Rüdiger Riesch
Michael Tobler
Martin Plath *Editors*

# Extremophile Fishes

## Ecology, Evolution, and Physiology of Teleosts in Extreme Environments

Springer

# Extremophile Fishes

Rüdiger Riesch • Michael Tobler • Martin Plath
Editors

# Extremophile Fishes

## Ecology, Evolution, and Physiology of Teleosts in Extreme Environments

Springer

*Editors*
Rüdiger Riesch
School of Biological Sciences
Centre for Ecology, Evolution
and Behaviour
Royal Holloway University of London
Egham, Surrey
UK

Michael Tobler
Division of Biology
Kansas State University
Manhattan, KS
USA

Martin Plath
Sino-German Animal Research Center
College of Animal Science and Technology
Northwest A&F University
Yangling, P.R. China

Evolutionary Ecology Group
Goethe University Frankfurt
Frankfurt a.M., Germany

ISBN 978-3-319-13361-4 ISBN 978-3-319-13362-1 (eBook)
DOI 10.1007/978-3-319-13362-1
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2015931075

© Springer International Publishing Switzerland 2015
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

# Preface

> *Editing is the very edge of your knowledge forced to grow—a test you can't cheat on.*—
> S. Kelley Harrell

This book project grew out of our long-standing fascination with the amazing adaptive potential of many organisms, which enables them to survive some of the most extreme conditions found on Earth. For the last decade, our research has been dominated by our collaborative projects on livebearing fishes of the family Poeciliidae living in, and adapting to, extreme environmental conditions due to the presence of toxic hydrogen sulfide ($H_2S$) or the absence of light in subterranean habitats. Nonetheless, upon commencing the editing of this book, it became clear how little we actually knew about the biology of fishes in other types of extreme habitats, as well as about the biology of non-poeciliid fishes from sulfidic waters. Thus, editing this book became a humbling learning experience for us; something we are truly grateful for.

Extreme environments are abundant on Earth, and the scientific literature on extreme environmental conditions and how organisms deal with them have blossomed over the last few decades. In parts these efforts were driven by the desire to not only understand the limits within which life itself is possible but also to aid in our understanding of the physiological, ecological, and evolutionary responses to a more recent phenomenon that exacerbated existing and created novel extreme environments: human-induced environmental change. Teleost fishes in particular, with their high evolutionary potential (e.g., due to repeated genome duplications), are excellent models to delve into these questions, as many of them have adapted to environments usually considered inhospitable. However, books on extreme environments traditionally focused on microbes and invertebrates, while only few books to date have focused on extremophile teleosts. These books usually had more of a natural history rather than a conceptual approach to the topic (i.e., fewer habitat types were being covered and the focus was less on general evolutionary and ecological patterns). The implications of adaptation to extreme environments for the formation of new species (i.e., speciation), for example, have also not yet been covered. *Extremophile Fishes* tries to fill this gap.

However, when one of us (RR) was first approached by Springer in August of 2012 about the possibility of writing or editing a book on the adaptations of fishes to extreme environments, the first response was rather tentative. RR was just about to finish his first postdoctoral position at North Carolina State University, soon-to-be followed by a move to the University of Sheffield to begin a 3-year postdoctoral project working on chemical communication in stick insects. How much time would there really be over the coming years to devote to such an important project that focuses on fishes and not insects? Nevertheless, the opportunity was also too good to pass up on, because for the last decade we often complained (to one another as well as to other scientists) about the apparent lack of communication between scientists working in different biological specialty areas, which often seemed to result in each specialty group having to reinvent the wheel (e.g., cave biologists vs. "general" evolutionary ecologists). Since Springer was open to the option of having this become a multi-editor book, the project soon involved all three of us, and over the next few months we went to work drafting an outline for the book, and getting in touch with potential authors we considered the best choice for some of the individual chapters on fishes from specific types of extreme environments. The responses we got were so overwhelmingly positive that we decided to go ahead with this project despite some previous reservations, and so we submitted our project proposal to Springer, who officially approved the book concept by May of 2013. As a result, most authors had begun drafting their respective chapters by August of the same year.

First and foremost, we would therefore like to express our eternal gratitude and appreciation to all the authors for their excellent contributions, as well as all the external reviewers that kindly provided us with additional expert opinions and evaluations of the different chapters on top of our own assessments. All of them were a pleasure to work with, and they made our job as editors fairly easy by being very responsive to our every whim (please read: scientific enquiries, suggestions for revisions, tight deadlines, etc.), and by meeting all deadlines in a timely manner. In fact, the delays that inevitably happened during such a long-term project were usually the result of all three of us editors starting new positions during the 2 years of working on this book, rather than being the result of anything happening on the side of the authors or reviewers. It was their enthusiasm, dedication, and hard work for our project, which made the present book possible.

We also thank Ingo Schlupp (University of Oklahoma, OK, USA), who was the Ph.D. supervisor for two of us (RR and MT). He helped and guided us to find our respective scientific identities and facilitated a lot of the initial collaborative projects on poeciliids from extreme environments. Similarly, we thank Jakob Parzefall (professor emeritus and Ph.D. supervisor of MP at the University of Hamburg, Germany) for pioneering the research on the cave molly, and thus, providing a well-established starting point for our own investigations into the evolutionary ecology of the cave molly and other extremophile poeciliids. We further thank our various postdoctoral advisors who were instrumental in helping us grow as independent scientists and allowed us the freedom to pursue our own research on extremophile fishes in a collaborative fashion while being members of their research groups.

Specifically, RR would like to thank Brian Langerhans (North Carolina State University, NC, USA) and Patrik Nosil (University of Sheffield, UK), MT would like to thank Kirk Winemiller and Gil Rosenthal (both Texas A&M University, TX, USA), and MP would like to express his gratitude to Ralph Tiedemann (University of Potsdam, Germany) and Bruno Streit (JW Goethe University of Frankfurt, Germany). Furthermore, RR would like to thank Bernard Crespi (Simon Fraser University, Canada) for his valuable advice on book editing and handling contributing authors.

Moreover, we would like to thank the American Livebearers Association, the Erwin Riesch-Stiftung, the Freunde und Förderer der Goethe-Universität Frankfurt, the German Academic Exchange Service (DAAD), the German Ichthyological Society (GfI), the German Research Foundation (DFG), the Herrmann Willkomm-Stiftung, the Human Frontier Science Program (HFSP), the National Geographic Society, the National Science Foundation of the USA (NSF), and the Swiss National Science Foundation for financial support over many years. Our research was only possible due to the collaborative support and substantial help rendered by Lenin Arias-Rodriguez and Jeane R. Indy (both Villahermosa, Mexico), Francisco J. García de León (La Paz, Mexico), and Carlos Rodriguez Peña (Santo Domingo, Dominican Republic) as well as a large number of undergraduate, graduate, and postdoctoral researchers that have worked with us or circled through our labs over the years.

This book would have never reached fruition without the incredible help of the excellent people at Springer Verlag that helped, guided, and accommodated us along the way. We are grateful to Lars Koerner, who first approached us with the idea for this book, and who subsequently was of tremendous help in bringing the book from concept to reality. We are also indebted to Anette Lindqvist, the Project Coordinator for our book at Springer, in particular for her remarkable calm and accommodating responses during some of the inevitable problems we encountered during the writing and editing phases of the book. We also have to thank Murugesan Tamilselvan, our Production Editor, Sheik Mohideen, our Project Manager, and the countless others who worked behind the scenes, but who we never got to meet or interact with directly, for their tireless work and helpfulness during the design, production, advertising, and editing of the book.

Finally, we would like to thank our parents and partners, as well as our extended families and friends for their lifelong support and patience with us. They put up with us storing questionable things in the home fridge, being gone for extended field trips and conference visits, or simply disappearing for days behind our computer screens whenever we were trying to meet the next grant or scholarship deadline.

Our repeated thanks to all contributors, and we hope that the reader will find this book a valuable source of information.

London, UK Rudy Riesch
Manhattan, USA Michi Tobler
Frankfurt a.M., Germany Martin Plath

# Contents

# List of Contributors

**Santiago Ceballos** Zoological Institute, University of Basel, Basel, Switzerland

Centro Austral de Investigaciones Científicas (CADIC), Ushuaia, Tierra Del Fuego, Argentina

**Lauren J. Chapman** Department of Biology, McGill University, Montreal, QC, Canada

**Marco Colombo** Zoological Institute, University of Basel, Basel, Switzerland

**Kevin W. Conway** Biodiversity Research and Teaching Collections and Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, USA

**Malte Damerau** Thünen Institute of Fisheries Ecology, Hamburg, Germany

**Reinhold Hanel** Thünen Institute of Fisheries Ecology, Hamburg, Germany

**Stanley D. Hillyard** School of Dental Medicine, University of Nevada, Las Vegas, NV, USA

**Gary Laverty** Department of Biological Sciences, University of Delaware, Newark, DE, USA

**Nathan K. Lujan** Department of Natural History, Royal Ontario Museum, Toronto, ON, Canada

Center for Systematic Biology and Evolution, Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA

**Michael Matschiner** Zoological Institute, University of Basel, Basel, Switzerland

Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway

**Cole W. Matson** Department of Environmental Science, Institute for Biomedical Studies, Baylor University, Waco, TX, USA

Center for Reservoir and Aquatic Systems Research (CRASR), Baylor University, Waco, TX, USA

**Jay A. Nelson** Department of Biological Sciences, Towson University, Towson, MD, USA

**Matthew L. Niemiller** Illinois Natural History Survey, University of Illinois Urbana-Champaign, Champaign, IL, USA

**Elias M. Oziolor** Department of Environmental Science, Institute for Biomedical Studies, Baylor University, Waco, TX, USA

Center for Reservoir and Aquatic Systems Research (CRASR), Baylor University, Waco, TX, USA

**Martin Plath** College of Animal Science and Technology, Sino-German Animal Research Center, Northwest A&F University, Yangling, PR China

Evolutionary Ecology Group, Goethe University Frankfurt, Frankfurt a.M., Germany

**Jason E. Podrabsky** Department of Biology, Portland State University, Portland, OR, USA

**Matej Polačik** Institute of Vertebrate Biology, v.v.i., Academy of Sciences of the Czech Republic, Brno, Czech Republic

**Rüdiger Riesch** School of Biological Sciences, Centre for Ecology, Evolution and Behaviour, Royal Holloway University of London, Egham, Surrey, UK

**Walter Salzburger** Zoological Institute, University of Basel, Basel, Switzerland

Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway

**Erik Skadhauge** Faculty of Health and Medical Sciences, Department of Veterinary Clinical and Animal Sciences, Copenhagen University, Frederiksberg C, Denmark

**Daphne Soares** Biological Sciences, New Jersey Institute of Technology, Newark, NJ, USA

**Michael Tobler** Division of Biology, Kansas State University, Manhattan, KS, USA

**Frank van Breukelen** School of Life Sciences, University of Nevada, Las Vegas, NV, USA

# Extremophile Fishes: An Introduction

**Martin Plath, Michael Tobler, and Rüdiger Riesch**

**Abstract** Extremophile organisms thrive under environmental conditions considered inhospitable for most eukaryotes due to the presence of physicochemical stressors. To cope with such stressors, extremophiles have often evolved complex adaptations. Naturally occurring extreme habitats can be regarded as evolutionary experiments that allow studying the ability of species to habituate and adapt to altered ecological conditions, which may allow generating projections about the potential of organisms to habituate and adapt to human-induced stressors as well. This introduction provides an overview of different chapters of this book, focusing on the ecology, evolution, and physiology of extremophile fishes from various extreme habitats. Chapters introduce the nature of the physicochemical stressors and the taxonomic diversity in the respective habitat type. Furthermore, each chapter reviews adaptations of fishes in terms of modification of biochemical, physiological, morphological, life-history, and/or behavioral traits. In several cases, evidence for reduced gene flow between different locally adapted populations, i.e., indications for incipient or ongoing ecological speciation, is being discussed.

## 1 Background

With an amazing number of more than 28,000 described species, modern bony fishes (Teleostei) are currently the most speciose clade of vertebrates (Nelson 2006; http://www.fishbase.org). The ray-finned fishes (Actinopterygii), which teleost fishes are a

---

M. Plath (✉)
College of Animal Science and Technology, Northwest A&F University, Yangling 712100, PR China
e-mail: mplath-zoology@gmx.de

M. Tobler
Division of Biology, Kansas State University, 116 Ackert Hall, Manhattan, KS 66506, USA

R. Riesch
School of Biological Sciences, Centre for Ecology, Evolution and Behaviour, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_1

member of, seem to have derived from freshwater ancestors (Vega and Wiens 2012; see also the chapter “Hypersaline Environments” for discussion), but teleosts are nowadays found in almost any kind of aquatic habitat on Planet Earth, including shallow and deep-sea marine habitats and diverse, fast-flowing to stagnant freshwater habitats (Wootton 1991; Matthews 1998; Sebert et al. 2008). This book focuses on the ecology, evolution, and physiology of fishes living in extreme environments, so-called extremophile fishes.

The term extremophile derives from Latin *extremus* and Greek *φιλία* (*philia*) and, thus, translates to something like “loving the extreme.” While this direct translation may carry a somewhat anthropomorphic connotation, the term is clearly defined in biology: extremophiles are organisms that thrive under environmental conditions considered inhospitable for most eukaryotes due to the presence of physicochemical stressors, and they exhibit complex adaptations to cope with the stressors (Begon et al. 1996; Townsend et al. 2003). The term “extremophile” frequently refers to prokaryotes (e.g., Priscu et al. 1999; Seckbach 1999; Rothschild and Mancinelli 2001; Horikoshi et al. 2011), and “extreme environment” or “extremophile” is sometimes used for invertebrates (e.g., McMullin et al. 2000; Amils Pibernat et al. 2007), but following the aforementioned definition, vertebrates also comprise a surprising number of extremophiles (e.g., Sebert et al. 2008; Gerday and Glansdorff 2009).

Some examples of extremophile fishes are widely known not only to scientists but also to laymen—like the enigmatic pale and blind cavefishes. It may come as a surprise though that teleost fishes readily occur also in waters nearly devoid of oxygen, in hot desert waters and at temperatures below freezing, at salinities far above concentrations found in sea water, or even in habitats that temporarily fall dry. While many of these habitat types are naturally extreme to teleost fishes and can only be inhabited by highly specialized forms, more and more habitats, terrestrial and aquatic, are currently becoming extreme due to novel stressors stemming from human activities. Indeed, a recent article argues that we may currently be facing the next big mass extinction (Dirzo et al. 2014), one reason being that many species cannot adapt to rapidly changing environmental conditions. Therefore, the following chapters—focusing on some major types of extreme habitats in which teleosts can be found—will provide taxonomic overviews of species and families occurring under the respective extreme conditions. This information will allow deducing what taxonomic groups may be more likely to adapt to a given (novel) stressor or combination of stressors.

What general insights can we gain from studying the ecology, evolution, and physiology of extremophile fishes? First, naturally occurring extreme habitats can be regarded as evolutionary experiments that allow studying the ability of species to habituate and adapt to altered ecological conditions, which may allow projections as to the potential of those species to habituate and adapt to human-induced stressors as well (see chapters “Pickled Fish Anyone?” and “Evolutionary Toxicology: Population Adaptation in Response to Anthropogenic Pollution”, respectively). Likewise, studying the occurrence of fish in extreme habitats that have existed for prolonged periods of time allows making reasonable inferences about the limits of adaptation, which is also of central interest in light of exponentially increasing human-induced habitat degradation. Besides that, several extremophile fishes are emerging as model organisms for general questions in disciplines as diverse as EvoDevo [e.g., eye and pigment reduction and sensory modifications in cavefish: Wilkens (1988), Protas et al. (2007), Yamamoto and Jeffery (2000), Jeffery (2008, 2009), Yamamoto et al. (2009), Soares and Niemiller (2013)], aging research [seasonal *Nothobranchius* killifishes: e.g., Genade et al. (2005), Terzibasi et al. (2007), Reichwald et al. (2009)], and especially evolutionary ecology (the focus of this book).

Extreme habitats, due to the strength of directional selection from novel, physicochemical stressors, provide some of the best cases for studying the predictability of evolution (Tobler and Plath 2011; Riesch et al. 2014). A major question in this context is whether parallel phenotypes always diversify by parallel genetic bases or if alternative genomic routes lead to the same phenotype (e.g., Wray 2002; Wood et al. 2005; Hohenlohe et al. 2010; Stapley et al. 2011; Soria-Carrasco et al. 2014), and replicated ecological gradients—e.g., of benign and extreme habitat types—may be particularly well-suited systems in which to study this set of questions [reviewed in Elmer and Meyer (2011)].

## 2 Focus of This Book

The different chapters of this book will provide an overview over the nature of the physicochemical stressors and the taxonomic diversity in the respective habitat type. Pertinent fish adaptations in terms of biochemical, physiological, morphological, life-history, and/or behavioral traits will be reviewed. Where such information is available, relevant ecological (e.g., predator–prey, host–parasite, trophic, or symbiotic interactions) and/or evolutionary patterns (e.g., phenotypic plasticity, underlying genetics, hybridization, or speciation) and coping mechanisms governing the particular systems will be discussed. As a unifying theme the following chapters—where appropriate—will discuss evidence for reduced gene flow between different locally adapted populations, i.e., indications for incipient or ongoing ecological speciation as a result of the adaptation process (see Nosil 2012; Langerhans and Riesch 2013). Due to the different backgrounds of the contributing authors and different emphases in the respective fields, the chapters of this book will have slightly different foci, some emphasizing physiological coping mechanisms and gene expression patterns, while others focus more on phylogenetic patterns or fitness costs associated with local adaptations. Also, some chapters will additionally provide information on non-teleost fishes, e.g., because sarcopterygian lungfishes (Dipnoi) are a dominant group in temporary water bodies in the tropics (see chapter “Temporary Environments” for details). While, of course, there are many more kinds of extreme aquatic habitats at least temporarily inhabited by teleosts, our book provides ten specific examples:

In the chapter “Low-Oxygen Lifestyles”, Lauren J. Chapman reviews our current knowledge about teleosts experiencing ambient oxygen concentrations below 2 mg $O_2$ $l^{-1}$ in their natural habitats. Low-oxygen habitats are currently increasing on a worldwide scale as a consequence of human activities (like eutrophication and other sources), and so understanding how aquatic hypoxia affects key physiological mechanisms (e.g., oxygen uptake and transport) and morphological traits, alters ecological interactions, and acts as a selective force is of vital interest not only for evolutionary ecologists, but also from a conservation-oriented perspective. The author scrutinizes the evidence that mosaics of hypoxic and normoxic areas in larger water bodies might promote local adaptation and result in reduced gene flow between locally adapted forms.

The chapter "The Adaptive Radiation of Notothenioid Fishes in the Waters of Antarctica" by Michael Matschiner and colleagues demonstrates how teleosts have adapted to life in permanently cold Antarctic waters. Living at subzero temperatures requires adaptations to prevent freezing (i.e., intracellular ice crystallization) and leads to unique adaptations, such as antifreeze glycoproteins. The authors highlight how these key innovations appear to have initiated the adaptive radiation of notothenioid fishes, as open ecological niches became available to these fishes in the absence of competing taxa. This may be a fascinating example of sympatric speciation in marine fishes despite long-distance larval dispersal by the strong Antarctic Circumpolar Current.

In the chapter "Desert Environments" Stanley D. Hillyard and colleagues focus on fishes in arid to hyperarid environments and demonstrate how patterns of dispersal and vicariance drove evolutionary diversification and speciation of teleosts adapted to life in desert waters, where fish can experience fluctuating temperatures, hypersalinity, or dessication and isolation of the remaining water bodies. It may come as a surprise to learn that several desert regions were fairly wet only few thousand years ago. A special emphasis is put on the particularly well-studied pupfishes of the genus *Cyprinodon* from North American deserts—like the famous Devils hole pupfish (*C. diabolis*)—that have inspired a wealth of studies on the phylogeography and diversification of these hardy fishes.

Changes in ambient salinity were frequent in the evolutionary history of numerous teleost lineages due to repeated transitions between marine and freshwater environments. The chapter "Hypersaline Environments" by Gary Laverty and Erik Skadhauge focuses on physiological mechanisms employed by teleosts to cope with salinities even far above sea level. The authors outline the role of the intestinal and branchial epithelia in adapting to hypersaline conditions, as encountered, e.g., in coastal lagoons or in landlocked saline lakes. Several physiological mechanisms are similar to those seen when freshwater-habituated fish move into the sea, and include increased drinking, secretory transport of ions through the gills, as well as ion excretion via urine and through the gut.

The chapter "Life in the Fast Lane: A Review of Rheophily in Fishes" by Nathan K. Lujan and Kevin W. Conway considers adaptations to life in fast-flowing waters, including convergent specializations of body shape or attachment organs that prevent downstream drift. The authors provide a conceptual model describing longitudinal shifts at the evolutionary versus ecological scale of processes controlling the taxonomic composition of rheophilic fish assemblages from headwaters to large river rapids, while contrasting the importance of vicariance speciation in (isolated) upland headwaters, and adaptive radiations in lowland rapids as drivers for evolutionary diversification.

Hydrogen sulfide ($H_2S$) is a potent respiratory toxicant often associated with geological activity or environmental pollution. In the chapter "Hydrogen Sulfide-Toxic Habitats", Rüdiger Riesch and colleagues highlight multifarious selective regimes arising not only from $H_2S$ toxicity itself, but also from correlated abiotic stressors (like hypoxia) and altered ecological parameters. The authors show how local adaptation in key molecular (like $H_2S$-insensitive protein complexes and improved detoxification mechanisms), morphological, and behavioral traits repeatedly translates into the emergence of reproductive isolation in evolutionarily replicated systems due to selection against non-adapted individuals migrating between habitat types.

The chapter "Cave Environments" by Matthew L. Niemiller and Daphne Soares describes the troglomorphic fishes found in caves and other subterranean habitats, especially in karst regions. Not only does lightlessness lead to a number of convergent phenotypic changes throughout diverse groups of fishes (most strikingly, eye and pigment reduction), but the challenges of orientation and navigation, as well as finding food and mating partners in complete darkness, led to the evolution of improved nonvisual sensory systems. The authors review two long-standing topics that have fascinated generations of scientists: first, to explain what evolutionary mechanisms explain the loss of eyes and pigmentation in the absence of stabilizing selection, and second, what processes affect speciation patterns of cave faunas.

Cultural acidification of freshwaters in many parts of the world in the latter half of the twentieth century, coincident with the decline of many economically important fish species, prompted research projects on the physiological mechanisms of fishes to cope with low pH. The chapter "Pickled Fish Anyone? The Physiological Ecology of Fish from Naturally Acidic Waters" by Jay A. Nelson highlights the fact that extensive freshwater bodies, e.g., South America's Río Negro system, are inhabited by speciose fish faunas, thus raising the question of whether naturally occurring low pH over evolutionary timescales may actually be a driver of speciation processes. Acidic environments challenge fishes with net losses of essential monovalent ions especially through the gills, and mechanisms to overcome problems of ion homeostasis are being discussed.

Matej Polačik and Jason E. Podrabsky's chapter "Temporary Environments" focuses on fishes in temporary aquatic habitats in arid and semiarid ecosystems across the globe. Few groups have evolved the ability to persist phases of desiccation of their aquatic habitats, and different strategies include estivation as adults (e.g., in lungfishes) and survival as diapausing eggs (as seen in the famous annual killifishes in Africa and South America). The authors highlight how temporary environments offer ecological opportunities (open niches and reduced competition and predation) but require various specific adaptations to successfully exploit them.

The chapter "Evolutionary Toxicology: Population Adaptation in Response to Anthropogenic Pollution" by Elias M. Oziolor and Cole W. Matson considers recent, anthropogenic environmental changes as a source for phenotypically plastic (i.e., habituation), epigenetic, and eventually genomic changes of fish populations. Human-induced changes that may add to naturally existing stressors include, amongst others, increased UV radiation, radionuclide or heavy metal contamination, and toxicity from novel organic compounds, like polycyclic aromatic hydrocarbons, dioxins, and polychlorinated biphenyls. Adaptive responses of Atlantic tomcod (*Microgadus tomcod*) and Atlantic killifish (*Fundulus heteroclitus*, *F. grandis*) showing reduced sensitivity to the adverse effects of those compounds are being discussed, and in several cases, the molecular mechanisms of reduced sensitivity have already been characterized.

Our book ends with a summary and discussion ["Extremophile Fishes: An Integrative Synthesis" by Michael Tobler and colleagues] in which the authors synthesize the general approaches used to investigate teleost fishes in extreme environments and highlight generalities that are evident across different study systems. The chapter concludes with a discussion of some major open questions in our understanding of the ecology and evolution of life in extreme environments.

## References

Amils Pibernat R, Ellis-Evans C, Hinghofer-Szalkay HG (eds) (2007) Life in extreme environments. Springer, Heidelberg

Begon ME, Harper JL, Townsend CR (1996) Ecology, 3rd edn. Blackwell Science, Oxford

Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B (2014) Defaunation in the Anthropocene. Science 345:401–406

Elmer KR, Meyer A (2011) Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends Ecol Evol 26:298–306

Genade T, Benedetti M, Terzibasi E, Roncaglia P, Valenzano DR, Cattaneo A, Cellerino A (2005) Annual fishes of the genus *Nothobranchius* as a model system for aging research. Aging Cell 4:223–33

Gerday C, Glansdorff N (eds) (2009) Extremophiles. Encyclopedia of life support systems (EOLSS), developed under the Auspices of the UNESCO, vol 1. EOLSS Publishers, Paris. http://www.eolss.net

Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA (2010) Population genomics of parallel adaptation in threespined stickleback using sequenced RAD tags. PLoS Genet 6:e1000862

Horikoshi K, Antranikian G, Bull AT, Robb FT, Stetter KO (eds) (2011) Extremophiles handbook. Springer, Heidelberg

Jeffery WR (2008) Emerging model systems in evo-devo: cavefish and microevolution of development. Evol Dev 10:265–272

Jeffery WR (2009) Evolution and development in the cavefish *Astyanax*. Curr Top Dev Biol 86:191–221

Langerhans RB, Riesch R (2013) Speciation by selection: a framework for understanding ecology's role in speciation. Curr Zool 59:31–52

Matthews WJ (1998) Patterns in freshwater fish ecology. Springer, Stuttgart

McMullin ER, Bergquist DC, Fisher CR (2000) Metazoans in extreme environments: adaptations of hydrothermal vent and hydrocarbon fauna. Gravit Space Biol Bull 13:13–23

Nelson JS (2006) Fishes of the world, 4th edn. Wiley, New York, NY

Nosil P (2012) Ecological speciation. Oxford University Press, Oxford

Priscu JC, Adams EE, Lyons WB, Voytek MA, Mogk DW, Brown RL, McKay CP, Takacs CD, Welch KA, Wolf CF, Kirshtein JD, Avci R (1999) Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. Science 286:2141–2144

Protas M, Conrad M, Gross JB, Tabin C, Borowsky R (2007) Regressive evolution in the Mexican cave tetra, *Astyanax mexicanus*. Curr Biol 17:452–454

Reichwald K, Lauber C, Nanda I, Kirschner J, Hartmann N, Schories S, Gausmann U, Taudien S, Schilhabel MB, Szafranski K, Glöckner G, Schmid M, Cellerino A, Schartl M, Englert C, Platzer M (2009) High tandem repeat content in the genome of the short-lived annual fish *Nothobranchius furzeri*: a new vertebrate model for aging research. Genome Biol 10:R16

Riesch R, Plath M, Schlupp I, Tobler M, Langerhans RB (2014) Colonisation of toxic environments drives predictable life-history evolution in livebearing fishes (Poeciliidae). Ecol Lett 17:65–71

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101

Sebert P, Onyango DW, Kapoor BG (eds) (2008) Fish life in special environments. Science Publishers, Enfield, NH

Seckbach J (ed) (1999) Enigmatic microorganisms and life in extreme environments. Springer, Heidelberg

Soares D, Niemiller ML (2013) Sensory adaptations of fishes to subterranean environments. BioScience 63:274–283

Soria-Carrasco V, Gompert Z, Comeault AA, Farkas TE, Parchman TL, Johnson JS, Buerkle CA, Feder JL, Bast J, Schwander T, Egan SP, Crespi BJ, Nosil P (2014) Stick insect genomes reveal natural selection's role in parallel speciation. Science 344:738–742

Stapley J, Reger J, Feulner PGD, Smadja C, Galindo J, Ekblom R, Bennison C, Ball AD, Beckerman AP, Slate J (2011) Adaptation genomics: the next generation. Trends Ecol Evol 25:705–712

Terzibasi E, Valenzano DR, Cellerino A (2007) The short-lived fish *Nothobranchius furzeri* as a new model system for aging studies. Exp Gerontol 42:81–89

Tobler M, Plath M (2011) Living in extreme environments. In: Evans J, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. Chicago University Press, Chicago, IL, pp 120–127

Townsend CR, Begon ME, Harper JL (2003) Essentials of ecology, 2nd edn. Blackwell, Oxford

Vega GC, Wiens JJ (2012) Why are there so few fish in the sea? Proc R Soc B 279:2323–2329

Wilkens H (1988) Evolution and genetics of epigean and cave *Astyanax fasciatus* (Characidae, Pisces) – support for the neutral mutation theory. Evol Biol 23:271–367

Wood TE, Burke JM, Rieseberg LH (2005) Parallel genotypic adaptation: when evolution repeats itself. Genetica 123:157–170

Wootton RJ (ed) (1991) Fish ecology. Springer, Heidelberg

Wray GA (2002) Do convergent developmental mechanisms underlie convergent phenotypes? Brain Behav Evol 59:327–336

Yamamoto Y, Jeffery WR (2000) Central role for the lens in cave fish eye degeneration. Science 289:631–633

Yamamoto Y, Byerly MS, Jackson WR, Jeffery WR (2009) Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cavefish evolution. Dev Biol 330:200–211

# Low-Oxygen Lifestyles

**Lauren J. Chapman**

**Abstract** Aquatic hypoxia (low oxygen) provides a useful system for exploring ecological and evolutionary consequences of living under extreme conditions. It is also an environmental stressor of accelerating interest due to human activities that have increased the extent of hypoxic waters on a global scale. This chapter characterizes the distribution of hypoxic habitats, reviews key adaptations of fishes to extreme hypoxia, and explores the role of hypoxia as a divergent selective factor. Trade-offs in the costs and benefits of living in hypoxic and normoxic habitats may contribute to faunal diversification by creating spatially divergent selection that leads to specialized phenotypes as illustrated in studies of African fishes from hypoxic swamps and associated normoxic sites. In these systems alternative dissolved oxygen (DO) environments provide a strong predictor of intraspecific variation, particularly in traits related to oxygen uptake efficiency or oxygen limitations, but also in characteristics indirectly affected through trait correlations. Studies of fish persisting under hypoxia highlight the importance of localized extreme habitats as model systems for studying divergent natural selection and more generally for exploring effects of physicochemical stressors on ecological and evolutionary processes.

## 1 Introduction

In some environments organisms are challenged by adverse physicochemical conditions like extreme levels of temperature, oxygen, pH, and toxicants. Some organisms are able to cope with extreme environmental conditions via unique adaptations giving rise to specialized phenotypes and distinctive ecological communities. It has been argued that such adaptations may be costly from an evolutionary and energetics perspective. Less is known about potential benefits that trade off with these costs. However, extreme habitats are often (though not always) less

---

L.J. Chapman (✉)
Department of Biology, McGill University, 1205 Dr. Penfield Avenue, Montreal, QC, Canada
H3A 1B1
e-mail: lauren.chapman@mcgill.ca

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_2

productive than adjacent non-extreme habitats and characterized by low species diversity, which may lead to reduced competition, predation, and/or disease (Tobler et al. 2007; Tobler 2008).

Extreme habitats can occur over broad scales such as in polar regions, but can also exist at patches nested within non-extreme habitats on small spatial scales, for example, hypoxic areas of lakes and swamps, or sulfidic springs in river systems (see chapter “Hydrogen Sulfide-Toxic Habitats”). Such localized extreme habitats allow us to explore the effects of physicochemical stressors on ecological and evolutionary processes without confounding effects of larger biogeographical trends. In particular, localized extreme habitats can inform our understanding of how populations adapt to heterogeneous environments and the role of extreme habitats as drivers of divergent natural selection (Chapman 2007; Tobler et al. 2008).

