estimates of abundance.

11.6 Advantages and Limitations of Acoustics

The main advantage of acoustic surveys over traditional net-based sampling methods for Antarctic silverfish is that the larger adult fish are unlikely to avoid the echosounder. Acoustics is a remote sampling method, layers of silverfish occur relatively deep (typically 150–450 m), and there was no detectable response of aggregations to the passage of the survey vessel (authors' personal observations). Acoustic surveys also allow relatively wide coverage over the whole water column in a short time period, because data are collected while the vessel is steaming at 8–10 knots. Compared to conventional methods, such as net-based sampling, acoustics can be operated from a diverse range of platforms. Acoustic methods provide a promising means to obtain the sort of high spatial and temporal resolution data required for improved ecosystem models at an affordable cost (Handegaard et al. 2013).

The key uncertainties, as with most acoustic survey, are target identification and target strength. Accurate estimates of silverfish biomass are strongly dependent on good estimates of TS and, despite two previous studies (Azzali et al. 2010; O'Driscoll et al. 2011), there is still considerable uncertainty, particularly for juvenile silverfish (see Fig. 11.8). There is a particular need for further *in situ* data collection to help resolve differences between model estimates.

In acoustic surveys in Antarctic waters, krill are routinely separated from fish on the basis of higher levels of scattering at 120 kHz than at 38 kHz (e.g., Madureira et al. 1993). This method of target identification works well where krill occur in conjunction with swim-bladder-bearing fish, but may not be appropriate for silverfish which have a similar frequency response to that of krill. In Antarctic regions where silverfish are common, further work is required to develop objective algorithms to separate silverfish from krill. The main problem occurs in distinguishing between Antarctic silverfish and *Euphausia crystallorophias* because they occur frequently in the same areas and same portions of the water column and because they have a very similar discrete frequency response (Azzali et al. 2010; O'Driscoll et al. 2011). This problem may be less severe with *Euphausia superba* that has a quite specific backscattering where peak response occurs at 120 kHz (Demer and Conti 2003).

11.7 Future Directions

Some of the limitations of acoustics (see previous section) may be reduced by technology advances. For example, one of the tools that could improve the identification of acoustic targets at sea is the use of broadband acoustic signals (Lavery et al. 2010). Broadband systems give a global view of the acoustic response of marine organisms without significant frequency gaps. Some data on Antarctic silverfish and Antarctic krill were collected using broadband Simrad EK80 echosounders on a joint New Zealand-Australian research voyage to the Ross Sea in February-March 2015.

Classification of silverfish and separation from krill might be improved by methods such as classification trees that combine information about school characteristics as well as multifrequency energetics (Fernandes 2009). For example, Gauthier et al. (2014) found that groups of mesopelagic fish on the Chatham Rise, New

Zealand could not be separated based on their frequency response alone, but could be distinguished based on their unique acoustic school characteristics (amplitude and dimension features of the volume backscatter), vertical distribution, and ancillary information. Classification results were synthesised in a decision tree model that was able to distinguish six fish assemblages. To apply such methods for silverfish would require a broader "training set" of acoustic data for where the classification is known.

Another source of useful information about silverfish could be to add acoustic instrumentation to moorings normally deployed in the area for the study of currents and environmental conditions. In this way, there could be a local characterization of the pelagic ecosystem and its changes in time such as vertical diurnal migrations or larger scale migration to or from a mooring site. An example is given in Brierley et al. (2006) who studied short term variability in abundance of *E. superba* at South Georgia, coupling echosounders operating at 125 kHz with an Acoustic Doppler Current Profiler operating at 300 kHz, allowing the identification of krill through the differences in echo intensity at the two frequencies. An upward-facing ASL 70 kHz echosounder was recently deployed by New Zealand and Italian researchers in Terra Nova Bay in the Ross Sea to record potential silverfish migration during winter 2015.

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Chapter 12
Impact of Climate Change on the Antarctic Silverfish and Its Consequences for the Antarctic Ecosystem

Katja Mintenbeck and Joseph J. Torres

Abstract *Pleuragramma antarctica* is the dominant forage fish of the coastal Antarctic, exhibiting a circumantarctic distribution and a well documented abundance in all shelf environments, from the high Antarctic Weddell and Ross Sea systems, to the milder waters of the western Antarctic Peninsula (WAP) shelf. Rapid regional warming on the WAP has produced a dichotomy in annual weather patterns between the high Antarctic systems and the WAP, resulting in swiftly rising midwinter air temperatures and fewer sea ice days during the annual winter cycle on the WAP, and little change in the Ross and Weddell Seas. The WAP shelf thus provides a model system for examining the potential effects of climate warming on an important Antarctic species.

Pleuragramma's life history is characterized by slow growth, late maturity, a high reproductive investment and an association with coastal sea ice for spawning and larval development. All those features will allow the species to weather episodic annual failures in recruitment, but not long term change.

Most effects of the increasing temperature associated with climate change will be indirect ones, as temperatures will not increase to the point where they are physiologically life-threatening in the short term. A recent survey of *Pleuragramma* distribution on the WAP shelf revealed a large break in its historical distribution in shelf waters, suggesting a collapse in the local population of silverfish there. The break occurred in the area that has been most heavily impacted by rapid regional warming: the northern mid-shelf including Anvers and Renaud Island. It may be that the multi-faceted effects of climate change are already at work in its local disappearance.

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Keywords Western Antarctic Peninsula • Global change • *Pleuragramma* distribution • Regional warming

12.1 Introduction

Clarke (1996) suggests three possible responses by a population of organisms experiencing an environmental challenge.

1. Migration: the species shifts to a more favorable area
2. Adaptation: the species evolves to shift the phenotypic reaction norm to better respond to the new environment (or can accommodate it within its existing phenotype)
3. Extinction: the species fails to adapt or migrate and becomes extinct (can be local extinctions if they reproduce in place and don't migrate appreciably)

The fact that polar species dwell in an end-member climatic regime limits the potential for migration. More favorable areas may be found within the polar biosphere itself due to regional variability, but excursions outside of the polar latitudes will not result in a more hospitable climate. In contrast, tropical species may shift poleward for temperatures more compatible with their "climate envelope" (Pearson and Dawson 2003).

The boundaries dictated by species' life history tactics, their behavior patterns such as foraging strategies, and their physiological breadth, or flexibility in dealing with warming temperatures, are important in assessing the vulnerability of individual species to changing climate. Once a species' boundaries are understood, they can be interpreted within the framework of observed and projected changes in climate to pinpoint vulnerabilities and evaluate the likelihood of survival.

The purpose of the present chapter is to identify the environmental challenges the Antarctic silverfish *Pleuragramma antarctica*, is facing now and likely to face in the immediate future. The western Antarctic Peninsula (WAP) shelf, currently the most rapidly warming region on the planet (Vaughan et al. 2003), is used as a reference and model system. The chapter is divided into a series of sections that initially summarize the distribution of *Pleuragramma* in the Antarctic system, the regional climatic differences it presently faces, and the challenges they present. Life history tactics are summarized and evaluated for vulnerabilities to the evolving physical and biotic environment of a changing climate. Physiological responses to changing seasonal temperatures and salinity fields are considered within the context of what is known about *Pleuragramma* and notothenioids in general. Potentially altered prey availability and composition are considered, as is the possibility of increased competition from oceanic pelagics in warming shelf waters. The chapter concludes with observed changes in the WAP shelf populations of silverfish, the importance of silverfish to the coastal Antarctic and recommendations for future research.

12.2 Geographic Distribution and Role as Prey

P. antarctica, given the common name Antarctic silverfish by DeWitt and Hopkins (1977), is the dominant pelagic fish in all regions of the coastal Antarctic (Kellerman 1996; Eastman 1993; DeWitt 1970; Ekau 1990). Unlike most notothenioids, which are associated with the benthos as adults, silverfish are found in the midwaters over the continental shelf from the surface to 500 m, and deeper in coastal canyons.

Pleuragramma's pelagic lifestyle makes it a key diet component of coastal Antarctic apex predators. The bottom fauna is inaccessible to many diving predators on the Antarctic continental shelf because its typical 500 m depth (Eastman 1993) is beyond their reach. Thus, because of their pelagic distribution, silverfish are an important prey source for flighted seabirds (Ainley et al. 1991) and the shallow-diving pygoscelid penguins (Bengtson et al. 1993; Williams 1995; Ainley 2002). They are also a significant part of the diet of deeper divers such as emperor penguins (Cherel and Kooyman 1998) and crabeater, Ross, Weddell, and leopard seals (Øritsland 1977; Lowry et al. 1988; Fuiman et al. 2002). Several species of fishes feed on *Pleuragramma* as well, including the notothenioids *Pagothenia borchgrevinki, Dissostichus mawsoni,* and *Gymnodraco acuticeps* (Eastman 1985), indicating that it represents a critical trophic link in the coastal system.

Pleuragramma is found in all coastal regions of the continental Antarctic, as well as several Antarctic and sub-Antarctic islands including those of the Scotia Arc (South Shetlands, South Orkneys, South Georgia, and South Sandwich islands) and the Balleny Islands of the Ross Sea region (MacDiamid and Stewart 2015, La Mesa and Eastman 2012). Its wide-ranging distribution and the vast size of the Antarctic continent means that the species experiences climate diversity within its natural range. Overall, the temperatures at which it is found range from −2 to 4 °C (Gordon et al. 1982), with the warmest in islands of the Scotia Arc and the coldest in all coastal regions of the Antarctic continent outside of the WAP shelf, where it encounters temperatures of −2 to 2 °C over the course of the year (Gordon et al. 1982). It is worth noting that the vast majority of its range has a year-round temperature of −2 °C throughout the water column and significant ice cover all year round (Zwally et al. 2002; Gordon et al. 1982). Since about 1950, climate records suggest a dichotomy in coastal climate between the Western Antarctic/Bellingshausen Sea region and the remainder of the Antarctic coastal system (Vaughan et al. 2003; Turner et al. 2005). Regional warming and loss of annual sea ice is far more pronounced in the WAP than the coastal Ross and Weddell Seas or the eastern Antarctic, which have remained cooler, retaining their high Antarctic character (Vaughan et al. 2003; Turner et al. 2005; Ducklow et al. 2007; Stammerjohn et al. 2008a) (Fig. 12.1).

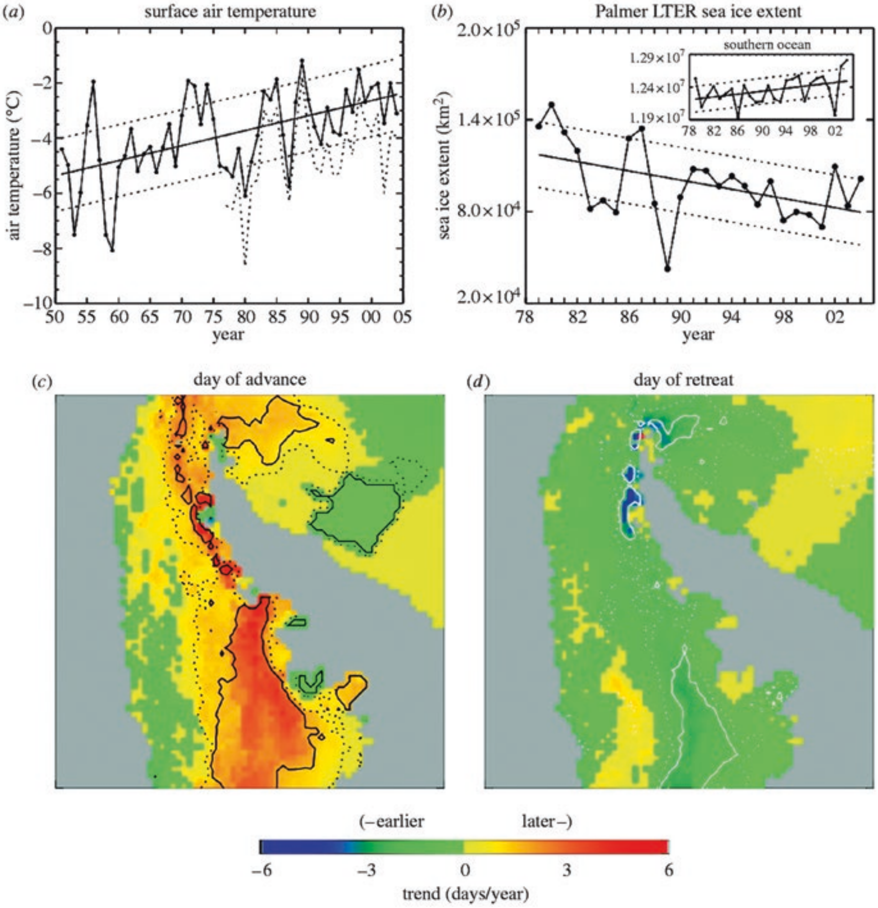

Fig. 12.1 (**a**) Annual average air temperature recorded at Faraday/Vernadsky Station (65°15′ S, 64°16′ W) from 1951 to 2004. The linear regression fit (*solid*) and ±1 standard deviation (*dotted*) about the fit are included. Annual average air temperature recorded at Rothera Station (67°34′ S, 68°08′ W) from 1977 to 2004 is shown by the dotted curve. The standard error and significance were determined using the effective degrees of freedom ($N_{eff} = 24.8$) present in the regression residuals. Also included are the ±1 standard deviation lines (*dotted*). (**b**) Annual average sea ice extent for the Palmer LTER region and for the Southern Ocean (*inset*) from 1979 to 2004. The linear regression fit (*solid*) and ±1 SD (*dotted*) about the fit are included. Spatial maps of linear trends (1979–2004) in (**c**) day of advance and (**d**) day of retreat in the greater AP region (From Ducklow et al., Marine pelagic ecosystems: the West Antarctic Peninsula, Philosphical Transactions B, 2007, vol 362, issue n. 1477, Fig. 2, page 69, by permission of the Royal Society)

12.3 Physical Environment and Regional Change

12.3.1 The High Antarctic

The most complete data sets describing *Pleuragramma* distribution, abundance, and general biology come from three areas of the coastal Antarctic: the Weddell and Ross Seas, and the WAP shelf. The coastal Weddell and Ross Seas may be characterized as high Antarctic, with an isothermal water column on the shelf predominantly composed of −2 °C ice-shelf waters (Gordon et al. 1982). Though each system has experienced either the calving of massive icebergs (B-15, C-16 in the Ross Sea) or the break-up of ice shelves (Larsen and Filchner Ice Shelves in the Weddell Sea) within their respective embayments, each has retained a high Antarctic water column and a minimal reduction in annual sea ice days (Smith et al. 2007; Zwally et al. 2002; Jacobs and Giulivi 1998). A non-significant warming trend in annual surface temperature (0.29 ± 0.36 °C decade^{-1}) has been observed at Scott Base in the Ross Sea and a non-significant cooling trend (−0.11 ± 0.47 °C decade^{-1}) at Halley Station in the Weddell Sea during the period from 1958–2000 (Vaughan et al. 2003). From a climatic standpoint, observed changes in the Ross Sea and Weddell Sea embayments would have minimal impact on the life history (La Mesa and Eastman 2012) of *P. antarctica* living within them.