Fish have colonized almost all aquatic environments on Earth including many habitats that test the limits of tolerance to physicochemical stressors—from deep oceans to sulfidic caves (see chapters “Hydrogen Sulfide-Toxic Habitats” and “Cave Environments”) to hypersaline lakes (see chapter “Hypersaline Environments”), and alkaline waters. Aquatic hypoxia (low dissolved oxygen, DO) also tests the limits of fish persistence, and provides a useful system for exploring ecological and evolutionary consequences of living under extreme conditions. It is also an environmental stressor of accelerating interest due to human activities that have increased the extent of hypoxic waters on a global scale (Diaz and Rosenberg 2008). The objectives of this chapter are to (1) review key responses to hypoxic stress that have permitted fish to persist under extreme hypoxia, (2) focus on hypoxia as a modulator of ecological interactions, in particular, predator–prey relationships, and (3) explore the role of extreme hypoxia as a divergent selective factor.

## 2 Hypoxic Habitats

Hypoxic habitats are often characterized as waters with concentrations below 2 mg $O_2\,l^{-1}$, a definition that aligns well with the mean lethal DO concentration ($LC_{50}$) of 206 species of aquatic organisms reviewed in Vaqyer-Sunyer and Duarte (2008). However, it has also been argued that the definition of hypoxia should reflect its impact on organisms, and include DO levels low enough to induce negative impacts on target species (Pollock et al. 2007). The oxygen profile in aquatic habitats is often reported as the DO concentration in the water, measured in mg $O_2\,l^{-1}$, ml $O_2\,l^{-1}$ (1 mg $O_2 = 0.7$ ml $O_2$), or % saturation. The amount of DO in water decreases with increases in temperature, salinity, and elevation. In fishes, oxygen from the environment is taken up at a site of gas exchange, typically (though not always) the gills. Since the rate of oxygen diffusion from water to blood is a function of the partial pressure gradient between the two media, physiological studies often report DO as the partial pressure of oxygen in the water in units of mm Hg (the air-saturated value at sea level is about 159 mm Hg).

All fish require oxygen for long-term survival; however, the physical properties of water make oxygen uptake a challenge even at high DO. Water holds approximately 1/30th the oxygen content of air at saturation, and oxygen diffuses about 10,000 times more slowly through water than air (Nikinmaa and Salama 1998). In addition to constraints of oxygen uptake imposed by the physical properties of water, there are many systems where water may not remain saturated with oxygen, leading to hypoxia. Oceanic oxygen minimum zones (OMZs) represent the largest areas of stable hypoxic water on the planet, and are located at intermediate depths (400–1,000 m) and low temperatures in most of the world's oceans (Childress and Seibel 1998). OMZs tend to form under areas of high surface productivity that sinks, decomposes, and consumes oxygen; and these zones are more likely to develop in areas of the ocean that lack a constant supply of well-oxygenated water such as the Arabian Sea and the eastern Pacific and Indian oceans (Kamykowski and Zentara 1990; Levin 2002; Helly and Levin 2004). In an area of the ocean containing an OMZ, upper waters are well oxygenated, but once the OMZ is entered, the DO drops very quickly, eventually stabilizing at less than 0.1 ml $l^{-1}$. DO may increase again in deeper waters below the OMZ; however, where these zones intersect continental margins one finds chronically hypoxic benthic habitats that are estimated to comprise approximately 1,148,000 $km^2$ of sea floor where DO is $<0.5$ ml $l^{-1}$ (31 % in the eastern Pacific and 59 % in the Indian Ocean, Helly and Levin 2004). OMZs are unique relative to many other hypoxic habitats in that they are characterized by extreme hypoxia over very large spatial areas and over very long time periods, thus hosting an assemblage of organisms with extraordinary adaptations to hypoxia (Childress and Seibel 1998; Levin 2002; Helly and Levin 2004). In coastal marine systems, hypoxia occurs naturally in salt marsh habitats and other intertidal zones where hypoxic conditions are generated by pool isolation and/or nocturnal respiration of plants and animals (Congleton 1980; Innes and Wells 1985; Timmerman and Chapman 2004a).

Hypoxia is also characteristic of many freshwater systems with low mixing and high rates of organic decomposition, and/or inadequate light for photosynthetic production of oxygen, such as heavily vegetated swamps, flooded forests, floodplains, the deep waters of lakes and ponds, ice-covered northern lakes, and some springheads. Chronic hypoxia and extensive anoxia are characteristic of some deepwater meromictic lakes such as Lake Tanganyika and Lake Malawi in Africa, driven by strong stratification, as well as sinking and decomposition of organic matter (Spigel and Coulter 1996). In other freshwater systems, strong seasonal variation in DO is associated with seasonal fluctuations in rainfall, mixing, incident light, and water temperature. In intermittent streams, habitats may shift from fast-flowing, well-oxygenated habitats in the wetter seasons, to small isolated hypoxic pools during drier periods (Chapman and Kramer 1991). In temperate lakes, the degree of hypolimnetic oxygen depletion depends on lake depth, primary productivity, and temperature with many eutrophic lakes experiencing summer oxygen depletion. In winterkill lakes, reduced aeration due to ice cover, reduced light due to snow cover, and a high organic matter supply relative to the amount of available DO can produce severe hypoxia or anoxia for extended periods (Kalff 2002). Nocturnal respiration in eutrophic lakes or small exposed pools often drive diel variation in DO from hyperoxia at midday to hypoxia at night (Congleton 1980; Kramer et al. 1978; Chapman and Chapman 1993), while pools characterized by little mixing under dense forest cover may be consistently hypoxic during periods of isolation (Chapman and Kramer 1991).

In tropical freshwaters, hypoxic conditions are often exacerbated by high temperatures that elevate rates of organic decomposition and reduce oxygen tension (Chapman et al. 2001). This can be particularly acute in dense tropical swamps where thick vegetation limits light and mixing, and fuels extraordinarily high rates of organic decomposition. The emergent sedge papyrus (*Cyperus papyrus*) dominates much of the permanent swamp on the African continent (Beadle 1981). In dense papyrus stands, which average 3–4 m in height (Thompson et al. 1979), the terminal brush-like umbels form a closed canopy producing dark, cool conditions. High rates of organic matter decomposition in papyrus swamps produce methane (60 %), carbon dioxide (30 %), and hydrogen ($H_2$, 10 %) gases (Visser 1993), and pH is slightly acidic (Chapman et al. 2001). The most striking characteristic of papyrus swamps is the very low DO, which results from high decomposition, low water flow rates, and negligible aquatic photosynthesis. In the Rwembaita Swamp, a valley papyrus swamp in the Mpanga River drainage of western Uganda (Fig. 1),

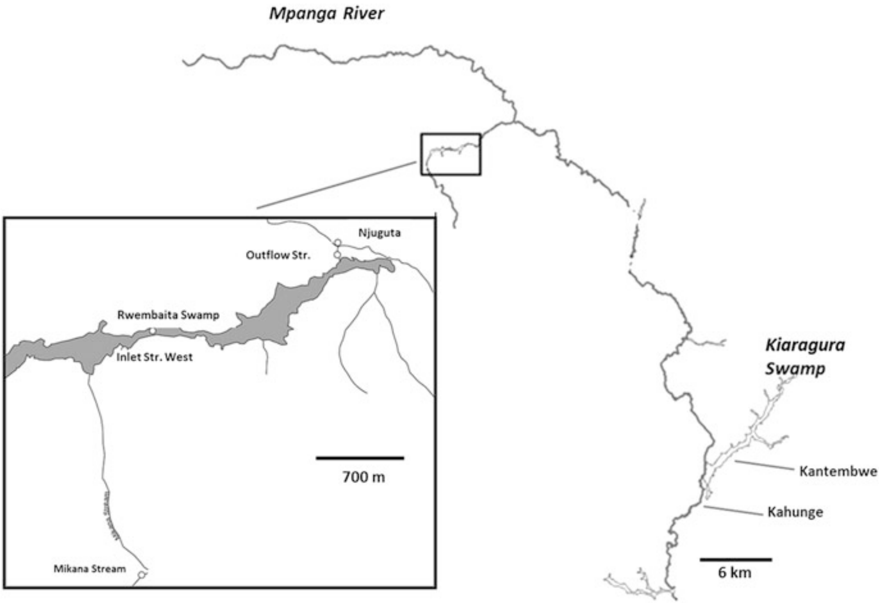
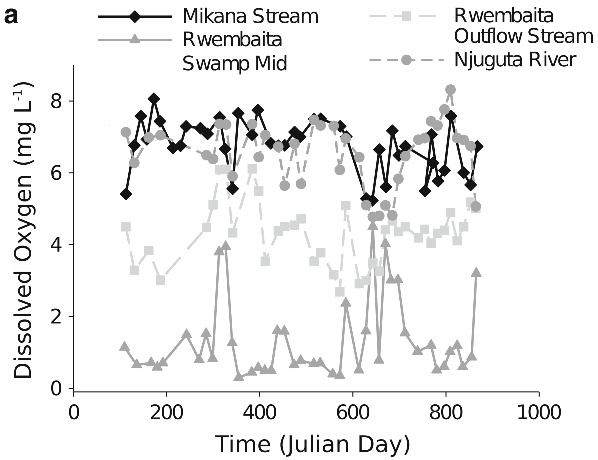


**Fig. 1** Map illustrating the locations of Rwembaita Swamp and Kiaragura Swamp and their respective rivers in western Uganda. (Reprinted from Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology Volume 165, J. Joyner-Matos and L. J. Chapman, Persisting in papyrus: Size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia, pp. 405–416, 2013, with permission from Elsevier)



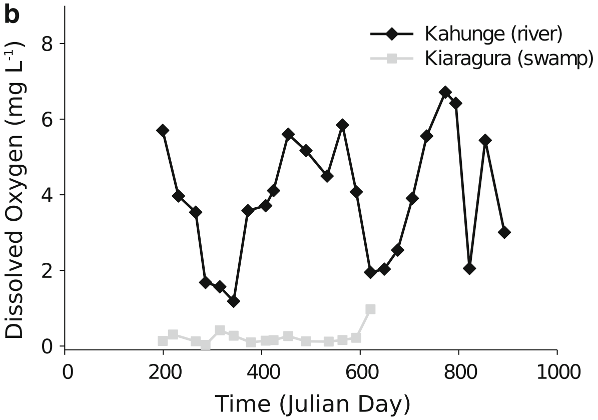


**Fig. 2** Patterns in DO in two valley swamp systems in western Uganda. (**a**) The Rwembaita Swamp: dissolved $O_2$ levels (in mg $O_2\,l^{-1}$) were taken biweekly from June 2001 to June 2003 at the Mikana Stream site (intermittent stream), Rwembaita Swamp Mid site, Rwembaita Outflow Stream, and the Njuguta River. See Fig. 1 for map of sites. Mean values were taken in the upper 20 cm of the water column, at approximately 2-week intervals at several micro-sites within each system. (**b**) The Mpanga swamp system: DO levels were taken monthly from July 2006 to December 2007 (Kantembwe site, a swamp site) and June 2008 (Kahunge site, a river site). Sampling sites are illustrated in Fig. 1. (Reprinted from Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology Volume 165, J. Joyner-Matos and L. J. Chapman, Persisting in papyrus: Size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia, pp. 405–416, 2013, with permission from Elsevier)

DO levels have been recorded since the early 1990s and averaged 1.5 mg $O_2\,l^{-1}$ in the 1993–1996 period (Chapman et al. 2000a) and 1.35 mg $O_2\,l^{-1}$ between 2001 and 2003 (range = 0.29–4.5; Fig. 2). In the Kiaragura Swamp, a more extensive valley swamp in the Mpanga River system, DO averaged only 0.29 mg $l^{-1}$ (Fig. 2), with low seasonal variation. The chronic hypoxia in the Kiaragura Swamp likely reflects its large size, with waters in the dense interior largely isolated from tributary influence. Interestingly, DO in the open waters of rivers into which both swamps drain declines (sometimes dramatically) during the rainy period when the swamp outflow mixes with the river water (Fig. 2). Extreme hypoxia has been documented in other papyrus swamp systems. Carter (1955) reported oxygen values averaging less than 0.1 mg $O_2 l^{-1}$ for the near shore areas of littoral papyrus swamps in Lake Victoria, and average values of 0.7 mg $O_2 l^{-1}$ in papyrus valley swamps of the Lake Victoria Basin near Jinja, Uganda.

Although hypoxic (and anoxic) environments occur naturally, human-induced environmental degradation is increasing the occurrence of hypoxia, as influxes of municipal wastes and fertilizer runoffs accelerate eutrophication and pollution of water bodies (Diaz 2001). Cultural eutrophication and associated hypoxia in freshwater systems have a long history, becoming widespread in the latter half of the twentieth century in both temperate (e.g., Lake Erie) and tropical (e.g., Lake Victoria) systems, though effective nutrient management has reversed the trend in some parts of the world (Jeppesen et al. 2005). In coastal and estuarine ecosystems, eutrophication driven primarily by anthropogenic fertilization has produced benthic hypoxia, which has led to population declines of inhabitants, mass mortality of inhabitants, and associated changes in community structure (Diaz 2001; Rabalais et al. 2002; Dybas 2005; Diaz and Rosenberg 2008). Dead zones have increased exponentially since the 1960s and are now reported for more than 400 systems that comprise an area of more than 245,000 km$^2$ (Diaz and Rosenberg 2008). Thus, spreading dead zones are creating extreme environments on a massive spatial scale, and the mortality that has occurred in some regions suggests that adaptive response on contemporary timescales may be limited or time lagged (Diaz and Rosenberg 1995). However, studies of adaptation to naturally occurring extreme hypoxia may provide important insights into the characteristics of organisms that may facilitate persistence and recovery.

## 3 Rising to the Challenge: Fish Under Extreme Hypoxia

In general, the problem of extreme aquatic hypoxia has led to one or more of the following strategies: (1) evolution of air-breathing organs, (2) movement to zones of higher DO, (3) evolution of mechanisms to maximize oxygen uptake from the water, (4) reduction of metabolic rate and/or activity to reduce oxygen requirements, and (5) use of anaerobic metabolism to bridge the difference between aerobic capacity and metabolic demands.

A number of bony fishes have evolved bimodal respiration, whereby they retain functional gills but can also gulp air at the water surface and store this in an air-breathing organ (ABO). ABOs show remarkable diversity in their structure and origins (Graham 1997), including diverticula of the branchial chambers (e.g., *Clarias*, *Ctenopoma*) and modifications of the air bladder (e.g., *Polypterus*, *Protopterus*). Air-breathing fishes combine the use of dissolved and atmospheric oxygen; however, there is great variation in the degree of dependence on atmospheric air and in gill development. Some species like the African lungfishes (*Protopterus* spp.) are obligatory air breathers and will die without access to the surface, while other species, including the air-breathing clariid catfishes (*Clarias* spp.), have well-developed gills and can meet their oxygen requirements using water breathing at higher oxygen levels (Chapman and Chapman 1994). A large number of highly derived intertidal marine teleosts are believed to have evolved air-breathing strategies and amphibious habits independently of the freshwater air breathers, and typically use skin, gills, and branchial chambers as ABOs (Graham 1997; Graham and Lee 2004; Sayer 2005; Lam et al. 2006).

Air breathing should, in theory, be more energetically efficient than water breathing for fishes, because air is so much richer in oxygen and requires much less effort to ventilate (Kramer 1983a, 1987; Graham 1997). However, air-breathing fishes comprise only 2 % of known fish species (Graham 1997), and most fishes that persist in extremely hypoxic waters are non-air breathers. For example, the majority of the fish species in dense papyrus and *Miscanthidium*-dominated wetlands in Africa are water breathers from a diversity of lineages including cichlids, cyprinids, mormyrids, and killifishes (Joyner-Matos and Chapman 2013). Thus, there must be significant physiological and ecological costs to air breathing, which likely include energetic costs and increased risk of predation associated with travel to the surface (Kramer 1983a, 1987; Kramer et al. 1983; Bevan and Kramer 1986).

Behavioral responses provide additional flexibility to deal with variation in DO that can occur temporally or spatially in extreme environments through diel or seasonal movements, decreases in activity, or use of aquatic surface respiration (ASR). Extreme hypoxia can induce changes in spontaneous swimming activity—either a reduction in activity or an increase in activity, depending upon the species and the context, with the former viewed as an energy-saving response and latter viewed as an avoidance response (reviewed in Chapman and McKenzie 2009). For example, one of the energy-saving strategies used by crucian carp (*Carassius carassius*) is to reduce spontaneous activity by 50 %, providing an estimated saving of 35 % of overall energy requirements in anoxia (Nilsson et al. 1993).

Habitat shifts or avoidance behavior in response to hypoxia has been observed in several studies (reviewed in Pollock et al. 2007). For example, Wannamaker and Rice (2000) characterized the hypoxia avoidance response in several species of estuarine fishes, and found that all species could detect and avoid waters of 1 mg $O_2\,l^{-1}$, the lowest concentration in their choice tests. In their study of pelagic fish distributions in the seasonally hypoxic coastal waters of the northern Gulf of Mexico, Zhang et al. (2009) found that fish avoided waters $<2$ mg $O_2\,l^{-1}$. They did so by moving vertically or horizontally to the edges of hypoxic areas, and spatial overlap between fish biomass and mesozooplankton biomass was reduced during years of severe hypoxia. Diel migrations are common in myctophid (lantern) fishes that encounter OMZs during the daytime and ascend to warmer and better-oxygenated surface waters during the night (Lopes et al. 2013). Interestingly, an anticipated challenge for organisms migrating from OMZs is the production of reactive oxygen species (ROS) driven by the transition between extreme hypoxia and reoxygenation states, as well as higher oxygen consumption demands driven by warmer temperatures. In their study of heat-shock responses and antioxidant enzyme activities of myctophids in the Eastern Pacific Ocean, Lopes et al. (2013) observed an increase in heat-shock protein levels under elevated temperatures, likely to prevent oxidative stress.

In shallow water systems that experience hypoxia, a widespread behavioral response is the use of ASR, whereby fish rise to the surface and ventilate their gills with the layer of water in contact with air, which is richer in DO than the underlying water (Lewis 1970; Gee et al. 1978; Kramer and McClure 1982; Kramer and Mehegan 1981; Kramer 1983b; Gee and Gee 1995; McNeil and Closs 2007). Aquatic surface respiration is more efficient than simply increasing ventilation under extreme hypoxia (Kramer and Mehegan 1981; Kramer 1983a, b). In addition, some fishes increase the efficiency of oxygen extraction during ASR by swimming continuously across the surface (Chapman et al. 1994, 1995; Rosenberger and Chapman 2000) or through the use of buccal bubble holding that may serve as a buoyancy compensation mechanism and/or to increase the oxygen content of the water passing over the bubble (Burggren 1982; Gee and Gee 1991). A number of species have morphological features, such as upturned mouths and flattened heads that seem to increase the efficiency of ASR (Lewis 1970; Cech et al. 1985), or dermal lip protuberances that facilitate access to water from the surface film (Winemiller 1989). ASR can, however, incur costs in terms of energy and predator risk, which may account for very low ASR initiation thresholds in many species, levels that are often close to the critical oxygen tension (reviewed in Chapman and McKenzie 2009). In a recent study, compared the ASR threshold of the cichlid *P. multicolor* from a chronically hypoxic swamp in Uganda to a literature review of ASR thresholds (81 values, Chapman and McKenzie 2009). *P. multicolor* was characterized by an average $ASR_{10}$ threshold ($PO_2$ at which fish spend 10 % of their time at the surface) in the lower 25th percentile of the distribution and exhibited the lowest $ASR_{50}$ threshold of all fish examined, indicating far-reaching adaptation to extreme hypoxia in this species.

Fishes relying on aquatic respiration in chronically hypoxic habitats use many strategies to increase oxygen transfer from the environment to their tissues and/or to evade problems associated with hypoxia. One mechanism to meet routine metabolic requirements under hypoxia is to increase oxygen uptake through an enlarged gas exchange surface. Several studies comparing populations of the same species from hypoxic swamp and high-oxygen environments in East Africa have demonstrated larger total gill size (surface area and/or total gill filament length) in swamp-dwelling populations including, as examples, the cyprinid *Barbus neumayeri* (Chapman et al. 1999; Langerhans et al. 2007) and the cichlid *Pseudocrenilabrus multicolor victoriae* (Chapman et al. 2000b; Wiens et al. 2014). In the poeciliid fishes *Poecilia mexicana* and *P. sulphuraria*, populations from sulfidic, hypoxic springs exhibit larger gills than populations from adjacent non-sulfidic habitats (Tobler et al. 2011; see chapter “Hydrogen Sulfide-Toxic Habitats”). Large gill surface area is also characteristic of some species of demersal fishes from oxygen minimum zones: Friedman et al. (2012) found that the OMZ-dwelling flatfish *Microstomus pacificus* had a larger gill surface area than comparably sized flatfishes from higher-oxygen waters outside the OMZ in Monterey Canyon, California. Similarly, in their comparison of two rattail (gadiform) species, *Nezumia liolepis* from the OMZ exhibited a larger gill surface area than *Corphaenoides acrolepis*, a species living below the OMZ in waters of higher oxygen content. In addition to interspecific and interdemic patterns in gill surface area, some fishes can alter their gill surface area by remodeling their gill morphology. Gill remodeling was first observed in crucian carp and goldfish (*Carassius auratus auratus*) (see review in Nilsson et al. 2012) and involves either the expansion or reduction of the cell mass between the lamellae on the gill filaments referred to as the interlamellar cell mass (ILCM). In response to increased oxygen uptake demands, the ILCM is reduced through apoptosis exposing the respiratory epithelium to water and increasing oxygen uptake capacity (Sollid and Nilsson 2006; Nilsson 2007; Tzaneva et al. 2011; Nilsson et al. 2012).

There is great diversity in blood oxygen transport traits of fishes that appear to have evolved in response to both functional hypoxia driven by metabolic demands and environmental hypoxia. Blood oxygen transport in most teleosts is dependent upon the protein hemoglobin and is increased by adjusting the affinity of hemoglobin for oxygen, increasing the number of erythrocytes in circulation, and/or increasing hemoglobin concentration [Hb] (Hughes 1973; Johansen et al. 1978; Jensen 1991; Brauner and Val 2006; Wells 2009). Wells (2009) suggested that the evolutionary success of teleost fishes may be due, in part, to an oxygen secretion mechanism involving Root effect hemoglobins, which are unique to teleosts. Short-term increases in Hct (volume percentage of red blood cells in blood) and [Hb] in response to seasonal or acclimation-induced hypoxia have been reported in several non-air-breathing and air-breathing fishes including, as examples, increased Hct in the characid *Prochilodus cf. nigricans* (Val et al. 1992), increased [Hb] in the characid *Piabucinae festae* (Graham 1985) and the loricariid catfishes *Hypostomus plecostomus* and *Ancistrus chagresi* (Graham 1985), and increased Hct and [Hb] in the notothenid *Pagothenia borchgrevinki* (Wells et al. 1989) and the poeciliid *Poecilia latipinna* (Timmerman and Chapman 2004b). However, these blood capacity changes are often modest, and some studies have detected no change at all (Marinsky et al. 1990).

Oxygen-binding properties of hemoglobin are also critical in meeting the challenges of environmental hypoxia. These properties are routinely described by the relationship between the partial pressure of oxygen and the fraction of the oxygen-bound Hb, referred to as the oxygen equilibrium curve (OEC). The shape of the relationship is quantified by Hill's coefficient that varies from 1 when hyperbolic to approximately 3 when the relationship is sigmoidal (see Fig. 1 in Wells 2009). The $P_{50}$ is an important diagnostic trait that represents the $PO_2$ at which 50 % of the hemoglobin is oxygenated. Increases in blood oxygen affinity of hemoglobin are frequently observed in response to hypoxia, and are mediated by increased pH (Bohr effect), decreased erythrocytic concentration of organic phosphates (adenosine and guanosine triphosphates, ATP and GTP, respectively), or variation in the organic phosphate ratio (e.g., Bartlett 1978; Monteiro et al. 1986; Val et al. 1992; Rutjes et al. 2007). The organic phosphates bind to specific sites on the Hb tetramer, and a decrease in phosphates results in increased hemoglobin oxygen affinity (Wells 2009). The synthesis of ATP and GTP proceeds via an aerobic pathway; thus hypoxia that drives a shift from aerobic to anaerobic metabolism can reduce ATP and GTP and therefore increase blood oxygen affinity (Val 2000; Wells 2009). Teleosts also often have complex hemoglobin systems, which can be important in determining hypoxia tolerance (Fyhn et al. 1979; Riggs et al. 1979; Perry and McDonald 1993; Perez et al. 1995). In their study of adaptations to lifelong hypoxia in the East African cichlid *Haplochromis ishmaeli* from Lake Victoria, Rutjes et al. (2007) reared offspring under normoxic and hypoxic conditions. They found that hypoxia-reared fish exhibited a different iso-Hb pattern compared to normoxia-reared sibs, which correlated with a higher Hb-$O_2$ blood oxygen affinity. In general, fish from hypoxic environments are characterized by high oxygen-carrying capacity, high blood $O_2$ affinities, low Hill coefficients, and a hemoglobin function that is modulated by both GTP and ATP (Wells 2009). This pattern is seen both among species and populations inhabiting low- and high-oxygen sites. For example, in their study of hypoxic swamps surrounding Lake Nabugabo in Uganda, Chapman et al. (2002) found that swamp-dwelling fish species were characterized by higher Hct and [Hb] than lake-dwelling species, and Graham (1985) reported a negative relationship between [Hb] and the DO content of the habitat among three populations of the catfish *Hypostomus plecostomus*.

Another approach to persisting under extreme hypoxia is to reduce metabolic demands. A lower metabolic rate under hypoxia may offset energetic constraints of high blood viscosity driven by high [Hb], but has the disadvantage of reducing aerobic metabolism and the amount of energy available for many biochemical processes. Nonetheless, a relatively low metabolic rate seems to be characteristic of several fishes that inhabit chronically hypoxic waters. Examples include the mormyrid *Petrocephalus catostoma* (Chapman and Chapman 1998) and the cichlid *P. multicolor* (Rosenberger and Chapman 2000) from hypoxic swamps in Uganda, and the sailfin molly (*P. latipinna*) from periodically hypoxic saltmarsh habitat in Florida. Low metabolic demands are also characteristic of some fish species inhabiting OMZs (Childress et al. 1990); however, Childress and Seibel (1998) argue that these low metabolic rates have not evolved in response to low DO, because pelagic taxa living at comparable depths outside of OMZs have comparably low rates. Nonetheless low metabolic rates in these fishes are functionally adaptive for their aerobic survival under the extreme hypoxic characteristic of OMZs.

Very low critical oxygen tensions are characteristic of many hypoxia-tolerant fishes. The majority of fish species can be described as metabolic oxygen regulators, i.e., they are capable of maintaining a constant metabolic rate over a range of DO. The minimum oxygen level required to maintain a constant metabolic rate is defined as critical tension ($P_c$), below which metabolism decreases linearly with oxygen tension. In their study on interior swamps in the Lake Victoria basin, Chapman et al. (2002) found that swamp-dwelling species were characterized by a lower average $P_c$ (12.2 mm Hg) than lake-dwelling fishes (18.3 mm Hg) reflecting the high-oxygen uptake efficiency of the swamp-adapted fishes. They also observed a positive relationship between metabolic rate and $P_c$ among the fishes in Lake Nabugabo; swamp-dwelling fishes with the lowest $P_c$ values were also characterized by very low metabolic rates. This seems consistent with the idea that fishes of oxygen-poor areas have evolved reduced oxygen demands and improved means of extracting what oxygen is available. A low critical oxygen tension reflects other adaptations to hypoxia that facilitate oxygen extraction at low-oxygen tensions. For example, in their study of a group of closely related sculpins (Cottidae) from a range of habitats with varying levels of oxygen availability, Mandic et al. (2009) showed a phylogenetically independent correlation between hypoxia tolerance ($P_c$) and Hb-$O_2$ binding affinity.

Another strategy to deal with life in low oxygen is the use of anaerobic metabolism to bridge the difference between the aerobic capacity and metabolic demands. Many hypoxia-tolerant fishes increase oxygen-independent ATP production to maintain cellular energy balance (Land et al. 1993; Jibb and Richards 2008; Davies et al. 2011), relying more heavily on the contribution of glycolysis to produce ATP (Hochachka et al. 1996; DeKoning et al. 2004; Pollock et al. 2007). However, the yield of ATP is very low during anaerobic glycolysis relative to oxidative metabolism (Hochachka and Somero 2002); thus the use of anaerobic metabolic pathways for anything beyond small bursts of activity in fishes requires large reserves and results in accumulation of deleterious end products (Seibel 2011). Fishes living under chronic hypoxia possess a suite of adaptations (as discussed above) to facilitate aerobic survival at low DO. As such, evidence in some stable hypoxic environments suggests that anaerobic metabolism is used at most to supplement aerobic metabolism. In reviewing evidence for anaerobic metabolisms in fishes of the OMZs, Childress and Seibel (1998) concluded that in OMZs with DO $>0.20$ ml $O_2\,l^{-1}$, anaerobic metabolism is important for supporting activity above routine levels; however, the primary adaptation to zones with DO $>0.20$ ml $O_2\,l^{-1}$ is a low $P_c$ facilitated by strategies for increasing oxygen uptake capacity at low DO. Most studies of fishes living in OMZs have found no evidence for increased anaerobic capacities in enzymatic profiles (Seibel 2011). In shallow-water systems characterized by chronic hypoxia, studies quantifying enzyme activities in natural populations are few, and results vary among study sites and species. In their study of the eurytopic cichlid *P. multicolor*, Crocker et al. (2013) reported little variation in metabolic capacities of populations from hypoxic swamps and high-oxygen sites within two drainages in Uganda. However, higher glycolytic capacity (phosphofructokinase in heart and skeletal muscle) and higher activity of liver lactate dehydrogenase were evident in the African cyprinid *B. neumayeri* from the hypoxic waters of a papyrus swamp relative to conspecifics from well-oxygenated tributary waters (Martinez et al. 2004, 2011).

Reliance on anaerobic pathways is critical for persistence of fish in anoxic environments, and the best-known example is the crucian carp, a species that can persist for days to months in anoxic ice-covered water bodies. The anoxia tolerance in this species has been well studied and reviewed by Vornanen et al. (2009). A critical factor contributing to its anoxia tolerance is its ability to produce ethanol as its major end product of anaerobic metabolism, which allows the species to endure anoxia for long periods without suffering lactic acidosis. To balance the loss of energy-rich hydrocarbons released into the water, the carp accumulates huge stores of glycogen before the winter months set in, a process induced by temperature acclimatization Vornanen et al. (2009). In addition, the crucian carp can reversibly adjust the surface of its gills (gill remodeling) to match oxygen availability (Sollid et al. 2003, 2005). Other strategies employed by crucian carp include high blood oxygen affinity (Sollid et al. 2005) and cardiovascular adjustments that extend the ability to extract oxygen from the water as ambient levels fall (Vornanen et al. 2009), and a remarkable ability to maintain brain ATP levels under anoxia by increasing brain blood flow, though activity levels of the brain are reduced and certain senses are suppressed (Johansson et al. 1997; Nilsson 2001).

## 4 A Cost-Benefit Approach

Fishes that persist under extreme hypoxia may incur a number of costs related to the low availability of oxygen. Hypoxia can affect growth by influencing metabolic pathways, metabolic rate, and allocation of energy resources, and growth depression is a widespread response to hypoxic stress (Wang et al. 2009). Hypoxia has also been observed to affect reproduction in fishes by altering the concentration and ratio of sex hormones, disrupting gonad formation and development, altering fertilization and spawning behavior, and impairing embryo development (e.g., Zhou et al. 2001; Wu et al. 2003; Gercken et al. 2006; Landry et al. 2007; Thomas et al. 2007; Hassell et al. 2008; reviewed in Wu 2009). For example, in the widespread mouth-brooding cichlid *P. multicolor*, populations from hypoxic environments are characterized by a smaller female size at maturity and smaller egg size than conspecifics from normoxic sites (Reardon and Chapman 2009).

Despite potential energetic constraints and reproductive costs of life in low oxygen, there may be benefits of these extreme habitats that offset physiological and/or biochemical costs such as reduced competition, predation, and/or disease. Several recent studies provide evidence for reduced predation pressure in hypoxic habitats. In the Lake Victoria basin, for example, hypoxic refugia have mitigated impacts of a large introduced piscivore, the Nile perch (*Lates niloticus*) on native fish species. The explosive speciation of haplochromine cichlid fishes in Lake Victoria is unrivaled among vertebrates; however, introduction of the predatory Nile perch and effects of eutrophication (e.g., loss of water transparency) coincided with the mass extinction of endemic cichlids (Kaufman 1992; Seehausen et al. 1997; Balirwa et al. 2003). A similar pattern of faunal collapse was observed in other lakes in the basin that host introduced Nile perch (e.g., lakes Kyoga, Nabugabo). Studies on Lake Nabugabo (historically a backwater of Lake Victoria) demonstrated that hypoxic wetlands serve as refugia for some fishes, because the

Nile perch is sensitive to hypoxic stress (Chapman et al. 1996, 2002, 2003; Schofield and Chapman 2000). Coincident with heavy fishing on Nile perch some species that were in retreat are reemerging in lakes Victoria, Nabugabo, and Kyoga, emphasizing the importance of hypoxic habitats in fostering resurgence of native species (Witte et al. 2000; Chapman et al. 2003). Extreme hypoxia also appears to mitigate predator–prey interactions in coral reef fishes where nocturnal hypoxia is common (Nilsson et al. 2007). Nilsson and Östlund-Nilsson (2004) reported a surprisingly high level of tolerance to hypoxia in 31 coral reef species off the coast of Australia. They suggested that the widespread tolerance in this group reflects the use of hypoxic spaces in the coral as nocturnal refugia from predators or use of isolated periodically hypoxic tide pools. In another Australian habitat, McNeil and Closs (2007) quantified hypoxia tolerance in fishes of the Ovens River floodplain where low-oxygen conditions are common in small lentic waters (billabongs). Behavioral studies indicated a high tolerance in most floodplain fish species with the exception of three species including the introduced predatory redfin perch (*Perca fluviatilis* L.), which may account for high abundance of native hypoxia-tolerant species in more deoxygenated billabongs. McKinsey and Chapman (1998) quantified fish community structure along a sharp DO gradient in a Florida spring, where they found eastern mosquitofish *Gambusia holbrooki* to be the most abundant species persisting under extreme hypoxia at spring boil where DO averaged 0.20 mg $O_2\,l^{-1}$. They suggested that the boil offers a refugium for predation and a reduced competitive environment for the mosquitofish in these springs where fish diversity (and number of piscivores) increases with distance from the boil (McKinsey and Chapman 1998). Physiological refugia are also evident in north temperate systems. The extraordinary anoxia tolerance of the crucian carp offers access to piscivore-free refugia in ice-covered shallow ponds in Northern Europe (Nilsson and Renshaw 2004; Vornanen et al. 2009). Moreover, Robb and Abrahams (2003) observed higher hypoxia tolerance of small yellow perch (*Perca flavescens*) and fathead minnows (*Pimephales promelas*), than large yellow perch, a potential predator. They suggested that prey may intentionally seek out low-oxygen habitats under risk of predation.

Robb and Abrahams (2003) proposed an ecological advantage of being small under hypoxia associated with the allometric relationship for mass-specific gill surface area (Muir 1969; Hughes 1984), and a fractal scaling relationship whereby larger fish may be limited by the fixed size of the red blood cells for gas exchange (West et al. 1997). However, relationships between fish size and hypoxia tolerance are not consistent across studies. Nilsson and Östlund-Nilsson (2008) argued that body size per se does not influence oxygen uptake ability because (1) gill surface area has a similar scaling relationship as metabolic rate, and (2) where anaerobic ATP production is required, large fish seem to benefit from a lower mass-specific metabolic rate. Interestingly, hypoxic habitats may still offer a predator or cannibalism refuge even when smaller individuals are not more tolerant: In Amazonian oscars (*Astronotus ocellatus*), Sloman et al. (2006) found evidence to suggest that juveniles are not more hypoxia tolerant than larger conspecifics, but accept a physiological cost to access hypoxic refugia. Interestingly, Everett and Crawford

(2010) reported a negative relationship between $P_c$ and body mass in their study of seven populations of *Fundulus grandis*, suggesting that (in this species) an increase in body size is an effective strategy to mitigate effects of hypoxia. Clearly, there is a need for additional studies on the relationship between body size and hypoxia tolerance both within and across species.

Another potential advantage of living in extreme habitats is that such habitats may serve as a refuge from parasites and disease (Tobler et al. 2007). The Atlantic molly (*Poecilia mexicana*) inhabits both surface freshwater systems and limestone caves with high concentrations of hydrogen sulfide. Tobler et al. (2007) found lower levels of parasitism by the trematode *Uvulifer* sp. in cave-dwelling mollies than in conspecifics from a non-sulfidic habitat (but see Tobler et al. 2014). Similarly, juvenile pumpkinseed sunfish (*Lepomis gibbosus*) from a periodically hypoxic wetland bay in Lac Hertel, Quebec, are characterized by a lower prevalence and load of *Uvulifer* sp. than conspecifics from well-oxygenated waters in the main lake (L. Chapman, unpublished data). In contrast, in their study of the monogenean diplozoon *Neodiplozoon polycotyleus* that parasitizes the gills of the African cyprinid *Barbus neumayeri*, Raymond et al. (2006) found that parasite occurrence was higher in the hypoxic papyrus swamp habitat than in stream and river sites in the same drainage (Raymond et al. 2006).

## 5 Divergent Selective Environments

Trade-offs in the costs and benefits of living in hypoxic and normoxic habitats may contribute to faunal diversification by creating spatially divergent selection that leads to specialized phenotypes. Hypoxic habitats like papyrus swamps can occur nested within normal habitats on small spatial scales and contribute to faunal diversification by creating heterogeneous landscapes. Such localized extreme habitats and their inhabitants are especially valuable for evolutionary ecology research, because they create divergent selective environments among populations and/or closely related species, and thus can inform our understanding of a fundamental question in evolutionary biology—how populations adapt to heterogeneous environments (Schluter 2000, 2001).

Hypoxic habitats that are connected to normoxic habitats provide excellent contrasting environments for exploring the role of hypoxia in driving divergence. For example, midwater OMZs grade into waters of higher oxygen content above and below, hypoxic spring boils are often extreme habitats nested within normoxic rivers, and valley papyrus swamps in East and Central Africa represent patches of hypoxic habitat in the matrix of the larger, flowing, and generally well-oxygenated rivers. Populations of species or closely related species can be compared across divergent DO environments to inform our understanding of DO as a driver of phenotypic divergence and microevolutionary change. Studies of East African fishes from swamp and associated normoxic sites have demonstrated that alternative DO environments provide a strong predictor of intraspecific phenotypic variation, particularly in respiratory traits (e.g., gill size), but also life-history traits, energetics, brain size, and biochemical traits. Observations comparing populations from low- and high-oxygen sites have reported larger gills in hypoxic populations of the eurytopic African cichlids *P. multicolor* (Chapman et al. 2000b, 2008; Wiens et al. 2014) and *Astatoreochromis alluaudi* (Chapman et al. 2007; Binning et al. 2010), the African cyprinid *B. neumayeri* (Chapman et al. 1999; Langerhans et al. 2007), and the African mormyrid *Petrocephalus catostoma* (Chapman and Hulen 2001). Interestingly, interdemic variation in gill size is also evident in the air-breathing African catfish *Clarias liocephalus* that exhibits a larger air-breathing organ and small gill size in a hypoxic papyrus swamp than in a connected normoxic tributary (Chapman 2007). Other traits related to maximizing oxygen uptake also differ across high- and low-oxygen populations. In the cyprinid *B. neumayeri*, swamp-dwelling populations are characterized by lower ASR thresholds (Olowo and Chapman 1996); higher hematocrit (Martinez et al. 2004; Chapman 2007), lower $P_c$ (Chapman 2007), higher liver LDH activities, and higher glycolytic capacity (phosphofructokinase in heart and skeletal muscle) than in *B. neumayeri* from connected high-DO sites (Martinez et al. 2011; Table 1).

It is intuitive that larger gills should be advantageous under hypoxic conditions to facilitate oxygen uptake; however, there may be costs associated with gill proliferation that negatively affect performance in oxygen-rich environments. For example, because of the compact, laterally compressed head morphology characteristic of most teleosts, the production of large gills may affect surrounding morphological structures that are involved in feeding or swimming (e.g., body shape). The consequences of such functional-morphological trade-offs may limit feeding performance, swimming efficiency, or other fitness-related traits, and therefore contribute to the maintenance of specialized local phenotypes in extreme environments. Large gill size of hypoxia-adapted *B. neumayeri* correlates with a reduction in the size of key trophic muscles and lower feeding performance than in normoxia-adapted conspecifics (Schaack and Chapman 2003; Langerhans et al. 2007). Similarly, in the cichlid *A. alluaudi,* Binning et al. (2010) found a negative correlation between composite morphological variables related to the size and shape of the gill apparatus and pharyngeal jaw size. Furthermore, in a study of nine populations of *B. neumayeri,* Langerhans et al. (2007) showed that DO had an effect on body shape, but this effect was largely driven by oxygen's effects on gill size (Table 1). These studies demonstrate links between hypoxia and divergence in non-respiratory traits and suggest that an optimal trait value in one environment (large gills in hypoxia) could be indirectly associated with liability in others through trait correlation or developmental constraints.

Phenotypic variation across DO gradients may reflect genetic and/or phenotypically plastic adaptation to local selective pressures. The cichlid *P. multicolor,* a species that persists under both extreme hypoxia and normoxia and exhibits high levels of phenotypic plasticity in several morpho-physiological traits, has provided a valuable tool for exploring the potentially diverse roles of plasticity in the process of adaptation and evolutionary change. Lab-rearing experiments with *P. multicolor* have demonstrated a high degree of developmental plasticity in its gill size, brain

**Table 1** Studies illustrating trait variation between populations of the African cyprinid *Barbus neumayeri* from high-DO and low-DO field sites, between fish held in cages in high-DO or low-DO field sites (acclimatization experiment), between fish held for short periods in high-DO and low-DO aquaria in the lab (acclimation experiment), or between fish reared under high-DO or low-DO conditions in the lab

| Trait | High DO | Low DO | Study design | Reference |
|---|---|---|---|---|
| Gill filament length | ↓ | ↑ | Field populations | Chapman et al. (1999), Langerhans et al. (2007) |
| Gill filament length | ↓ | ↑ | Lab rearing under low vs high DO | Chapman (unpublished) |
| Aquatic surface respiration thresholds | ↑ | ↓ | Field populations | Olowo and Chapman (1996) |
| Critical oxygen tension | ↑ | ↓ | Field populations | Chapman (2007) |
| Hematocrit | ↓ | ↑ | Field populations | Martinez et al. (2004) |
| Lactate dehydrogenase (liver) | ↓ | ↑ | Field populations | Martinez et al. (2004, 2011) |
| Aerobic enzyme CCO (heart) | ↑ | ↓ | Field acclimatization | Martinez et al. (2011) |
| Glycolytic enzyme PFK (heart/muscle) | ↓ | ↑ | Field acclimatization | Martinez et al. (2011) |
| Growth rate | ↑ | ↓ | Field acclimatization | Martinez et al. (2011) |
| Body condition | ↑ | ↓ | Lab acclimation | Barrow and Chapman (2006) |
| Parasite frequency [Afrodiplozoon Polycotyleus (Monogenea)] | ↓ | ↑ | Field populations | Raymond et al. (2006) |
| Head size | ↓ | ↑ | Field populations | Schaack and Chapman (2003), Langerhans et al. (2007) |
| Body depth | ↓ | ↑ | Field populations | Schaack and Chapman (2003), Langerhans et al. (2007) |
| Geniohyoideus length; sternohyoidus height | ↑ | ↓ | Field populations | Schaack and Chapman (2003) |
| Feeding rate | ↑ | ↓ | Lab acclimation | Barrow and Chapman (2006) |
| Feeding performance | ↑ | ↓ | Field populations | Schaack and Chapman (2003) |

Arrows indicate that the trait value is higher or lower

size, and body shape in response to growth of full sibs under either low DO or high DO, suggesting that selection against dispersers may be weak. However, population level differences were also detected in some traits (e.g., brain mass), suggesting genetic components to trait divergence (Chapman et al. 2008; Crispo and Chapman 2010a, 2011). Mitochondrial DNA and microsatellite studies provided evidence for high gene flow among *P. multicolor* populations from divergent DO environments in the Mpanga and Nabugabo River drainages of Uganda (Crispo and Chapman 2008, 2010b). Thus, in this system, evolutionary divergence in ecologically important traits seems to occur despite high levels of gene flow. The cyprinid *B. neumayeri* displays similar differences in behavioural, morphological, and biochemical traits between high- and low-DO sites (Olowo and Chapman 1996; Schaack and Chapman 2003; Martinez et al. 2004, 2011; Langerhans et al. 2007; Table 1). However, a combination of long-term acclimation and allozyme studies suggest the possibility of genetic differentiation between oxygen regimes over small spatial scales (Chapman et al. 1999; Martinez et al. 2004). Harniman et al. (2013) used nuclear introns to examine whether populations of *B. neumayeri* within swamp/river systems of western Uganda are genetically structured by DO regime (hypoxia or normoxia), physical barriers such as geographical distance, or both oxygen regime and geography. They found that over a large scale (between drainages), geographical distance affected the genetic structure of *B. neumayeri* populations; however, within the Rwembaita-Njuguta system (Fig. 1), gene flow was high between locations of similar DO regimes, but low between areas of divergent oxygen regimes, suggesting that DO can act as a selective force limiting gene flow among populations of some fish species. These findings are supported by earlier studies on the biochemistry of *B. neumayeri* in this system that detected differences between DO regimes, and demonstrated that these differences were maintained under long-term acclimation, conclusions consistent with some degree of genetic isolation between low- and high-oxygen populations (Martinez et al. 2004, 2011; Table 1). Together these studies highlight the importance of localized extreme habitats as model systems for studying divergent natural selection and more generally for exploring effects of physicochemical stressors on ecological and evolutionary processes.

## 6 Conclusions and Outlook

Hypoxia habitats are useful systems for exploring ecological and evolutionary consequences of living under extreme conditions. Hypoxia occurs naturally in habitats characterized by low mixing and/or light limitation, such as heavily vegetated swamps, flooded forests, floodplain lakes and ponds, ephemeral pools, spring boils, tidal pools, coral reef crevices, and profundal zones of deep lakes. Oceanic oxygen minimum zones (OMZs) represent the largest areas of stable hypoxic water on the planet characterized by extreme hypoxia over very large spatial areas and over very long time periods, thus hosting an assemblage or organisms with extraordinary adaptations to hypoxia. In tropical freshwaters, hypoxic conditions are often exacerbated by high temperatures, and hypoxia can be both chronic and severe in dense tropical swamps where thick vegetation limits light and mixing and fuels extraordinarily high rates of organic decomposition. Human-induced environmental degradation is increasing the occurrence of hypoxia to the point where hypoxia is now recognized as an environmental issue of global importance to fresh and coastal waters. Spreading dead zones are creating extreme environments on a massive spatial scale, and mass mortality of fishes and other organisms characteristic of some recent eutrophication-induced hypoxic events suggests that adaptive response on contemporary timescales is challenging in these systems.

Fishes have evolved a variety of solutions to hypoxic stress, including morphological adaptations, physiological adjustments, and biochemical and molecular defenses. Behavioral responses provide additional flexibility to deal with variation in DO that can occur temporally or spatially in extreme environment. In general, fishes persisting under extreme hypoxia use one or more of the following strategies: (1) evolution of air-breathing organs, (2) movement to zones of higher DO, (3) evolution of mechanisms to maximize oxygen uptake from the water, (4) reduction of metabolic rate and/or activity to reduce oxygen requirements, and (5) use of anaerobic metabolism. Future studies that fully integrate the molecular basis for hypoxia adaption will ultimately be critical to understanding the extraordinary abilities of many non-air-breathing fish species to colonize and persist in naturally occurring extreme hypoxia (see reviews by Nikinmaa and Rees 2005; Richards 2009).

Despite potential energetic constraints and reproductive costs of life in low oxygen, there may be benefits of these extreme habitats that offset physiological and/or biochemical costs; these include reduced competition, predation, and/or disease. Several recent studies provide compelling evidence for reduced predation pressure in hypoxic habitats and the significance of hypoxic refugia for hypoxia-tolerant species in the face of piscine predators. However, there is much need for additional studies of parasite communities in extreme environments (Tobler et al. 2006). Trade-offs in the costs and benefits of living in hypoxic and normoxic habitats may contribute to faunal diversification by creating spatially divergent selection that leads to specialized phenotypes as illustrated in studies of East African fishes from swamps and associated normoxic sites. In these systems alternative DO environments provide a strong predictor of intraspecific variation, particularly in traits related to oxygen uptake efficiency or oxygen limitations (e.g., gill size, brain mass), but also in traits that are indirectly affected through trait correlations (e.g., body shape, trophic muscles). The integration of rearing experiments, field studies, and population genetics tools in a modest number of extremophilic fishes demonstrates that phenotypic variation across DO gradients reflects genetic and/or plastic adaptation to local selective pressures with trait-specific variation in plasticity. Together these studies highlight the importance of localized extreme habitats as model systems for studying divergent natural selection and more generally for exploring effects of physiochemical stressors on ecological and evolutionary processes.

**Acknowledgments** The author would like to acknowledge financial support provided by the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chair program.

## References

Balirwa JS, Chapman CA, Chapman L, Cowx I, Geheb K, Kaufman L, Lowe-McConnell RH, Seehausen O, Wanink JH, Welcomme RL, Witte F (2003) Biodiversity and fishery sustainability in the Lake Victoria Basin: an unexpected marriage? Bioscience 53:703–715

Barrow L, Chapman LJ (2006) Foraging costs of hypoxia acclimation in the swamp dwelling African cyprinid *Barbus neumayeri*. Copeia 2006:552–557

Bartlett GR (1978) Water soluble phosphates of fish red blood cells. Can J Zool 56:870–877

Beadle LC (1981) The inland waters of Africa. Longman, London

Bevan DJ, Kramer DL (1986) The effect of swimming depth on respiratory behavior of the honey gourami. *Colisa chuna* (Pisces, Belontiidae). Can J Zool 64:1893–1896

Binning SA, Chapman LJ, Dumont J (2010) Feeding and breathing: trait correlations in an African cichlid fish. J Zool 282:140–149

Brauner CJ, Val AL (2006) Oxygen transfer. In: Val LV, Almeida-Val VMF, Randall DJ (eds) Tropical fishes, vol 21, Fish physiology. Academic Press/Elsevier, San Diego, CA, pp 277–306

Burggren WW (1982) "Air Gulping" improves blood oxygen transport during aquatic hypoxia in the goldfish, *Carassius auratus*. Physiol Zool 55:327–334

Carter GS (1955) The papyrus swamps of Uganda. Heffner, Cambridge

Cech JJ, Massingill MJ, Vondracek B, Linden AL (1985) Respiratory metabolism of mosquitofish, *Gambusia affinis*: effects of temperature, dissolved oxygen, and sex difference. Environ Biol Fish 13:297–307

Chapman LJ (2007) Morpho-physiological divergence across oxygen gradients in fishes. In: Fernandes MN, Rantin FT, Glass ML, Kapoor BG (eds) Fish respiration and the environment. Science Publishers, Enfield, NH, pp 14–39

Chapman LJ, Chapman CA (1993) Desiccation, flooding, and the behaviour of *Poecilia gillii* (Pisces: Poeciliidae). Ichthyol Explor Freshw 4:279–287

Chapman LJ, Chapman CA (1994) Observations on synchronous air breathing in *Clarias liocephalus*. Copeia 1994:246–249

Chapman LJ, Chapman CA (1998) Hypoxia tolerance of the mormyrid *Petrocephalus catostoma*: implications for persistence in swamp refugia. Copeia 1998:762–768

Chapman LJ, Hulen KJ (2001) Implications of hypoxia for the brain size and gill surface area of mormyrid fishes. J Zool 254:461–472

Chapman LJ, Kramer DL (1991) Limnological observations of an intermittent tropical dry forest stream. Hydrobiologia 226:153–166

Chapman LJ, McKenzie D (2009) Behavioural responses and ecological consequences. In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia, vol 27, Fish physiology. Elsevier, Amsterdam, pp 26–77

Chapman LJ, Kaufman LS, Chapman CA (1994) Why swim upside down? A comparative study of two mochokid catfishes. Copeia 1994:130–135

Chapman LJ, Kaufman LS, Chapman CA, McKenzie FE (1995) Hypoxia tolerance in twelve species of East African cichlids: potential for low oxygen refugia in Lake Victoria. Conserv Biol 9:1274–1288

Chapman LJ, Chapman CA, Ogutu-Ohwayo R, Chandler M, Kaufman L, Keiter AE (1996) Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. Conserv Biol 10:554–561

Chapman LJ, Chapman CA, Brazeau D, McGlaughlin R, Jordan M (1999) Papyrus swamps and faunal diversification: geographical variation among populations of the African cyprinid *Barbus neumayeri*. J Fish Biol 54:310–327

Chapman LJ, Chapman CA, Crisman TL, Prenger J (2000a) Predictors of seasonal oxygen levels in a Ugandan swamp/river system: a 3-year profile. Verhandlungen Internationale Vereinigung Limnologie 27:3048–3053

Chapman LJ, Galis F, Shinn J (2000b) Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. Ecol Lett 3:388–393

Chapman LJ, Balirwa J, Bugenyi FWB, Chapman CA, Crisman TL (2001) Wetlands of East Africa: biodiversity, exploitation, and policy perspectives. In: Gopal B, Junk WJ, Davis JA (eds) Wetlands biodiversity. Backhuys Publisher, Leiden, pp 101–132

Chapman LJ, Chapman CA, Nordlie FG, Rosenberger AE (2002) Physiological refugia: swamps, hypoxia tolerance, and maintenance of fish biodiversity in the Lake Victoria Region. Comp Biochem Physiol A 133:421–437

Chapman LJ, Chapman CA, Olowo JP, Schofield PJ, Kaufman LS, Seehausen O, Ogutu-Ohwayo R (2003) Fish faunal resurgence in Lake Nabugabo, East Africa. Conserv Biol 7:500–511

Chapman LJ, DeWitt, TJ, Tzenava V, Paterson J (2007) Interdemic variation in the gill morphology of a eurytopic African cichlid. In: Proceedings of the 9th international symposium on fish physiology, toxicology, and water quality. EPA/600/R-07/010, pp 209–225

Chapman LJ, Albert J, Galis F (2008) Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. Open Evol J 2:75–88

Childress JJ, Seibel BA (1998) Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. J Exp Biol 201:1223–1232

Childress JJ, Cowles DL, Favuzzi JA, Mickel TJ (1990) Metabolic rates of benthic deep-sea decapod crustaceans decline with increasing depth primarily due to the decline in temperature. Deep-Sea Res 20:1093–1109

Congleton JL (1980) Observations on the responses of some southern California tidepool fishes to nocturnal hypoxia stress. Comp Biochem Physiol A 66:719–722

Crispo E, Chapman LJ (2008) Population genetic structure across dissolved oxygen regimes in an African cichlid fish. Mol Ecol 17:2134–2148

Crispo E, Chapman LJ (2010a) Temporal variation in the population genetic structure of a riverine African cichlid fish. J Hered 101:97–106

Crispo E, Chapman LJ (2010b) Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. J Evol Biol 23:2091–2103

Crispo E, Chapman LJ (2011) Hypoxia drives divergence in cichlid body shape. Evol Ecol 25:949–964

Crocker CD, Chapman LJ, Martinez ML (2013) Natural variation in enzyme activity of the African cichlid *Pseudocrenilabrus multicolor victoriae*. Comp Biochem Physiol B 164:53

Davies R, Moyes CD, Wang YS (2011) Intra- and inter-specific variation in metabolic gene expression in relationship to environmental hypoxia. Comp Biochem Physiol A Mol Integr Physiol A150:25–31

DeKoning ABL, Picard DJ, Bond SR, Schulte PM (2004) Stress and inter-population variation in glycolytic enzyme activity and expression in a teleost fish, *Fundulus heteroclitus*. Physiol Biochem Zool 77:18–26

Diaz RJ (2001) Overview of hypoxia around the world. Environ Qual 30:275–281

Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr Mar Biol Ann Rev 33:245–303

Diaz JR, Rosenberg R (2008) Spreading dead zones and consequences of marine ecosystems. Science 321:926–929

Dybas CL (2005) Dead zones spreading in world oceans. BioScience 55:552–557

Everett MV, Crawford DL (2010) Adaptation versus allometry: population and body mass effects in hypoxic metabolism in *Fundulus grandis*. Physiol Biochem Zool 83:182–190

Friedman JR, Condon NE, Drazen JC (2012) Gill surface area and metabolic enzyme activities of demersal fishes associated with the oxygen minimum zone off California. Limnol Oceanogr 57:1701–1710

Fyhn UEH, Fyhn HJ, Davis BJ, Powers DA, Fink WL, Garlick RL (1979) Hemoglobin heterogeneity in Amazonian fishes. Comp Biochem Physiol A 62:39–66

Gee JH, Gee PA (1991) Reactions of gobioid fishes to hypoxia: buoyancy control and aquatic surface respiration. Copeia 1991:17–28

Gee JH, Gee PA (1995) Aquatic surface respiration, buoyancy control and the evolution of air-breathing in gobies (*Gobiidae*; Pisces). J Exp Biol 198:79–89

Gee JH, Tallman RF, Smart HJ (1978) Reactions of some Great Plains fishes to progressive hypoxia. Can J Zool 56:1962–1966

Gercken J, Forlin L, Andersson J (2006) Developmental disorders in larvae of eelpout (*Zoarces viviparus*) from German and Swedish Baltic coastal waters. Mar Pollut Bull 53:497–507

Graham JB (1985) Seasonal and environmental effects on blood haemoglobin concentrations of some Panamanian air-breathing fishes. Environ Biol Fish 12:291–301

Graham JB (1997) Air breathing fishes: evolution, diversity, and adaptation. Academic Press, San Diego, CA

Graham JB, Lee HG (2004) Breathing air in air: in what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? Physiol Biochem Zool 77:720–731

Harniman R, Merritt TJS, Chapman LJ, Lesbarreres D, Martinez ML (2013) Population differentiation of the African cyprinid *Barbus neumayeri* across dissolved oxygen regimes. Ecol Evol 3:1495–1506

Hassell KL, Coutin PC, Nugegoda D (2008) Hypoxia impairs embryo development and survival in black bream (*Acanthopagrus butcheri*). Mar Pollut Bull 57:302–306

Helly JJ, Levin LA (2004) Global distribution of naturally occurring marine hypoxia on continental margins. Deep-Sea Res I 51:1159–1168

Hochachka PW, Somero GN (2002) Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press, Oxford

Hochachka P, Buck LT, Doll CJ, Land SC (1996) Unifying theory of hypoxia tolerance: molecular/metabolic defence and rescue mechanisms for surviving oxygen lack. Proc Natl Acad Sci U S A 93:9493–9498

Hughes GM (1973) Respiratory responses to hypoxic in fish. Am Zool 13:475–489

Hughes GM (1984) Scaling of respiratory areas in relation to oxygen consumption of vertebrates. Experientia 41:519–652

Innes AJ, Wells RFG (1985) Respiration and oxygen transport functions of the blood from an intertidal fish, *Helicogramma medium* (Tripterygiidae). Environ Biol Fish 14:213–226

Jensen FB (1991) Multiple strategies in oxygen and carbon dioxide transport by haemoglobin. In: Woakes AJ, Grieshaber MK, Bridges CR (eds) Physiological strategies for gas exchange and metabolism. Cambridge University Press, Cambridge, pp 55–78

Jeppesen E, Sondergaard M, Jensen JP et al (2005) Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies. Freshw Biol 50:1747–1771

Jibb LA, Richards JG (2008) AMP-activated protein kinase activity during metabolic rate depression in the hypoxic goldfish, *Carassius auratus*. J Exp Biol 211:3111–3122

Johansen K, Mangum CP, Lykkeboe G (1978) Respiratory properties of the blood of Amazon fishes. Can J Zool 56:898–906

Johansson D, Nilsson GE, Doving KB (1997) Anoxic depression of light-evoked potentials in retina and optic tectum of crucian carp. Neurosci Lett 237:73–76

Joyner-Matos J, Chapman LJ (2013) Persisting in papyrus: size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia. Comp Biochem Physiol A 165:405–416

Kalff J (2002) Limnology. Prentice Hall, Englewood Cliffs, Upper Saddle River, NJ

Kamykowski D, Zentara SJ (1990) Hypoxia in the world ocean as recorded in the historical data set. Deep-Sea Res 37:1861–1874

Kaufman LS (1992) Catastrophic change in species-rich freshwater ecosystems: the lessons of Lake Victoria. BioScience 42:846–858

Kramer DL (1983a) The evolutionary ecology of respiratory mode in fishes: an analysis based on the costs of breathing. Environ Biol Fish 9:145–158

Kramer DL (1983b) Aquatic surface respiration in fishes of Panama: distribution in relation to risk of hypoxia. Environ Biol Fish 8:49–54

Kramer DL (1987) Dissolved oxygen and fish behavior. Environ Biol Fish 18:81–92

Kramer DL, McClure M (1982) Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. Environ Biol Fish 7:47–55

Kramer DL, Mehegan JP (1981) Aquatic surface respiration, an adaptive response to hypoxia in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). Environ Biol Fish 6:299–313

Kramer DL, Lindsey CC, Moodie GEE, Stevens ED (1978) The fishes and the aquatic environment of the central Amazon basin, with particular reference to respiratory patterns. Can J Zool 56:717–729

Kramer DL, Manley D, Bourgeois R (1983) The effects of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. Can J Zool 61:653–665

Lam K, Tsui T, Nakano K, Randall DJ (2006) Physiological adaptations of fishes to tropical intertidal environments. In: Val LV, Almeida-Val VMF, Randall DJ (eds) Tropical fishes, vol 21, Fish physiology. Academic Press/Elsevier, San Diego, CA, pp 502–582

Land SC, Buck LT, Hochachka PW (1993) Response of protein-synthesis to anoxia and recovery in anoxia-tolerant hepatocytes. Am J Physiol 265:R41–R48

Landry CA, Steele SL, Manning S, Cheek AO (2007) Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. Comp Biochem Physiol A 148:317–323

Langerhans RB, Chapman LJ, DeWitt TJ (2007) Complex phenotype-environment associations revealed in an East African cyprinid. J Evol Biol 20:1171–1181

Levin LA (2002) Deep-ocean life where oxygen is scarce. Am Sci 90:436–444

Lewis WM (1970) Morphological adaptations of cyprinodontoids for inhabiting oxygen deficient waters. Copeia 1970:319–325

Lopes AR, Trubenbach K, Teixera T, Lopes VM, Pires V, Baptista M, Repolho T, Calado R, Diniz M, Rosa R (2013) Oxidative stress in deep scattering layers: heat shock response and antioxidant enzymes activities of myctophid fishes thriving in oxygen minimum zones. Deep-Sea Res I 82:10–16

Mandic M, Todgham AE, Richards JG (2009) Mechanisms and evolution of hypoxia tolerance in fish. Proc Roy Soc B 276:735–744

Marinsky CA, Houston AH, Murad A (1990) The effect of hemoglkobin isomorph abundances in rainbow trout, *Salmo gairdneri*. Can J Zool 68:8840–8888

Martinez MS, Chapman LJ, Grady JM, Rees BB (2004) Interdemic variation in hematocrit and lactate dehydrogenase in the African cyprinid *Barbus neumayeri*. J Fish Biol 65:1056–1069