12.3.2 The Western Antarctic Peninsula (WAP) Shelf: A Natural Experiment

The hydrography of the WAP shelf is quite different from that of the Ross and Weddell Sea embayments. In contrast to the isothermal −2 °C water column found in the midwaters of the Ross and Weddell Sea continental shelves (Gordon et al. 1982), periodic intrusions of warm (0−2 °C) circumpolar deep water onto the WAP shelf give it a consistently warmer pelagic environment (Hofmann et al. 1996, 1998). The WAP shelf has experienced dramatic changes in climate over nearly the same time frame (1951–2000) as the more limited change observed in the Ross and Weddell Seas. Designated as one of the three most rapidly warming regions of the world, the WAP shelf has increased in mean annual surface temperature at a rate of 0.56 ± 0.43 °C decade^{-1} since 1951 (Faraday/Vernadsky Station record, Turner et al. 2005); winter temperatures have increased even more profoundly (1.09 ± 0.88 °C decade^{-1}), resulting in present midwinter temperatures about 6 °C higher than those observed in the 1950s. On the WAP shelf, sea ice advance and retreat shows coupling to the recent rapid regional warming of the system (Vaughan et al. 2003; Stammerjohn et al. 2008a), with a total of about 10 fewer sea ice days over the same time frame (Ducklow et al. 2007).

The WAP shelf provides a natural experiment, or model system, for examining the effects of climate change on silverfish. The major changes observed in the WAP system due to rapid regional warming include:

- Mean annual air temperature on the WAP shows an increase of 3.7 ± 1.6 °C century^{-1} (X ± SD; Vaughan et al. 2003). Its effects are important to marine life in that the increase in air temperature shows a coupling with the pattern of sea-ice advance, retreat and concentration (Weatherly et al. 1991; Vaughan et al. 2003; Turner et al. 2005; Stammerjohn et al. 2008a).
- Seasonal sea ice is advancing later in autumn and retreating earlier in spring, resulting in approximately 10 fewer sea ice days per year in the vicinity of Palmer Station (Ducklow et al. 2007). Between the years of 1979 and 2004 sea ice retreated a total of 31 ± 10 days earlier overall in the WAP/Bellingshausen Sea region (Stammerjohn et al. 2008b). In addition, reductions in sea ice concentrations have been noted within the pack ice (Vaughan et al. 2003). As might be expected, a North-South gradient in sea-ice advance and retreat dates exists along the shore on the WAP with the southernmost regions showing the least change and the northern mid-shelf region the greatest change (Stammerjohn et al. 2008a).
- Summer sea surface temperatures have risen by about 1 °C since 1950 (Meredith and King 2005).
- Seven ice shelves are in retreat or have already broken up and 87% of the glaciers on the WAP are in retreat (Vaughan et al. 2003; Schofield et al. 2010).
- Increasing temperatures result in reduced surface water salinity due to increasing glacial melt-water runoff and melting ice shelves (Moline et al. 2004, 2008). Vast areas west of the Antarctic Peninsula are covered by low salinity water (33.4–33.6‰; Moline et al. 2004), and the melt water plume extends to depths as great as 50 m (Dierssen et al. 2002).
- Increased landward influence of warm upper circumpolar deep water (UCDW) along the WAP over the last two decades described by Suprenand et al. (2015); Piñones et al. (2013b); Smith et al. (1999); Martinson et al. (2008); Martinson (2012) and Dinniman et al. (2012), effectively making the outer shelf a more oceanic system, particularly at depths below 200 m.
- Significant spatiotemporal changes in phytoplankton and zooplankton community structure were observed, with recurrent shifts from large diatoms to small cryptophytes (Moline et al. 2004) and episodic annual changes from a krill-dominated zooplankton community to a salp-dominated community (Loeb et al. 1997; Nicol et al. 2000; Atkinson et al. 2004).

12.4 Life History and Vulnerability to a Changing Climate

Species' life history traits provide an excellent framework for interpreting vulnerability to climate change (Pearson et al. 2014). Stearns (1992) lists the principal life history traits as: size at birth, growth pattern, size at maturity, age at maturity, number, size, and sex-ratio of offspring, age and size-specific reproductive investments, age and size-specific mortality schedules, and length of life. Data collected in the Ross and Weddell Seas as well as in the waters of the Antarctic Peninsula provide valuable information on a number of *Pleuragramma*'s life history traits. We summarize them here as a useful reference for interpreting climate change effects. For a more comprehensive treatment, see La Mesa and Eastman (2012).

12.4.1 Size at Birth

Pleuragramma hatch at an average length of 9.3 mm in the Ross Sea, with a range of 8–10 mm (Vacchi et al. 2004). Like most notothenioid fishes (Loeb et al. 1993; Kock 1992), it has a functional mouth at hatching and is theoretically capable of feeding right away. Hubold (1990) reported an average length of 9 mm for newly hatched *Pleuragramma* in the Weddell Sea, suggesting that variability in size at hatch is minimal from place to place.

12.4.2 Spawning

Spawning behavior of *Pleuragramma* is unknown. However, two lines of evidence provide suggestions. First, the data of Vacchi et al. (2004, 2012) confirm that *Pleuragramma* has pelagic eggs, and that they are found in high concentrations underneath the land-fast sea ice in Terra Nova Bay in the Ross Sea. Second, the observations of Daniels (1982) that thousands of *Pleuragramma* were observed under land-fast sea ice on three occasions in the June–October period suggests that *Pleuragramma* may form spawning aggregations under coastal sea ice in winter. At the very least, since Daniels (1982) observed the fishes feeding under the ice in Arthur Harbor vic. Palmer Station, it may be that the behavior of *Pleuragramma* in the winter months includes shoaling and movement inshore. It is believed that spawning takes place in the late winter, in the August/September time frame, and that embryos develop over a period of 60–75 days prior to hatching (Kock and Kellermann 1991; Ghigliotti et al. 2017a, b). Clearly, the presence of coastal sea ice at the time of spawning is important. At present, sea-ice is present at the time of spawning throughout the species' range.

In the one place where eggs were definitively located and collected, Terra Nova Bay in the Ross Sea (Vacchi et al. 2004, 2012), *Pleuragramma* eggs were found floating in the platelet ice underneath 1.3 m thick sea ice. They were found to be hatching within the ice starting in mid-November. This was a very important finding, since it suggests that sea ice cover is important to *Pleuragramma*'s early life history. In addition, it confirmed that *Pleuragramma* has pelagic eggs, a conclusion first reported by Faleyeva and Gerasimchuk (1990) in a histological study on the gonads of *Pleuragramma.* As late as 1996 (Kellermann 1996) it was assumed that *Pleuragramma* had demersal eggs like many of the other notothenioids (Kock 1992). *Pleuragramma* eggs had been found earlier in the stomach of a primarily benthic species, *Trematomus scotti* (Hubold 1990), giving credence to the idea that the species had demersal eggs.

12.4.3 Growth and Ontogeny of Diet

In the Weddell Sea region, first year or age class 0 (AC 0) *Pleuragramma* appear in the plankton in late November to early December (Hubold and Tomo 1989); it is assumed that their hatch date is about 1 November. AC 0 *Pleuragramma* appear in the plankton a little later in the Terra Nova Bay region: December-early January (Granata et al. 2002; Guglielmo et al. 1998). Kellermann (1986) assumed a hatch date of early December for the *Pleuragramma* larvae captured in Antarctic Peninsula waters based on length distributions of post-larvae captured in the plankton there. Like the size at hatch, time of hatching is consistently in the November–December period in the three areas of the Antarctic where data are available: the Antarctic Peninsula, and the Ross and Weddell Seas (Vacchi et al. 2004; Kellermann and Kock 1991; Hubold and Tomo 1989; Kellermann 1986).

Post-larval *Pleuragramma* (10–30 mm – AC 0) are distributed largely in the upper 50 m where they feed on small *Limacina*, *Oncaea*, and *Oithona* (Hubold and Hagen 1997; Hopkins 1987; Hubold 1985) as well as nauplii and copepodites of calanoids. Koubbi et al. (2007) also found diatoms and tintinnids in the guts of young silverfish larvae, suggesting that omnivory in early life is another dietary option.

AC 1 larvae (30–55 mm) are found primarily below 50 m in the upper 200 m (Guglielmo et al. 1998) and still feed primarily on cyclopoids. However, once *Pleuragramma* reach a size of 60 mm a diet shift occurs, with the primary prey being *Calanoides acutus* and *Calanus propinquus* (Hubold and Hagen 1997; Hopkins 1987; Kellermann 1987). Fishes greater than 60 mm (Age 2+ – adult) are found primarily below 200 m, but some fraction (15–20%) of the age 2+ population can always be captured in the upper 200 m (Hubold 1984). Larger size classes of *Pleuragramma* (>100 mm) feed on furcilia, *C. acutus* and *C. propinquus, Metridia gerlachei, Rhincalanus gigas, Euphausia crystallorophias*, and *E. superba* (Hopkins 1987; Hubold 1985; Lancraft et al. 2004; Mintenbeck 2008).

Pleuragramma don't reach their juvenile "silverfish" appearance until in their third year (AC 2+) of life at lengths of 60–90 mm. As they grow larger, their diet shifts, their vertical distribution deepens and their eye diameter as a percentage of body size grows larger (Hubold 1985) suggesting a developmental adjustment to promote visual acuity at greater depth. Data on length-at-age obtained using annular rings of otoliths (sagittae) suggest that *Pleuragramma* reach a size of about 200 mm in their 14th year of life (Reisenbichler 1993; Hubold and Tomo 1989). The annular rings of *Pleuragramma* sagittae suggested that the fish's largest sizes (245–250 mm) were about 21 years old (Hubold and Tomo 1989). At the moment, what is most certain is that *Pleuragramma* is a slow-growing, long-lived fish, even when considered among Antarctic fishes (La Mesa and Eastman 2012).

12.4.4 Buoyancy and Vertical Distribution

Despite its lack of a swim bladder, *Pleuragramma* is neutrally buoyant, allowing it to minimize energy expended in maintaining its vertical position within the water column. The lack of a swim bladder is compensated for by a reduction in bone mass and density, and by the static lift provided by large lipid deposits stored in subcutaneous and intramuscular lipid sacs (Eastman and DeVries 1982; Eastman 1985). Whether the functional role of lipids in the lipid sacs is limited to buoyancy (see Maes et al. 2006) or if they also serve as an energy reserve (see Eastman and De Vries 1989, Hagen and Kattner 2017) is still not clear.

Vertical distribution within the water column differs with age. Two elements of *Pleuragramma*'s vertical distribution are clear. First, fishes AC 2+ and younger are found primarily in the upper 200 m, with the youngest fishes found highest in the water column. Second, vertical distribution of fish 3+ and older is uncertain**,** other than the fact that they are found deeper in the water column. Data from Southern Ocean GLOBEC (Donnelly and Torres 2008) were in good agreement with older literature in that AC1+ fish were found mainly in the upper 100 m and AC2+ fish in the 100–200 m depth stratum in Marguerite Bay.

Limited data are available on diel periodicity during seasons where there is a diel period. During the austral fall in the Croker Passage, north of Anvers Island in the Gerlache Strait on the WAP shelf, juvenile and adult *Pleuragramma* (length greater than 60 mm) performed a diel vertical migration, changing their center of distribution from approximately 600 m during the day to approximately 300 m at night. A sizable fraction of the population (ca 20%) reached the 100–200 m depth stratum at night (Lancraft et al. 2004). The same was observed in the eastern Weddell Sea: here, adult *Pleuragramma* were found to spend most of the day close to the sea floor at water depths greater than 350 m. At night the shoals moved up into the upper water column to feed on zooplankton at the pycnocline (Plötz et al. 2001; Mintenbeck 2008).

12.4.5 Growth and Diet in a Changing Environment

The change in the timing of sea-ice retreat observed over the last 25 years (Stammerjohn et al. 2008a) on the northern mid-Peninsula coincides roughly with the time of *Pleuragramma* hatching – episodically leaving the hatching eggs and young larval fish without a sea-ice refugium and more vulnerable to predation. Key parameters for the condition and survival of pelagic fish larvae are prey abundance, prey type and seasonal timing (Beaugrand et al. 2003). It is very likely that changes in sea-ice dynamics will involve alterations in all of these three parameters.