Martinez ML, Raynard EL, Rees BB, Chapman LJ (2011) Oxygen limitation and tissue metabolic potential of the African fish *Barbus neumayeri*: roles of native habitat and acclimatization. BMC Ecol 11:2

McKinsey DM, Chapman LJ (1998) Dissolved oxygen and fish distribution in a Florida spring. Environ Biol Fish 53:211–223

McNeil DL, Closs GP (2007) Behavioural responses of a south-east Australian floodplain fish community to gradual hypoxia. Freshw Biol 52:412–420

Monteiro PLC, Val AL, Almeida-Val VMF (1986) Biological aspects of Amazonian fishes. Hemoglobin, hematology, intraerythrocytic phosphates, and whole blood Bohr effect of *Mylossoma duriventris*. Can J Zool 65:1805–1811

Muir BS (1969) Gill dimensions as a function of size. J Fish Res Bd Can 26:165–170

Nikinmaa M, Rees BB (2005) Oxygen-dependent gene expression in fishes. Am J Physiol 288: R1079–R1090

Nikinmaa M, Salama A (1998) Oxygen transport in fish. In: Perry SF, Tufts BL (eds) Fish respiration. Academic Press, New York, NY, pp 141–170

Nilsson GE (2001) Surviving anoxia with the brain turned on. News Physiol Sci 16:218–221

Nilsson GE (2007) Gill remodeling in fish – a new fashion or an ancient secret? J Exp Biol 201:2403–2409

Nilsson GE, Östlund-Nilsson S (2004) Hypoxia in paradise: widespread hypoxia tolerance in coral reef fishes. Proc R Soc Lond B (Suppl) 271:S30–S33

Nilsson GE, Östlund-Nilsson S (2008) Does size matter for hypoxia tolerance in fish? Biol Rev 83:173–189

Nilsson GE, Renshaw GMC (2004) Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. J Exp Biol 207:3131–3139

Nilsson GE, Rosen P, Johansson D (1993) Anoxic depression of spontaneous locomotor activity in crucian carp quantified by a computerized imaging technique. J Exp Biol 180:153–162

Nilsson GE, Hobbs J-PA, Östlund-Nilsson S (2007) Tribute to P.L. Lutz: respiratory ecophysiology of coral-reef teleosts. J Exp Biol 210:1673–1686

Nilsson GE, Dymowska A, Stecyk JAW (2012) New insights into the plasticity of gill structure. Respir Physiol Neurobiol 184:214–222

Olowo JP, Chapman LJ (1996) Papyrus swamps and variation in the respiratory behaviour of the African fish *Barbus neumayeri*. Afr J Ecol 34:211–222

Perez JE, Rylander K, Nirchio M (1995) The evolution of multiple haemoglobins in fishes. Rev Fish Biol Fisheries 5:304–319

Perry SF, McDonald G (1993) Gas exchange. In: Evans DH (ed) The physiology of fishes, Marine science series. CRC, Boca Raton, FL, pp 251–278

Pollock MS, Clarke LMJ, Dubé MG (2007) The effects of hypoxia on fishes: from ecological relevance to physiological effects. Environ Rev 15:1–14

Rabalais NN, Eugene Turner R, Wiseman WJ Jr (2002) Gulf of Mexico hypoxia A.K.A. The dead zone. Ann Rev Ecol Syst 33:235–261

Raymond K, Chapman LJ, Lanciani C (2006) Host, macrohabitat, and microhabitat specificity in the gill parasite *Neodiplozoon polycotyleus* [Monogenea]. J Parasit 92:1211–1217

Reardon ER, Chapman LJ (2009) Hypoxia and life-history traits in a eurytopic African cichlid. J Fish Biol 75:1683–1699

Richards JG (2009) Metabolic and molecular responses of fish to hypoxia. In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia, vol 27, Fish physiology. Elsevier, Amsterdam, pp 443–485

Riggs A, Fyhn HJ, Fyhn UEH, Noble RW (1979) Studies of functional properties of hemoglobin of *Hoplias malabaricus* and *Hoplerythrunus unitaeniatus*. Comp Biochem Physiol A 62:189–193

Robb T, Abrahams MV (2003) Variaiton in tolerance to hypoxia in a predatory and prey species: an ecological advantage of being small? J Fish Biol 62:1067–1081

Rosenberger AE, Chapman LJ (2000) Respiratory characters of three haplochromine cichlids: implications for persistence in wetland refugia. J Fish Biol 57:483–501

Rutjes HA, Nieveen MC, Weber RE, Witte F, Van den Thillart GEEJM (2007) Multiple strategies of Lake Victoria cichlids to cope with lifelong hypoxia include hemoglobin switching. Am J Physiol Regul Integr Comp Physiol 293:R1376–R1383

Sayer MDJ (2005) Adaptations of amphibious fish for surviving life out of water. Fish Fisheries 6:186–211

Schaack SR, Chapman LJ (2003) Interdemic variation in the African cyprinid *Barbus neumayeri*: correlations among hypoxia, morphology, and feeding performance. Can J Zool 81:430–440

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, Oxford

Schluter D (2001) Ecology and the origin of species. Trends Ecol Evol 16:372–380

Schofield PJ, Chapman LJ (2000) Hypoxia tolerance of introduced Nile perch: implications for survival of indigenous fishes in the Lake Victoria basin. Afr Zool 35:35–42

Seehausen O, van Alphen JJM, Witte F (1997) Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277:1808–1811

Seibel BA (2011) Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. J Exp Biol 214:326–336

Sloman KA, Wood CM, Scott GR, Wood S, Kajiumura M, Johannsson OE, Almeida-Val VMF, Val D (2006) Tribute to R.G. Boutilier: the effect of size on the physiological and behavioural responses of oscar, *Astronotus ocellatus*, to hypoxia. J Exp Biol 209:1197–1205

Sollid J, De Angelis P, Gundersen K, Nilsson GE (2003) Hypoxia induces adaptive and reversible gross-morphological changes in crucian carp gills. J Exp Biol 206:3667–3673

Sollid J, Weber RE, Nilsson GE (2005) Temperature alters the respiratory surface area of crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*). J Exp Biol 208:1109–1116

Sollid J, Nilsson GE (2006) Plasticity of respiratory structures – adaptive remodeling of fish gills induced by ambient oxygen and temperature. Respir Physiol Neurobiol 154:241–251

Spigel RH, Coulter GW (1996) Comparison of hydrology and physical limnology of the East African Great Lakes: Tanganyika, Malawi, Victoria, Kivu and Turkana (with references to some North American Great Lakes). In: Johnson TC, Odada EO (eds) The limnology, climatology, and paleoclimatology of the East African lakes. Gordon and Breach Publishers, Amsterdam, pp 103–135

Thomas P, Rahman MS, Khan IA, Kummer JA (2007) Widespread endocrine disruption and reproductive impairment in an estuarine fish population exposed to seasonal hypoxia. Proc Biol Sci 274:2693–2702

Thompson K, Shewry PR, Woolhouse HW (1979) Papyrus swamp development in the Upemba Basin, Zaire: studies of population structure in *Cyperus papyrus* stands. Bot J Linn Soc 78:299–316

Timmerman CM, Chapman LJ (2004a) Patterns of hypoxia in a coastal salt marsh: implications for ecophysiology of resident fishes. Flor Sci 67:80–91

Timmerman CM, Chapman LJ (2004b) Behavioral and physiological compensation for chronic hypoxia in the live-bearing sailfin molly (*Poecilia latipinna*). Physiol Biochem Zool 77:601–610

Tobler M (2008) Divergence in trophic ecology characterizes colonization of extreme habitats. Biol J Linn Soc 95:517–528

Tobler M, Schlupp I, Heubel KU, Riesch R, Garcia de Leon FJ, Giere O, Plath M (2006) Life on the edge: hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters. Extremophiles 10:577–585

Tobler M, Schlupp I, Garcia de Leon FJ, Glaubrecht M, Plath M (2007) Extreme habitats as refuge from parasite infections? Evidence from an extremophile fish. Acta Oecol 31:270–275

Tobler J, DeWitt TJ, Schlupp I, Garcia de Leon FJ, Herrmann R, Feulner PGD, Tiedemann R, Plath M (2008) Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. Evolution 10:2643–2659

Tobler M, Palacios M, Chapman LJ, Mitrofanov I, Bierbach D, Plath M, Arias-Rodriguez L, Garcia de Leon FJ, Mateos M (2011) Replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. Evolution 65:2213–2228

Tobler M, Plath M, Riesch R, Schlupp I, Grasse A, Munimanda GK, Setzer C, Penn DJ, Moodley Y (2014) Selection from parasites favours immunogenetic diversity but does not promote speciation. J Evol Biol 27:960–974

Tzaneva V, Bailey S, Perry SF (2011) The interactive effects of hypoxemia, hyperoxia, and temperature on the gill morphology of goldfish (*Carassius auratus*). Am J Physiol Regul Integr Comp Physiol 300:R1344–R1351

Val AL (2000) Organic phosphates in the red blood cells of fish. Comp Biochem Physiol 125:417–435

Val AL, Affonso EG, De Almeida-Val VM (1992) Adaptive features of Amazon fishes: blood characteristics of Curimata (*Procilodus cf. nigricans*, Osteighthyes). Physiol Zool 65:832–843

Vaqyer-Sunyer R, Duarte CM (2008) Thresholds of hypoxia for marine biodiversity. PNAS 105:15452–15457

Visser NW (1993) Wetlands and tourism. In: Crafter SA, Njuguna SG, Howard GW (eds) Wetlands of Kenya, Proceedings of a seminar on wetlands of Kenya. IUCN, Gland, Switzerland, pp 135–138

Vornanen M, Stecyk JAW, Nilsson GE (2009) The anoxia-tolerant crucian carp (*Carassius carassius* L.). In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia, vol 27, Fish physiology. Elsevier, Amsterdam, pp 397–441

Wang T, Lefevre S, Huong DTT, Cong NV, Bayley M (2009) The effects of hypoxia on growth and digestion. In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia, vol 27, Fish physiology. Elsevier, Amsterdam, pp 361–396

Wannamaker CM, Rice JA (2000) Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. J Exp Mar Biol Ecol 249:145–163

Wells RMG (2009) Blood-gas transport and haemoglobin function: adaptations for functional and environmental hypoxia. In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia, vol 27, Fish physiology. Elsevier, Amsterdam, pp 255–299

Wells RMG, Grigg GC, Beard LA, Summers G (1989) Hypoxic responses in a fish from a stable environment: blood oxygen transport in the antarctic fish *Pagothenia borchgrevinki*. J Exp Biol 141:97–111

West GB, Brown JH, Enquist BJ (1997) A general model for the origin of allometric scaling laws in biology. Science 276:122–126

Wiens K, Crispo E, Chapman LJ (2014) Phenotypic plasticity is maintained despite geographical isolation in an African cichlid fish, *Pseudocrenilabrus multicolor*. Int Zool 9:85–96

Winemiller KO (1989) Development of dermal lip protuberances for aquatic surface respiration in South America characid fishes. Copeia 1989:382–390

Witte F, Msuku BS, Wanink JH, Seehausen O, Katunzi EFB, Goudswaard KPC, Goldschmidt T (2000) Recovery of cichlid species in Lake Victoria: an examination of factors leading to differential extinction. Rev Fish Biol Fisheries 10:233–241

Wu RSS (2009) Effects of hypoxia on fish reproduction and development. In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia, vol 27, Fish physiology. Elsevier, Amsterdam, pp 255–299

Wu RSS, Zhou BS, Randall DJ, Woo NYS, Lam PKS (2003) Aquatic hypoxia is an endocrine disruptor and impairs fish reproduction. Environ Sci Technol 37:1137–1141

Zhang H, Ludsin SA, Mason DM, Adamack AT, Brandt SB, Zhang X, Kimmel DG, Roman MR, Boicourt WC (2009) Hypoxia-driven changes in the behavior and spatial distribution of pelagic fish and mesozooplankton in the northern Gulf of Mexico. J Exp Mar Biol Ecol 381:S80–S91

Zhou B, Wu RSS, Randall DJ, Lam PKS (2001) Bioenergetics and RNA/DNA ratios in the common carp (*Cyprinus carpio*) under hypoxia. J Comp Physiol B 171:49–57

# The Adaptive Radiation of Notothenioid Fishes in the Waters of Antarctica

**Michael Matschiner, Marco Colombo, Malte Damerau, Santiago Ceballos, Reinhold Hanel, and Walter Salzburger**

**Abstract** Fishes of the perciform suborder Notothenioidei, which dominate the ichthyofauna in the freezing waters surrounding the Antarctic continent, represent one of the prime examples of adaptive radiation in a marine environment. Driven by unique adaptations, such as antifreeze glycoproteins that lower their internal freezing point, notothenioids have not only managed to adapt to sub-zero temperatures and the presence of sea ice, but also diversified into over 130 species. We here review the current knowledge about the most prominent notothenioid characteristics, how these evolved during the evolutionary history of the suborder, how they compare between Antarctic and non-Antarctic groups of notothenioids, and how they could relate to speciation processes.

## 1 Antarctic Waters: An Extreme Environment

Antarctica represents an isolated "continental island," separated from other continental shelves by the Antarctic Circumpolar Current (ACC) that reaches the ocean floor (Foster 1984) and transports more water than any other ocean current on Earth (Tomczak and Godfrey 2003). The Antarctic Polar Front (APF), located between 50 and 60°S, thermally isolates the continent (Gordon 1971) and poses an

---

M. Matschiner (✉) • W. Salzburger (✉)
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway
e-mail: michaelmatschiner@mac.com; walter.salzburger@unibas.ch

M. Colombo
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

M. Damerau • R. Hanel
Thünen Institute of Fisheries Ecology, Palmaille 9, 22767 Hamburg, Germany

S. Ceballos
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

Centro Austral de Investigaciones Científicas (CADIC), Bernardo A. Houssay 200, cp 9410
Ushuaia, Tierra Del Fuego, Argentina

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_3

additional physical barrier to marine organisms (Shaw et al. 2004). As a result, Antarctic waters are unique marine environments, characterized by sub-zero temperatures and the widespread presence of sea ice. At high latitudes, temperatures remain close to the freezing point of seawater at −1.86 °C throughout the year (Eastman 1993). Due to the weight of the continental ice cap, the Antarctic shelf is eight times deeper than the world average (Anderson 1999). Many potential shallow water habitats are covered by ice foots and anchor ice, and gigantic icebergs regularly rework the bottom topography as deep as 550 m below sea level, so that these habitats are constantly in a state of change or recovery (Barnes and Conlan 2007). Even at depths below 400 m, water temperatures can remain near the freezing point throughout the year (Cheng and Detrich 2007). As a consequence, Antarctic waters are among the thermally most stable habitats on Earth. Nevertheless, they are subject to strong seasonality in light conditions, which in turn influences primary production and nutrient availability (Clarke 1988). Taken together, sub-zero temperatures, the continuous presence of sea ice, and extreme seasonality pose great ecophysiological challenges for marine organisms living in Antarctic waters.

Due to the harsh environment and the isolation by the APF, only a few groups of teleost fishes have managed to successfully colonize Antarctic waters. Out of a diversity of about 28,000 teleost species worldwide (Nelson 2006), less than 400 are known to occur in Antarctica (Eastman 2005). The bulk of the Antarctic fish diversity (~90 %; Eastman 2005) belongs to three different taxonomic groups, which have all been assigned to the recently redefined order Perciformes (Betancur-R et al. 2013): the suborder Notothenioidei (107 species; see below), the family Liparidae (~150 species; Stein 2012), and family Zoarcidae (28 species; Matallanas 2008). The two largest of these groups (Notothenioidei and Liparidae) occupy mostly non-overlapping habitats, as liparids are almost exclusively found in the deep sea below ~800 m depth and are of low abundance (Stein 2012), whereas Antarctic notothenioids dominate the continental shelf and upper slope in terms of vertebrate species number (~50 %) and biomass (90–95 %) (Eastman and Clarke 1998). As most scientific sampling to date has focused on depths shallower than 1,000 m, the Antarctic liparid diversity is greatly understudied, and new species are still frequently described (Stein 2012). Nevertheless, it seems clear that Antarctic Liparidae represent a polyphyletic group resulting from multiple independent invasions from the north (Balushkin 2012), so that they are considered a secondary Antarctic group (Stein 2012). In contrast, the similarly species-rich Antarctic notothenioids apparently evolved in situ on the continental shelf and have been described as a rare example of a marine “species flock.”

The species flock concept was developed more than 100 years ago by botanists to describe assemblages of closely related taxa that “flock together,” i.e., coexist in the same area, and later adopted by ichthyologists for the particularly diverse cichlid fishes of the East African Great Lakes and other lacustrine evolutionary radiations (Salzburger et al. 2014). The key features of a species flock are thus, besides species richness, the common ancestry of its members, a clear-cut geographic circumscription, and, hence, high levels of endemicity. Most, if not all, species flocks are the product of adaptive radiations (Eastman and McCune 2000; Salzburger and Meyer 2004), and as we will describe below in more detail, Antarctic notothenioid fishes represent what is arguably the most spectacular example of an extant adaptive radiation in the marine realm.

## 2 Taxonomy of Notothenioids

The Notothenioidei have been taxonomically classified into 8 different families and 136 species (Eastman and Eakin 2000; Table 1, continuously updated by Eastman and Eakin and available at http://www.oucom.ohiou.edu/dbms-eastman/; version Oct. 18, 2013). Five families are predominantly Antarctic and three occur in the coastal waters of New Zealand, Australia, South America, and subantarctic islands (Fig. 1a). The most widely distributed family is Bovichtidae, consisting of nine

**Table 1** All non-Antarctic notothenioids with presumed Antarctic ancestry and presence of AFGP

| Family/genus and species | Occurrence | AFGP |
|---|---|---|
| Nototheniidae | | |
| *Dissostichus eleginoides* | SA, NZ, SG | No |
| *Notothenia angustata* | NZ | Yes |
| *Notothenia microlepidota* | NZ | Yes |
| *Paranotothenia magellanica* | SA | Yes |
| *Lepidonotothen macrophthalma* | SA | ? |
| *Patagonotothen brevicauda* | SA | ? |
| *Patagonotothen canina* | SA | ? |
| *Patagonotothen cornucola* | SA | ? |
| *Patagonotothen elegans* | SA | ? |
| *Patagonotothen guntheri* | SA, SG | No |
| *Patagonotothen jordani* | SA | ? |
| *Patagonotothen kreffti* | SA | ? |
| *Patagonotothen longipes* | SA | ? |
| *Patagonotothen ramsayi* | SA | No |
| *Patagonotothen sima* | SA | ? |
| *Patagonotothen squamiceps* | SA | ? |
| *Patagonotothen tessellata* | SA | No |
| *Patagonotothen trigramma* | SA | ? |
| *Patagonotothen thompsoni* | SA | ? |
| *Patagonotothen wiltoni* | SA | ? |
| Harpagiferidae | | |
| *Harpagifer bispinis* | SA | ? |
| Channichthyidae | | |
| *Champsocephalus esox* | SA | Yes |

*SA* South America, *NZ* New Zealand, *SG* South Georgia, ? AFG possession unknown species in three genera. Only one of these, *Bovichtus elongatus*, is found in Antarctica at the tip of the Antarctic Peninsula, while all other bovichtid species occur north of the ACC. Two notothenioid families are monotypic, and limited to temperate habitats. *Pseudaphritis urvillii* is the only member of the family Pseudaphritidae and occurs in southeast Australia and Tasmania. It is one of very few species of notothenioids that inhabits freshwaters such as slow-flowing streams and estuaries. The second monotypic family, Eleginopsidae, is represented by *Eleginops maclovinus*, which is distributed on the shelf areas of Patagonia and the Falkland Islands and commonly fished commercially in Chile and Argentina, where it is known as "róbalo." The remaining five families Nototheniidae, Bathydraconidae, Harpagiferidae, Artedidraconidae, and Channichthyidae represent the bulk of the notothenioid species diversity, including at least 125 species, most of which occur only in Antarctic waters. The exception to this are 22 non-Antarctic species of the genera *Lepidonotothen*, *Notothenia*, *Paranotothenia*, *Champsocephalus*, *Harpagifer*, *Dissostichus,* and *Patagonotothen* (see Table 1), which secondarily escaped the Southern Ocean to colonize the coastal waters of New Zealand and South America (Cheng et al. 2003).

## 3 Characteristics of Notothenioids

The diversification of Notothenioidei has been accompanied by a number of physiological innovations. In their ice-laden environment, the greatest ecophysiological challenge for ectotherm organisms is to prevent freezing of blood and body tissue. As marine teleost fishes have a higher colligative freezing point than seawater, contact with sea ice would lead to rapid freezing of body fluids (Cheng and Detrich 2007), which is lethal for almost all vertebrates. Thus, arguably the most important innovation of Antarctic notothenioids are antifreeze glycoproteins (AFGPs) that effectively lower their freezing point and thus prevent freezing upon contact with sea ice. AFGPs are present in all notothenioids of the five predominantly Antarctic families, with the exception of the nototheniid genus *Patagonotothen* that secondarily escaped to continental shelves of South America (Near et al. 2012). The AFGPs evolved from a pancreatic trypsinogen gene and are usually composed of 4–56 repeats of a threonine–alanine–alanine tripeptide, with threonine residues being O-glycosylated by disaccharides (Hsiao et al. 1990; Chen et al. 1997a). According to size differences, AFGPs are grouped into eight distinct types, with molecular weights between 2.6 and 33.7 kDa (DeVries and Cheng 2005). They are synthesized in the exocrine pancreas as large polyprotein precursors that are cleaved post-translationally to produce the eight different types of AFGPs (Hsiao et al. 1990; Evans et al. 2012). From the exocrine pancreas, AFGPs are discharged into the gastrointestinal tract (Cheng et al. 2006), where they bind to ice crystals ingested with food or water, and inhibit their growth until they are excreted along with feces (see Fig. 1b). Free AFGPs are resorbed via the rectal epithelium and enter the blood and the interstitial fluid. Blood-borne AFGPs reach

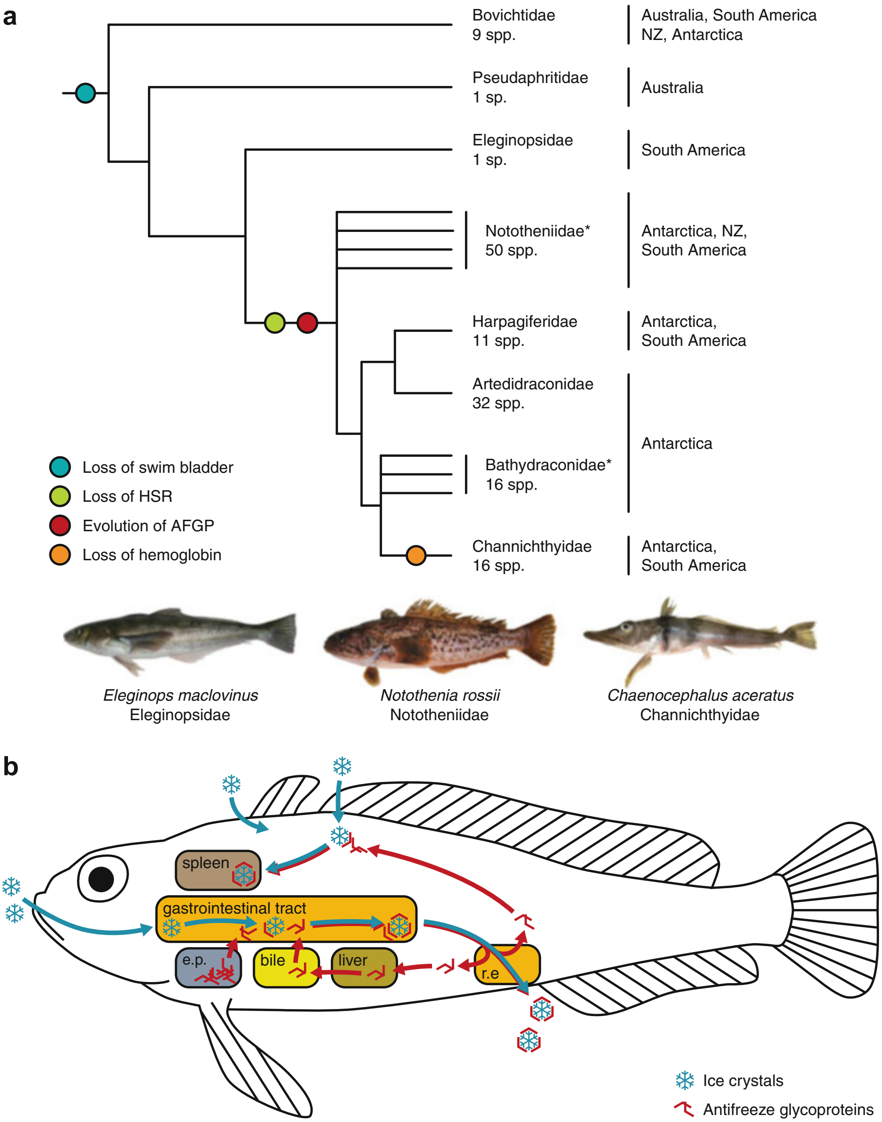


**Fig. 1** (**a**) A simplified cladogram showing relations between notothenioid families, their species richness, and distribution. Major evolutionary innovations and losses (see main text) are marked by *circles* (*Asterisks* indicate presumably non-monophyletic families. *HSR* heat-shock response, *AFGP* antifreeze glycoproteins). (**b**) Schematic representation of the function of AFGP in notothenioids (*Blue-green arrows* indicate points of entry and transport of ice crystals, *red arrows* show AFGP pathways. For details see main text)

the bile via the liver and are discharged again into the gastrointestinal tract. Ice crystals present in the circulatory system usually enter the body through the epithelium, as endogenous ice nucleators are apparently absent in notothenioid fishes (Evans et al. 2011). It has been shown that larvae with low levels of AFGPs survive in ice-laden waters as long as the epithelium is intact, thus highlighting the important role of the epithelium as a protection against freezing (Cziko et al. 2006). In addition, external mucus of adult notothenioids contains AFGPs, which inhibit ice growth on the body surface, and thus prevent injury of the epithelium (albeit the mechanisms by which AFGPs are incorporated in the mucus are still unknown; Evans et al. 2011). If, despite these protective mechanisms, ice enters through the body surface, it is adsorbed by AFGPs of the blood and the interstitial fluid and transported to the spleen, where it is stored in ellipsoidal macrophages (Præbel et al. 2009; Evans et al. 2012). As no mechanism is known for the disposal of AFGP-bound ice from the spleen, it is assumed that ice accumulates in the spleen until seasonal warming events allow melting (Evans et al. 2011).

In a remarkable case of convergent evolution, near-identical AFGPs have independently emerged in at least seven Arctic species of the family Gadidae (Chen et al. 1997b; Zhuang 2013). As in notothenioids, gadid AFGP contains a large number of threonine–alanine–alanine tripeptide repeats, but apparently evolved from noncoding DNA through recruitment of an upstream regulatory sequence, rather than from a precursor gene as in notothenioids (Zhuang 2013). Different types of non-glycosylated antifreeze proteins (AFP) are known from distantly related Arctic and Antarctic fish groups, such as Zoarcidae (eelpouts), Labridae (cunner), Cottidae (sculpins), Hemitripteridae (sea ravens), Osmeridae (smelt), and Clupeidae (herring) (Cheng and DeVries 1989; Fletcher et al. 2001). The latter three lineages possess highly conserved sequences in both exons and introns of AFP genes despite an evolutionary distance of ~250 million years (Ma) (Betancur-R et al. 2013), which led Graham et al. (2008) to suggest sperm-mediated lateral gene flow as the mean of AFP acquisition. In this scenario, fish sperm would absorb foreign DNA from seawater, followed by partial integration into the sperm nucleus. Regardless of the mode of transfer, the presence of highly conserved AFP genes in distantly related lineages highlights the strong natural selection for freeze protection in sub-zero environments.

Besides AFGPs, another general feature found in all notothenioids is the lack of a swim bladder. For this reason, most notothenioids are heavier than seawater and dwell on or near the seafloor. However, several notothenioid lineages, including the genera *Aethotaxis*, *Pleuragramma*, and *Dissostichus*, have independently colonized the water column in a trend termed pelagization (Klingenberg and Ekau 1996; Rutschmann et al. 2011). If these fishes were not neutrally buoyant, continuous investment of muscular energy would be required to provide hydrodynamic uplift. Therefore, these species evolved a plethora of morphological adaptations to compensate for the lack of a swim bladder and attain neutral buoyancy (see below). To name but a few of these adaptations, some pelagic species have reduced ossification of the vertebral column and other body components, the scales of *Pleuragramma* and *Dissostichus* are only weakly mineralized in order to save weight, and *Pleuragramma* further deposits lipids in large assemblages of adipose cells to decrease overall density and to produce static uplift (Eastman 1993; Fernández et al. 2012).

In addition to the loss of the swim bladder, at least some notothenioid species have lost a second trait that is otherwise ubiquitous: The expression of heat-shock proteins (HSPs) as a response to elevated temperatures is regarded a universal characteristic of nearly all organisms, but is absent in the highly cold-adapted stenothermal nototheniid *Trematomus bernacchii* (Hofmann et al. 2000). Further research revealed that the absence of the heat-shock response (HSR) in *T. bernacchii*, as well as in a second member of the same genus, *T. borchgrevinki*, is not due to a loss of HSPs itself, but, on the contrary, due to a constitutive upregulation of Hsp70, which is attributed to permanent cold-stress conditions (Place et al. 2004; Place and Hofmann 2005). Subsequently, this finding has been extended to a representative of the Harpagiferidae, *Harpagifer antarcticus*, suggesting that the loss of the HSR affects most notothenioids and occurred just once during their diversification (Clark et al. 2008). Despite the lack of the classic heat-shock response, notothenioids have recently been shown to retain the ability to acclimatize to elevated temperatures of up to 13–18 °C, yet the molecular mechanisms of this heat hardening remain unknown (Bilyk and DeVries 2011; Bilyk et al. 2012).

Another exceptional loss affecting part of the notothenioid radiation, namely the members of the most derived family, the Channichthyidae, is the lack of the ability to synthesize hemoglobin (Ruud 1954; Eastman 1993). The Channichthyidae are thus the only vertebrate group without oxygen-bearing blood pigments. While the absence of hemoglobin apparently results from the loss of the β-globin subunit gene due to a single deletion event (di Prisco et al. 2002), truncated and inactive remnants of the α-globin gene are retained in the channichthyid genomes (Cocca et al. 1995; Near et al. 2006). Since the oxygen-carrying capacity of the hemoglobin-less phenotype is reduced by a factor of 10, the Channichthyidae evolved compensational features such as an increased blood volume that is 2–4 times that of comparable teleosts, a large stroke volume and cardiac output, and relatively large diameters of arteries and capillaries (Eastman 1993). The adaptive value and evolutionary cause of the loss of hemoglobin remain uncertain (Sidell and O’Brien 2006), but could potentially be related to low iron availability in the Southern Ocean (von der Heyden et al. 2012).

## 4 Notothenioid Phylogeography

The sister lineages of Notothenioidei have long been uncertain (Dettaï and Lecointre 2004), but molecular phylogenetic analyses that have recently become available support the placement of notothenioids within a redefined order of Perciformes that also contains the suborders Serranoidei, Percoidei, Scorpaenoidei, Triglioidei, Cottioidei, and the two families Percophidae and Platycephalidae (Betancur-R et al. 2013; Lautrédou et al. 2013). Within this order, relationships remain poorly resolved, but close affinities of Notothenioidei with Percophidae, Trachinidae, and Percidae have repeatedly been suggested (Matschiner et al. 2011; Lautrédou et al. 2013). Of the three families, Trachinidae and some members of Percidae are also characterized by the lack of a swim bladder (Lewis 1976; Evans and Page 2003), which could thus represent a shared loss between notothenioids and their sister lineage(s), depending on the precise interrelationships of these groups.

Within notothenioids, all molecular phylogenies to date agree on the sequence of the basal splits: the basal Bovichtidae are the sister group of all other notothenioid families, and the monotypic families; Pseudaphritidae and Eleginopsidae diverged before the diversification of the five predominantly Antarctic families (Balushkin 1992; Bargelloni et al. 2000; Near et al. 2004; Near and Cheng 2008; Matschiner et al. 2011; Rutschmann et al. 2011; Near et al. 2012; Betancur-R et al. 2013). Uncertainty remains only regarding the placement of the monotypic genus *Halaphritis*, which appears to be endemic to southeastern Australia and Tasmania. Only seven specimens are known of *H. platycephala*, and DNA could not be extracted from this species (Last et al. 2002). Morphologically, *H. platycephala* superficially resembles the sympatrically occurring pseudaphritid *Pseudaphritis urvillii*, but was provisionally assigned to the Bovichtidae, as it shares almost all diagnostic characters defining this family (Last et al. 2002).

Regardless of the exact affinities of *Halaphritis* with Bovichtidae and Pseudaphritidae, three out of the four most ancestral genera of notothenioids (the monotypic bovichtid genus *Cottoperca* being the exception) occur in, or are even endemic to Australian waters, suggesting that the initial diversification of the suborder took place in this region (Balushkin 2000; Matschiner et al. 2011). This scenario was supported by the time-calibrated molecular phylogeny of Matschiner et al. (2011), who found that the separation of bovichtid and pseudaphritid ancestors may have coincided with shelf area fragmentation between Australia and New Zealand around 70 Ma ago, and that the divergence between Pseudaphritidae and more derived Notothenioidei could have been caused by the breakup of Australia and Antarctica that became complete only around 32 Ma (Barker et al. 2007). According to this hypothesis, individual bovichtid lineages that occur in South America and the island of Tristan da Cunha could have arrived with paleogene currents, owing to their extended pelagic larval durations (Balushkin 2000; Matschiner et al. 2011). The same time-calibration further supports a vicariant separation of the South American Eleginopsidae from the five predominantly Antarctic families in the Eocene, before the opening of the Drake Passage around 41 Ma (Scher and Martin 2006).

Subsequent to the opening of both the Tasman Gateway and the Drake Passage, the onset of the ACC led to thermal isolation of the Antarctic continent and, in combination with declining atmospheric carbon dioxide (DeConto and Pollard 2003; Scher and Martin 2006), to a decrease in water temperatures by up to 4 °C (Nong et al. 2000), resulting in widespread Antarctic continental glaciation at the time of the Eocene–Oligocene transition 34–33 Ma. Whereas the drop to sub-zero temperatures may have been delayed in the marine environment compared to continental Antarctica, there is evidence for sea ice since the early Oligocene. Deposits in offshore drill cores show that since that time, glaciers have repeatedly extended well onto the continental shelf (Cape Roberts Science Team 2000). Sea ice-dependent diatoms have been found in Oligocene sediments (Olney et al. 2009), and widespread ice-rafting occurred as early as 33.6 Ma (Zachos et al. 1996). Freezing conditions in Antarctic waters have been episodic before the middle Miocene climate transition (MMCT) around 14 Ma (Shevenell et al. 2004); however, even seasonal presence of sea ice during cold events of the Oligocene and early Miocene (Naish et al. 2001) must have had a strong impact on the marine fauna of Antarctica.

Fossil evidence from the La Meseta Formation of Seymour Island, off the Antarctic Peninsula, shows that a diverse temperate ichthyofauna existed in the Late Eocene, when Antarctic water temperatures ranged between 10 and15 °C (Eastman 1993; Claeson et al. 2012). Even though ancestral notothenioid lineages were probably present in Antarctic waters during the Eocene, only a single putative notothenioid fossil is known from the La Meseta Formation. *Proeleginops grandeastmanorum* has originally been described as a gadiform (Eastman and Grande 1991), but was subsequently claimed to represent an early member of Eleginopsidae (Balushkin 1994). The fossil has been used to time-calibrate the molecular phylogeny of Near (2004); however, its taxonomic assignment remains questionable. The type locality is specified as RV-8200 and reported to be about 40 Ma (Eastman and Grande 1991). However, according to Long (1992), RV-8200 lies in the lower section of "Tertiary Eocene La Meseta" (Telm) 4, the age of which has recently been reevaluated and is now considered to be 52.5–51.0 Ma (Ivany et al. 2008). This age is substantially older than the mean molecular date estimate for the origin of Eleginopsidae (42.9 Ma) in the study of Matschiner et al. (2011). In their molecular analysis, Matschiner et al. (2011) deliberately excluded *P. grandeastmanorum* as a time constraint due to its debated taxonomic assignment. The presumed fit of their results with the fossil's age (there assumed to be 40 Ma) supported the interpretation of *P. grandeastmanorum* as a notothenioid; however, this does not hold if the fossil is in fact 52.5–51 Ma old. Thus, Notothenioidei may not be represented at all in the Eocene fossil record of the La Meseta Formation, even though a large number of other fishes are found at the same location.

According to the time-calibrated molecular phylogeny of Matschiner et al. (2011), the diversification of the five predominantly Antarctic notothenioid families began near the Oligocene–Miocene boundary, about 24 Ma. Their study agrees with almost all other molecular phylogenies of notothenioids in finding the most basal divergences of Antarctic notothenioids within a paraphyletic family Nototheniidae (Bargelloni et al. 2000; Near and Cheng 2008; Rutschmann et al. 2011; Near et al. 2012; Dettai et al. 2012; Betancur-R et al. 2013). However, uncertainty remains regarding the sister group of all other Antarctic notothenioids, with different analyses recovering either the genus *Gobionotothen* (Matschiner et al. 2011; Near and Cheng 2008; Near et al. 2012), *Aethotaxis* (Rutschmann et al. 2011; Betancur-R et al. 2013), a clade composed of *Aethotaxis* and *Dissostichus* (Near and Cheng 2008; Near et al. 2012; Dettai et al. 2012), or the combined genera *Pleuragramma*, *Aethotaxis*, and *Dissostichus*, in this position (Near et al. 2012).

Individual groups within Nototheniidae receive overwhelming support from molecular analyses, such as the species-rich Trematominae that are composed of the genera *Trematomus*, *Lepidonotothen*, *Patagonotothen*, *Pagothenia* (now included in *Trematomus*; Near et al. 2012), and *Cryothenia* (Janko et al. 2011; Lautrédou et al. 2012), or the clade combining *Notothenia* and *Paranotothenia* (Dettai et al. 2012). Similarly, the more derived families Artedidraconidae, Harpagiferidae, and Channichthyidae appear nested within the paraphyletic Nototheniidae, but are themselves strongly supported to be monophyletic (Derome et al. 2002; Johnston et al. 2003; Rutschmann et al. 2011; Near et al. 2012; Dettai et al. 2012). The same cannot be claimed for the family Bathydraconidae. Monophyly of a clade combining Bathydraconidae and Channichthyidae has not been questioned; however, most analyses recover Channichthyidae nested within Bathydraconidae, thus rendering the latter family paraphyletic (Derome et al. 2002; Near et al. 2012; Dettai et al. 2012; Betancur-R et al. 2013).

## 5 The Adaptive Character of the Notothenioid Radiation

Adaptive radiation is the rapid origin of an array of morphologically and ecologically distinct species from a common ancestor, as a consequence of the adaptation to distinct ecological niches (Schluter 2000; Gavrilets and Losos 2009). Adaptive radiations typically occur after an ancestral species conquers a new, island-type environment with many open niches ("ecological opportunity"), after the extinction of antagonists, liberating previously occupied niches (another form of opportunity), or following the evolution of a novel trait (a so-called key innovation) allowing to effectively exploit new niches (Schluter 2000; Gavrilets and Vose 2005; Yoder et al. 2010). Schluter (2000) defined four main criteria of an adaptive radiation: common ancestry, rapid diversification, phenotype-environment correlation, and trait utility. In the following, we discuss these criteria with respect to the notothenioid species flock:

The first two criteria, common ancestry and rapid diversification, were highlighted by several studies investigating notothenioid phylogeny and diversification rates (Eastman 2005; Matschiner et al. 2011; Near et al. 2012). However, diversification rates seem to be lower in notothenioids compared to other adaptive radiations like the East African cichlid fishes (Rutschmann et al. 2011). It has been suggested that this inequality is due to the lack of habitat heterogeneity, the absence of certain prime inshore habitats in the Antarctic shelf area, enhanced long-range migration ability of pelagic larval stages (Damerau et al. 2014), the absence of genetic population structuring over large distances (see below), and that the notothenioid radiation may not yet have reached its final stage (see Rutschmann et al. 2011, and references therein).

Phenotype–environment correlation and trait utility in notothenioids are best understood with regard to pelagization (the shift from a benthic to a pelagic lifestyle) that has arisen independently in several notothenioid clades (Klingenberg and Ekau 1996; Rutschmann et al. 2011). This shift, referred to as "the hallmark of the notothenioid radiation" (Eastman 2000), was facilitated by adaptations enabling various species to exploit previously unoccupied niches in the water column. Starting from a benthic ancestor, substantial morphological diversification led to phenotypes suited for foraging modes of pelagic or partially pelagic zooplanktivory and piscivory (Eastman 2000). Notothenioids diversified to fill these niches while at the same time also remaining the dominant benthic group of vertebrates (Eastman 2000).

Various morphological and physiological adjustments were needed for species to be able to colonize the water column, mainly to achieve effective swimming performance and to compensate the lack of a swim bladder that most other teleosts use to regulate their buoyancy (Klingenberg and Ekau 1996). Several notothenioid species achieved neutral buoyancy by reducing the mineralization of the skeleton and scales (a pedomorphic trait; Balushkin 2000; Eastman 2000), and by the accumulation of lipid deposits (Eastman 2000). While pelagization has occurred independently in several notothenioid clades (see e.g. Rutschmann et al. 2011), the most complete examples can be found within the family Nototheniidae, where about half of the species occupy the ancestral benthic habitat, whereas the other half adopted a semipelagic, epibenthic, cryopelagic, or pelagic lifestyle (Eastman 2005). Pelagization may be best depicted by *Pleuragramma antarctica,* a sardine-like zooplankton feeder. Morphological adaptations to a life in the water column are highly pronounced in this species, and it evolved to become the dominant species in the water column and the key species in the high-Antarctic food web (Eastman 2005), with several species of channichthyids (*Chionodraco hamatus, Chionodraco myersi, Dacodraco hunteri, Neopagetopsis ionah*) feeding almost exclusively on this species (La Mesa et al. 2004). On the High Antarctic shelf, *Pleuragramma antarctica* is the most important prey item for *Dissostichus mawsoni*, and top predators like penguins, Weddell seals (*Leptonychotes weddellii*), and minke whales (*Balaenoptera bonaerensis*) also greatly rely on *Pleuragramma antarctica* as a food source (Eastman 1985; La Mesa et al. 2004). *Pleuragramma antarctica* has become the dominant species of the midwater fish fauna, with over 90 % both in abundance and biomass (La Mesa et al. 2004), and this dominance can be attributed to a wide range of highly specialized morphological adaptations.

*Pleuragramma antarctica* evolved neutral buoyancy by driving the abovementioned adaptations (reduced mineralization of the skeleton and lipid deposits) to a degree of completion unreached by any other notothenioid species. Lipid, more precisely triglyceride, is stored in intermuscular and subcutaneous sacs. Translucent sacs containing lipid are present between the muscle masses at the bases of the dorsal and anal fins. Furthermore, smaller subcutaneous sacs can be found at the sides of the body (DeVries and Eastman 1978; Eastman 1993).

The skeleton of *P. antarctica* is pedomorphic and reduced, including the reduction of vertebrae and the persistence of the notochord in adult specimens (Eastman 1993).

Adaptations for a life in the water column other than the reduction of buoyancy include morphological changes to alter feeding and swimming performance. *Pleuragramma antarctica* possesses short, protractile jaws featuring a single row with few but large oral teeth suited for suction feeding on planktonic prey (Albertson et al. 2010). Notothenioids living in the water column generally tend to have more elongated, slimmer bodies, but smaller heads than benthic feeders (Klingenberg and Ekau 1996)—the latter probably due to planktonic prey generally being smaller than benthic prey (Klingenberg and Ekau 1996, and references therein). Analyses of the shape of the operculum (Wilson et al. 2013) have furthermore shown that members of the Channichthyidae and Nototheniidae evolved broadly similar opercle shapes in relation to their position along the benthic-pelagic axis and that benthic species generally have an extended posterior margin of the opercle compared to pelagic species, probably reflecting the generally larger head width of benthic notothenioids.

Ecological diversification along the benthic–pelagic axis is also reflected in carbon isotope levels, which can be used to approximate the habitat type. The lowest $\partial^{13}$C values are found in more pelagic species like *Chaenodraco wilsoni*, *Champsocephalus gunnari*, and *Pleuragramma antarctica*, while strictly benthic notothenioids like *Gobionotothen gibberifrons*, *Lepidonotothen nudifrons*, and *Pogonophryne scotti* occupy the upper end of the range (Rutschmann et al. 2011). Carbon isotopic levels further correlate with nitrogen isotope amounts in notothenioids, indicating a connection between habitat and trophic levels. With the exception of the pelagic top predator *Dissostichus mawsoni*, the highest $\partial^{15}$N values are found almost exclusively in benthic species. Remarkably, very similar ranges of isotope signatures are present in at least two notothenioid families, Nototheniidae and Channichthyidae, suggesting convergent ecological evolution along habitat and trophic axes, which is considered characteristic for adaptive radiation (Muschick et al. 2012).

Other than buoyancy adaptations, a second trait that serves well to illustrate both phenotype–environment correlation and trait utility in notothenioids are AFGPs. As these proteins are present in all Antarctic notothenioid clades they are commonly thought to have evolved only once prior to the notothenioid radiation (Chen et al. 1997a; Cheng et al. 2003). The utility of AFGPs in the Antarctic environment is obvious, as these proteins are essential to prevent the formation of ice crystals within the fish's body, and thus are needed for the survival of notothenioids in sub-zero waters (as described above). A correlation between the phenotype and the environment could also be demonstrated in a case study of 11 channichthyid species, where freeze avoidance due to AFGP expression was found to be greater in species occurring at higher latitudes (and thus at colder water temperatures; Bilyk and DeVries 2010). Thus, the four criteria outlined by Schluter (2000) for the detection of adaptive radiation are all fulfilled by Antarctic notothenioids, whereby the latter two criteria (phenotype–environment correlation and trait utility) apply to even more than one notothenioid characteristic.

The evolution of AFGPs is often viewed as a "key-innovation" (see Schluter 2000 for more details on the term), meaning that the emergence of this trait allowed notothenioids to effectively exploit new niches and therefore triggered the notothenioid adaptive radiation (Matschiner et al. 2011). It has been hypothesized that the drop to sub-zero water temperatures around Antarctica led to the extinction of most of the previously existing ichthyofauna (Eastman 1993), which enabled notothenioids to diversify and occupy the subsequently vacant niches (Matschiner et al. 2011). However, diversification rate analyses have recently suggested that major pulses of lineage diversification within notothenioid clades, responsible for a large share of the notothenioid species richness, occurred substantially later than the origin of AFGPs, thus suggesting that the key innovation of AFGPs may not have been the only driver of the notothenioid radiation (Near et al. 2012).

## 6 Non-Antarctic Notothenioids

The non-Antarctic notothenioids comprise two main groups of fishes: basal lineages that diverged before the isolation of Antarctica (families Eleginopsidae, Pseudaphritidae, and Bovichtidae), which therefore never experienced the "Antarctic permanent cold conditions" during their evolutionary history, and a more derived group that presumably originated from northward dispersal events of Antarctic ancestors (belonging to families Nototheniidae, Harpagiferidae, and Channichthyidae). The comparison between Antarctic and non-Antarctic notothenioids may be important to better understand the numerous unique traits that notothenioids have evolved in Antarctic waters. In addition, the specific comparison with non-Antarctic notothenioids of Antarctic ancestry may allow the identification of features that allow them to inhabit cold-temperate waters outside the APF. The knowledge about these latter traits may be a key to better understand how evolution in the stable cold waters of Antarctica has constrained the ability of Antarctic notothenioids to deal with environmental changes and global warming.

So far, phylogenetic analyses have identified seven genera (represented by 22 species) that are nested within the Antarctic notothenioid clade, but occur north of the border drawn by the APF (Table 1). There are still many uncertainties about the phylogenetic relationships between and within these seven genera, though. Nonetheless, there is some evidence that supports monophyly of a clade combining *Paranotothenia magellanica* and the two non-Antarctic *Notothenia* species (Cheng et al. 2003). Likewise, *Lepidonotothen macrophthalma*, the only non-Antarctic representative of the genus, has never been included in a molecular phylogeny, but is morphologically closely related to *L. squamifrons* (Balushkin 2000; Pequeño 2000), which was found to be the sister taxon of the genus *Patagonotothen* (Dettai et al. 2012; Near et al. 2012). Thus, there is the possibility that *L. macrophthalma* and the genus *Patagonotothen* form a monophyletic group as well. The remaining components of the group of non-Antarctic notothenioids are strongly supported as non-monophyletic (Rutschmann et al. 2011; Dettai et al. 2012; Near et al. 2012). Therefore, the most parsimonious explanation would involve at least five putative ancestors that dispersed northward across the APF. Three would belong to the family Nototheniidae, the most basal one of the five Antarctic notothenioids families, and the other two are members of the more derived high-Antarctic families Harpagiferidae and Channichthyidae.

Some authors have suggested that the "escapes" of these ancestors from the Antarctic waters may be linked to temporally northwards movements of the APF (Bargelloni et al. 2000; Cheng et al. 2003; Coppes Petricorena and Somero 2007). If the five putative escapes are linked to paleoceanographic events, it seems that at least two different events would be involved. The divergence between the Antarctic and non-Antarctic sister lineages of the family Nototheniidae apparently took place more than 7 Ma (Near 2004; Near et al. 2012), whereas the divergence of *Champsocephalus esox* and its Antarctic sister taxon *C. gunnari* was estimated at around 4–1.7 Ma (Near et al. 2004; Stankovic et al. 2002). An estimation of the divergence time between the South American *Harpagifer bispinis* and its closest relative from Antarctica, *H. antarcticus,* is still lacking, albeit their very similar morphology (Gon and Heemstra 1990) may suggest a recent divergence, probably closer in time to the separation of non-Antarctic Channichthyidae than to that of non-Antarctic nototheniids.

The Antarctic ancestry of these non-Antarctic notothenioids led to the prediction that these species might have AFGP genes or at least its remnants in their genomes. The occurrence of AFGP in non-Antarctic notothenioids from South America and New Zealand waters has already been examined in eight species (Cheng and Detrich 2007), confirming its presence in four of them (Table 1). The most parsimonious explanation for the apparent absence of AFGP in *Dissostichus eleginoides* and three species of *Patagonotothen* involves at least two independent losses or severe mutations of this gene. On the other hand, whereas the Antarctic notothenioids lost the HSR, the New Zealand notothenioid *Notothenia angustata* is able to upregulate the transcription of hsp70 in response to heat shock (Hofmann et al. 2005).

One of the main differences in the evolutionary history between non-Antarctic and Antarctic notothenioids is that the former evolved in the presence of fish groups that are absent or uncommon in Antarctic waters. Therefore, these lineages likely experienced more competition compared with the Antarctic notothenioids, limiting the occupation of niches distinct from the original benthic one. In agreement with this idea, no evidence for diverse static buoyancy values was found in non-Antarctic notothenioids that would allow them to occupy different areas in the water column in the same way as Antarctic notothenioids (Fernández et al. 2012). Comparison of Antarctic and non-Antarctic sister taxa with modern genomic technology may help to identify the genetic changes underlying the transition across the APF, and reveal whether or not they led to adaptations in a similar fashion in different notothenioid families.

The main radiation of notothenioids occurred in an Antarctic environment, and thus the bulk of notothenioids species inhabit the Southern Ocean within the APF. Nonetheless, the second-most species-rich genus is found almost exclusively in non-Antarctic waters: the genus *Patagonotothen* with so far 15 described species is only surpassed in diversity by the Antarctic genus *Pogonophryne* that contains 24 species. The 15 *Patagonotothen* species occur in southern South America with the only exception being *P. guntheri,* which has a trans-APF distribution and it is also found in South Georgia. Morphological analysis suggests that *P. guntheri* may be considered as a derived species within the genus (Balushkin 1992). Thus its presence within the APF is probably a derived character rather than an ancestral one.

The age of the most recent common ancestor of the *Patagonotothen* genus was estimated to be around 5 Ma (Near et al. 2012). This implies a rather rapid radiation of the 15 species, whereby the drivers of this radiation remain unknown but are likely unrelated to the key innovation hypothesis for AFGP. The inshore fish fauna of southern South America seems to be characterized by generally low diversity (Ojeda et al. 2000), which could have facilitated the *Patagonotothen* expansion. A similar radiation in the same region is the one exhibited by the species of the mollusc genus *Nacella*. In this case it has been proposed that the currently overlapping distributions of *Nacella* species and their close genetic relationships could be explained by allopatric speciation, or at least incipient separation, in separate refugia during glaciations, followed by geographical re-expansion and ecological separation (González-Wevar et al. 2011). A similar scenario could explain the *Patagonotothen* radiation; however, more research on this group will be needed to support this hypothesis.

## 7 Demography and Population Structure in Antarctic Notothenioids

Whereas phylogenies can inform about the macroevolutionary history of Antarctic notothenioids, the underlying forces of speciation processes are commonly linked to ecological factors that often act on a far more microevolutionary timescale (Nosil 2012). Understanding the population dynamics of species, especially the factors modulating demography and gene flow among populations, is therefore crucial for the understanding of the adaptive radiation of notothenioids. Molecular genetic signatures left by past and present demographic events, such as population size changes or migration, allow us to disentangle the importance of biotic and abiotic factors that influence differentiation processes on the population level.

For polar organisms including notothenioid fishes, it has often been hypothesized that population size changes are driven by glaciation cycles associated with severe implications for species' survival and distribution (Kennett 1982; Eastman 1993; Petit et al. 1999). During major glacial periods, the Antarctic ice sheet extended as far as the edge of the continental shelf (Ingólfsson 2004; Gersonde et al. 2005), sometimes eradicating Antarctic marine bottom communities on the large scale (Thatje et al. 2005) and "bulldozing the surviving fauna to the deep continental margin" (Barnes and Conlan 2007). As a result, populations were periodically isolated in remaining ice-free refugia (Barnes et al. 2006), which was suggested as a key mechanism for allopatric speciation (Hewitt 1996; Rogers 2007), and is expected to result in population expansions subsequent to glacial retreat. The use of population level molecular data allows the investigation of past population size changes, and in fact has provided evidence for demographic expansions in multiple notothenioid fishes (Zane et al. 2006; Janko et al. 2007; Matschiner et al. 2009), which highlights the impact of glacial cycles on notothenioid populations.

The extent to which population fragmentation leads to differentiation and allopatric speciation in notothenioid fishes remains unclear, but is strongly linked to their potential for long-distance gene flow. While distances between isolated notothenioid populations are on the order of thousands of kilometers (Matschiner et al. 2009), notothenioid fishes are characterized by extended pelagic larval stages that may last between a few months and more than 1 year (Loeb et al. 1993; La Mesa and Ashford 2008). This, in combination with the strong current of the ACC endows the propagules of many species with a great potential for long-distance dispersal (Damerau et al. 2014). Hence, high levels of gene flow could be expected between distant notothenioid populations, which might counteract differentiation and allopatric speciation events.

Since the advent of DNA sequencing and genotyping technologies, estimates of population connectivity have been inferred based on population genetic tools, which measure the distribution of genetic variation among populations. To date, at least 29 population genetic studies have been published for 22 notothenioid species (see references in Volckaert et al. 2012, as well as Carvalho and Warren 1991; Smith and Gaffney 2000; Damerau et al. 2012; Agostini et al. 2013; Damerau et al. 2014). The results of these studies were highly variable and depended clearly on sampling designs and applied marker types (see Table 3 in Matschiner et al. 2009). Nonetheless, an overall trend uncovered by these studies is the decrease of genetic homogeneity among populations with distance, indicating an isolation-by-distance relationship. On a regional scale (within a few hundred kilometers), the vast majority of species showed genetic homogeneity. Even population differentiations within ocean sectors are predominantly insignificant, sometimes over several thousand kilometers. Although a marginal majority of studies revealed significant population differentiations between ocean sectors, many populations of species with circum-Antarctic distributions showed no significant differentiation, as, for example, in the bentho-pelagic Antarctic toothfish *Dissostichus mawsoni* (Smith and Gaffney 2005) or the truly pelagic Antarctic silverfish *Pleuragramma antarctica* (Zane et al. 2006). Moreover, populations of strictly benthic species, such as *Gobionotothen gibberifrons*, which is confined to shelf areas as adults, were not significantly differentiated over their distribution range on subantarctic islands (Matschiner et al. 2009).

The seemingly high levels of gene flow among populations separated by deep ocean over large geographic scales, but connected by currents like the ACC, regardless of the adult life strategy, suggest that gene flow is mediated via dispersal of pelagic developmental stages such as eggs, larvae, or juveniles. This finding is corroborated by genetic breaks that have been found over much shorter geographic distances, where oceanographic barriers exist. For example, populations of *D. eleginoides* are not significantly differentiated over large parts of its circumpolar distribution range, whereas populations that are geographically close but separated by the APF show little connectivity (Shaw et al. 2004; Rogers et al. 2006). Hence, oceanographic features are an important factor regulating population connectivity of notothenioids by either enhancing or attenuating larval dispersal, as has also been shown in species from warmer waters with distinctly shorter pelagic larval stages (e.g. Taylor and Hellberg 2003; Bay et al. 2006; Cowen and Sponaugle 2009). However, the general validity of the observed patterns is limited by varying sampling designs, genetic marker types, and species' biogeography, what makes general inferences about gene flow by larval dispersal a challenging task.

## 8 Conclusions and Outlook

It has been 60 years since notothenioid fishes were first brought to the attention of a broader scientific community, with Johan T. Ruud's (1954) publication in *Nature* demonstrating the loss of hemoglobin as the cause of the colorlessness of the blood of channichthyids. Our knowledge about the nature of the notothenioid evolution has greatly increased over the recent decades, especially since the advent of molecular sequencing technology, but important questions of the notothenioid radiation remain to be answered. While recent phylogenetic work (Rutschmann et al. 2011; Near et al. 2012; Lautrédou et al. 2012) helped to identify multiple well-supported clades such as Trematominae, Artedidraconidae, and Channichthyidae, the same studies also disagreed with respect to more basal notothenioid relationships and thus highlight the need for more comprehensive sequence data sets. Due to ongoing sampling efforts in combination with rapid improvements in sequencing technologies and methodological advances, we may soon be able to address these questions. Through combination of population level and species level sequence data, approaches like the multi-marker coalescent model implemented in *BEAST (Heled and Drummond 2010) are able to account for incomplete lineage sorting, which is common in rapidly diversifying clades (Koblmüller et al. 2010), and could be the cause of incompatibilities between published phylogenies. To date, family-level relationships within Bovichtidae and Harpagiferidae have not been investigated in detail, but could provide valuable insights into the geographic origin and the early phylogeography of the notothenioid radiation. Finally, thanks to the rapidly decreasing cost of next generation sequencing, genome-size data sets may soon be available for notothenioid fishes and permit investigations into the molecular basis of notothenioid adaptations.

Other than molecular data, recent studies have begun to systematically quantify morphological and physiological characteristics (Rutschmann et al. 2011; Near et al. 2012; Wilson et al. 2013), a trend that will continue to give us increasingly well-resolved descriptions of ecological niches occupied by notothenioid taxa. In addition, more behavioral data can be acquired through continuing field expeditions equipped with remotely operated underwater vehicles. In combination, these data will allow us to better understand the axes along which the notothenioid radiation has proceeded (and continues to proceed), as well as the molecular adaptations that enabled their tremendous evolutionary success.

**Acknowledgements** We thank the editors and Joseph Eastman for valuable comments on the manuscript. The authors of this book chapter have been supported by funding from the Swiss National Science Foundation (SNF grants PBBSP3-138680 to MM and CRSII3-136293 to WS), the European Research Council (Starting Grant "INTERGENADAPT" to WS), the Volkswagen Foundation (grant I/83 548 to MM), and the German Research Foundation (grant HA 4328/4 to RH).