The prey taxa of early *Pleuragramma* larvae, like *Pleuragramma,* exhibit a circumantarctic distribution (Everson 1984; Smith and Schnack-Schiel 1990). However, prey taxa also have species-specific phenologies, with reproduction tied to photoperiod, sea-ice retreat, and phytoplankton production (Atkinson 1991). Cyclopoids, like *Oncaea* and *Oithona*, and the calanoid *M. gerlachei*, remain active in the winter months (Hopkins et al. 1993b) and are able to reproduce when food in the form of phytoplankton comes available. Most of the important calanoids (*C. propinquus, C. acutus, R. gigas*) exhibit a fairly strict phenology of winter dormancy or diapause (Hopkins et al. 1993b; Atkinson 1991). The breaking of diapause, reproduction, and appearance of eggs and copepodites must match up with the hatch time of *Pleuragramma* or they will be unavailable as fuel. Thus, it would appear that timing of prey reproduction is a key element for survival of young *Pleuragramma* larvae. If it occurs too early, prey sizes may exceed those that are optimum for survival.

12.4.6 Size at Maturity

Agreement on size at sexual maturity is a little better than that for age at maturity. Visual and histological studies (Faleeva and Gerasimchuk 1990) suggest that fish collected in the Mawson Sea spawn at 13–16 cm standard length for the first time, with more variability in size at first reproduction in males than females. Hubold (1985) reported that fish in the Weddell Sea first show gonadal development at a size of 12.5 cm and Reisenbichler (1993) reported that McMurdo Sound fish did not show appreciable gonad development until approximately 16 cm. Agreement between those three studies and others (La Mesa and Eastman 2012) is quite good, with a consensus figure of 13–16 cm.

12.4.7 Age at Maturity

If the size at sexual maturity, 13–16 cm SL, is applied to the Hubold and Tomo (1989), Reisenbichler (1993), or Sutton and Horn (2011) growth curve, *P. antarctica* begins reproduction at an age of 6–9 years or about 55% of its maximum size

(Kock and Kellermann 1991). Gerasimchuk (1992) as reported in La Mesa and Eastman (2012) observed an age at maturity of 4–5 years for silverfish of 13–16 cm in the Mawson Sea. However, all studies reporting a size at sexual maturity consistently place it at 13–16 cm, which in at least three studies of length at age (Hubold and Tomo 1989; Reisenbichler 1993; Sutton and Horn 2011) corresponds to an age at maturity of 6–9 years. The species thus shows little difference in size at maturity between regions. Differences in reproductive age between studies are likely due to differences in aging techniques, but three of four give a consensus age of 6–9 years. Deferring reproduction until midway through life allows for greater initial fecundity when reproduction occurs and higher later fecundity through growth (Stearns 1992). For a fish with high natural mortality such as *Pleuragramma* (La Mesa and Eastman 2012), delayed maturity does increase the risk of individual reproductive failure, but the trade-off apparently confers increased individual fitness.

12.4.8 Age-Specific Reproductive Investments

GSI (Gonadosomatic index: the percentage of total fish weight made up by the gonad) for *Pleuragramma* varies between 13 and 46 (Kock and Kellermann 1991), suggesting that, though variable, reproductive investments are quite high. For comparison, GSI's of other nototheniids, *Pleuragramma*'s benthic dwelling relatives, vary between 9 and 29 with a mean of about 16. Other data support a case for high reproductive investment. Relative fecundity of *Pleuragramma* has been reported as 124.9 ± 4.4 eggs g^{-1} (X ± SD; Gerasimchuk 1987) and 70–190 oocytes g^{-1} wet mass (La Mesa et al. 2014), which is 3–5 times that of any of the other nototheniids (Kock and Kellermann 1991) but 4–8 times less than the relative fecundity of other small pelagic fish species such as clupeids (e.g. Alheit and Alegre 1986). Table 12.1 is an estimate of the energy invested in reproduction relative to that invested in metabolism and in somatic growth for three calculated levels of GSI: 15, 25, and 45. Investments in somatic growth were obtained from equations in Reisenbichler (1993) who determined caloric values for *Pleuragramma* tissues through proximate analysis (protein, lipid, carbohydrate, water, ash). Reisenbichler's growth curve, virtually identical to that of Hubold and Tomo (1989), was used to estimate growth in mass. Data for *Pleuragramma* metabolism are from Martinez and Torres (2017).

Table 12.1 An estimate of energy (Kcal/year) invested in reproduction relative to that invested in metabolism and in somatic growth for three calculated levels of GSI: 15, 25, and 45

			Reproductive energy		
Age (year)	Somatic growth	Metabolism	GSI 15	GSI 25	GSI 45
7	8.81	47.98	3.51	5.86	10.54
8	11.92	62.25	5.30	8.84	15.91
9	15.56	78.49	7.64	12.73	22.91
10	19.75	96.70	10.60	17.67	31.80

Reproductive energy was calculated using the conservative approach, that energy invested in gonadal development was equivalent in caloric value to somatic tissues. Clearly, reproductive investment by *Pleuragramma* is quite high; at a GSI of 45, it eclipses the energy invested in somatic growth per year.

Metabolism and growth command the lion's share of the assimilated energy of carnivorous fishes (Brett and Groves 1979), generally about 75%. In the case of *Pleuragramma*, within the total energy devoted to growth and metabolism, reproduction commands between 6 and 27% depending on the year and GSI. Maintaining such a high reproductive output requires a high level of feeding success and excellent physiological condition. Reproductive output will most likely decrease or cease altogether (skip spawning – see Ghigliotti et al. 2017a) if the spawning population is directly or indirectly stressed due to environmental alterations or disturbances.

12.4.9 Mortality Schedules

Estimates of the instantaneous rates of natural mortality (M) vary from 0.25 to 2.21 (La Mesa and Eastman 2012), which is on the high end for notothenioids in general (Kock, 1992; La Mesa and Eastman 2012), reflecting its role as a linchpin prey item for apex predators (Eastman 1985) in the coastal Antarctic. As for most species, it is assumed that mortality decreases with increasing size. Multiple years of recruitment failure, or absence of spawning activity due to poor feeding conditions, coupled with a high natural mortality make silverfish particularly susceptible to local population extinctions (Parker et al. 2015).

12.4.10 Length of Life

It is believed that *Pleuragramma* are capable of living to at least 14 years of age (La Mesa and Eastman 2012; Sutton and Horn 2011; Reisenbichler 1993; Hubold and Tomo 1989). The maximum size is not at issue; it is about 25 cm (La Mesa and Eastman 2012). A long life coupled with reproductive maturity at about the halfway point allows the fish multiple opportunities (7–8 years at minimum) for successful reproduction, a valuable attribute in an environment that is highly variable even without the added uncertainties of climate change. A long life thus may be considered a strength rather than a vulnerability in a high Antarctic climate pattern. If a warming environment results in direct competition for a limiting resource (Lancraft et al. 2004) with a species that exhibits earlier maturity and a shorter generation time, e.g. *Electrona* on the WAP shelf, the species could eventually be displaced.

12.4.11 Capsule Summary of Life History

Pleuragramma is a long-lived, slow growing fish with a tie to coastal sea ice for spawning and early larval development. Sexual maturity occurs at a length of 13–16 cm, which, by conventional reading of otoliths yields an age of 6–9 years (La Mesa and Eastman 2012), and in favorable conditions are capable of reproducing yearly after maturity, though skip spawning is also possible. Young larvae are primarily distributed in the upper 100 m. Dispersal of silverfish is considered to be maximal in the first 2 years of life. Center of distribution deepens as the fish age. Fish exhibit vertical migrations as juveniles and adults (Lancraft et al. 2004; Plötz et al. 2001) and shoaling behavior has been observed both by divers and with "critter cams" mounted on seals (Fuiman et al. 2002). Slow growth, late maturity, and an apparent tie to coastal sea ice all contribute to the species' vulnerability to a warming regional climate on the WAP shelf.

12.5 Effects of Climate Change

Life history traits including reproductive output, individual growth, fitness, development and survival are vulnerable to environmental alterations and may be affected directly or indirectly by changes in temperature, salinity, prey availability and composition, competition and predation.

12.5.1 Temperature and Salinity

Ambient temperature is one of the major drivers controlling the rate of metabolic processes in ectothermic organisms such as fish. The temperature range where physiological functions perform at optimal level, is species-specific (and sometimes also region-specific). Though not necessarily lethal, temperatures outside the optimum range may affect physiological efficiency, thereby impairing fish growth rate and lifetime fecundity (Pörtner et al. 2001; Pörtner and Peck 2010). Moreover, in some fish species ambient water temperature determines the sex-ratio of the offspring (e.g. Brown et al. 2015; Ospina-Alvarez and Piferrer 2008) which may impact reproductive output in the longer term. Temperature has been shown to affect development and growth rate of the early developmental stages of fishes (embryos and larvae) (Fukuhara 1990; Hufnagl and Peck 2011).

There are no experimental data describing temperature and/or salinity effects on *Pleuragramma*, except for one study on freezing resistance of larvae (Cziko et al. 2006). Nothing is known about upper temperature limits. A number of studies have addressed temperature effects on closely related notothenioids and several species were found to be highly stenothermal with an upper lethal temperature of 4–6 °C

(Somero and DeVries 1967; Robinson 2008), while the cryopelagic *P. borchgrevincki* exhibits high thermal tolerance and acclimation capacities to elevated temperatures of >4 °C (Seebacher et al. 2005; Robinson 2008; Robinson and Davison 2008).

In the Ross and Weddell Seas, *Pleuragramma* is found exclusively in the very cold ice-shelf waters (−2 °C) typical of its high Antarctic coastal distribution (Hubold and Ekau 1987; DeWitt 1970; Donnelly et al. 2004). On the WAP shelf, silverfish experience temperatures ranging from −2 to 2 °C (Lancraft et al. 2004; Donnelly and Torres 2008). Two single adult individuals were caught far north, off South Georgia Island and the South Shetland Islands (Trunov 2001), suggesting a certain degree of thermal flexibility; however, water temperatures close to the sea floor where these fish were caught were about 1.5 °C, which is within the range fish are also exposed to off the WAP. At present, the best data available suggest that temperatures over the known range of *Pleuragramma* are not physiologically life-threatening. However, species often exhibit thermal preferenda (Brett 1952), that may be due to physiological trade-offs between metabolism and growth or simply prey distributions. In Marguerite Bay on the WAP, *Pleuragramma* was found in highest abundance in the most neritic environments and not on the outer shelf (Donnelly and Torres 2008).

Experimental studies on the impact of salinity changes on notothenioid fish are extremely scarce and limited to few species, which were found to be tolerant towards large fluctuations in salinity (O'Grady and DeVries 1982).

Data from non-Antarctic fish species show ontogenetic differences in vulnerability towards changes in temperature (Pörtner and Farrell 2008; Pörtner and Peck 2010) and sea water salinity (Varsamos et al. 2005), with early developmental stages such as eggs and larvae being more sensitive than adult fish. In the case of *Pleuragramma*, there is some indirect evidence that larvae and juveniles might be vulnerable to changes in temperature and salinity. Larvae and juveniles are mostly found associated with water masses of particular temperature and salinity (e.g. Hubold 1984; Kellermann 1986; Slósarczyk 1986; Guglielmo et al. 1998; Granata et al. 2002). West of the Antarctic Peninsula, larvae and juveniles were clearly confined to cold and high salinity water masses of Weddell Sea origin (Slósarczyk 1986). Based on combined datasets of fish abundances in water of Weddell Sea and Bellinghausen Sea origin, Slósarczyk (1986) calculated the range of approximate optimum water temperature (−0.50 to +0.45 °C) and salinity (~34.10–34.62‰) for *Pleuragramma* larvae and juveniles in the Bransfield Strait. Though changes in salinity induced by climate change might be a locally restricted phenomenon, dense aggregations of larvae and juveniles that are both concentrated close to the coast/shelf ice in the upper water layers might be affected. Moline et al. (2004) found vast areas west of the Antarctic Peninsula covered by low salinity water (33.4–33.6‰), and the melt water plume extended to depths as great as 50 m (Dierssen et al. 2002).

However, whether the occurrence of larvae and juveniles in particular water masses really reflects thermal and salinity optima or preferences, or maybe just major transport pathways, remains to be verified. Nevertheless, there is some evidence that larvae are actually highly sensitive to increasing temperatures from observations on board a research vessel: *Pleuragramma* larvae from the southern Weddell Sea, held in aquaria on board, were observed to shrink and to die immediately at water temperatures >0 °C (Hubold 1990).

12.5.2 Prey Availability and Composition

The environmental alterations off the Antarctic Peninsula are causing significant spatiotemporal changes in phytoplankton and zooplankton community structure. Recurrent shifts are observed from large diatoms to small cryptophytes (Moline et al. 2004) and from a krill-dominated zooplankton community to a salp-dominated community (Loeb et al. 1997; Nicol et al. 2000; Atkinson et al. 2004). Unlike krill, salps are able to feed efficiently on very small particles ±1 µm (Madin 1974; Kremer and Madin 1992; Fortier et al. 1994). Alterations in zooplankton community composition due to climate forcing have been observed world-wide, and often include a shift from larger to smaller zooplankton species, especially in copepods (e.g. in the southern North Sea, Helaouët and Beaugrand 2007; and in the Humbold Current ecosystem, reviewed in Alheit and Niquen 2004). Shifts in size distribution from large to small phytoplankton organisms in the Southern Ocean may thus favor the prevalence of smaller zooplankton species such as the cyclopoid copepods. Fishes, particularly small pelagic zooplankton conssumers, are known to be highly sensitive to indirect effects of climate forcing such as alterations in prey abundance, prey composition, and prey size (see McFarlane et al. 2001; Benson and Trites 2002; Beaugrand et al. 2003; Alheit and Niquen 2004).

Pleuragramma mainly preys upon copepods and different life stages of krill (see 12.4.3). There are indications for size selective feeding in larvae as well as in adults from studies on stomach contents (Hubold and Ekau 1990; Lancraft et al. 2004; Mintenbeck unpublished data), with adults neglecting prey below a certain size limit, but it is unknown whether the selective feeding behaviour is obligate or facultative. Moreover, compared to most other notothenioids, *Pleuragramma* is a specialist feeder with a narrow food spectrum (Mintenbeck et al. 2012). *Pleuragramma* might therefore be extremely vulnerable to changes in zooplankton composition in general, and to changes in zooplankton size-structure in particular.