## References

Agostini C, Papetti C, Patarnello T et al (2013) Putative selected markers in the *Chionodraco* genus detected by interspecific outlier tests. Polar Biol 36:1509–1518

Albertson RC, Yan Y-L, Titus TA et al (2010) Molecular pedomorphism underlies craniofacial skeletal evolution in Antarctic notothenioid fishes. BMC Evol Biol 10:4

Anderson JB (1999) Antarctic marine geology. Cambridge University Press, Cambridge

Balushkin AV (1992) Classification, phylogenetic relationships, and origins of the families of the suborder Notothenioidei (Perciformes). J Ichthyol 32:90–110

Balushkin AV (1994) Fossil notothenioid, and not gadiform, fish *Proeleginops grandeastmanorum* gen. sp. nov. (Perciformes, Notothenioidei, Eleginopidae) from the late Eocene found in Seymour Island (Antarctica). Voprosy Ikhtiologii 34:298–307

Balushkin AV (2000) Morphology, classification, and evolution of notothenioid fishes of the Southern Ocean (Notothenioidei, Perciformes). J Ichthyol 40:S74–S109

Balushkin AV (2012) *Volodichthys* gen. nov. new species of the primitive snailfish (Liparidae: Scorpaeniformes) of the southern hemishpere. Description of new species V. *Solovjevae* sp. nov. (Cooperation Sea, the Antarctic). J Ichthyol 52:1–10

Bargelloni L, Marcato S, Zane L, Patarnello T (2000) Mitochondrial phylogeny of notothenioids: a molecular approach to Antarctic fish evolution and biogeography. Syst Biol 49:114–129

Barker PF, Filippelli GM, Florindo F, Martin EE, Scher HD (2007) Onset and role of the Antarctic circumpolar current. Deep Sea Res Pt II 54:2388–2398

Barnes DKA, Conlan KE (2007) Disturbance, colonization and development of Antarctic benthic communities. Philos Trans R Soc B 362:11–38

Barnes DKA, Hodgson DA, Convey P, Allen CS, Clarke A (2006) Incursion and excursion of Antarctic biota: past, present and future. Global Ecol Biogeogr 15:121–142

Bay LK, Crozier RH, Caley MJ (2006) The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Mar Biol 149:1247–1256

Betancur-R R, Broughton RE, Wiley EO et al (2013) The Tree of Life and a new classification of bony fishes. PLoS Curr 5:18

Bilyk KT, DeVries AL (2010) Freezing avoidance of the Antarctic icefishes (Channichthyidae) across thermal gradients in the Southern Ocean. Polar Biol 33:203–213

Bilyk KT, DeVries AL (2011) Heat tolerance and its plasticity in Antarctic fishes. Comp Biochem Physiol A 158:382–390

Bilyk KT, Evans CW, DeVries AL (2012) Heat hardening in Antarctic notothenioid fishes. Polar Biol 35:1447–1451

Carvalho GR, Warren M (1991) Genetic population structure of the mackerel icefish, *Champsocephalus gunnari*, in Antarctic waters. Document WG-FSA-91/22. CCAMLR working paper

Chen L, DeVries AL, Cheng C-HC (1997a) Evolution of antifreeze glycoprotein gene from a trypsinogen gene in Antarctic notothenioid fish. Proc Natl Acad Sci U S A 94:3811–3816

Chen L, DeVries AL, Cheng C-HC (1997b) Convergent evolution of antifreeze glycoproteins in Antarctic notothenioid fish and Arctic cod. Proc Natl Acad Sci U S A 94:3817–3822

Cheng C-HC, Detrich HW III (2007) Molecular ecophysiology of Antarctic notothenioid fishes. Philos Trans R Soc B 362:2215–2232

Cheng C-HC, DeVries AL (1989) Structures of antifreeze peptides from the Antarctic eel pout, *Austrolycicthys brachycephalus*. Biochim Biophys Acta 997:55–64

Cheng C-HC, Chen L, Near TJ, Jin Y (2003) Functional antifreeze glycoprotein genes in temperate-water New Zealand nototheniid fish infer an Antarctic evolutionary origin. Mol Biol Evol 20:1897–1908

Cheng C-HC, Cziko PA, Evans CW (2006) Nonhepatic origin of notothenioid antifreeze reveals pancreatic synthesis as common mechanism in polar fish freezing avoidance. Proc Natl Acad Sci U S A 103:10491–10496

Claeson KM, Eastman JT, MacPhee RDE (2012) Definitive specimens of Merlucciidae (Gadiformes) from the Eocene James Ross Basin of Isla Marambio (Seymour Island), Antarctic Peninsula. Antarct Sci 24:467–472

Clark MS, Fraser KPP, Burns G, Peck LS (2008) The HSP70 heat shock response in the Antarctic fish *Harpagifer antarcticus*. Polar Biol 31:171–180

Clarke A (1988) Seasonality in the Antarctic marine environment. Comp Biochem Physiol B 90:461–473

Cocca E, Ratnayake-Lecamwasam M, Parker SK et al (1995) Genomic remnants of alpha-globin genes in the hemoglobinless Antarctic icefishes. Proc Natl Acad Sci U S A 92:1817–1821

Coppes Petricorena ZL, Somero GN (2007) Biochemical adaptations of notothenioid fishes: comparisons between cold temperate South American and New Zealand species and Antarctic species. Comp Biochem Physiol A 147:799–807

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Ann Rev Mar Sci 1:443–466

Cziko PA, Evans CW, Cheng C-HC, DeVries AL (2006) Freezing resistance of antifreeze-deficient larval Antarctic fish. J Exp Biol 209:407–420

Damerau M, Matschiner M, Salzburger W, Hanel R (2012) Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the southern Scotia Arc, Antarctica. Polar Biol 35:1073–1086

Damerau M, Matschiner M, Salzburger W, Hanel R (2014) Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae). Mol Ecol 23:284–299

DeConto RM, Pollard D (2003) Rapid Cenozoic glaciation of Antarctica induced by declining atmospheric $CO_2$. Nature 421:245–249

Derome N, Chen W-J, Dettaï A, Bonillo CÉ, Lecointre G (2002) Phylogeny of Antarctic dragonfishes (Bathydraconidae, Notothenioidei, Teleostei) and related families based on their anatomy and two mitochondrial genes. Mol Phylogenet Evol 24:139–152

Dettaï A, Lecointre G (2004) In search of notothenioid (Teleostei) relatives. Antarct Sci 16:71–85

Dettai A, Berkani M, Lautrédou A-C et al (2012) Tracking the elusive monophyly of nototheniid fishes (Teleostei) with multiple mitochondrial and nuclear markers. Mar Genomics 8:49–58

DeVries AL, Cheng CH (2005) Antifreeze proteins and organismal freezing avoidance in polar fishes. In: Farrell AP, Steffensen JF (eds) Fish physiology, vol 22. Academic Press, San Diego, CA, pp 155–201

DeVries AL, Eastman JT (1978) Lipid sacs as a buoyancy adaptation in an Antarctic fish. Nature 271:352–353

di Prisco G, Cocca E, Parker SK, Detrich HW III (2002) Tracking the evolutionary loss of hemoglobin expression by the white-blooded Antarctic icefishes. Gene 295:185–191

Eastman JT (1985) The evolution of neutrally buoyant notothenioid fishes: their specializations and potential interactions in the Antarctic marine food web. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, pp 430–436

Eastman JT (1993) Antarctic fish biology: evolution in a unique environment. Academic Press, San Diego, CA

Eastman JT (2000) Antarctic notothenioid fishes as subjects for research in evolutionary biology. Antarct Sci 12:276–287

Eastman JT (2005) The nature of the diversity of Antarctic fishes. Polar Biol 28:93–107

Eastman JT, Clarke A (1998) A comparison of adaptive radiations of Antarctic fish with those of nonAntarctic fish. In: di Prisco G, Pisano E, Clarke A (eds) Fishes of Antarctica. A biological overview. Springer, Milano, pp 3–26

Eastman JT, Eakin RR (2000) An updated species list for notothenioid fish (Perciformes; Notothenioidei), with comments on Antarctic species. Arch Fish Mar Res 48:11–20

Eastman JT, Grande L (1991) Late Eocene gadiform (Teleostei) skull from Seymour Island, Antarctic Peninsula. Antarct Sci 3:87–95

Eastman JT, McCune AR (2000) Fishes on the Antarctic continental shelf: evolution of a marine species flock? J Fish Biol 57:84–102

Evans JD, Page LM (2003) Distribution and relative size of the swim bladder in *Percina*, with comparisons to *Etheostoma*, *Crystallaria*, and *Ammocrypta* (Teleostei: Percidae). Environ Biol Fish 66:61–65

Evans CW, Gubala V, Nooney R et al (2011) How do Antarctic notothenioid fishes cope with internal ice? A novel function for antifreeze glycoproteins. Antarct Sci 23:57–64

Evans CW, Hellman L, Middleditch M et al (2012) Synthesis and recycling of antifreeze glycoproteins in polar fishes. Antarct Sci 24:259–268

Fernández DA, Ceballos SG, Malanga G, Boy CC, Vanella FA (2012) Buoyancy of sub-Antarctic notothenioids including the sister lineage of all other notothenioids (Bovichtidae). Polar Biol 35:99–106

Fletcher GL, Hew C-L, Davies PL (2001) Antifreeze proteins of teleost fishes. Annu Rev Physiol 63:359–390

Foster TD (1984) The marine environment. In: Laws RM (ed) Antarctic ecology, vol 2. Academic Press, London, pp 345–371

Gavrilets S, Losos JB (2009) Adaptive radiation: contrasting theory with data. Science 323:732–737

Gavrilets S, Vose A (2005) Dynamic patterns of adaptive radiation. Proc Natl Acad Sci U S A 102:18040–18045

Gersonde R, Crosta X, Abelmann A, Armand L (2005) Sea-surface temperature and sea ice distribution of the Southern Ocean at the EPILOG Last Glacial Maximum—a circum-Antarctic view based on siliceous microfossil records. Quaternary Sci Rev 24:869–896

Gon O, Heemstra PC (1990) Fishes of the Southern Ocean. J.L.B. Smith Institute of Ichthyology, Grahamstown

González-Wevar CA, Nakano T, Cañete JI, Poulin E (2011) Concerted genetic, morphological and ecological diversification in *Nacella* limpets in the Magellanic Province. Mol Ecol 20:1936–1951

Gordon AL (1971) Oceanography of Antarctic waters. In: Reid JL (ed) Antarctic oceanology I. American Geophysical Union, Washington, DC, pp 169–203

Graham LA, Lougheed SC, Ewart KV, Davies PL (2008) Lateral transfer of a lectin-like antifreeze protein gene in fishes. PLoS One 3:e2616

Heled J, Drummond AJ (2010) Bayesian inference of species trees from multilocus data. Mol Biol Evol 27:570–580

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc 58:247–276

Hofmann GE, Buckley BA, Airaksinen S, Keen JE, Somero GN (2000) Heat-shock protein expression is absent in the Antarctic fish *Trematomus bernacchii* (family Nototheniidae). J Exp Biol 203:2331–2339

Hofmann GE, Lund SG, Place SP, Whitmer AC (2005) Some like it hot, some like it cold: the heat shock response is found in New Zealand but not Antarctic notothenioid fishes. J Exp Mar Biol Ecol 316:79–89

Hsiao K-C, Cheng C-HC, Fernandes IE, Detrich HW III, DeVries AL (1990) An antifreeze glycopeptide gene from the antarctic cod *Notothenia coriiceps neglecta* encodes a polyprotein of high peptide copy number. Proc Natl Acad Sci U S A 87:9265–9269

Ingólfsson Ó (2004) Quaternary glacial and climate history of Antarctica. Dev Quaternary Sci 2C:3–44

Ivany LC, Lohmann KC, Hasiuk F et al (2008) Eocene climate record of a high southern latitude continental shelf: Seymour Island, Antarctica. Geol Soc Am Bull 120:659–678

Janko K, Lecointre G, DeVries AL et al (2007) Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. BMC Evol Biol 7:220

Janko K, Marshall C, Musilová Z et al (2011) Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): phylogenetic approach and test of the early-radiation event. Mol Phylogenet Evol 60:305–316

Johnston IA, Fernández DA, Calvo J et al (2003) Reduction in muscle fibre number during the adaptive radiation of notothenioid fishes: a phylogenetic perspective. J Exp Biol 206:2595–2609

Kennett JP (1982) Marine geology. Prentice Hall, Englewood Cliffs, NJ

Klingenberg CP, Ekau W (1996) A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). Biol J Linn Soc 59:143–177

Koblmüller S, Egger B, Sturmbauer C, Sefc KM (2010) Rapid radiation, ancient incomplete lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe Tropheini. Mol Phylogenet Evol 55:318–334

La Mesa M, Ashford J (2008) Age and early life history of juvenile Scotia Sea icefish, *Chaenocephalus aceratus*, from Elephant and the South Shetland Islands. Polar Biol 31:221–228

La Mesa M, Eastman JT, Vacchi M (2004) The role of notothenioid fish in the food web of the Ross Sea shelf waters: a review. Polar Biol 27:321–338

Last PR, Balushkin AV, Hutchins JB (2002) *Halaphritis platycephala* (Notothenioidei: Bovichtidae): a new genus and species of temperate icefish from Southeastern Australia. Copeia 2002:433–440

Lautrédou A-C, Hinsinger DD, Gallut C et al (2012) Phylogenetic footprints of an Antarctic radiation: the Trematominae (Notothenioidei, Teleostei). Mol Phylogenet Evol 65:87–101

Lautrédou A-C, Motomura H, Gallut C et al (2013) New nuclear markers and exploration of the relationships among Serraniformes (Acanthomorpha, Teleostei): the importance of working at multiple scales. Mol Phylogenet Evol 67:140–155

Lewis DB (1976) Studies of the biology of the lesser weever. J Fish Biol 8:127–138

Loeb VJ, Kellermann AK, Koubbi P, North AW, White MG (1993) Antarctic larval fish assemblages: a review. Bull Mar Sci 53:416–449

Long DJ (1992) Sharks from the La Meseta Formation (Eocene), Seymour Island, Antarctic Peninsula. J Vertebr Paleontol 12:11–32

Matallanas J (2008) Description of *Gosztonyia antarctica*, a new genus and species of Zoarcidae (Teleostei: Perciformes) from the Antarctic Ocean. Polar Biol 32:15–19

Matschiner M, Hanel R, Salzburger W (2009) Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. Mol Ecol 18:2574–2587

Matschiner M, Hanel R, Salzburger W (2011) On the origin and trigger of the notothenioid adaptive radiation. PLoS One 6:e18911

Muschick M, Indermaur A, Salzburger W (2012) Convergent evolution within an adaptive radiation of cichlid fishes. Curr Biol 22:2362–2368

Naish TR, Woolfe KJ, Barrett PJ et al (2001) Orbitally induced oscillations in the East Antarctic ice sheet at the Oligocene/Miocene boundary. Nature 413:719–723

Near TJ (2004) Estimating divergence times of notothenioid fishes using a fossil-calibrated molecular clock. Antarct Sci 16:37–44

Near TJ, Cheng C-HC (2008) Phylogenetics of notothenioid fishes (Teleostei: Acanthomorpha): inferences from mitochondrial and nuclear gene sequences. Mol Phylogenet Evol 47:1–9

Near TJ, Pesavento JJ, Cheng C-HC (2004) Phylogenetic investigations of Antarctic notothenioid fishes (Perciformes: Notothenioidei) using complete gene sequences of the mitochondrial encoded 16S rRNA. Mol Phylogenet Evol 32:881–891

Near TJ, Parker SK, Detrich HW III (2006) A genomic fossil reveals key steps in hemoglobin loss by the Antarctic icefishes. Mol Biol Evol 23:2008–2016

Near TJ, Dornburg A, Kuhn KL et al (2012) Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proc Natl Acad Sci U S A 109:3434–3439

Nelson JS (2006) Fishes of the world. John Wiley, Hoboken, NJ

Nong GT, Najjar RG, Seidov D, Peterson WH (2000) Simulation of ocean temperature change due to the opening of Drake Passage. Geophys Res Lett 27:2689–2692

Nosil P (2012) Ecological speciation. Oxford University Press, New York, NY

Ojeda FP, Labra FA, Muñoz AA (2000) Biogeographic patterns of Chilean littoral fishes. Revista Chilena de Historia Natural 73:625–641

Olney MP, Bohaty SM, Harwood DM (2009) *Creania lacyae* gen. nov et sp. nov and *Synedropsis cheethamii* sp. nov., fossil indicators of Antarctic sea ice? Diatom Res 24:357–375

Pequeño RG (2000) Peces del crucero Cimar-Fiordo 3, a los canales del sur de Magallanes (ca. 55°S), Chile. Ciencia y Tecnología del Mar 23:83–94

Petit JR, Jouzel J, Barkov NI et al (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399:429–436

Place SP, Hofmann GE (2005) Constitutive expression of a stress-inducible heat shock protein gene, hsp70, in phylogenetically distant Antarctic fish. Polar Biol 28:261–267

Place SP, Zippay ML, Hofmann GE (2004) Constitutive roles for inducible genes: evidence for the alteration in expression of the inducible hsp70 gene in Antarctic notothenioid fishes. Am J Physiol-Reg I 287:R429–R436

Præbel K, Hunt B, Hunt LH, DeVries AL (2009) The presence and quantification of splenic ice in the McMurdo Sound Notothenioid fish, *Pagothenia borchgrevinki* (Boulenger, 1902). Comp Biochem Physiol A 154:564–569

Rogers AD (2007) Evolution and biodiversity of Antarctic organisms: a molecular perspective. Philos Trans R Soc B 362:2191–2214

Rogers AD, Morley S, Fitzcharles E, Jarvis K, Belchier M (2006) Genetic structure of Patagonian toothfish (*Dissostichus eleginoides*) populations on the Patagonian Shelf and Atlantic and western Indian Ocean Sectors of the Southern Ocean. Mar Biol 149:915–924

Rutschmann S, Matschiner M, Damerau M et al (2011) Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Mol Ecol 20:4707–4721

Ruud JT (1954) Vertebrates without erythrocytes and blood pigment. Nature 173:848–850

Salzburger W, Meyer A (2004) The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. Naturwissenschaften 91:277–290

Salzburger W, Van Bocxlaer B, Cohen AS (2014) Ecology and evolution of the African Great Lakes and their faunas. Annu Rev Ecol Evol Syst 45:519–545

Scher HD, Martin EE (2006) Timing and climatic consequences of the opening of Drake Passage. Science 428:428–430

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, New York, NY

Shaw PW, Arkhipkin AI, Al-Khairulla H (2004) Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. Mol Ecol 13:3293–3303

Shevenell AE, Kennett JP, Lea DW (2004) Middle Miocene Southern Ocean cooling and Antarctic cryosphere expansion. Science 305:1766–1770

Sidell BD, O'Brien K (2006) When bad things happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes. J Exp Biol 209:1791–1802

Smith P, Gaffney PM (2000) Toothfish stock structure revealed with DNA methods. Water Atmos 8:17–18

Smith PJ, Gaffney PM (2005) Low genetic diversity in the Antarctic toothfish (*Dissostichus mawsoni*) observed with mitochondrial and intron DNA markers. CCAMLR Sci 12:43–51

Stankovic A, Spalik K, Kamler E, Borsuk P, Weglenski P (2002) Recent origin of sub-Antarctic notothenioids. Polar Biol 25:203–205

Stein DL (2012) Snailfishes (Family Liparidae) of the Ross Sea, Antarctica, and closely adjacent waters. Zootaxa 3285:1–120

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–109

Team CRS (2000) Studies from the Cape Roberts project, Ross Sea, Antarctica. Initial report on CRP-3. Terra Antarctica 7:1–209

Thatje S, Hillenbrand C-D, Larter R (2005) On the origin of Antarctic marine benthic community structure. Trends Ecol Evol 20:534–540

Tomczak M, Godfrey JS (2003) Regional oceanography: an introduction. Daya Publishing House, Delhi

Volckaert FAM, Rock J, Putte AP (2012) Connectivity and molecular ecology of Antarctic fishes. In: di Prisco G, Verde C (eds) Adaptation and evolution in marine environments, vol 1. Springer, Berlin, pp 75–96

von der Heyden BP, Roychoudhury AN, Mtshali TN, Tyliszczak T, Myneni SCB (2012) Chemically and geographically distinct solid-phase iron pools in the Southern Ocean. Science 338:1199–1201

Wilson LAB, Colombo M, Hanel R, Salzburger W, Sánchez-Villagra MR (2013) Ecomorphological disparity in an adaptive radiation: opercular bone shape and stable isotopes in Antarctic icefishes. Ecol Evol 3:3166–3182

Yoder JB, Clancey E, Des Roches S et al (2010) Ecological opportunity and the origin of adaptive radiations. J Evol Biol 23:1581–1596

Zachos JC, Quinn TM, Salamy KA (1996) High resolution ($10^4$ years) deep-sea foraminiferal stable isotope records of the Eocene-Oligocene climate transition. Paleoceanography 11:251–266

Zane L, Bargelloni L, Bortolotto E et al (2006) Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. Mol Ecol 15:4499–4511

Zhuang X (2013) Creating sense from non-sense DNA: de novo genesis and evolutionary history of antifreeze glycoprotein gene in northern cod fishes (Gadidae). Dissertation, School of Integrative Biology, University of Illinois at Urbana-Champaign

# Desert Environments

**Stanley D. Hillyard, Jason E. Podrabsky, and Frank van Breukelen**

**Abstract** Desert fishes may seem paradoxical, but there is an extensive and diverse ichthyofauna associated with deserts around the globe. Examining the distribution and biology of extant species allows the generation of hypotheses that address questions about how desert fishes evolved and persist in what appear to be inhospitable environments. Desert fishes are usually hardy fish that persisted through the desertification process. Patterns of dispersal and vicariance can vary between sympatric taxa suggesting differential exploitation of stochastic events. For instance, one fish species may have used a dispersal event to occupy a new region whereas another species did not. Oftentimes, stepwise patterns of dispersal and vicariance occur that correlate with profound geomorphological changes. Fish that evolve rapidly radiate into distinct taxa whereas fish that more robustly avail themselves of dispersal opportunities persist with wider distributions. The accelerated and profound alterations of anthropogenic influences on dispersal, vicariance, and environmental change for desert fishes represent severe threats to their continued survival.

## 1 Introduction

> Any division of the earth's surface into zoogeographical regions which starts exclusively from the present distribution of animals without considering its origin must be unsatisfactory (Ortmann 1902).

---

S.D. Hillyard
School of Dental Medicine, University of Nevada, Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154, USA

J.E. Podrabsky
Department of Biology, Portland State University, 1719 SW 10th Avenue, SRTC rm 246, Portland, OR 97201, USA

F. van Breukelen (✉)
School of Life Sciences, University of Nevada, Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154, USA
e-mail: frank.vanbreukelen@unlv.edu

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_4

Deserts generally receive less than 250 mm of precipitation per year, and finding fish in a desert may seem paradoxical. However, numerous fish species are found in deserts around the world. Oftentimes, the waters these fish inhabit are seemingly inhospitable. This leads to the questions of how fish colonized those habitats in the first place, and how they manage to survive. An important aspect in understanding extant desert fishes is an understanding of their evolutionary history and ecology. Although we are frequently biased by our current observations, note should be taken that many desert habitats were recently much wetter than they are today and, importantly, have an evolving topography and drainage patterns. Fossil remains reveal the widespread distribution of fish in what are now deserts (Smith et al. 2002; Lévêque 1990). Drainage patterns are modified by tectonic activity and continue to affect current fish distributions (Spencer 2008). The entire Southwest of the United States of America was dotted with largely interconnected streams and lakes until pluvial waters receded about 10,000 years ago, at the end of the Pleistocene (Echelle 2008; Lowenstein 2002; Roskowski et al. 2010). The Mojave Desert in southwestern North America was named for a local Native American word, “Hamakhaave,” which means “by the water” (http://www.bigorrin.org/mojave_kids.htm). One only needs to look at the tremendous shift in human populations as civilizations left Africa for Europe and beyond to understand that the Sahara Desert *recently* supported much more life than it does today (Armitage et al. 2007; Drake et al. 2011). A look back at the Sahara’s hydrological history reveals a land that was characterized by interconnected streams and lakes as recently as 10,000 years ago. Another unifying idea behind these deserts (or perhaps better “under these deserts”) is the availability of aquifers that continue to feed spring-fed oases where fish populations persist. Indeed, the largest known aquifer underlies the Sahara Desert belying its characterization as a dry environment.

The cyclical nature of water availability as a function of an ever-changing climate and geology punctuated opportunities for dispersal of fishes. Wet climates created connections between water sources that allowed dispersal. Dry climates allowed water sources to become isolated as interconnecting water receded and created vicariance or barriers to dispersal. Similarly, tectonic movements may alter the availability of barriers. Hubbs and Miller (1948) recognized these processes and utilized the distribution and taxonomic characteristics of desert fishes to infer patterns of dispersal and vicariance. These authors attempted to relate their observations with those of geologists who proposed models for the creation of large lakes based on mineral deposits (termed lacustrine) and evidence of flow (fluvial) patterns between the lakes (Smith et al. 2002). More recently, molecular techniques such as sequencing of mitochondrial (mtDNA) and nuclear DNA and application of mutation rates estimates have created biological clocks to estimate dates of divergence that could arise from vicariance (Echelle 2008). Further, geologists have used stable isotope ratios to infer climatic conditions and patterns of water flow (Roskowski et al. 2010) that would permit or restrict dispersal. The challenge has been to correlate the chronology of species divergence with the geomorphology to better understand the mechanisms driving the evolution of desert fishes (Smith et al. 2002; Spencer et al. 2013). We should note that much of these data and their interpretations are not set in stone. For instance, the historical interpretation of the Bouse Embayment as an extension of the Gulf of California versus a more modern view as an outflow of the Colorado River has tremendous implications in the interpretation of biogeography.

As might be expected, the survival of a fish in the face of climate change and vicariance often means having to survive in very different habitats. In particular, desertification may result in warm and/or highly saline waters (see chapter "Hypersaline Environments"). For instance, pupfish in Cottonball Marsh in Death Valley may experience salinities above the 96 hr-$LC_{50}$ of ~100 ppt (sea water is ~35 ppt) reported by Naiman et al. (1976). Where desertification has been rapid, e.g., in the Sahara Desert and Southwest North America, little time has been available for adaptation. Most species present in deserts were likely preadapted for the harsh environments, that is, they had already evolved adaptations to tolerate extremes of temperature and salinity. Conversely, other species persist in rivers and streams that flow into or across deserts, where adaption to variable stream flow is a prerequisite to survival (see also chapter "Temporary Environments"). The ever-changing habitats often impose novel selection pressures on physiological functions of the fish at some or all life stages.

In this chapter, we illustrate mechanisms for the evolution of desert fishes and discuss how adaptation of changing ecological conditions established the extant species of today. We have reviewed the literature on desert fishes of the world to reveal some interesting biogeographic patterns. However, this process has also made it clear that our knowledge of desert fishes and their ecology is limited for many deserts, especially those in Asia. Thus, we have chosen to use exemplary accounts that help illustrate how fishes evolved for life in the desert.

## 2 Desert Aquatic Habitats

Desert aquatic habitats are diverse (Fig. 1). On one extreme, there are large, permanent rivers such as the Colorado River that originate in wetter regions and flow through deserts, some of them disappearing into sands. Seasonal or ephemeral rivers and their associated floodplain pools are common in many areas such as the wadis, gueltas, and pools of Arabia and the Sahara. Permanent lakes are also present in some deserts, although they are often very salty, and sometimes far too saline to support permanent populations of fish (see chapter "Hypersaline Environments"). Springs and oases are critical permanent or semi-permanent sources of water in deserts supporting "islands" of biodiversity, both terrestrial and aquatic. As we will discuss below (4.5 to 4.7), many desert habitats are highly variable environments that fluctuate daily and seasonally. However, many other desert environments, especially springs, are quite stable environments that offer very little daily or seasonal variations. The high diversity of isolated aquatic habitats has very likely played a large role in the evolution of desert fishes.

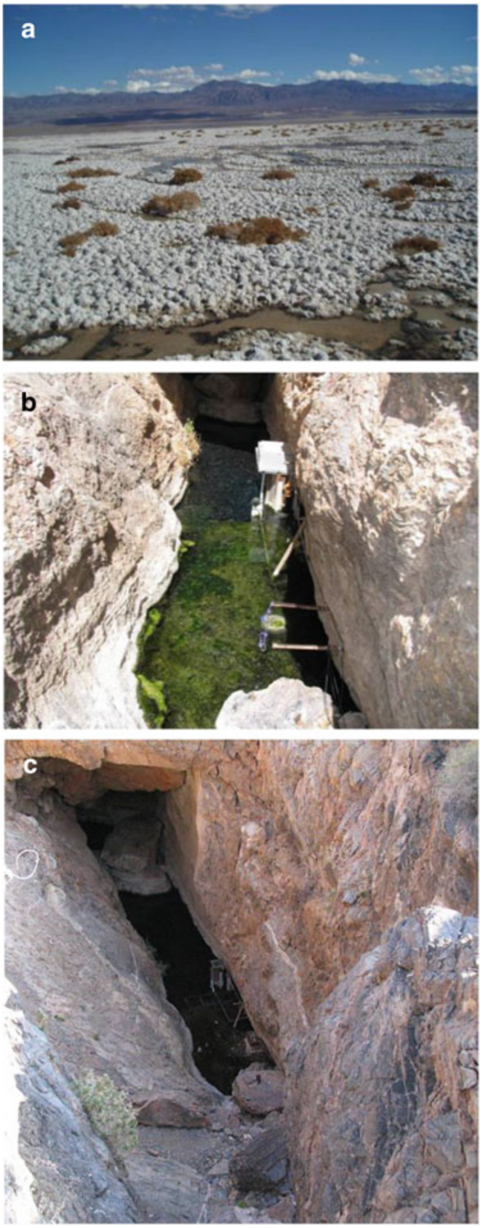


**Fig. 1** Examples of desert pupfish habitats. Cottonball Marsh (**a**) in Death Valley is an extremely variable and harsh environment, while Devils Hole (**b**, **c**) is a rather stable but isolated environment

## 3 Fishes of the Major World Deserts

A survey of the literature reveals that around 460 species or subspecies of fish from 51 families inhabit desert waters (Fig. 2; Table 1). However, most of the diversity exists in a small number of families. Over 43 % of the species belong to the family Cyprinidae, 10 % are from the family Cyprinodontidae, and all of the other families each represent less than 4 % of desert species. It is important to note that in some deserts, such as the North American deserts, much of the diversity can be found in isolated springs, while in others such as the Thar of India, much of the diversity that has been described is found in rivers passing through the desert. A quick glance at the distribution of fish families that occur in deserts across the globe (Fig. 2) clearly

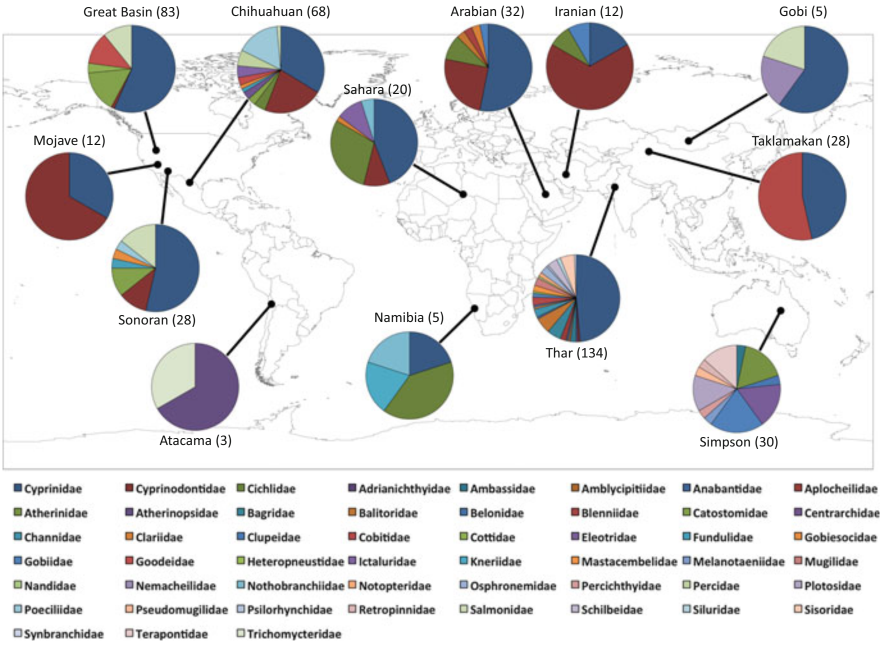


**Fig. 2** Distribution of fish Families found in major deserts of the world. Each desert is composed of a unique assembly of taxa that is related to the general distribution of fishes in the area. This pattern supports evolution of extant desert species through vicariance, rather than a suite of specific adaptations that allow particular groups of fish to invade desert habitats. *Numbers* in *parentheses* indicate the number of species or subspecies. Assignments of taxa to each desert were taken from the literature. Arabian Peninsula: Ageili et al. (2013), Al-Daham et al. (1977), Al-Ghamdi and Abu-Zinadah (1998), Alkahem et al. (1990), Borkenhagen and Krupp (2013), Feulner (1998), Hamidan (2004) and Reichenbacher et al. (2009). Simpson Desert: Unmack (2001). Iranian Desert: Coad (1982) and Esmaeili et al. (2012). Sahara: Kara (2012), Lévêque (1990) and Trape 2009. Namibia: Curtis et al. (1998) and Skelton (1990). Gobi: Ocock et al. (2006). Atacama: North American Deserts: Bernardi et al. (2007), Olden et al. (2008) and Williams et al. (1985). Taklamakan: Yuan (1988), Wang et al. (2010) and Wu and Tan (1991). Thar: Yazdani (1996)

**Table 1** Families of fishes found in major deserts of the world and the number of taxa found in each family on each continent

| Order | Family | Environment | | | N. America | Africa | Arabia/Iran | Australia | Asia | S. America |
|---|---|---|---|---|---|---|---|---|---|---|
| *Marine* | | F[a] | B[b] | M[c] | | | | | | |
| Atheriniformes | Atherinidae | X | X | X | | | | 5 | | |
| Clupeiformes | Clupeidae | X | X | X | | | | 1 | 1 | |
| Cyprinodontiformes | Belonidae | X | X | X | | | | | 1 | |
| Gobiesociformes | Gobiesocidae | X | X | X | 2 | | | | | |
| Perciformes | Ambassidae | X | X | X | | | | 1 | 3 | |
| Perciformes | Blenniidae | X | X | X | | | 1 | | | |
| Perciformes | Eleotridae | X | X | X | | | | 5 | | |
| Perciformes | Gobiidae | X | X | X | | | 2 | 6 | 2 | |
| Perciformes | Mugilidae | X | X | X | | | | | 4 | |
| Perciformes | Terapontidae | X | X | X | | | | 4 | | |
| Siluriformes | Plotosidae | X | X | X | | | | 4 | | |
| *Peripheral* | | | | | | | | | | |
| Atheriniformes | Atherinopsidae | X | X | X | | | | | | 2 |
| Osmeriformes | Retropinnidae | X | X | X | | | | 1 | | |
| Perciformes | Percichthyidae | X | X | | | | | 1 | | |
| Salmoniformes | Salmonidae | X | X | X | 14 | | | | 1 | |
| Scorpaeniformes | Cottidae | X | X | X | 3 | | | | | |
| Synbranchiformes | Synbranchidae | X | X | | | | | | 1 | |
| *Secondary freshwater* | | | | | | | | | | |
| Atheriniformes | Melanotaeniidae | X | X | | | | | 1 | | |
| Atheriniformes | Pseudomugilidae | X | X | | | | | 1 | | |
| Beloniformes | Adrianichthyidae | X | X | | | | | | 1 | |
| Cyprinodontiformes | Aplocheilidae | X | X | | | | | | 3 | |
| Cyprinodontiformes | Cyprinodontidae | X | X | X | 27 | 2 | 16 | | 1 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cyprinodontiformes | Fundulidae | X | X | X | 2 | | | | | |
| Cyprinodontiformes | Goodeidae | X | X | | 12 | | | | | |
| Cyprinodontiformes | Poeciliidae | X | X | | 12 | | | | | |
| Perciformes | Cichlidae | X | X | | 3 | 8 | 4 | | | |
| *Primary freshwater* | | | | | | | | | | |
| Cypriniformes | Balitoridae | X | | | | | 1 | | 8 | |
| Cypriniformes | Catostomidae | X | | | 18 | | | | | |
| Cypriniformes | Cobitidae | X | | | | | | | 19 | |
| Cypriniformes | Cyprinidae | X | X | | 89 | 10 | 19 | | 81 | |
| Cypriniformes | Nemacheilidae | X | X | | | | | | 1 | |
| Cypriniformes | Psilorhynchidae | X | | | | | | | 1 | |
| Cyprinodontiformes | Nothobranchiidae | X | | | | 2 | | | | |
| Gonorynchiformes | Kneriidae | X | | | | 1 | | | | |
| Osteoglossiformes | Notopteridae | X | X | | | | | | 2 | |
| Perciformes | Anabantidae | X | X | | | | | | 1 | |
| Perciformes | Centrarchidae | X | | | 2 | | | | | |
| Perciformes | Channidae | X | | | | | | | 4 | |
| Perciformes | Nandidae | X | X | | | | | | 1 | |
| Perciformes | Osphronemidae | X | | | | | | | 4 | |
| Perciformes | Percidae | X | X | | 4 | | | | | |
| Siluriformes | Amblycipitidae | X | | | | | | | 1 | |
| Siluriformes | Bagridae | X | X | | | | | | 7 | |
| Siluriformes | Clariidae | X | | | | 2 | 1 | | 1 | |
| Siluriformes | Heteropneustidae | X | | | | | | | 1 | |
| Siluriformes | Ictaluridae | X | | | 3 | | | | | |
| Siluriformes | Schilbeidae | X | | | | | | | 5 | |
| Siluriformes | Siluridae | X | | | | | | | 2 | |

(continued)

**Table 1** (continued)

| Order | Family | Environment | | | N. America | Africa | Arabia/Iran | Australia | Asia | S. America |
|---|---|---|---|---|---|---|---|---|---|---|
| Siluriformes | Sisoridae | X | | | | | | | 7 | |
| Siluriformes | Trichomycteridae | X | | | | | | | | 1 |
| Synbranchiformes | Mastacembelidae | X | | | | | | | 3 | |

The following designations refer to members of each family in respect to where genera and species may be found. References for taxa locations are presented in the legend of Fig. 2

[a] *F* freshwater

[b] *B* brackish water

[c] *M* marine

illustrates that each desert is home to a unique assembly of fishes that are more related to those from the surrounding regions than they are to each other. This pattern suggests evolution of desert fishes through the isolation and adaptation of locally derived species, rather than the invasion of fishes with superior adaptive abilities specifically suited to desert habitats.

## 4 Evolution of Desert Pupfishes

Perhaps the best-studied desert fishes are the pupfishes (*Cyprinodon* species) of the Southwest United States of America and Northern Mexico. The term pupfish was coined by Hubbs and Miller (1948) because of their behavioral pattern of rapid movements punctuated by periods of inactivity that resembled puppies at play. These killifishes (order Cyprinodontiformes, family Cyprinodontidae) represent a large group of secondary freshwater fishes (i.e., fishes normally occurring in inland aquatic systems, but being tolerant of brackish waters and capable of occasionally crossing narrow sea barriers). Pupfishes have a long evolutionary history of dispersal and vicariance that provides insights as to their success in desert habitats. Geological records, fossil evidence, and phylogenetic comparisons of cyprinodontids suggest their origins in the shallow seas and estuarine environments of the west Tethys Ocean (Parker and Kornfield 1995). With the breakup of the North American and Eurasian land masses some 80 million years ago (mya), one cyprinodontid lineage remained in what was to become the Mediterranean/Middle Eastern region, while another moved with the North American land mass.

Desert pupfishes in North America are derived from a common ancestor of what is now *Cyprinodon variegatus*. This species occupies coastal and estuarine habitats of the eastern and gulf coast of North America (Riggs and Deacon 2002). These fish are extremely tolerant of temperature and salinity extremes and have been called “the toughest fish on earth” (Haney and Nordlie 1997). Based on the current species distributions and sequence divergence in mitochondrial DNA, pupfish from the Gulf of Mexico are proposed to have migrated up the Rio Grande 6.3–5.4 mya and followed its tributaries into the Río Conchos and Guzman basins (Echelle 2008; Fig. 3). Climatic and tectonic changes allowed migration through water courses to the Colorado River. From there, fish moved into the Death Valley System an estimated 3.8–3.6 mya.

There remains controversy regarding the route or routes used. Inferences from geomorphic and phylogenetic data are often contradictory (Smith et al. 2002) and change with the acquisition of new information. For example, it was previously believed that the Gulf of California extended northward as the Bouse Embayment. This saltwater marsh had potential connections that allowed pupfishes from the lower Colorado River to disperse into the Death Valley System. Despite the presence of closely related pupfishes in Death Valley and the lower Colorado River, Brown and Rosen (1995) could not find any geomorphic evidence that such a connection occurred during the late Miocene to early Pleistocene.

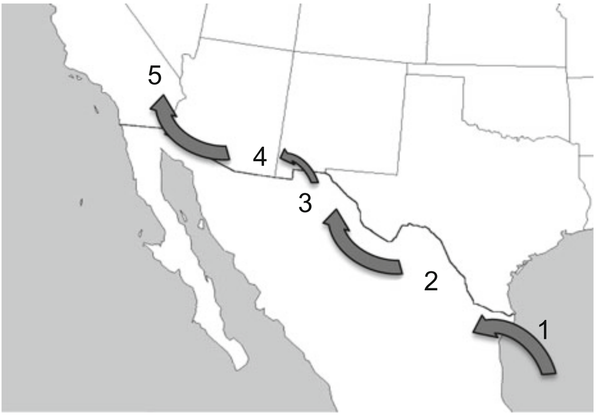


**Fig. 3** Proposed route of invasion of Southwest North America by pupfish based on relict populations combined with molecular dating estimates. The invasion likely occurred in a stepwise fashion. Initial invasion of Rio Grande river system by the ancestor of *C. variegatus* occurred ~6.3–5.4 mya (denoted by *arrow* 1). The fish presumably used this water system to invade the Rio Conchos (2 on map) and Guzman Basin (3) drainages. From there, additional invasions of the Quitobaquito (4) and Death Valley systems are thought to have occurred ~3.8–3.6 mya

They suggested that fish must have dispersed into Death Valley prior to 4 mya in several stages or events. This possibility was addressed by studies that examined ratios of stable isotopes of Strontium ($^{sr}87/^{sr}86$) to indicate that the Bouse Formation was, in fact, the result of massive flow of freshwater from the upper Colorado River forming extensive freshwater lakes beginning ~4.9 mya (Spencer et al. 2013). These lakes were as deep as 500 m and are thought to have sequentially overflowed as water accumulated upstream. Evaporation resulted in salt concentrations comparable to seawater giving the impression that the water source was marine rather than freshwater. The lower Colorado River is proposed to have reached the Gulf of California about 5 mya (Spencer et al. 2013). It remains to be elucidated exactly what route or routes pupfish may have taken as these water sources became connected to the Death Valley System and how species within the system may have become separated and rejoined as the result of climatic and geological events. Nevertheless, potential scenarios suggested from the analysis of current pupfish habitats and proposed phylogenies (Echelle 2008) are instructive for understanding the distribution of fishes in desert habitats.

The proposed route for pupfish migration from the Rio Grande Drainage to the Death Valley System may be retraced by examining extant species. These species survive as mostly relict populations in isolated springs as a result of desertification and recession of pluvial waters. Mitochondrial DNA sequence divergence between populations of *C. macularius* from the Salton Sea and Colorado River Delta indicates some isolation despite periods in recorded history of flood events that should have allowed mixing (Echelle et al. 2000). To the east, pupfish in Quitobaquito springs in Organ Pipe National Monument in southern Arizona and the associated Rio Sonoyta are thought to be separated from the Salton

Sea/Colorado River Delta populations for approximately 100,000 years. The current interpretation is that these populations warrant classification as distinct species (e.g., *C. eremus* was previously classified as *C. macularius eremus*). Further east, several pupfish species have been identified in the Guzman Basin related drainages of Northern Mexico, including *C. fontinalis*. Obfuscating our understanding is that mtDNA analyses of *C. fontinalis* suggest a closer relationship to species living in the Amargosa Valley (adjacent valley to the east of Death Valley) than to either *C. macularius* (Salton Sea area) or *C. radiosus* (Owens River drainage to the west of Death Valley). This result is consistent with multiple dispersal/vicariance events, one connecting the Owens River with the Colorado River and another connecting the Colorado River to the Death Valley/Amargosa River/Ash Meadows areas (Echelle 2008). Further to the east of Guzman Basin, the Río Conchos drainage flows from the Sierra Madre Occidental in México to the Rio Grande, passing through the Chihuahuan Desert. Warm springs at Baños de San Diego and Julimes along the course of the Río Conchos provide habitats for pupfish. At the southern edge of the Chihuahuan Desert lies Cuatro Cienegas, a system of springs with two recognized endemic pupfish species (*Cyprinodon bifasciatus* and *Cyprinodon atrorus*; Tobler and Carson 2010). One might readily surmise then a stepwise invasion of the west by the ancestors of the extant desert pupfishes tempered by some mixing of species or populations.

The Death Valley System consists of the Lower Colorado River and Salton Sea drainage to the south, the Owens River to the northwest, and the Death Valley–Amargosa River–Ash Meadows region to the east (Smith et al. 2002). While most of us are familiar with the dry conditions of Death Valley, it may be surprising to learn that Death Valley was recently inundated with a large lake. During the late Pleistocene (12,000–10,000 years ago), Lake Manley covered Death Valley with 100 m of water. Even 2,000 years ago, there was some 10 m of water. Moreover, much of what is now the Mojave Desert was covered by various lakes and interconnecting streams (Stoffer 2004). The time course and duration of these water connections were inferred from geological data. Importantly, extant geological data do not usually include stochastic events such as flooding. Therefore, interpretations that a given population was isolated may be subject to future revision, as we begin to reconcile these stochastic events. Indeed as we will see later, some desert fishes have been noted to swim considerable distances in shallow waters.

Similarly, the use of biological clock data to infer phylogenetic relationships and their timing may also be subject to future revision. A *lineage* is an evolutionary sequence from an ancestral species through the intermediate species and finally to a particular descendent species. *Incomplete lineage sorting* may complicate these phylogenetic interpretations when one population maintains more similarity to a more distantly related population than to a more closely related population in a subset of characteristics. In addition, *hybridization* may occur when separated populations are reconnected (e.g., Carson et al. 2012). The impression then might be that the population is polyphyletic. Importantly too, mutation rates are greater when warmer temperatures drive an increase in tissue metabolism and when increased temperatures shorten generation times (Martin and Palumbi 1993; Gillooly et al. 2001). Given these caveats, Echelle (2008) suggested that inferred phylogenies should be seen “as approximations” that “remain as crude estimates.” Nonetheless, these analyses allow testable hypotheses to be formulated that improve our understanding of the origins of these fish.

A review of the extant Death Valley System pupfishes may illustrate these points. Mitochondrial DNA studies indicate *C. radiosus* to be distinct from other species in the Death Valley, having diverged from *C. macularius* about 3.8–2.7 mya (Echelle et al. 2005; Echelle 2008). We note that allozyme data suggest a very different and more recent speciation process (see Riggs and Deacon 2002). Any divergence requires a historical connection between the Owens River and Colorado River drainages and separation from Lake Manley. This connection, while supported by phylogenetic data, is not supported by the available geomorphic data and requires assumptions about patterns of dispersal and vicariance. Similarly, *C. salinus* in the northern part of Death Valley is estimated to have diverged from *C. nevadensis* from Saratoga Springs in the southern part of Death Valley over 1 mya based on mtDNA divergences, despite the availability of Lake Manley as recently as a few thousand years ago. Another example is that of the Devils Hole pupfish, *C. diabolis*. Devils Hole is essentially a pool of an underground aquifer that opened to the surface when the overlying structure collapsed some 65 kya (Riggs and Deacon 2002). However, mtDNA analyses suggest divergence from *C. nevadensis* as long as 500 kya (Echelle et al. 2005; Echelle 2008). Sympatric speciation events and lineage sorting could readily have contributed to the apparent genetic divergence between Saratoga Springs and Salt Creek fish. In fact, Strecker (2006) demonstrated complex sympatric speciation in *Cyprinodon* fishes in Laguna Chichancanab. However, such concepts are less viable in the context of Devils Hole, where the biological clock data suggest a point of divergence much earlier than what the geomorphological data would support. Our personal bias on why biological clocks and geomorphological data are often at odds is that there are accelerated evolutionary processes. However, additional data will be needed before any such conclusion may be made.

## 5 Thermal Ecology of Desert Pupfishes

Much attention has been paid to how desert pupfishes cope with what are considered extreme temperatures. Certainly, some species like *C. julimes* or *C. pachycephalus* experience extreme temperatures (Montejano and Absalon 2009). These species live in hot springs associated with the Río Conchos system of Mexico. *Cyprinodon julimes* lives in an arsenic-laden habitat where the coolest water is 38 °C, but has been observed swimming for significant periods in water that is 44 °C and occasionally through water as hot as 46 °C. Similarly, another related member of the *C. eximius* group, *C. atrorus*, has been noted in 47.2 °C water

(Deacon and Minckley 1974). Little is known of the ecology and ecophysiology of these fishes.

Assumptions have been made of Death Valley System pupfishes of extreme thermal tolerance that may be premature. Importantly, one must note that salt inclusions indicate that temperatures of Lake Manley were 4–15 °C cooler than Death Valley waters are today, i.e., below 20 °C (Lowenstein 2002). Thus, much of the recent evolutionary history of Death Valley System fishes may have been spent in cool water. Only after pluvial waters receded in the past 10 kya have pupfish been isolated to the warm springs that typify their current habitats.

Physiologically, temperature extremes are considered to be important stressors that contribute to natural selection (Brown and Feldmeth 1971). This finding is not surprising when one considers that a typical $Q_{10}$ value (fold difference in biological rates for every 10 °C change in body temperature) is 2–3 for most biological processes, including oxidative metabolism. Therefore, an increase of just 1 °C is predicted to result in 20–30 % greater energetic demand. Physiological responses of desert pupfishes to thermal stress have been mostly limited to the determination of critical thermal maxima, quantified by exposing fish from different habitats and acclimation groups to acute increases in ambient temperature, typically 0.2–0.3 °C/h, until they lose equilibrium and can no longer swim normally (Otto and Gerking 1973; Feldmeth et al. 1974; Shrode and Gerking 1977; Stuenkel and Hillyard 1981). Brown and Feldmeth (1971) measured upper lethal thermal tolerance limits (measured as cessation of opercular ventilation) for six species of *Cyprinodon* from the Death Valley System. Species inhabiting thermally constant springs and thermally variable streams were found to have similar thermal tolerances (Feldmeth et al. 1974). In all cases, loss of equilibrium or cessation of ventilation occurred at temperatures ~40 °C, only slightly above the temperatures that may be normally encountered in native habitats; e.g., the breeding shelf in Devils Hole may reach 39 °C during the summer (Riggs and Deacon 2002). Unfortunately, little is known about the effects of chronic thermal stress on pupfish although our personal experiences with Ash Meadows species indicate that just 3 weeks of exposure to 36 °C may be very stressful and results in cessation of reproduction.

Importantly, reproductive capacity and recruitment of young into the population are more sensitive to temperature extremes. The number of breeding attempts and eggs per spawning by *C. n. nevadensis* bred in the laboratory peaks at approximately 30 °C, but falls precipitously at temperatures above 32 °C (Shrode and Gerking 1977). Oogenesis and hatching begin to decline at temperatures above 30 °C and are essentially zero by 36 °C. Furthermore, Gustafson and Deacon (1998) indicated that successful breeding in early spring in Devils Hole only occurs on the shelf (and not in the 33–34 °C main pool), and that while 50 % of larvae are found where temperatures are more moderate, only 13 % of larvae are found in areas with warmer water. These data suggest that both eggs and adults of Death Valley System fishes experience chronic stress at temperatures that may be encountered in their native habitats. Even long-term acclimation to temperature does not rescue the poor reproductive performance at higher temperatures (Gerking et al. 1979).

## 6 Oxygen Ecology of Desert Pupfishes

In addition to thermal stress, solubility of oxygen in warm water is low (see chapter "Low-oxygen Lifestyles") and photosynthesis, especially in Devils Hole, is seasonally limited and produces highly variable degrees of oxygen saturation that can range from near saturation to marked hypoxia (Gustafson and Deacon 1998). Indeed, small aquatic systems in general may approach anoxia at night as algal blooms respire. The few data on the effects of hypoxia in pupfish used acute exposures (e.g., Hirschfield et al. 1980). Our preliminary data indicate a critical $PO_2$ of approximately 62 Torr (tension of $O_2$ where a marked decline in oxygen consumption is noted; Heuton et al. 2013) and $O_2$ tensions are typically ~51 Torr in Devils Hole (Gustafson and Deacon 1998). Given these data, one must then question the role for oxidative vs. anaerobic metabolism in these fishes. Can fish adequately supply oxygen demand when living below the critical $PO_2$?

## 7 Salinity Ecology of Desert Pupfishes

Some Death Valley System fishes have apparently retained their ancestor's ability to withstand high salt conditions (see chapter "Hypersaline Environments"). *Cyprinodon salinus* is found in Salt Creek that flows from McClain Springs in the northern part of Death Valley National Park (Hillyard 2011). Temperatures near the head spring are in the 15–30 °C range, but salinity is approximately 15 ppt. From the head spring, water flows about 3 km and ultimately disappears into the desert floor. During the summer months, flow is greatly reduced, air temperatures may approach 50 °C, and water temperatures in downstream pools may reach 40 °C. Not surprisingly, salts are concentrated as the result of evaporation. It remains to be seen whether fish exposed to these high temperatures and salinity actually survive and reproduce.

A subspecies, *C. s. milleri*, occurs in Cottonball Marsh fed by a series of spring pools and likely fed by Salt Creek during local floods. The etymology of Cottonball Marsh denotes the white crusts of salt that are formed when water evaporates. Salinity has been reported to be as low as 10 ppt in the winter and as high as 160 ppt in the summer (LaBounty and Deacon 1972; Naiman et al. 1973; Hunt et al. 1966). Air temperatures in excess of 55 °C—the highest ever recorded in North America—have been recorded at Furnace Creek, near Cottonball Marsh. Because of the remote nature of Cottonball Marsh, few records of water temperature exist. However, evaporation may result in cooler water temperatures. During the course of one day in August, water temperatures ranged from 23 to 36 °C (Naiman et al. 1973). Water temperatures were 9 °C in December (Otto and Gerkin 1973). Little is known of the ecology and ecophysiology of these fish.

## 8 Variation in Dispersal Patterns

The dispersal pattern of pupfish is not the only pattern seen in North American desert fishes. While many other desert fish taxa utilized connections to the lower Colorado River drainage, some also exploited connections from the upper Colorado River. *Rhinichthys osculus* (speckled dace) is the most abundant and widespread species of native fish in the Colorado River drainage (Smith and Dowling 2008). Phylogenies based on mtDNA dating, adjusted for fossil records and metabolic rates, indicate that this species diverged from sister taxa in the Columbia-Snake River drainages an estimated 6.3 mya and appeared in the Colorado River drainage about 3.6 mya. At that time, large lakes filled the Lahontan and Bonneville basins. Lake spillovers in addition to tectonic events that separate drainages have been proposed as mechanisms for molecular divergences. A stepwise vicariance leading to different taxa is evident. A separation of northern and southern haplotypes by the Grand Canyon can be dated to 1.34 mya. Further divergences occurred in the southern haplotypes within the Virgin River drainage some 105-45 kya. Divergences of lower Colorado River populations are accompanied by morphological changes related to the ability to swim in rapidly flowing water. Notably, the small population of *R. osculus* in Ash Meadows, perhaps the most extreme desert habitat where this species persists, is found in a rapidly flowing spring outlet that was recently restored to accommodate the historical habitat requirement. In contrast to *Cyprinodon* spp., populations of *R. osculus* are also found in streams of the Los Angeles basin that is currently separated from the lower Colorado River drainage by considerable mountain and desert barriers. This population is most closely related to the population in the Gila and Salt River drainages of Arizona with divergence times estimated over a broad range of time estimates, $1.90 \pm 1.18$ mya. An interesting question is why pupfish and dace populations are only partly sympatric. A pattern of vicariance and dispersal based both on serendipitous exploitation of stochastic and long-lived interconnections appears evident in both species. Temporal and spatial exploitation of connections are further complicating the patterns. The examples of pupfish and dace evolution may be models for the evolution of other desert fishes.

Even in riverine environments, the stochastic nature of a changing environment may influence distribution patterns, ecology, and evolution of desert fishes. Chubs in the genus *Gila* and suckers in the genus *Catostomus* are widespread with some relict populations persisting in desert habitats (Spencer et al. 2008). Analysis of rapidly evolving mitochondrial genes of flannelmouth suckers, *Catostomus latipinnis*, shows very low levels of genetic diversity within and divergence between populations. It was proposed that a severe drought sometime during the late Pleistocene/early Holocene (ca 24-10 kya) caused a genetic bottleneck in these fish (Douglas et al. 2003). Presumably the end of the drought restored water flow so the surviving individuals could reestablish much of their historical range. A similar scenario has been proposed for the razorback sucker, *Xyrauchen texanus* (Dowling et al. 2012).

## 9 Similarities of the Sahara Desert to the Mojave Desert

A look back at the Sahara Desert's hydrological history reveals a land that was characterized by interconnected streams and lakes as recently as 10,000 years ago (Armitage et al. 2007). Recent analyses of lacustrine sediments reveal the existence of large mega lakes that were filled by streams leading into what is now desert. Spillovers from these lakes and merging of stream outflows created a continuous pathway for dispersal of fishes across Northern Africa. This observation has created particular interest since it provides new insights regarding the migration of human populations into the "green Sahara" (Drake et al. 2011). Fossil fish remains from late Miocene deposits show affinities with those of Egypt and tropical regions to the south, indicating a broad distribution of freshwater fish from tropical Africa into what is now the Sahara Desert (Lévêque 1990). Isolation of extant fish populations is thought to have occurred with drying of the lakes and streams about 6-5 kya resulting in a relatively rich ichthyofauna. Relict fish populations include the cichlid *Tilapia zilli*, a member of a large genus of fish that are well known to be tolerant of high temperature and osmotic stress (see chapter "Hypersaline Environments"), and two clariid species, *Clarias anguillaris* and *C. gariepinus*, that are members of a genus of air-breathing catfish. Because of their hardiness and ability to rapidly reproduce, both *Tilapia* and *Clarias spp.* are used extensively for aquaculture. Tropical species also found in the Sahara are several species of the cyprinid genera *Barbus* and *Labeo* (the Nile Carp) (Lévêque 1990; Trape 2009), both tolerant of extreme temperatures and hypoxia (see chapter "Low-oxygen lifestyles").

Much of the North American deserts overlie very large aquifers deposited when water was more abundant. Similarly, the largest known aquifer underlies the Sahara Desert. The North-West Sahara Aquifer System often referred to as the Système Aquifère du Sahara Septentrional (SASS) is one of two major North African transboundary groundwater basins. As a result, the aquifer system spreads under large areas of Algeria, Tunisia, and Libya (Gonçalvès et al. 2013). Remarkably, there are multiple accounts of fish ejected in water from artesian wells at isolated water sources (Schroeder 1879; Heggen 2013). Connections between these water sources are suspected to exist just below the desert surface. Such interconnections could provide for additional avenues of dispersal.

Not all Saharan desert fishes are derived from tropical Africa. Ancestral stock of both North American *Cyprinodon* species and Mediterranean *Aphanius* species inhabited the west Tethys Sea. Upon the breakup of Pangea, these fishes were separated. The current distribution of *Aphanius* species ranges from the Iberian Peninsula to Iran including coastal and inland populations of the Sahara Desert (Hrbek and Meyer 2003). The distribution of *Aphanius* species involves complex geological and climatic events that include connection between the Mediterranean Sea and Atlantic Ocean through the Rifian Corridor in what is now Morocco and Algeria, ca 8 mya. This corridor was closed ca. 6 mya by tectonic activity that created the Atlas Mountains. Lack of connections to the ocean resulted in evaporation of the Mediterranean Sea to produce a greatly reduced volume and increased salinity an estimated 6 mya (Krijgsman et al. 1999). The opening of the Strait of Gibraltar ca. 5.3 mya allowed refilling of the Mediterranean basin and created a barrier for dispersal. This has provided opportunities for separate colonization events seen with *A. apodus* and historical records of *A. iberus* in the Atlas Mountains (Hrbek and Meyer 2003; Blanco et al. 2006). On the Iberian Peninsula molecular evidence separates *A. iberus* in Mediterranean drainages from *A. baeticus* in Atlantic drainages. The most isolated pupfish, *A. saourensis*, was recently described from an oasis system in the Saouro Valley of northwestern Algeria (Blanco et al. 2006). It is a sister species to *A. iberus* and *A. baeticus* with divergence time, based on sequence differences in the cytochrome b gene, estimated as about 5.3 mya. This places all three species along the existing coastline of the late Miocene at which time they may have constituted a single panmictic population. Like many North American *Cyprinodon* species, the *A. sauorensis* population is highly endangered by its small population size, human impacts including agricultural water use, and the introduction of exotic species.

## 10 Australian Deserts Inform on Differences in Dispersal Ability of Desert Fishes

As with North American and African Deserts, Australian deserts were once much wetter. Fossil plant material indicates they were covered with forests 40-25 mya (Alley 1998). The Simpson Desert of central Australia contains the Lake Eyre drainage, an internally draining (*endorheic*) basin that consists of an upper and lower component and associated lake beds (Unmack 2001). Lake Eyre, the lowest point in Australia (15.2 m below sea level), was a permanent saline lake 10-5 kya but has been dry for an estimated 5-3 kya except for exceptional flooding events in 1950 and 1974 (Kotwicki and Isdale 1991). These authors suggest periodic flooding occurs as a result of heavy rainfall associated with the El Niño Southern Oscillation. Of interest is that Lake Eyre may now serve as a barrier to dispersal. Many of the feeding streams rarely reach the lake bed itself. Furthermore, upon inundation with water, salt deposits in the lake bed may result in such hypersaline conditions as to be an effective barrier to fish dispersal. More likely, dispersal strategies are afforded by the generally flat topography. As adjacent streams overflow, connections and downstream convergences may allow for dispersal of species. Some of the streams flow through regions where geomorphic features such as scouring of basins lined by bedrock promote retention of water in permanent or semi-permanent pools. More transient pools may also harbor species that survive and move to other pools during wetter periods. In all there are 18 endemic fish species in the Lake Eyre Basin, 8 of which are restricted to desert springs (Unmack 2013) that are fed by the Great Artesian Basin aquifer that underlies the Lake Eyre Basin. Salinities in these springs range from 0.7 to 1.4 ppt (Kotwicki 1988), but this may increase as spring outflow evaporates.

Persistence of fishes in the Simpson Desert has been ascribed to the capacity of fish to disperse during wetter periods (Unmack 2001, 2013). Successful fishes must be capable of tolerating extreme temperatures and salinity to survive in the unpredictable environment of a desert. However, a widely distributed fish must also be able to migrate quickly when water becomes opportunistically available. An example is given of the spangled perch (*Leiopotherapon unicolor*) that has been reported to have migrated 10.6 km in 6 h in shallow water of a tire rut following rainfall. It is the most widely distributed fish in the Lake Eyre drainage basin. One might imagine that as a local population is extirpated, the ability to widely disperse would allow for the repopulation of the area in a metapopulation type of system. In so doing, the species would remain relatively homogeneous because of the extensive mixing of the gene pool. *Leiopotherapon unicolor* is in the family Terapontidae that also has species that live in shallow coastal waters of the Indian Ocean and western Pacific where they encounter a range of salinities from saltwater to brackish water to freshwater. Three other genera of the Terapontidae are found in Australian deserts (http://desertfishes.org/Australia).

Other tough fishes that are not as widely dispersed as a single species include the desert gobies (*Chlamydogobius* species, family Gobiidae), which are found in a number of springs and waterholes where they are able to tolerate temperatures between 5 and 40 °C, salinities of 60 ppt, and oxygen concentrations as low as 0.8 mg/l (Unmack 2001, 2013). Although one might argue that these fish are very hardy and—as a group—widely dispersed, their persistence as distinct taxa in various drainage systems might suggest a more limited dispersal ability than that of *L. unicolor*. Four species of *Chlamydogobius* are found in specific drainages of the Lake Eyre basin.

As their common name (hardyhead) implies, *Craterocephalus* species (family Atherinidae) are tolerant of extreme environmental conditions in the restricted habitats where they occur (Unmack 2001, 2013). *Craterocephalus dalhousiensis* has been observed to make brief excursions into water as hot as 41.8 °C. Other fishes, e.g., Plotosidae (eel tailed catfishes), may sometimes be represented by separate taxa in individual drainages. The differences in dispersal patterns of fish like *Leiopotherapon unicolor* and these other hardy fish lead one to speculate if perhaps fish that are less capable of wide dispersal through the exploitation of stochastic opportunities end up speciating into multiple taxa whereas fish that excel at dispersal remain as a single species.

Temporal and spatial considerations of dispersal in the Lake Eyre Basin have similarities to the North American and Sahara Desert environments. Other fish families in the area include the Clupeidae (bony herrings), Ambassidae (glassfishes), Eleotridae (gudgeons), and Melanotaeniidae (rainbowfishes; http://desertfishes.org/Australia). Connections with the Gulf of Carpentaria to the north likely allowed exchange of glassfish, bony herring, and desert rainbow fish with those of the Gulf drainages (Unmack 2013). There also have been transfers of hardyhead, desert rainbowfish, carp gudgeon, and glassfish species between the

Lake Eyre drainages and the Burdekin River that flows approximately 200 km to the Pacific Ocean. Possible connections with the Murray–Darling drainages remain to be fully elucidated.

## 11 Timescales for Evolutionary Change in Desert Fishes

In the sections above, we reviewed the ecology and evolution of selected desert fishes. From this, we can deduce what makes a fish successful in desert habitats. Fish that are hardy are able to persist despite the extremes of a changing environment. Fish that avail themselves well of the opportunity to disperse are able to persist as a metapopulation when local populations are stochastically extirpated. Fish that evolve rapidly may accommodate environmental changes *or* may continue to persist as distinct taxa. Importantly, fishes that are found in the desert appear to have persisted in the environment during the desertification process. Furthermore, these processes occurred over thousands of years.

Anthropogenic influence on ecosystems may result in extremely rapid changes and/or changes that negatively impact the natural evolution and ecology of desert fishes. Global climate change will influence desertification (Lal 2014). Of concern is that the remarkably rapid changes in climate may not allow some species to adapt or persist. Census data show the percentage of the Devils Hole pupfish population observed on the breeding ledge (where temperatures vary to as high as 39 °C during summer) ranged from over 60 % during the winter to 40 % during the summer between 1974 and 1981 (Devils Hole Pupfish Recovery Team). However, between 1993 and 2003, the percentage on the ledge declined to about 30 % during the winter and less than 10 % during the summer. This change in localized distribution of the fish coincides with a trend towards warmer ambient temperatures (National Oceanic and Atmospheric Agency; http://www.NOAA.gov). The National Climate Data Center of NOAA reported that the mean temperatures in the USA for 2006 exceeded the 30-year average, with Nevada having among the greatest increases. We note that the population of fish in 2006 dipped to a then all-time low of 38 individuals. Desert fishes may be the proverbial canary in a coalmine wherein taxa that relied on slow desertification processes in order to persist, and are already surviving at or near the apparent environmental limits for vertebrate life, may face extirpation with rapid environmental changes.