Pleuragramma larvae strongly depend on time of appearance, abundance and type of prey. A mismatch between hatching and prey availability due to climate change-induced phenological shifts might significantly compromise larval condition and survival (see, e.g. Wöhrmann et al. 1997; La Mesa et al. 2010; La Mesa and Eastman 2012). Early postlarvae exhibit only low levels of reserve lipids, early stages therefore depend on the availability of prey and thus on synchronous hatching of copepod offspring (Wöhrmann et al. 1997). However, despite low lipid levels, larval *Pleuragramma* can starve for about 3 weeks (Hubold 1992) and adults are also assumed to be able to cope with periods of food deprivation due to their low metabolic requirements (Wöhrmann 1998), so there is a certain degree of plasticity. Larvae and juveniles, however, were observed to feed all year long (Kellermann and

Schadwinkel 1991) and overall lipid content of adult individuals shows no pronounced seasonal variation (summer-winter), both indicating that the large lipid deposits are primarily used as buoyancy aid and not as energy reserve during winter months (Hubold and Hagen 1997), as e.g. in Antarctic myctophid fish (Donnelly et al. 1990). However, the functional role of lipids in *Pleuragramma* is still not fully understood. If the functional role of lipids in Antarctic silverfish is exclusively restricted to buoyancy (see, e.g. Maes et al. 2006), starvation capability is extremely low. If lipid stores, or at least parts of them are used as energy reserves in case of food deprivation (see, e.g., Eastman and DeVries 1989), the reduced lipid content will negatively affect buoyancy and thereby energetics underlying dispersal and migration (vertical and horizontal).

Prey quality may be as important as prey availability to young *Pleuragramma*; condition of *Pleuragramma* larvae varied strongly with food composition (copepods vs. diatoms; Koubbi et al. 2007). Likewise, condition of adult fish may be affected by a shift in zooplankton towards a salp-dominated community, as has been observed off the WAP. Gelatinous zooplankton, such as salps, are occasionally consumed by fishes (including notothenioids) and flighted seabirds (Ainley et al. 1986), acting as "survival food" when preferred zooplankton prey are not abundant (Kashkina 1986; Mianzan et al. 2001). Energy density and nutritive value of gelatinous species are extremely low when compared to crustaceans such as krill and copepods (e.g. Clarke et al. 1992; Donnelly et al. 1994; Torres et al. 1994). Low energy food may affect survival, growth, body condition and reproductive output of consumers such as *Pleuragramma*, in turn making the species a low quality prey item for its warmblooded predators (see Österbloom et al. 2006, 2008; Ruck et al. 2014).

12.5.3 Competition and Predation

In vast areas around the Antarctic continent an oceanic, offshore pelagic fish community and a neritic pelagic fish community are readily distinguished (e.g. in the Weddell and Ross Seas; DeWitt 1970; Hubold and Ekau 1987; Lancraft et al. 1991; Donnelly et al. 2004). The oceanic community is typically dominated by myctophid fish and the neritic fish community is almost exclusively composed of *Pleuragramma* and early developmental stages of other notothenioid fish species. As the oceanic and neritic communities are usually clearly separated at the shelf break, there is no pelagic fish species seriously competing with *Pleuragramma* for prey in the high Antarctic. However, on the West Antarctic Peninsula (WAP) shelf, the neritic and oceanic communities overlap, resulting in a mixed pelagic community (Donnelly and Torres 2008).

The oceanic fraction of the mixed community is dominated by the myctophids *Electrona antarctica* and *Gymnoscopelus* spp., and the neritic fraction is mainly represented by *Pleuragramma*. *E. antarctica* and *Gymnoscopelus* spp. both mainly feed on copepods, in particular *C. acutus, C. propinquus* and *M. gerlachei*, and on ostracods and euphausiids (Hopkins et al. 1993a; Saunders et al. 2015), so there is very high overlap in prey composition between the myctophids and *Pleuragramma*, making competition for food in this region likely.

The degree of overlap between oceanic and neritic communities was found to be directly correlated to local hydrodynamic conditions, i.e. to the mixture of cold shelf water masses and warmer and more saline circumpolar deep water (Donnelly and Torres 2008; Cullins et al. 2011). The absence of myctophid fish in other coastal Antarctic regions where cold (−2 °C) and less saline ice shelf water prevails (e.g. Ross and Weddell Seas), is explained by the absence of antifreeze glycoproteins (AFGPs) in the oceanic species (Cullins et al. 2011). The occurrence of oceanic species on the WAP with its warmer mid-depths (2 °C), suggests that, with more widespread warming of shelf water masses due to climate change, oceanic myctophid fish may increasingly invade other high Antarctic regions, perhaps becoming a serious competitor of *Pleuragramma* for zooplankton on a larger spatial scale. Growth and time to first reproduction happen much faster in myctophid fish than notothenioid species (Greely et al. 1999; Linkowski 1987), so population density may increase rapidly. Ongoing climate change might thus result in competitive suppression of *Pleuragramma* populations.

However, populations may not exclusively be suppressed by competition, but also by top-down pressure, namely predation. Predation on *Pleuragramma* is pervasive. All developmental stages are important food sources and are heavily preyed upon by a multitude of predators, including piscivorous fish, seals, seabirds and penguins (Castellini et al. 1984; Eastman 1985; Green 1986; Plötz 1986; Klages 1989; Hureau 1994; Burns et al. 1998; Cherel and Kooyman 1998; Daneri and Carlini 2002; La Mesa et al. 2004). Under stable conditions the populations of *Pleuragramma* can sustain this exploitation, but in a changing Southern Ocean, where krill are in decline and salps are more pervasive (as observed at the WAP; Loeb et al. 1997; Nicol et al. 2000; Atkinson et al. 2004), krill consumers may rely more heavily on pelagic fish resulting in increased predation pressure on *Pleuragramma*.

Competition and predation alone are unlikely to cause a collapse of *Pleuragramma* populations. However, the cumulative effects of several stressors working in tandem, e.g. competition, predation, changing prey spectrum, reduced sea ice, episodic recruitment, and the physiological effects of warm temperature work together to put a high level of pressure on the species.

12.6 *Pleuragramma* on the WAP Shelf: Mechanisms Underlying Its Response to Rapid Regional Warming

Pleuragramma has been extensively sampled on the WAP shelf as part of large multi-investigator programs and smaller individual efforts, providing a historical record of its larval and adult distribution since 1976 that is unique in the Antarctic

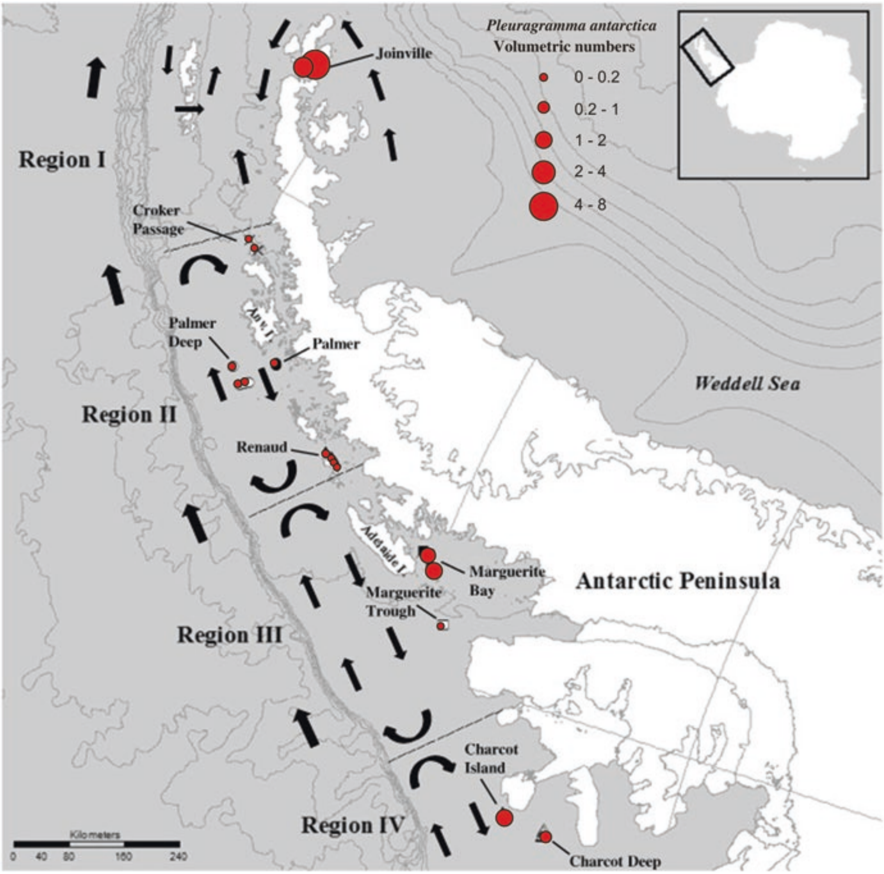

Fig. 12.2 Distribution of *P. antarctica* (number × 10^4 m^{-3} in the upper 500 m) on the western Antarctic Peninsula shelf in the austral fall of 2010. Results from 32 trawls (Data from Parker et al. 2015)

system. Taken together, the historical information confirmed a continuous distribution of *P. antarctica* from the Antarctic Sound in the Northern Peninsula to Marguerite Bay in the south up to at least 1990, when targeted sampling for *Pleuragramma* became less frequent (Parker et al. 2015; Ross et al. 2014; Kellermann 1996).

In an effort to assess the current status of *Pleuragramma* on the WAP shelf, a study was undertaken in austral fall of 2010 (Parker et al. 2015). Samples were collected at nine sites within the historic distribution of *Pleuragramma* along the WAP (Fig. 12.2) with a 10-m^2 Multiple Opening and Closing Net and Environmental Sampling System (MOCNESS) outfitted with six 3-mm mesh nets (Wiebe et al. 1976, 1985). A total of 32 trawls were successfully completed, with 7 conducted during the day and 25 at night.

Sites were located within four hypothesized hydrographic regimes (Fig. 12.2), based on observed shelf circulation patterns (Parker et al. 2015; Hofmann et al. 1996; Stein 1983). The regimes, designated as peninsular regions, were delineated as follows. Region I comprised the northern peninsula including Joinville Island, Antarctic Sound, and the Bransfield Strait. It is strongly influenced by cold-water flow from the Weddell Sea. Region II was the northern mid-peninsula, including Anvers Island and the Biscoe Islands community where regional warming has produced a marked decline in spring (November) sea ice (Ducklow et al. 2007; Stammerjohn et al. 2008a, b). Region III was the southern mid-peninsula (Marguerite Bay) community including the Marguerite Bay system. Region IV was the southern peninsula (Charcot Island) community roughly defined by Lazarev Bay on the north and an as yet undefined boundary well south of Charcot Island. Model results suggest that the gyral circulation in Region III reaches closure near Lazarev Bay, and a third gyral circulation is set up just north of Charcot Island, reaching closure further south (Piñones et al. 2011, 2013a, b). For purposes of discussion, the different regions described above (shown in Fig. 12.2) will be used for convenience in discussing peninsular locations.

Silverfish were captured at Joinville Island in Region I, Marguerite Bay in Region III, and in the vicinity of Charcot Island in Region IV. One fish was captured in Croker Passage, an area in Region II where they were formerly abundant (88 individuals 10^3 m^{-2} in the upper 200 m; Lancraft et al. 2004). A continuous distribution of juvenile and adult silverfish along the WAP shelf in the 1970s and 1980s (Daniels and Lipps 1982; Reisenbichler 1993) was highly discontinuous in early fall of 2010. The question is: what does a gap in a formerly continuous distribution mean for silverfish on the WAP?

12.6.1 Paleohistory of Silverfish on the WAP

Part of the answer to *Pleuragramma*'s disappearance from Region II is provided by observed shifts in paleo-climate. Two studies of abandoned penguin rookeries along the WAP show evidence of shifts in paleo-diet in the Adélie penguin (*Pygoscelis adeliae*) in response to past warming and cooling periods. The first study, located in the vicinity of Palmer Station in Region II (Emslie et al. 1998), and covering a time span from approximately 644 to 250 years BP (Before Present), shows a shift in the non-euphausiid component of Adélie diets from primarily silverfish during cool periods to primarily squid (*Psychroteuthis glacialis*) during warm periods of the WAP's environmental history. Abandoned rookeries of Marguerite Bay (Region III) showed an identical trend in a study encompassing 6000 years of paleohistory (6000 years BP to modern), with diets shifting from primarily silverfish in cooler periods to squid in warmer (Emslie and McDaniel 2002). Clearly, silverfish have exhibited up and down trends in population numbers on the WAP throughout the Holocene.

12.6.2 Mechanisms Underpinning the Disappearance of Pleuragramma in Region II: Alternate Hypotheses

Ultimately, *Pleuragramma*'s absence from Region II reflects an absence in recruitment of young fish accompanied by attrition in the older size classes due to predation or migration. In the late 1980s, larval silverfish showed an abrupt and persistent drop in numbers in the northern mid-peninsula region (Region II) (Kellermann 1996), which persists to the present day (Ross et al. 2014). Ross et al. (2014) attribute the loss of larval *Pleuragramma* to three possible causes: (1) loss of connectivity to a distant source population, (2) loss of a local source population, or (3) decrease of larval survival in the northern mid-peninsula. Any of the three alternatives would potentially result in an absence of recruitment, leading eventually to local extinction. It will be useful to consider each possibility in light of what is presently known about *Pleuragramma* on the peninsula.

12.6.2.1 The "Larval Pump" Hypothesis

Much thought has been given as to how a high-Antarctic species such as *Pleuragramma* has succeeded in the warmer waters of the WAP shelf. Youngest post-larvae (10–12 mm) were rarely captured on the peninsula in the many larval surveys conducted from the 1970s through the early 1990s (Kellermann 1996), and then not in high abundance (Kellermann 1986), which argued against local source populations, particularly when compared to the numbers found in the Weddell and Ross Seas. Instead, recruitment of *Pleuragramma* to the waters of the WAP shelf was attributed to larval dispersal, a "larval pump", from spawning centers associated with the ice shelves in the western Weddell Sea (e.g. Larsen Bay) and the continental Bellingshausen Sea southwest of the Peninsula (Kellermann and Schadwinkel 1991). In the larval-pump hypothesis, larvae originating in the Weddell Sea, notably in Larsen Bay, are transported from the Weddell Sea via the Weddell Gyre/Antarctic Peninsula Coastal Current (APCC) as it flows through the Antarctic Sound and as it bends around Joinville Island (Fig. 12.2; Stein 1983; Hubold 1992; Hofmann et al. 1996; La Mesa et al. 2015) and into the eastern Bransfield Strait, eventually proceeding south with the APCC. Larvae originating in the other hypothetical spawning site, the continental Bellingshausen south of the peninsula, would be transported northeast in the upper 100 m of the Antarctic Circumpolar Current (ACC), merging with the general northeasterly flow at the shelf break along the peninsula, and making their way further inshore via the cross-shelf gyral flows in the vicinity of Alexander Island, Renaud Island, and the southern Bransfield Strait (Fig. 12.2; Hofmann et al. 1996; Piñones et al. 2011, 2013a, b).

Pleuragramma larvae have been present in most samples taken from the Bransfield Strait in the 1970s through the 1990s (Kellermann 1986, 1996; Loeb 1991; Morales-Nin et al. 1998) and more recently (Lipsky 2006; Loeb et al. 2006; Jones et al. 2006; La Mesa et al. 2015) though numbers have been highly variable.

Recent modeling of larval transport for *Pleuragramma* originating in the Weddell Sea corroborates the dispersal path described above, both in terms of trajectory and timing relative to hatch-date (La Mesa et al. 2015). Once in the Bransfield Strait, larvae could theoretically be transported south via the APCC, potentially to the Palmer Archipelago, the Biscoe Islands, and Marguerite Bay (Niiler et al. 1991; Moffat et al. 2008). Flow in the upper 200 m of the Gerlache Strait east of Anvers Island is generally north (Savidge and Amft 2009), so dispersal of larvae further south via the Gerlache Strait would be severely constrained by the circulation. Successful transport south would require that larvae be in the west central Bransfield Stait (Niiler et al. 1991; Moffat et al. 2008).

Whether the "larval pump" was ever a major supplier of *Pleuragramma* larvae from either the Weddell Sea or from the Bellingshausen for recruitment to the WAP shelf is a matter of conjecture, but it is highly unlikely that it has been contributing any in recent years. Ross et al. (2014) report that no *Pleuragramma* larvae were captured on the slope or outer shelf from 1993–2008, ruling out any input from the Bellingshausen via the Antarctic Circumpolar Current (ACC). Similarly, in samples from the Anvers Island region (Region II of Parker et al. 2015) no larvae were present from 1996–2008. The implication is that few larvae originating in the Weddell Sea survived the transit from the cold waters of the Bransfield Strait to the warmer region near Anvers Island. There is no evidence for present connectivity between the Weddell Sea and Region II.

Further evidence ruling out strong connectivity from the Northern to Southern peninsula is provided by genetics and otolith microchemistry of fish collected in Region I and Regions III and IV. Genetic analyses performed on fish captured in the 2010 survey described in Parker et al. (2015) showed that Marguerite Bay and Charcot Island fish comprised a single, panmictic population that was differentiated from the Joinville Island fish (Agostini et al. 2015). Microchemical analyses of otolith nuclei showed similar results. No significant differences were detected in isotopic ratios between fish captured in Marguerite Bay and near Charcot Island (Ferguson 2012) suggesting that *Pleuragramma* from Marguerite Bay and Charcot Island are part of one contiguous population. A separate population of silverfish was found at Joinville Island using the same techniques. Results of those studies coupled with the fish distributions observed in Parker et al. (2015) provide three lines of evidence that there is little connectivity between silverfish populations in the northernmost region at Joinville Island and the two southernmost regions at Marguerite Bay and Charcot Island.

12.6.2.2 Reproducing Populations on the WAP Shelf

An alternate hypothesis to the continuous replenishment of WAP silverfish populations by larvae from distant sources is that reproduction has been occurring in local populations on the WAP shelf, supplementing the larvae supplied by advection (Parker et al. 2015). Candidates for spawning locations would include Palmer Station's Arthur Harbor (cf. Daniels 1982), Crystal Sound, and Marguerite Bay.

Each may have hosted reproducing populations of *Pleuragramma*, but changing seasonal sea ice dynamics presently leave only Crystal Sound and Marguerite Bay as possibilities for local reproduction. Recruitment in local populations would be highly vulnerable to unfavorable sea-ice conditions brought about by a changing climate.

Larval captures in the Marguerite Bay region reported by Ross et al. (2014) and the presence of fish sufficiently large to be of reproductive age captured in Parker et al. (2015) support reproduction on the shelf. Larvae captured by Ross et al. (2014) were of the correct size to have been spawned there.

If the three hypotheses for absence of silverfish larvae in Region II stated in Ross et al. (2014) are revisited in combination with results from Parker et al. (2015), what is happening to silverfish on the peninsula becomes a bit clearer. Essentially, all three reasons for silverfish disappearance have converged in Region II, driven by the poleward movement of the ACC. The increased landward influence of warm upper circumpolar deep water (UCDW) along the WAP over the last two decades described by Suprenand et al. (2015), Piñones et al. (2013b), Smith et al. (1999a), Martinson et al. (2008), Martinson (2012) and Dinniman et al. (2012), has made the outer shelf a more oceanic system, particularly at depths below 200 m (Ashford et al. 2017). Nowhere has this eastward encroachment been more pronounced than in Region II.

12.6.2.3 Loss of Connectivity to a Distant Source Population

A potential larval source in the Weddell Sea is well established based on recent (La Mesa et al. 2015) and multiple older references (Kellermann 1996) that considered the Bransfield Strait as a nursery ground for *Pleuragramma*. Circulation in the Bransfield Strait, though complex, would allow some southward transport of larvae though the strait itself and into Region II. However, in recent years, once transported into the increasingly oceanic waters of Region II, they apparently do not survive (Ross et al. 2014). Any Weddell Sea larvae drifting south past Anvers Island and the Biscoe Islands will encounter a warmer water column and an altered prey spectrum. Clearly, hypothesis 3, decrease of larval survival, applies here as well.

Northward larval transport from the Bellingshausen via the ACC is also not supported by the Ross et al. (2014) larval survey, which found no silverfish larvae in outer shelf or slope samples. Though alluded to repeatedly (e.g. Kellermann 1996), a location for the source populations in the Bellingshausen Sea was never provided in the literature.

Northward transport of larvae produced in the Marguerite Bay region would also be possible, but the demonstrated absence of larvae in shelf/slope stations coupled with the highly episodic appearance of any northeasterly coastal flow (Moffat et al. 2008) precludes any likelihood of serious northward larval transport. Region II is effectively cut off from larvae originating to the north and south of it.

12.6.2.4 Loss of a Local Source Population

P. antarctica is a cold-water, neritic species, not found in oceanic waters during its juvenile or adult stages (De Witt 1970; Lancraft et al. 1989, 1991; Donnelly et al. 2004; Donnelly and Torres 2008) despite an entirely pelagic life history (La Mesa and Eastman 2012) and a rich prey spectrum in oceanic waters. Increasing influence of warm oceanic waters (upper circumpolar deep water) on the shelf will likely push its distribution further toward the inner shelf, a trend observed in Marguerite Bay during the 2001, 2002 GLOBEC program (Donnelly and Torres 2008).

Historical observations in the vicinity of Palmer Station and the Biscoe Islands, e.g. Daniels (1982) and Daniels and Lipps (1982), strongly suggest that Antarctic silverfish were quite abundant in Region II during the 1970s (Kellermann 1996). Region II shows the most pronounced effects of rapid regional warming on the peninsula, including mean annual temperatures that have increased by 5.7 ± 2.0 °C century^{-1} since 1951 (Vaughan et al. 2003) and a consistently earlier sea ice retreat at about the time-of-hatch for *Pleuragramma* eggs (Fig. 12.1; Stammerjohn et al. 2008a). Region II also showed a virtual absence of silverfish during Parker et al. (2015). The fact that *Pleuragramma* spawns under coastal sea ice and that eggs apparently develop and hatch in a nursery of platelet ice when it is available (Vacchi et al. 2004, 2012) points to regional warming and its effects on seasonal sea ice dynamics as an important element in the species' absence in Region II. Phenology of reproduction in the local silverfish population may have been disrupted by early ice retreat, resulting in the absence of a sea ice refugium prior to hatching, with an accompanying change in predation pressure and altered prey spectrum for larvae, resulting in multiple years of poor local recruitment. It is highly likely that a local breeding population formerly existed in Region II, but that it is no longer present, either due to attrition or migration.

12.7 Consequences for the Ecosystem

Pleuragramma is a shoaling fish (Fuiman et al. 2002) that can attain very high densities. For example, in the southern Weddell Sea the biomass of silverfish was estimated to be 1 ton km^{-2} (Hubold 1992). Adults undertake nocturnal migrations from deeper waters into upper water layers, where dense shoals provide an easily accessible food source for warmblooded animals living on the ice (Fuiman et al. 2002; Plötz et al. 2001; Lancraft et al. 2004; Mintenbeck and Knust unpublished data). This key species in the high Antarctic food web is threatened by climate change on several levels, making a reduction in its population density probable in the long run.

The ecosystem response to climate forcing detected so far off the WAP may be observed in other marine ecosystems worldwide. A common pattern emerging from the climate-driven alterations in the world's oceans is that the pelagic realm is always the first and/or the strongest to respond (reviewed in Mintenbeck et al. 2012). Among fishes, pelagic species in short plankton-based food chains often undergo strong fluctuations in stock density (e.g clupeids; Alheit and Niquen 2004), with severe consequences for warm-blooded apex predators (e.g. Alheit 2009; Arntz 1986; Cury et al. 2000).

Something similar may be expected in the long run for the Southern Ocean. If climate change proceeds and extends towards other regions of the coastal Antarctic, it is very likely that *Pleuragramma* will be affected by direct and/or indirect climate forcing. The potential consequences involve a decrease in nutritive value of the fish for predators and a decline in population density due to reduced reproductive output or increased mortality. As a polar species, *Pleuragramma* has no option for escape to a more favorable climate, nor is an evolutionary adaptation that keeps pace with the rate of change very likely (see Somero 2010). Notothenioid fish are evolutionarily adapted to stable (cold) environmental conditions without major disturbances, which is also reflected in *Pleuragramma*'s life history traits such as slow growth and low relative fecundity (compared to non-notothenioid pelagic fish, reviewed in Mintenbeck et al. 2012). A recovery of the population after the stock has declined is accordingly questionable. If *Pleuragramma*, in the worst case, disappears from the ecosystem, many species will lose their main food source.

Whether such a loss can be replaced within the food web by, e.g., invading myctophid fish or demersal notothenioid fish remains to be seen. Demersal notothenioids also contribute to the diet of Antarctic warm-blooded animals (Casaux et al. 2006; Coria et al. 2000; Plötz et al. 1991), but they neither occur in shoals, nor do they undertake regular vertical migrations into upper water layers, making their exploitation in vast areas on the deep shelf more costly in terms of capture efficiency and time and energy investment. Myctophids, which also undertake daily vertical migrations (e.g. Lancraft et al. 2004), might be able to substitute for Antarctic silverfish in the high Antarctic marine food web. One possible candidate is *Electrona antarctica*. Its circumantarctic distribution in waters seaward of the shelf break and south of 60° S (e.g. Lancraft et al. 1989, 1991) make it attractive as a potential replacement for *Pleuragramma* in the coastal system. Currently, its lack of antifreeze proteins physiologically exclude it from the ice-shelf waters of the Antarctic coast (Cullins et al. 2011), but it is found in abundance at typical shelf depths and in deeper coastal canyons in the warmer waters of the WAP (Parker et al. 2015; Donnelly and Torres 2008; Lancraft et al. 2004). It has a maximum age of 4 years, and is believed to broadcast spawn in multiple batches in its last year of life (Greely et al. 1999). The diet of *E. antarctica* is similar to that of *Pleuragramma*, with copepods being its main prey in its early years and juvenile krill as it reaches its maximum size (Lancraft et al. 2004).

E. antarctica undertakes strong diel vertical migrations; in oceanic waters it is found in the upper 300 m at night and between 600 and 1000 m during the day (Lancraft et al. 1989, 1991). Morphologically, it is well suited to the nearly constant reduced light levels typifying the habitat of a vertically migrating mesopelagic fish. It has a dark dorsum and a full suite of ventral and lateral photophores in addition to large, well developed eyes. Further, it exhibits little plasticity in its diel behavior with no evidence of swarming, or shoaling. Unlike *Pleuragramma*, it is not found over shallow bottom depths.

Piñones et al. (2016) note an increasing presence of circumpolar deep waters in cross-shelf canyons in the Ross Sea shelf area, so eventually *E. antarctica* may be able to make its way onto the Ross Sea shelf via coastal canyons as on the WAP shelf. In past climatic regimes, the Ross Sea has been an open water marine environment (45,000 to 27,000 years BP; Emslie et al. 2007), which would suit the species' life strategy well. However, this scenario is far in the future, even at the current rate of warming.

A fully functional substitution requires not only an alternative species similar in terms of diet, abundance, size, and energetic value, but also in terms of availability and accessibility. The dense shoals of *Pleuragramma* provide an easily accessible food source for warm-blooded predators, in particular when shoals migrate up into upper water layers. *E. antarctica* does not occur in such dense shoals, and its success as a potential substitute for *Pleuragramma* in the diet of apex predators also depend on their vertical migration pattern at high latitudes; e.g. during 24 h daylight in austral summer, longer periods of time are spent at great depth (Donnelly et al. 2004), out of the reach of most predators.

Clearly, systemic shifts affecting *Pleuragramma* will cause strong responses in food web structure. The long-term consequences for the ecosystem's top predators and for overall ecosystem functioning remains unclear, so far. However, if no species will be able to provide functional compensation within the food web, the consequences for the entire system are most likely severe.

12.8 Future Research

There are still many gaps and uncertainties in our knowledge on *Pleuragramma*'s fundamental ecology and life history traits that needs to be filled or verified. Future research, e.g., should verify the role of sea ice in *Pleuragramma*'s ecology and reproductive ecology and spawning sites.

The standard method for analyzing the effects of environmental alterations on fish species is the experimental approach, where single variables or even variable combinations can be manipulated to test for a response. Experimental approaches would definitely help to verify the assessment of *Pleuragramma*'s vulnerability to climate change inferred so far only from indirect evidence and knowledge on closely related species.

Experimental studies on Antarctic silverfish are extremely scarce due to its soft and fragile body structure, making the capture and cultivation of live specimens extremely difficult. Some of the few successful experimental approaches include studies on the freezing resistance of eggs and larvae (Cziko et al. 2006), on yolk resorption in starving yolk-sac larvae (Hubold 1992), haemoglobin system of adult fish (Tamburrini et al. 1997), and feeding behavior and prey preferences of juvenile *P. antarctica* (Fanta 1999).

Sampling of adults in good condition from research vessels using traditional fishing methods, such as trawls, is very difficult. However, careful sampling of early stages through holes drilled in the ice, as successfully done in Terra Nova Bay (Vacchi et al. 2004), may provide the best solution to collect *Pleuragramma* individuals alive for experimental studies. Such experimental approaches should test for the direct effect of changes in water temperature and salinity on fitness and survival and the acclimation capacities of this species. This will allow for further insights into actual sensitivity of these vulnerable life stages to alterations in environmental parameters. Feeding experiments with different life stages may help to elucidate selectivity in prey choice (size and type of prey) and the impact of prey quality on fish condition.

Also studies focusing on spatial and/or temporal variability in life history traits or fish condition could further elucidate the role of particular environmental parameters (abiotic and biotic) in controlling the state of individuals and populations (e.g., Ruck et al. 2014). For such an approach specimens from regions with different environmental regimes might be compared based in traditional measures (e.g. stomach contents, fatty acid composition, condition indices, etc.), but also using advanced methods. A recent study investigated the internal structure of preserved *Pleuragramma* using Magnetic Resonance Imaging (MRI; Bock et al. 2017). This modern tool may provide novel insights into, e.g. the structure of *Pleuragramma*'s lipid sacs, and may open new opportunities for studies on the impact of climate change on Antarctic silverfish.

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Chapter 13

Conservation and Management of Antarctic Silverfish *Pleuragramma antarctica* Populations and Habitats

Philippe Koubbi, Susie Grant, David Ramm, Marino Vacchi, Laura Ghigliotti, and Eva Pisano

Abstract One of the main conservation objectives for marine systems is to identify areas of ecological importance for biodiversity and essential species habitats which can be used as scientific reference areas for monitoring global change in the absence of major human impacts. The Antarctic silverfish, *Pleuragramma antarctica* is a keystone pelagic species, that has been assessed on the IUCN Red list of threatened species as a species with "Least Concern". However, this species is unique as it is placed at one extreme of the notothenioid evolutionary/ecological axis that ranges from benthic to secondary pelagic life style. Its different life stages occur in unique environments such as the platelet ice for eggs, some of the inner shelf canyons for

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young larvae and at the shelf break with Antarctic krill swarms for juveniles and adults. The winter habitats are not known. In addition to the effects of climate change, a threat to this species is its bycatch in krill fisheries; however it has not been directly harvested since the 1980s. As a midtrophic species, *P. antarctica* is sensitive to environmental changes and should be monitored in protected scientific reference areas to obtain information on global change. For a midtrophic fish in a supposed wasp-waist ecosystem, strict regulation of bycatch and monitoring should be carried out in parallel with the monitoring of Antarctic krill and ice krill. The designation of protected areas is an important mechanism for preserving the essential habitats of *P. antarctica* in the Southern Ocean.

Keywords Conservation • CCAMLR • Antarctic Treaty System • Midtrophic fish • Antarctic fish

13.1 Introduction

13.1.1 Marine Conservation in the Southern Ocean

Understanding the biogeography of species and the distributional patterns of communities and ecosystems in the Southern Ocean (de Broyer and Koubbi 2014) is essential for robust conservation and management (Leathwick et al. 2006; Grant et al. 2014) in the context of global change (Constable et al. 2014; Koubbi et al. 2015). The Convention on Biological Diversity (CBD) targets the objective of protecting at least 10% of marine areas by 2020. There are 7 major CBD criteria that were defined for conservation which includes the designation of Ecologically and Biologically Significant Areas (EBSA) and Marine Protected Areas (MPA).

The origins and status of these criteria are described by Dunn et al. (2014), they are:

- uniqueness or rarity;
- special importance for life history of species;
- importance for threatened, endangered or declining species and/or habitats;
- vulnerability, fragility, sensitivity, slow recovery;
- biological productivity;
- biological diversity;
- naturalness.

The aim is to identify areas of ecological importance for biodiversity and essential species habitats which can be used as scientific reference areas for monitoring global change with minimal human impacts. The CDB criteria can be used to determine what important biological and environmental features can be used to identify areas for protection. Most of them deal with protecting the diversity of species, ecosystems and seascapes, however some can be used for keystone species such as

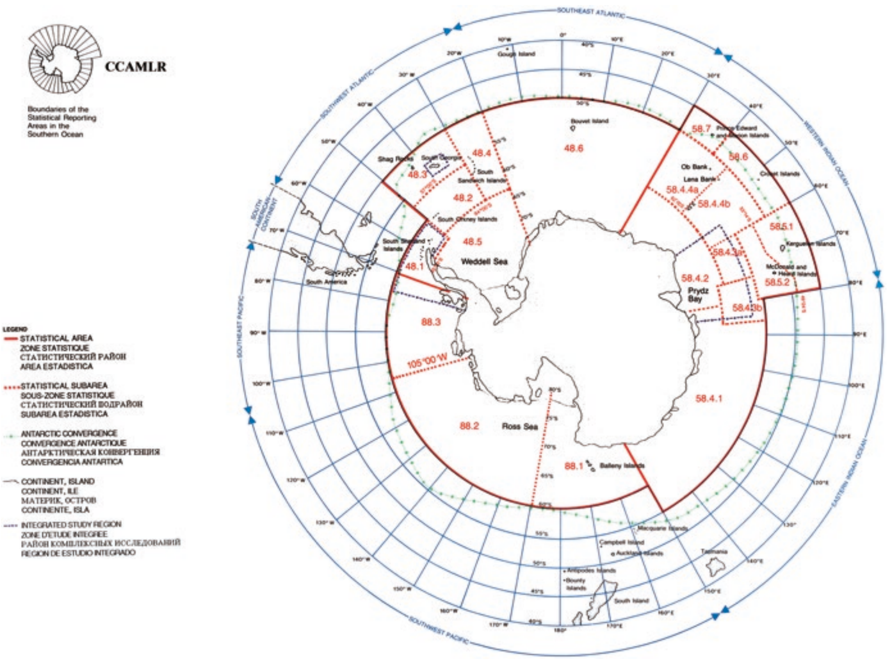


Fig. 13.1 Convention Area of CCAMLR with statistical subareas and divisions (www.ccamlr.org)

Pleuragramma antarctica. Our knowledge of the distribution, function in the ecosystem and evolution of this abundant species may justify new conservation approaches. It is also important to estimate the level of threat from fisheries on this species.

13.1.2 The Commission for the Conservation of Antarctic Marine Living Resources and the Committee for Environmental Protection

The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) was established by international convention in 1982 with the objective of conserving Antarctic marine life (Fig. 13.1). CCAMLR takes a precautionary, ecosystem-based management approach to the conservation of Antarctic marine ecosystems (www.ccamlr.org). It aims to regulate the harvesting of resources in a sustainable manner, with consideration of the potential effects of fishing on other ecosystem components, particularly those which are dependent upon, or related to, harvested species (CAMLR Convention 1982: Article 2; Grant et al. 2014).

CCAMLR employs a system of collection and assessment of scientific data through the input of Members into its Working Groups and Scientific Committee. Decisions made by the Commission are by consensus, and Conservation Measures giving effect to CCAMLR's objectives are formulated, adopted and revised on the basis of the best scientific evidence available (CAMLR Convention 1982: Article 9, and CCAMLR Resolution 31/XXXVIII, 2009).

Each year during its annual meeting, CCAMLR revises and implements a comprehensive suite of conservation measures in order to fulfil the Commission's objectives for the conservation of Antarctic marine living resources. Conservation measures cover a range of fishery, research and conservation matters such fishery compliance, fishery notifications, gear regulations, data reporting, research and experiments, minimisation of incidental mortality arising from fishing, fishery regulations including closed areas, prohibition and catch limits for target and by-catch taxa and protected areas (www.ccamlr.org). Some of these measures are limited in time or spatially, or concern only some species, mainly the two species of toothfish (*Dissostichus eleginoides* and *D. mawsoni*), Antarctic krill (*Euphausia superba*) and icefish (*Champsocephalus gunnari*) that are commercially harvested. Other measures related to the impact of fisheries on the Southern Ocean species (by catch, incidental mortality of birds and environmental protection), general prohibitions on fishing in closed areas, and conservation of marine ecosystems (including vulnerable marine ecosystems, VMEs).

In the Southern Ocean, a number of MPAs have been established around sub-Antarctic islands under national jurisdiction. CCAMLR has now established two high seas MPAs; the first on the South Orkney Islands southern shelf (CCAMLR, 2009 – Commission report CCAMLR-XXVIII), and recently the large Ross Sea region MPA (CCAMLR, 2016 – Commission report CCAMLR-XXXV). CCAMLR is working towards establishing a representative system of MPAs in the Southern Ocean (CCAMLR, 2011 – Commission report CCAMLR-XXX) and proposals are in various stages of development for MPAs in the Weddell Sea, western Antarctic Peninsula and East Antarctica. All these areas support high densities of *P. antarctica* which is broadly distributed around the Antarctic shelf (Duhamel et al. 2014).

The Committee for Environmental Protection (CEP) was established by the Protocol on Environmental Protection to the Antarctic Treaty, and formulates advice on environmental issues to the Antarctic Treaty Consultative Meeting (ATCM). The CEP's focus is on assessing the environmental impact of human activities, designating specially protected and managed areas, protecting fauna and flora, preventing pollution or other disturbances, the impacts of climate change, and the introduction of non-native species (Grant et al. 2014). The Protocol on Environmental Protection applies to both marine and terrestrial areas, and Antarctic Specially Protected Areas (ASPAs) and Antarctic Specially Managed Areas (ASMAs) may be designated anywhere south of 60°S, including the marine area.

At a joint meeting of the CEP and the CCAMLR Scientific Committee in 2009, it was agreed that CCAMLR should be the 'lead body' on work to establish marine protected areas in waters south of 60° S (CEP XII Report). However, the CEP also has its own mechanism by which it can designate marine areas for special protection or management, with the approval of CCAMLR where relevant (ATCM Decision 9, 2005). There are currently six entirely marine ASPAs, and a further seven ASPAs which contain both marine and terrestrial components (Grant et al. 2014). The geographic coverage of marine ASPAs is limited, with all sites located either along the Antarctic Peninsula or Victoria Land (Ross Sea), and the majority of these cover small coastal areas (Grant et al. 2012). Antarctic Specially Managed Areas (ASMAs) can also be designated in marine areas to coordinate human activities and minimise environmental impacts; currently three ASMAs include marine components (Grant et al. 2014).

13.2 What Conservation Criteria Apply to *P. antarctica*?

P. antarctica (family Nototheniidae) has been assessed on the IUCN (International Union for Conservation of Nature) Red list of threatened species as a species with "Least Concern". This Red list category indicates that "this species has no major threats acting against it at present", and it has been reported to be the most dominant pelagic fish species in areas of its broad distribution (Gon and Vacchi 2010). It is not commercially harvested; however because this species plays an important role in the Antarctic ecosystem food web, continued monitoring of the population numbers is needed.

13.2.1 Uniqueness

P. antarctica is the dominant pelagic fish over the Antarctic shelf (Duhamel et al. 2014; Ashford et al. 2017). Most notothenioids are demersal species with only a small part of their initial life cycle as ichthyoplankton. The Antarctic silverfish is the only known holopelagic species with all developmental stages, from egg to adult, living in the water column (e.g. DeWitt et al. 1990; Vacchi et al. 2004, 2012; La Mesa and Eastman 2012).

Pelagic eggs, produced in high quantities (Faleeva and Gerasimchuk 1990; Vacchi et al. 2012) according to an r-strategy style, lead to a high production of larvae that dominate in the neritic domain, locally reaching more that 99% of the ichthyoplankton (e.g. Loeb et al. 1993; Koubbi et al. 2009, 2011a). Juveniles and adults populate the water column where they live in shoals vertically moving for foraging (Plötz et al. 2001; Mintenbeck 2008). The capability of the Antarctic silverfish to live in the water column emerged at an evolutionary time scale during the Antarctic notothenioid adaptive radiation (e.g. Eastman 2005). After the acquisition of antifreeze proteins, enabling the survival in a cooling and icy marine environment (Cheng and Detrich 2007), although ancestrally devoid of a swim bladder and potentially advantaged by living near the bottom, some Antarctic notothenioids moved from benthic to epibenthic, semipelagic, cryopelagic and pelagic niches, profiting from the reduced competition and novel ecological opportunities that resulted from deep reshaping of near-shore Antarctic icy habitats (Near et al. 2012; Eastman et al. 2014).

The Antarctic silverfish is placed at one extreme of the notothenioid evolutionary/ecological axis which ranges from benthic to secondary pelagic life style. The capability of pelagic habitat utilization (secondary pelagization) has been accompanied by important morphological and physiological modifications including compensatory changes in body density and buoyancy, mostly achieved by a reduction of bone mass and changes in skeletal features (Voskoboinikova et al. 2017) and lipid accumulation (Eastman 1993; De Vries and Eastman 1978; Hagen and Kattner 2017).

13.2.2 Essential Fish Habitats

The Magnuson-Stevens Fishery Conservation and Management Act from US legislation defines Essential Fish Habitat (EFH) as being "all waters and substrate necessary for fish for spawning, breeding, feeding, or growth to maturity". EFH are readily identifiable for demersal fish species with segregated habitats of their ecophases as described by Harden-Jones (1968). Koubbi et al. (2009) showed that this scheme of life stages segregation can also apply to some Southern Ocean fishes. Fish ecophases include the different life stages of fish within their environment such as: eggs from spawning areas to hatching zones, larvae in the pelagic zones, juveniles in nursery grounds or adults from trophic areas to spawning grounds. Each of these phases are subject to environmental and anthropic pressures that influence survival to varying extent. Options for conservation measures will depend on the sensitivity of an ecophase to a particular pressure. Some measures may apply to reproductive habitats and others to nursery grounds for the juveniles. The level of protection may vary through a seasonal closure of fisheries (e.g. during the reproduction stage or juvenile growth) to the prohibition of fishing in coastal zones where nursery areas occur. Ultimately, such measures for protection would need to be supported by the objectives of MPAs.

Is such an approach suitable for conserving abundant midtrophic pelagic fish such as *P. antarctica*? The determination of ecophases for pelagic fish species is more complex as these species live in a dynamic 3-dimensional environment that is also responding to global change. Vacchi et al. (2012) and Guidetti et al. (2015) described the platelet ice environment for egg development for *P. antarctica*. There are very few fish species that have an obligate use of the sea-ice environment, the other example being *Pagothenia borchgrevinki* (a cryopelagic species) (Gutt 2002). The spatial segregation of life stages of *P. antarctica* was described in relation to gyres and currents in the Weddell Sea (Kellermann 1987; Hubold 1984, 1985). In East Antarctica in the D'Urville Sea, Koubbi et al. (2011a) showed the importance of coastal deep canyons for the development of young larvae. ROV (Remotely Operated Vehicle) observations indicated that these canyons support appropriate habitat for the fish larvae and provide a stable deep-water environment. Life stages were also separated according to their depth in the water column with adults occurring deeper than larvae and juveniles (Koubbi et al. 2011a). However, there is insufficient information to describe the ecology of this species during the winter.

Hjort (1914) estimated that the fluctuations of fisheries in Northern Europe were linked to the survival of larvae, the most critical stage for fish. For *P. antarctica*, Giraldo et al. (2011) showed inter-annual variations in the trophic condition of larvae according to their development with the younger larvae being more sensitive to starvation than the older ones which are able to adapt to lower abundance of prey items. Protection may be needed for the larval stage habitats as it will influence the yearly recruitment of the species. This question is of major importance worldwide for midtrophic fish as we observe important decreases of clupeids recruitment, and *P. antarctica* (also known as Antarctic herring) can be considered as ecologically equivalent to clupeids. Bakun's fundamental triad is the way of looking at early life-stages habitats of midtrophic fish. Recruitment of *P. antarctica* might depend on the three conditions stated by Bakun (1996):

- enrichment of surface layers by nutrient-rich waters;
- concentration of planktonic organisms on which young stages feed;
- retention of young stages within favourable areas.

Enrichment will depend on upwellings and other mixing events such as katabatic winds and other extreme meteorological conditions that will bring up deep-water nutrients to the surface layers where they will be consumed by phytoplankton. Concentration of planktonic preys depends on convergence or dynamics of fronts such as typically occurs at the shelf-break. Some areas show high plankton abundances that are not only linked to hydrodynamic features but also to the sea ice dynamics. The importance of polynyas is obvious for the Southern Ocean as these features help the offspring of the pelagic production during spring when *P. antarctica* larvae have hatched. The match between new foraging larvae and plankton production is very important. Retention prevents large emigration of larvae to the oceanic area and depends on eddies and also on circulation linked to topographical features which is the case with inner-shelf depressions. Protection of areas responding to the Bakun's triad is then obvious for maintaining conditions favourable for the survival of larvae of *P. antarctica*.

During the juvenile and adult stage, individuals shift their diet from phytoplankton and small zooplankton to larger zooplankton such as ice krill (*Euphausia crystallorophias*), Antarctic krill or large copepods (Giraldo 2012). This change in trophic behaviour is related to a change of habitat with individuals moving to the shelf and shelf break and away from the coastal zone. However, it is difficult to determine clear nursery areas for juveniles. One threat is linked to the association of Antarctic silverfish with Antarctic krill as fish can be taken as by-catch in the krill fisheries (see *13.3.3*).

13.2.3 Biological Productivity and Diversity Supported by **P. antarctica**

Some pelagic ecosystems such as upwellings are often dominated by a few schooling species which are representative of the crucial intermediate trophic level. *P. antarctica*, krill species (ice krill and Antarctic krill), myctophid fish and amphipods play this role in the Southern Ocean. The abundance of these species varies according to global change as demonstrated for Antarctic krill (Atkinson et al. 2004; Flores et al. 2012). We assume that the pelagic ecosystem of the Antarctic shelf functions as a "wasp-waist" ecosystem where species such as *P. antarctica* exert a control on the trophic dynamics of the pelagic ecosystem by influencing the lower trophic levels as well as the top predators which are foraging on them (Bakun 2006). If this is the case, environmental changes can drastically affect population abundances of these species, with major consequences on the pelagic community structure and on top predators. Future studies are needed to understand if *P. antarctica* can support the needs of higher trophic levels in the case of a decrease in the abundance of Antarctic krill. The CCAMLR Ecosystem Monitoring Programme (CEMP) aims to detect significant changes in critical components of the Antarctic marine ecosystem, and to distinguish between changes due to harvesting and those due to physical or biological environmental variability (Agnew 1997, and http://www.ccamlr.org/en/science/ccamlr-ecosystem-monitoring-program-cemp).

13.2.4 Other Criteria

We have poor knowledge on the long term trends in abundance of *P. antarctica* around the continent that could provide evidence that the stock is threatened, endangered or in decline. There are few surveys specifically dedicated towards addressing this issue. In East Antarctica, Koubbi et al. (2011a) have monitored *P. antarctica* fish larvae from 2004 to 2011. Although inter-annual variations of abundances or condition index were observed (Giraldo et al. 2011), it is not sufficient to describe trends. Unfortunately, the calving of the Mertz Glacial Tongue in 2010 prevented further surveys.

A broad and long-term dataset exists from the Western Antarctic Peninsula (WAP) where *P. antarctica* has been extensively surveyed since 1976. Comparison of historical and present data indicated a discontinuity in the distribution of juvenile and adult *P. antarctica* along the WAP (Parker et al. 2015). This information, along with the fact that the WAP shelf is considered the most rapidly warming region on the planet (Vaughan et al. 2003), makes this area a suitable model system to evaluate the vulnerability of *P. antarctica* to a changing environment (Mintenbeck and Torres 2017).

As to the biological diversity criterion, *P. antarctica* is an endemic Antarctic notothenioid. It is part of a unique fish species flock on the Antarctic shelf that generated the current biodiversity pattern in the high Antarctic region (Lecointre et al. 2013).

13.3 Fisheries

13.3.1 Historical Catches

P. antarctica was targeted by Soviet trawlers from 1978 to 1989 (Table 13.1). The total reported catch of *P. antarctica* was 5282 tonnes, most of which was taken in Division 58.4.2 (3316 t) (Table 13.1). Catches were also reported from Subarea 48.2 in 1983 (110 t), Division 58.4.1 in 1983–84 (339 t) and Area 88 in 1981 (split-year) (1517 t). Data available from the fishery in Division 58.4.1 and 58.4.2 indicated that vessels operated mid-water trawls in depth ranging from 50–620 m, with most of the catches taken between 400–620 m in Division 58.4.1 and 200–300 m in Division 58.4.2 (Fig. 13.2).

Table 13.1 Catches (tonne) of *P. antarctica* reported by Soviet trawlers during target fishing in the Southern Ocean in Subarea 48.2, Divisions 58.4.1 and 58.4.2 and Area 88 between 1978 and 1989. Seasons spans the 12-month period from 1 December of one year to 30 November of the following year

	Areas			
Season	48.2	58.4.1	58.4.2	88
1978			286	
1979			35	
1980			31	
1981[a]				1517
1982			46	
1983	110	95	307	
1984		244	905	
1985			984	
1986			596	
1987			30	
1988			67	
1989			29	
All seasons	110	339	3316	1517

Source: CCAMLR Statistical Bulletin

[a] 12-month period July 1980–June 1981

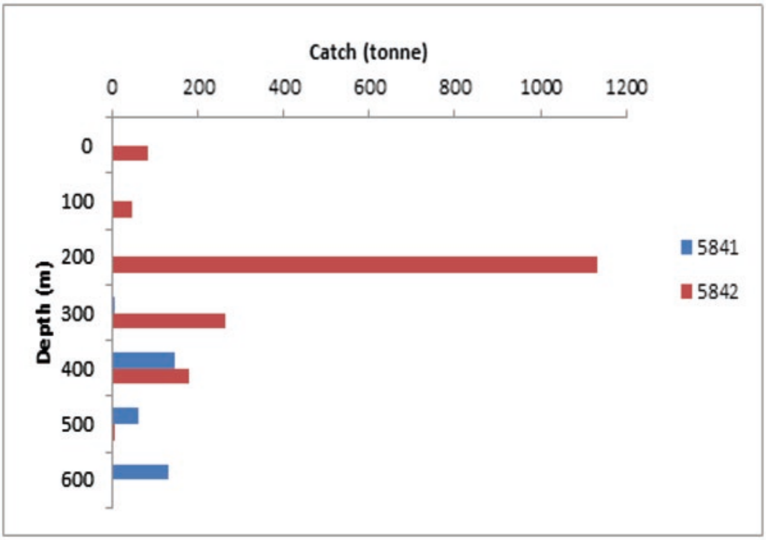


Fig. 13.2 Catches (tonne) of *P. antarctica* by 100 m depth interval reported by Soviet trawlers during target fishing in Divisions 58.4.1 and 58.4.2 between the CCAMLR seasons 1978 and 1989. (Source: CCAMLR catch and effort data)

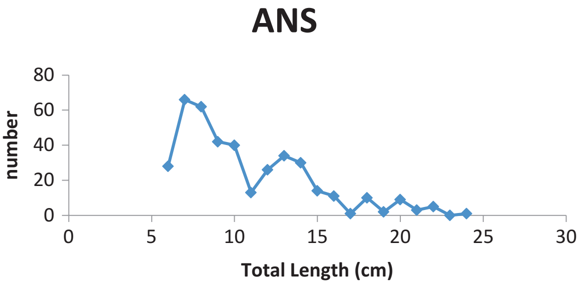
13.3.2 CCAMLR Conservation Measures

Since the catches reported from 1978 to 1989, CCAMLR received notifications for a targeted mid-water trawl fishery on *P. antarctica* in Division 58.4.2 between 1999 and 2003. This led to Conservation Measure 186/XVIII (and subsequent updates until 2003) which set annual catch limits for the species of 300–500 t in Division 58.4.2. However, this fishery was never undertaken by the proponents and the fishery lapsed. At present CCAMLR does not have any specific measures citing *P. antarctica* directly, as this species is not targeted by fisheries and has been infrequently reported as by-catch. However, conservation measures dealing with krill fisheries are also relevant as *P. antarctica* is a bycatch species of these fisheries.

13.3.3 Bycatch in Krill Fisheries

P. antarctica has been reported in the by-catch from the krill fisheries conducted by Soviet trawlers in Area 88 in 1982, and since 2005 by vessels targeting krill in Subareas 48.1 and 48.2. Annual catches of *P. antarctica* in those subareas is generally less than 1 tonne in total, however 14 tonnes were reported in 2006 (CCAMLR Statistical Bulletin).

Fig. 13.3 Length frequency of *P. antarctica* (subsample) in the by-catch from krill fisheries in Subareas 48.1 and 48.2



The lengths of *P. antarctica* recorded in the by-catch in Subareas 48.1 and 48.2 range from 6–24 cm (total length) (Fig. 13.3).

13.4 Special Protection for Silverfish

13.4.1 ASPAs

One ASPA is specifically concerned with *P. antarctica,* ASPA No. 173 (Cape Washington and Silverfish Bay) located in the coastal zone of Terra Nova Bay, Ross Sea.

ASPA No. 173 encompasses an approximate area of 286 km^2 comprising Cape Washington and Silverfish Bay (Fig. 13.4). The designation is based on the outstanding ecological and scientific values represented by the presence of one of the largest emperor penguin (*Aptenodytes forsteri*) colonies in Antarctica, made up of around 20000 pairs that breed on the sea-ice adjacent to Cape Washington, and of the nursery area for Antarctic silverfish located at Silverfish Bay (approximately 20 km west of Cape Washington). There, large quantities of embryonated eggs of the species are found entrapped in the platelet ice beneath the solid sea-ice during the austral Spring and mass hatching occurs (Vacchi et al. 2012). Silverfish Bay and its surrounding areas are the first documented hatching and nursery area for *P. antarctica*. This discovery has expanded our understanding of the early life-history of this species, and the proximity of the site to scientific stations makes it of outstanding scientific value for continuing study (Vacchi et al. 2004, 2012; Ghigliotti et al. 2015; Guidetti et al. 2015). Recent surveys from the sea-ice showed that the spatial distribution of the silverfish nursery area can be wide in some years, ranging from Cape Washington to Gerlache Inlet, over the eastern side of the Campbell Glacier Tongue outside the ASPA (Fig.13.4). Moreover, the surveys indicated that in the area the distribution pattern of eggs, and the abundance of newly hatched larvae, change at spatial scale of kilometres, with the eggs not homogeneously distributed under the solid ice, and with differences from year to year (Vacchi et al. 2012; Guidetti et al. 2015).

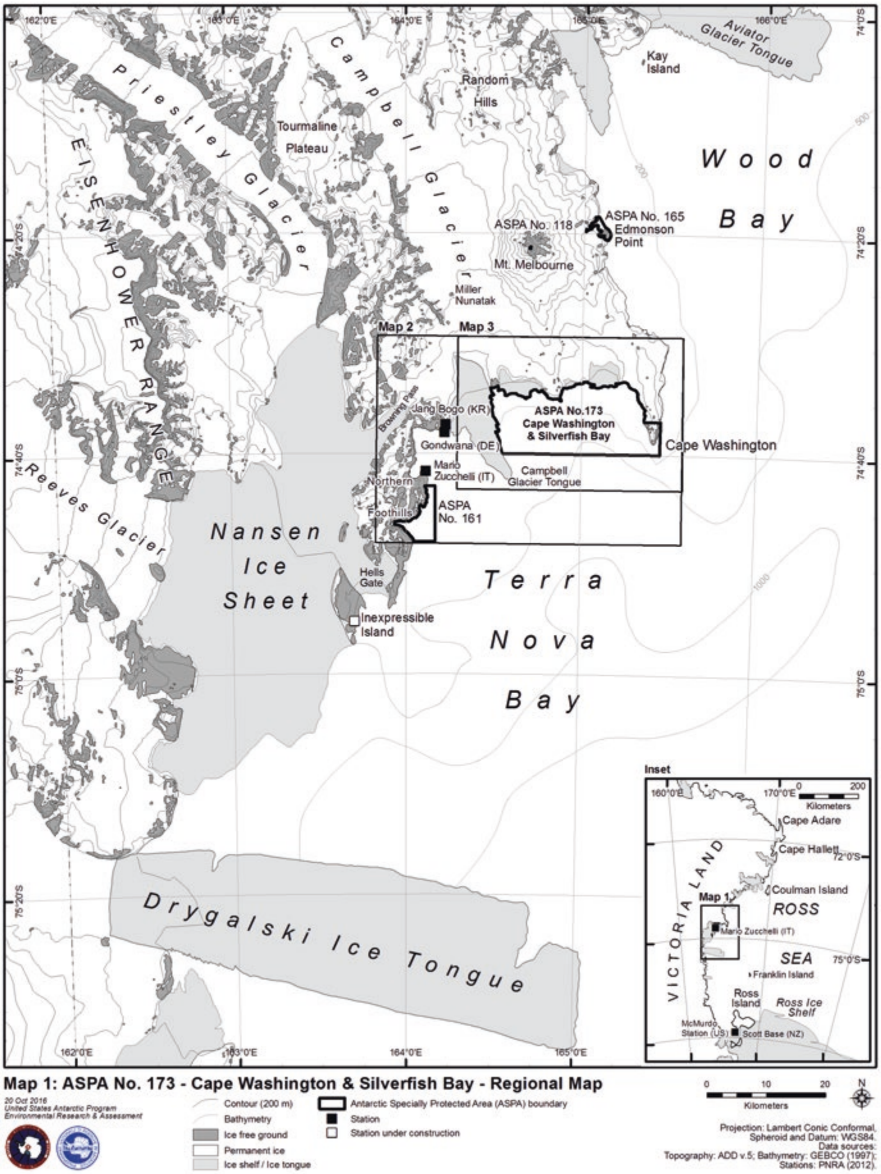


Fig. 13.4 ASPA No. 173: Cape Washington and Silverfish Bay from the “Management plan for Antarctic Specially Protected Area (ASPA) No. 173 Cape Washington & Silverfish Bay Northern Terra Nova Bay Ross Sea” (2013)

The scientific value of the ASPA is increased by the diversity of other species occurring in a relatively small area accessible to scientists thanks also to the support from nearby research stations. This body of characteristics makes it a good location to study the ecosystem structure and functioning, including the predator/prey relationships between different members of the marine ecosystem. Cape Washington itself is a nesting area for several flying birds, such as south polar skuas and snow petrels. Adélie penguins (*Pygoscelis adeliae*) are present in the emperor colony and on the sea-ice edge daily from November to mid-January. Large groups of killer whales and other cetaceans, such as Antarctic minke whales, are regularly observed foraging in this area, as well as Weddell and leopard seals. The embayment where the Antarctic silverfish nursery is located is an important haul-out and breeding area for Weddell seals, with several hundred typically congregating along the sea ice. Crabeater seals and Arnoux's beaked whales are occasionally seen at the sea ice edge in the region. Cape Washington is the only place known where the interaction between leopard seals and emperor penguins can be so reliably observed.The boundaries of the ASPA were defined according to an integrated approach that considered also the inclusion of several components of the local ecosystem. The management plan for the ASPA stresses the need for long-term special protection owing to the outstanding ecological and scientific values, as well as the potential vulnerability to disturbance from scientific, logistic and tourist activities in the region.

ASPA No. 173 is among only three ASPAs designated specifically to protect fish species or habitats, together with ASPA Nos. 152 and 153, which are located in the western Antarctic Peninsula region.

ASPA No. 152 (Western Bransfield Strait) is recognized as an important spawning ground for several fish species, including the rockcod *Notothenia coriiceps* and the icefish *Chaenocephalus aceratus*. For the latter species, the importance of conservation of the spawning grounds is highlighted by the discovery of the nesting and parental care behavior of *C. aceratus* (Detrich et al. 2005) that makes this fish particularly vulnerable during the reproductive phase.

ASPA No. 153 (Eastern Dallmann Bay) is considered an important habitat for juvenile fish species including the rockcod *N. coriiceps* and the icefish *C. aceratus*. In addition the fish specimens of this ASPA area are used for comparative studies with those of the more heavily impacted Arthur Harbor Area where the Bahia Paraiso wreckage and oil-spill occurred in 1989.

13.4.2 CCAMLR MPAs

CCAMLR Conservation Measure 91-04 (2011) gives the general framework for the establishment of MPAs which can be designated to protect:

- representative examples of marine ecosystems, biodiversity and habitats;
- key ecosystem processes, habitats and species, including populations and life history stages;

- scientific reference areas for monitoring natural variability and long-term reference areas or for monitoring the effects of harvesting and other human activities;
- areas vulnerable to impacts by human activities, including unique, rare or highly biodiverse habitats and features;
- features critical to the function of local ecosystems;
- areas to maintain resilience or the ability to adapt to the effects of climate change.

Some of these criteria follow the CDB criteria. *P. antarctica* can be considered for all the points listed. As a keystone midtrophic pelagic species, *P. antarctica* is representative of the neritic pelagic ecosystem and is critical for the functioning of the pelagic food web. With euphausiids, it is a key species between plankton and top predators. As a midtrophic species, *P. antarctica* is sensitive to environmental changes and should be monitored in designated scientific reference areas to monitor global change. But, as one of the bycatch taxa taken in krill fisheries, the abundances observed from these fisheries can also provide estimates of mortality of fish bycatch.

13.4.2.1 South Orkney Islands Southern Shelf MPA

The South Orkney Islands southern shelf MPA (SOISS MPA) was established by CCAMLR in 2009 (Conservation Measure 91-03) with the objective of conservation of biodiversity of representative examples of five pelagic bioregions and six geomorphic zones of the subarea 48.2 of CCAMLR (SC-CAMLR-XXXIII/BG/19, Delegation of the European Union, 2014). The MPA includes an area of key importance for winter penguin foraging. *P. antarctica* may occur on the South Orkney shelf, however the MPA conservation measure does not focus on this species. Nevertheless, one of the specific objectives of the MPA is the protection of representative examples of pelagic marine ecosystems, biodiversity and habitats.

13.4.2.2 Ross Sea Region MPA

The Ross Sea Region is one of the most productive areas of the Southern Ocean, with important populations of top predators such as penguins, flying birds, seals and cetaceans. A proposal for designating the Ross Sea Region MPA was first discussed in 2012, and then considered at CCAMLR-XXXIV (CCAMLR, 2012 – Commission report CCAMLR-XXXI and CCAMLR-XXXIV/29 Rev. 1, Delegation of New Zealand and the USA (2015)). After various refinements in terms of supporting scientific evidence and boundary details, the establishment of the Ross Sea Region MPA was approved by CCAMLR countries in October 2016 (Commission report CCAMLR – XXXV and Conservation Measure 91-05) to come into force in December 2017. Extending to 1.55 million km^2, this will be the world's largest

Marine Protected Area. One of the main objectives of this MPA is to conserve natural ecological structure, dynamics and function throughout the Ross Sea Region at all levels of biological organization, by protecting habitats that are important to native mammals, birds, fishes, and invertebrates. In order to meet specific conservation goals and habitat protection, ecosystem monitoring and fisheries management objectives, some activities will be limited or entirely prohibited. Seventy-two percent of the MPA will be a 'no-take' zone, which forbids all fishing, while other zones will permit some harvesting of fish and krill for scientific research. Specific actions are directed towards fish, for instance protecting important areas in the life cycle of Antarctic toothfish, but also towards the pelagic realm with the aim to protect trophically dominant pelagic prey species such as Antarctic krill, ice krill and *P. antarctica*. The research and monitoring plan details scientific projects that should be carried out by using acoustic surveys to map distribution of these pelagic species including dedicated research on silverfish in Terra Nova Bay and surveys around the Balleny Islands as a potential nursery area for Antarctic silverfish and Antarctic toothfish. The protection of representative pelagic and benthic ecosystems is also considered by including not only the diversity of species and habitats but also a functional approach including species ecophases and trophic ecology.

13.4.2.3 East Antarctica Representative System of MPA

CCAMLR is considering a proposal to establish an East Antarctic Representative System of Marine Protected Areas (EARSMPA) (CCAMLR-XXXIV/30, Delegations of Australia, France and the European Union (2015)– Commission report CCAMLR-XXXIV). This proposal, which was first discussed in 2012, identifies three candidate MPAs in the different biogeographic provinces along East Antarctica. It includes general conservation objectives that have relevance to protection of the pelagic ecosystem, as well as specific conservation objectives relating to *P. antarctica*. It is important to note that Antarctic krill is not as abundant in this area as it is in the Atlantic sector of the Southern Ocean where it is commercially harvested. One of the general objectives is "to protect key ecosystem processes, habitats including vulnerable marine ecosystems and species in East Antarctica, such as reproductive and early life-cycle phases nursery grounds of *Euphausia superba*, *Dissostichus mawsoni* and *Pleuragramma antarctica*". For the D'Urville Sea-Mertz MPA, it is proposed "to protect nursery areas for Antarctic silverfish" following the results of research on fish larvae, juveniles and adults in this area from the CEAMARC project of the Census of Antarctic Marine Life (International Polar Year project 53) (Hosie et al. 2011; Koubbi et al. 2011a, b).

This proposal identifies the need for maintaining the biodiversity and productivity within the MPAs. For pelagic communities, it includes plankton, krill species, *P. antarctica* and the epipelagic and mesopelagic fish, all of which are important for land-based predators. This informs us of the importance to consider in marine protection not only the species diversity but also ecosystem functioning and particularly trophic webs. In addition, the MPA Research and Monitoring Plan encourages research in East Antarctica to study ecosystem change in areas where the impacts of fishing are minimised.

13.4.2.4 Other MPA Proposals

Two further proposals are being developed for MPAs around the Antarctic continent in areas where *P. antarctica* occurs. The first concerns MPA Planning Domain 1 (Southern Scotia Arc and the Western Antarctic Peninsula) (WG-EMM-15/42, Second WS-MPA Domain 1, 2015). The proposal seeks to protect important areas for fish life stages, concentrating on species that were overexploited during the late 1970s and the 1980s.

The second proposal concerns the Weddell Sea which has as one of its objectives the protection of Antarctic krill, ice krill and Antarctic silverfish. *P. antarctica* is the dominant pelagic fish of this area and is an important prey species for other fish, seals and penguins. The life history of this species in the Weddell Sea is well documented from hatching of larvae in October–November to the drift of larvae along the shelf towards the coastal polynyas (Hubold 1984, 1992; SC-CAMLR-XXXIV, SC-CAMLR-XXXIV/BG/15, Delegation of Germany (2015)).

13.5 Concluding Remarks

Midtrophic level fish species are abundant globally and include herring in the North Sea and the various species of sardines and anchovies in upwelling ecosystems. Major changes in their abundances can either be explained by climatic variations or, in some cases, overfishing as it is observed in the Benguela current ecosystem. There is also a high impact on top predators. Conservation and marine protection should not only focus on rare species or habitats but should also consider ecosystem functionality and protection of abundant species, particularly when these are keystone species. Research and monitoring needs to be conducted on these midtrophic species as they are sentinels of the changes of the pelagic ecosystem. Like the Antarctic krill, *P. antarctica* is a keystone species. The strength of its larval cohorts should be monitored annually to obtain abundance indexes, as currently done for herring in the North Sea by ICES (International Council for the Exploration of the Sea). Trophic condition index can complete our understanding of the trophic web quality.

For midtrophic fish in a supposed wasp-waist ecosystem, such as *P. antarctica*, a strict regulation of bycatch and monitoring should be carried out in parallel with the monitoring of Antarctic krill and ice krill. Essential habitats of these species' sensitive ecophases (eggs, larvae and juveniles) should be afforded protection not only by limiting catches but by also, and importantly, by preserving the abiotic and biotic characteristics of these habitats through spatial conservation and management measures including protected areas.

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