Underlying the North American, Saharan, and Australian deserts are large aquifer systems. Agricultural and developmental demands have resulted in tremendous pumping of these aquifers that exceed their capacity for recharge. In Devils Hole, drawdown of the aquifer resulted in partial exposure of the breeding shelf. The intervention of the US Supreme Court limited the aquifer pumping and allowed for some recovery of the fish (Riggs and Deacon 2001). Continued development worldwide will likely place similar stresses on spring systems that seem vital to the persistence of desert fishes.

Dispersal of fishes has allowed persistence in a temporal scale. Prior to Ash Meadows being incorporated as a National Wildlife Refuge in 1984, much of the area was used for cattle ranching and alfalfa farming. Natural flooding was restricted through construction of levees and irrigation canals. The result was changes to dispersal patterns of pupfish during stochastic flooding events. Much effort has been geared towards restoring these natural patterns.

On a larger scale, construction of dams and reservoirs has had a major impact on the distribution of desert fishes. For instance, construction of dams along the Colorado River changed water availability. Prior to damming, the Colorado River underwent seasonal patterns of flooding. Periods of high flow alternated with periods of low flow. Water temperatures fluctuated with flow. In this habitat, a group of "large river fishes" evolved morphologies specialized for swimming in rapid currents. The razorback sucker (*Xyrauchen texanus*, Catostomidae) may be as large as 1 m and live for >40 years (Dowling et al. 2012). The razorback sucker is named for its sharp-edged bulge on its back just behind the head that is an adaptation for living in fast flowing water. Historically, razorbacks were found in the upper and lower Colorado River basins and headwater streams including those of the Gila and Salt rivers. These fish may be adversely affected by changes to the thermal environment as a result of damming. Damming created deep reservoirs that continuously discharge relatively cold water. While small numbers of mature fish persist, there is a significant concern that these fish may not have had significant reproduction since construction of Hoover Dam. A captive breeding program now exists at Willow Beach National Fish Hatchery. Similar programs exist for *Gila cypha* and *G. elegans*. *Gila elegans* is thought to be functionally extinct with no natural reproduction (Hedrick et al. 2000).

The largest of the Colorado River fishes is the Colorado pikeminnow (*Ptychocheilus lucius*), which may reach 1.8 m and weigh over 50 kg. These now threatened fish were once very abundant in the upper and lower Colorado River drainages. Growth rates and survivorship studies have shown that reproduction and recruitment of young into the population are more successful in the warmer waters that were available prior to dam construction (Kaeding and Osmundson 1988).

Human development has also resulted in direct extirpation of desert fish species and populations. Tecopa pupfish (*C. nevadensis calidae*) lived in two outflows of hot springs in California (Miller et al. 1989). Construction of bathhouses and the enlargement of the hot spring pools altered those outflows. In 1965, the outflows of the two springs were merged. The resulting swifter currents caused downstream water temperatures to rise above hospitable levels. These modifications also allowed the related Amargosa River pupfish (*C. n. amargosae*) to migrate upstream from the Amargosa River and hybridize with the Tecopa pupfish. Specimens of *C. n. calidae* were last collected on February 2, 1970. The fish was automatically included in the Endangered Species Act (ESA) of 1973 even though it was probably already extinct by that point. Indeed, the Tecopa pupfish has the dubious distinction of being the first animal to be delisted in 1982 from the ESA due to extinction.

Non-native species were cited as contributing factors to the extinction of the Tecopa pupfish and remain a real threat to a variety of desert fishes. Throughout the

Ash Meadows region, introduced species such as crayfish (*Procambarus clarkii*), bullfrogs (*Lithobates catesbeianus*), largemouth bass (*Micropterus salmoides*), western mosquitofish (*Gambusia affinis*), convict cichlid (*Amatitlania nigrofasciata*), green sunfish (*Lepomis cyanellus*), black *bullhead* (*Ameiurus melas*), and sailfin mollies (*Poecilia latipinna*) threaten pupfishes and dace (Scoppettone et al. 2011). Even within the Colorado River, introduction of fish like striped bass for sport fishing threatens smaller fish including suckers. Management of these resources seems critical if desert fishes are to persist. While we highlighted the issues of the local North American deserts, we note similar conservation concerns for virtually all desert fishes throughout the world.

## 12 Conclusions and Outlook

Desert fishes oftentimes live in isolated habitats that were once interconnected. Hubbs and Miller (1948) noted current species distributions needed to be reconciled with putative climatic and geomorphologic changes. Disturbingly, models of biological evolution oftentimes contradict the extant geomorphological data. Since that time many studies have sought to resolve this issue (Smith et al. 2002; Lowenstein 2002; Spencer et al. 2008). However, controversies still exist, especially the degree to which stochastic flooding events might lead to connections and gene flow that would not be evident from longer term changes in geomorphology. Much of these controversies might be settled with better reconciliation on what factors affect evolutionary process. For instance, changes in temperature and food supply can affect morphometric and meristic characteristics (e.g., body shape, fin ray counts, etc.) that have traditionally been used to identify species (Lema and Nevitt 2006). Thus, specific habitats may affect phenotypic expression as much as effects of natural selection on the genotype itself. Future research directions should include discussion of what we deem as biological species and the mechanisms that give rise to those species.

Introduced species, altered water flows, and climate change are likely contributing to the extirpation of desert fishes. Groundwater pumping from aquifers appears to be exceeding the capacity for recharge. Captive breeding programs are likely to play an important role in the conservation of affected desert fishes. Sadly, the African pupfish *Aphanius sauorensis* has recently been declared extinct in the wild due to human modification of the habitat and the introduction of non-native species. Hope exists that a captive bred population might preserve the species from total extinction (Bacha et al. 2014). Captive breeding programs, while essential for preservation of species whose habitats have become inhospitable, present problems that must be considered if they are designed to provide progeny that can eventually be reintroduced to their native habitats. As noted above, trait expression can be affected by environmental conditions so each successive generation may appear different both anatomically and physiologically from the native population.

Understanding how plasticity, epigenetics, and genomic evolution intersect will be vital to understanding how best to conserve a given species.

The remote nature of much of the world's deserts likely means that we have an incomplete understanding of the microhabitats, species, and ecology of the desert fishes. When combined with the issues above, it would come as no surprise that many fish species will be extinct before they become known to science.

**Acknowledgments** The authors would like to acknowledge members of their laboratory for their diligent efforts and inspiration. FvB and SDH are supported by grants from the United States Fish and Wildlife Service and the Nevada Department of Wildlife. JEP is supported by NIH grant R01 HL095454.

## References

Ageili IM, Al-Akel AS, Suliman EM (2013) Seasonal variations in food selectivity, condition factor and hepatosomatic and gonadosomatic indices in the endangered killifish *Aphanius dispar dispar* (Teleostei: Cyprinodontidae) in Alhasa, Saudi Arabia. Life Sci J 10:443–449

Al-Daham NK, Huq MF, Sharma KP (1977) Notes on the ecology of fishes of the genus Aphanius and Gambusia affinis in southern Iraq. Freshwater Biol 7:245–251

Al-Ghamdi HS, Abu-Zinadah OA (1998) Study on freshwater fish fauna of the mid-western region of Saudi Arabia. JKAU: Science 10:39–45

Alkahem HF, Behnke RJ, Ahmad Z (1990) Some osteological distinction among four Arabian cyprinid species. Jap J Ichthyol 36:477–482

Alley NF (1998) Cainozoic stratigraphy palaeoenvironments and geological evolution of the Lake Eyre Basin. Palaeogeogr Palaeoclimatol Palaeoecol 144:239–263

Armitage SJNA, Drake S, Stokes A et al (2007) Multiple phases of North African humidity recorded in lacustrine sediments from the Fazzan Basin Libyan Sahara. Quat Geochronol 2:181–186

Bacha M, Benamirouche C, Freyhof J (2014) Critically endangered freshwater fish species of North Africa likely to be extinct in the wild. Newsletter IUCN SSC/WI Freshwater Fish Specialist Group 4:29

Bernardi G, Ruiz-Campos G, Camarena-Rosales F (2007) Genetic isolation and evolutionary history of oases populations of the Baja California killifish, *Fundulus lima*. Cons Genet 8:547–554

Blanco JL, Hrbek T, Doadrio I (2006) A new species of the genus *Aphanius* (Nardo 1832) (Actinopterygii Cyprinodontidae) from Algeria. Zootaxa 1158:39–53

Borkenhagen K, Krupp F (2013) Taxonomic revision of the genus *Carasobarbus* Karaman, 1971 (Actinopterygii, Cyprinidae). ZooKeys 339:1–53

Brown JH, Feldmeth CR (1971) Evolution in constant and fluctuating environments: thermal tolerances of desert pupfish (*Cyprinodon*). Evolution 25:390–398

Brown WJ, Rosen MR (1995) Was there a Pliocene-Pleistocene fluvial-lacustrine connection between Death Valley and the Colorado River? Quat Res 43:286–296

Carson EW, Tobler M, Minckley WL, Ainsworth RJ, Dowling TE (2012) Relationships between spatio-temporal environmental and genetic variation reveal an important influence of exogenous selection in a pupfish hybrid zone. Mol Ecol 21:1209–1222

Coad BW (1982) A new genus and species of cichlid endemic to southern Iran. Copeia 1982:28–37

Curtis B, Roberts KS, Griffin M et al (1998) Species richness and conservation of Namibian freshwater macroinvertebrates, fish and amphibians. Biodiv Conserv 7:447–466

Deacon JE, Minckley WL (1974) Desert fishes. In: Brown GW (ed) Desert biology, vol II. Academic Press, New York, NY, pp 385–488

Douglas MR, Brunner PC, Douglas ME (2003) Drought in an evolutionary context: molecular variability in flannelmouth sucker (*Catostomus latipinnis*) from the Colorado River Basin of western North America. Freshwater Biol 48:1254–1273

Dowling TE, Saltzgiver MJ, Marsh PC (2012) Genetic structure within and among populations of the endangered razorback sucker (*Xyrauchen texanus*) as determined by analysis of microsatellites. Conservat Genet 13:1073–1083

Drake NA, Blench RM, Armitage SJ et al (2011) Ancient watercourses and biogeography of the Sahara explain the peopling of the desert. Proc Natl Acad Sci 108:458–462

Echelle AA, Van Den Bussche RA, Malloy TP et al (2000) Mitochondrial DNA variation in pupfishes assigned to the species *Cyprinodon macularius* (Atherinomorpha: Cyprinodontidae): taxonomic implications and conservation genetics. Copeia 2000:353–364

Echelle AA, Carson EW, Echelle AF et al (2005) Historical biogeography of the new-world pupfish genus *Cyprinodon* (Teleostei: Cyprinodontidae). Copeia 2005:320–339

Echelle AA (2008) The western North American pupfish clade (Cyprinodontidae: *Cyprinodon*): mitochondrial DNA divergence and drainage history. Spec Papers Geol Soc Am 439:27–38

Esmaeili HR, Teimori A, Gholami Z et al (2012) Re-validation and re-description of an endemic and threatened species, *Aphanius pluristriatus* (Jenkins, 1910) (Teleostei, Cyprinodontidae), from southern Iran. Zootaxa 3208:58–67

Feldmeth CR, Stone EA, Brown JH (1974) An increased scope for thermal tolerance upon acclimating pupfish (*Cyprinodon*) to cycling temperatures. J Comp Physiol 89:39–44

Feulner GR (1998) Wadi fish of the UAE. Tribulus 8(2):16–22

Gerking SD, Lee R, Shrode JB (1979) Effects of generation-long temperature acclimation on reproductive performance of the desert pupfish *Cyprinodon n. nevadensis*. Physiol Zool 52:113–121

Gillooly JF, Brown JH, West GB et al (2001) Effects of size and temperature on metabolic rate. Science 293:2248–2251

Gonçalvès J, Petersen J, Deschamps P et al (2013) Quantifying the modern recharge of the "fossil" Sahara aquifers. Geophys Res Lett 40:2673–2678

Gustafson ES, Deacon JE (1998) Distribution of larval Devils Hole pupfish *Cyprinodon diabolis* (Wales) in relation to dissolved oxygen concentration in Devils Hole. Final report, Death Valley National Park

Hamidan N (2004) The freshwater fish fauna of Jordan. Denisia 14:385–394

Haney DC, Nordlie FG (1997) Influence of environmental salinity on routine metabolic rate and critical oxygen tension of *Cyprinodon variegatus*. Physiol Biochem Zool 70:511–518

Hedrick PWTE, Dowling WL, Minckley CA et al (2000) Brief communication: Establishing a captive broodstock for the endangered Bonytail chub (*Gila elegans*). J Hered 91:35–39

Heggen RJ (2013) Underground rivers. eBook. Available from Googlebooks and http://www.unmedu/~rheggen/UndergroundRiver.shtml. Accessed 31 Jan 2014

Heuton M, Hillyard S, Steinberg S et al (2013) Paradoxical anaerobism in desert pupfish. FASEB J 27:114910

Hillyard SD (2011) Life in hot water: the desert pupfish. In: Farrell AP, Stevens D (eds) Encyclopedia of fish physiology: from genome to environment. Academic, London, pp 1831–1842

Hirschfield MF, Feldmeth CR, Soltz DL (1980) Genetic differences in physiological tolerances of Amargosa pupfish (*Cyprinodon nevadensis*) populations. Science 207:999–1000

Hrbek T, Meyer A (2003) Closing of the Tethys Sea and the phylogeny of Eurasian killifishes (*Cyprinodontiformes*: *Cyprinodontidae*). J Evol Biol 16:17–36

Hubbs CL, Miller RR (1948) The Great Basin, with emphasis on glacial and postglacial times. II. The zoological evidence, correlation between fish distribution and hydrographic history in the desert basins of western United States. Univ Utah, Salt Lake City

Hunt CB, Robinson TW, Bowles WA, et al (1966) Hydrologic basin Death Valley California. Geol Survey Prof Paper 494-B

Kaeding LR, Osmundson DB (1988) Interaction of slow growth and increased early-life mortality: an hypothesis on the decline of Colorado squawfish in the upstream regions of its historic range. Environ Biol Fish 22:287–298

Kara HM (2012) Freshwater fish diversity in Algeria with emphasis on alien species. Eur J Wild Res 58:243–253

Kotwicki V, Isdale P (1991) Hydrology of Lake Eyre Australia: El Nino link. Palaeogeogr Palaeoclimatol Palaeoecol 84:87–98

Kotwicki V (1988) Hydrology of wetlands in arid central Australia. In: Proceedings of the international symposium on Hydrol wetlands semiarid arid region 95–98

Krijgsman WCG, Langereis WJ, Zachariasse M et al (1999) Late Neogene evolution of the Taza–Guercif Basin (Rifian Corridor Morocco) and implications for the Messinian salinity crisis. Mar Geol 153:147–160

LaBounty JF, Deacon JE (1972) *Cyprinodon milleri*, a new species of pupfish (family Cyprinodontidae) from Death Valley California. Copeia 1972:769–780

Lal R (2014) Desertification and soil erosion. In: Freedman B (ed) Global environmental change. Springer, Dordrecht, pp 369–378

Lema SC, Nevitt GA (2006) Testing an ecophysiological mechanism of morphological plasticity in pupfish and its relevance to conservation efforts for endangered Devils Hole pupfish. J Exp Biol 209:3499–3509

Lévêque C (1990) Relict tropical fish fauna in Central Sahara. Ichthyol Explor Freshwaters 1:39–48

Lowenstein TK (2002) Pleistocene Lakes and paleoclimates (0 to 200 Ka). In: Hershler R, Madsen DB, Currey DR (eds) Smithsonian contributions to the Earth Sciences Number 33, Great Basin aquatic systems history. Smithsonian Institution Press, Washington, DC, pp 109–112

Martin AP, Palumbi SR (1993) Body size, metabolic rate, generation time, and the molecular clock. Proc Natl Acad Sci 90:4087–4091

Miller RR, Williams JD, Williams JE (1989) Extinctions of North American fishes during the past century. Fisheries 14:22–38

Montejano G, Absalon IB (2009) Reporte del Proyecto: Caracterización del hábitat acuático asociado al pez *Cyprinodon julimes*. World Wildlife Fund Report. http://amigosdelpandeno.org/ligas.html. Accessed 31 Jan 2014

Naiman RJ, Gerking SD, Ratcliff TD (1973) Thermal environment of a Death Valley pupfish. Copeia 1973:366–369

Naiman RJ, Gerking SD, Stuart RE (1976) Osmoregulation in the Death Valley pupfish *Cyprinodon milleri* (Pisces: Cyprinodontidae). Copeia 1976:807–810

Ocock J, Baasanjav G, Baillie JEM et al (2006) Mongolian red list of fishes. Regional red list series, vol 3. Zoological Society of London, London

Otto RG, Gerking SD (1973) Heat tolerance of a Death Valley pupfish (genus *Cyprinodon*). Physiol Zool 46:43–49

Olden JD, Poff NL, Bestgen KR (2008) Trait synergisms and the rarity, extirpation, and extinction risk of desert fishes. Ecology 89:847–856

Ortmann AE (1902) The geographic distribution of freshwater decapods and its bearing upon ancient geography. Proc Am Philos Soc 171:267–400

Parker A, Kornfield I (1995) Molecular perspective on evolution and zoogeography of cyprinodontid killifishes (Teleostei; Atherinomorpha). Copeia 1995:8–21

Reichenbacher B, Feulner GR, Schulz-Mirbach T (2009) Geographic variation in otolith morphology among freshwater populations of *Aphanius dispar* (Teleostei, Cyprinodontiformes) from the southeastern Arabian peninsula. J Morphol 270:469–484

Riggs AC, Deacon JE (2002) Connectivity in desert aquatic ecosystems: The Devils Hole story. In: Conference proceedings: Spring-fed wetlands: important scientific and cultural resources of the intermountain region, vol 11.

Roskowski JA, Patchett PJ, Spencer JE et al (2010) A late Miocene–early Pliocene chain of lakes fed by the Colorado River: evidence from Sr, C, and O isotopes of the Bouse Formation and related units between Grand Canyon and the Gulf of California. Geol Soc Am Bull 122:1625–1636

Schroeder S (1879) Artesian wells and the great Sahara. Pop Sci Month 16:530–539

Scoppettone GG, Rissler P, Johnson D et al (2011) Relative abundance and distribution of fishes and crayfish at Ash Meadows National Wildlife Refuge, Nye County, Nevada. Open-File Report 1017, United States Geol Surv, pp 1–6

Shrode JB, Gerking SD (1977) Effects of constant and fluctuating temperatures on reproductive performance of a desert pupfish *Cyprinodon n. nevadensis*. Physiol Zool 50:1–10

Skelton P (1990) Status of fishes from sinkholes and caves in southwest Africa/Namibia. Namibia Scientific Society, Windhoek, Namibia, pp 75–83

Smith GR, Dowling TE, Gobalet KW et al (2002) Biogeography and timing of evolutionary events among Great Basin fishes. In: Hershler R, Madsen DB, Currey DR (eds) Smithsonian contributions to the earth sciences number 33. Great basin aquatic systems history. Smithsonian Institution Press, Washington, DC, pp 175–234

Smith GR, Dowling TE (2008) Correlating hydrographic events and divergence times of speckled dace (*Rhinichthys*: Teleostei: Cyprinidae) in the Colorado River drainage. Spec Paper Geol Soc Am 439:301–318

Spencer JE (2008) Middle to late Cenozoic geology, hydrography, and fish evolution in the American southwest. Spec Paper Geol Soc Am 439:279–299

Spencer JE, Pearthree PA, House PK (2008) An evaluation of the evolution of the latest Miocene to earliest Pliocene Bouse lake system in the lower Colorado River valley southwestern USA. Spec Paper Geol Soc Am 439:375–390

Spencer JEP, Patchett J, Pearthree PA et al (2013) Review and analysis of the age and origin of the Pliocene Bouse Formation lower Colorado River Valley southwestern USA. Geosphere 9:444–459

Stoffer P (2004) Changing climates and ancient lakes, Desert landforms and surface processes in the Mojave National Preserve and vicinity. Open-File Report 1007 US Geol Surv

Strecker U (2006) Genetic differentiation and reproductive isolation in a *Cyprinodon* fish species flock from Laguna Chichancanab, Mexico. Mol Phylogen Evol 39:865–872

Stuenkel EL, Hillyard SD (1981) The effects of temperature and salinity acclimation on metabolic rate and osmoregulation in the pupfish *Cyprinodon salinus*. Copeia 1981:411–441

Tobler M, Carson EW (2010) Environmental variation, hybridization, and phenotypic diversification in Cuatro Ciénegas pupfishes. J Evol Biol 23:475–1489

Trape S (2009) Impact of climate change on the relict tropical fauna of central Sahara: threat for the survival of Adrar mountains fishes, Mauritania. PLoS One 4:e4400

Unmack PJ (2001) Biogeography of Australian freshwater fishes. J Biogeogr 28:1053–1089

Unmack PJ (2013) Biogeography. In: Humphries P, Walker K (eds) The ecology of Australian freshwater fishes. CSIRO Publishing, Collingwood, pp 25–48

Wang Z-c, Jiang Z-f, Zhang C-z et al (2010) Checklist literature study and compilation of bracketed keys for indigenous fishes Tarim River basin. Progr Fish Sci 31:15–18

Williams JE, Bowman DB, Brooks JE et al (1985) Endangered aquatic ecosystems in North American deserts with a list of vanishing fishes of the region. J Ariz-Nev Acad Sci 20:1–62

Wu Y-F, Tan Q-J (1991) Characteristics of the fish-fauna of the characteristics of Qinghai-Xizang plateau and its geological distribution and formation. Acta Zool Sinica 37:152–165

Yazdani GM (1996) Fish diversity in the Thar desert. In: Ghosh AK, Baqri QH, Prakash I (eds) Faunal diversity in the Thar Desert: gaps in research. Scientific Publishers, Jodhpur, India, pp P285–295

Yuan G-y (1988) The vertebrates in middle-lower reaches of Hotan River. Arid Land Res 11:45–50

# Hypersaline Environments

**Gary Laverty and Erik Skadhauge**

**Abstract** A surprising number of diverse fish species are able to tolerate extreme conditions of salinity (hypersalinity) of even 2-3 times above seawater. This chapter explores some of the adaptations that allow for this tolerance. In some cases hypersaline tolerance appears to be further development of traits that are important for seawater adaptation; for others, novel adaptations may have arisen to deal with these extreme conditions. In particular, data have accumulated that support the presence of an elaborate system for bicarbonate secretion in the intestine, allowing for reduced luminal osmolality, precipitation of insoluble calcium carbonate and increased capacity for NaCl-driven water absorption from ingested hypersaline fluids. There are intriguing data suggesting reduced branchial water permeability under hypersaline conditions, possibly through the regulation of aquaporin channels on the gill epithelium, as well as data suggesting a reduction in whole-body ion permeabilities. Finally, both anatomical and physiological changes to the ion-secreting cells of the gill are essential for eliminating the large salt loads associated with seawater and hypersaline environments. There are new efforts to identify the molecular correlates of increasing secretory capacity by the gills, including changes in the selectivity of tight junctions that help to minimize diffusive salt uptake. It is important that future work continue to investigate the individual and integrated responses of fish to hypersaline conditions, providing new insights into osmoregulatory adaptation.

## 1 Introduction

The evolutionary history of teleost fishes is complex, punctuated by multiple, large-scale events, including extinctions, genome duplication, and rapid species diversification (Brunet et al. 2006; Santini et al. 2009; Vega and Wiens 2012). Recent studies suggest that actinopterygian clade (ray-finned fishes representing the vast

G. Laverty (✉)
Department of Biological Sciences, University of Delaware, Newark, DE 19716, USA
e-mail: laverty@udel.edu

E. Skadhauge
Faculty of Health and Medical Sciences, Department of Veterinary Clinical and Animal Sciences, Copenhagen University, Dyrlaegevej 16, DK 1870, Frederiksberg C, Denmark

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_5

**Table 1** Fish species known to tolerate hypersaline conditions. For references see main text

| Order | Common name | Genus and species |
|---|---|---|
| Anguilliformes | European eel | *Anguilla anguilla* |
| | Japanese eel | *Anguilla japonica* |
| Batrachoidiformes | gulf toadfish | *Opsanus beta* |
| Cyprinodontiformes | Arabian killifish | *Aphanius dispar* |
| | sheepshead minnow | *Cyprinodon variegatus* |
| | mummichog | *Fundulus heteroclitus* |
| | sailfin molly | *Poecilia latipinna* |
| Gonorynchiformes | milkfish | *Chanos chanos* |
| Perciformes | seabass | *Dicentrarchus labrax* |
| | Mozambique tilapia | *Oreochromis mossambicus* |
| | black-chin tilapia | *Sarotherodon melanontheron* |
| | gilthead seabream | *Sparus aurata* |
| | silver seabream | *Sparus sarba* |
| Pleuronectiformes | Senegalese sole | *Solea senegalensis* |
| Tetraodontiformes | spotted green puffer fish | *Tetraodon nigroviridis* |

majority of current marine fishes) actually derived from freshwater ancestors (Vega and Wiens 2012), supporting a long-standing but often-debated hypothesis, especially regarding the salmonid fishes (Day 1887, cited in McDowell 2002). This highlights the remarkable adaptive transitions that have allowed fish species to survive in various environments. Even more surprising is the ability of a number of species that can maintain osmotic homeostasis under hypersaline conditions of two or three times seawater, or even higher (Nordlie 2006). Such conditions can occur in landlocked saline lakes (e.g., Dead Sea), mangrove swamps, coastal lagoons, or estuaries (see Lorin-Nebel et al. 2012). Table 1 lists 15 species from 7 different orders of actinopterygians that are known to tolerate hypersaline conditions. This diversity suggests that salinity tolerance may be an important feature in the adaptive radiation of fishes.

Adaptation to hypersaline environments is partly characterized by further development of features characteristic of seawater teleosts, as well as other more specific responses to extreme conditions. This chapter will focus primarily on the role of the intestinal and branchial epithelia in adapting to hypersaline conditions. To provide context, some of the changes that take place during the transition from freshwater to seawater in euryhaline teleosts will also be reviewed. Recent reviews on the subject of adaptation to hypersaline environments have been published (Gonzalez 2012; Laverty and Skadhauge 2012); this chapter adds detail for some aspects of hypersalinity tolerance and attempts to provide a more integrated picture of the physiological mechanisms involved.

## 2 Water, Water Everywhere: Drinking Responses and Osmotic Permeability Changes

Figure 1 is a simplified view of the challenges and adaptive strategies of freshwater- and seawater-adapted fish. Both freshwater and seawater teleosts osmoregulate by coordinating activities of the intestines, kidneys, and gills. In freshwater, net water uptake occurs by osmotic flux primarily across the gill epithelium. For most species studied there is little or no drinking (Karnaky 1998; Evans et al. 2005; Evans 2008). The kidneys excrete this water as dilute urine, and mitochondrion-rich cells (MRC or chloride cells) of the gills mediate the active absorption of NaCl, possibly via several different pathways, to offset diffusional loss (Karnaky 1998; Evans et al. 2005; Hwang and Lee, 2007; Evans 2008; Hwang et al. 2011). In seawater, on the other hand, there is a constant loss of body water across permeable surfaces (primarily branchial epithelium), as well as diffusive accumulation of electrolytes. These fish must therefore drink continuously, further increasing the electrolyte load, with high intestinal fluid concentrations of not only NaCl but also divalent ions ($Mg^{++}$, $Ca^{++}$, and $SO_4^{--}$). Some of the $Mg^{++}$ and $SO^{--}$ ions are excreted in the very low urinary output, but most ions pass through the intestine (Karnaky 1998; Evans et al. 2005; Grosell 2006; Whittamore 2012). Water absorption from the ingested seawater is driven by active NaCl absorption by the intestinal epithelium. This absorbed salt load, together with that from diffusive uptake, is actively secreted by the gill epithelium (Karnaky 1998; Evans et al. 2005; Whittamore 2012). Thus, the coordination of drinking rate, intestinal absorption, urine excretion, and branchial salt excretion combine to allow for an effective desalination process (Fig. 1).

Seawater has an average salinity of 35 g $kg^{-1}$ water (ppt) with a corresponding osmotic pressure of just over 1,000 mOsm $kg^{-1}$ (Whittamore 2012). A number of fish species have been found to tolerate 2× or even 3× seawater. As pointed out by Gonzalez (2012), this would theoretically increase the NaCl transport needed to absorb water across the intestine and maintain the overall water balance by fourfold. Many of these species continue to show increased drinking rates, a measure of osmotic water loss in the steady state, as salinity increases (Potts et al. 1967; Maetz and Skadhauge 1968; Skadhauge 1980; Sardella et al. 2004; Marshall and Grosell 2005; Genz et al. 2008). In a study of trained (lower stress) *Aphanius dispar* from the Dead Sea, however, drinking rates did not increase in 2× seawater and actually decreased slightly in 3× seawater (Skadhauge and Lotan 1974, Skadhauge 1980). Similarly, sailfin mollies (*Poecilia latipinna*) and Mozambique tilapia ("California") (*Oreochromis mossambicus*) in hypersaline conditions have increased drinking rates less than predicted by the increased osmotic gradients (Gonzalez et al. 2005; Sardella et al. 2004). Assuming osmotic steady state, these findings suggest a reduction in water loss in hypersaline environments. The data in Table 2 illustrate this for *A. dispar*. As fish are exposed to increased salt loads, intestinal salt absorption, obtained by measuring salt content of the intestinal lumen directly or during in situ perfusion, increased (Lotan and Skadhauge 1972; Skadhauge 1980).

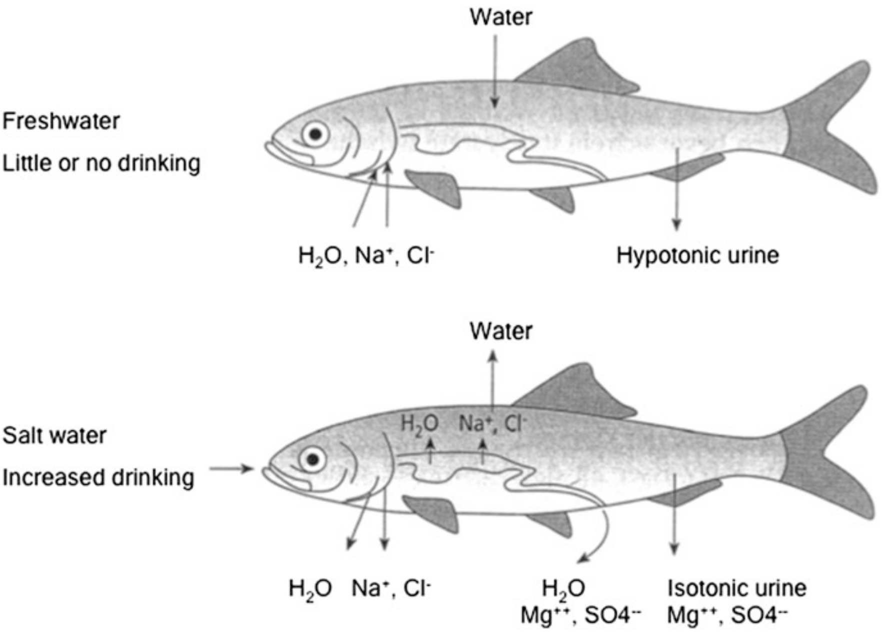


**Fig. 1** Major routes of water and ion intake and output in teleosts adapted to either freshwater or seawater. Note that with seawater adaptation, drinking increases, branchial transport of NaCl switches to net outward (secretory) transport, and both gut and kidney become important routes of divalent ion excretion. With hypersaline adaptation, many of these changes are further developed. *Arrows* indicate osmotic water movements, active uptake or extrusion of $Na^+$ and $Cl^-$, and urinary and intestinal excretion of divalent ions

**Table 2** *Aphanius dispar*: Adaptation to waters of high salinity

| | Osmolality | | | Intestinal absorption | | Permeability |
|---|---|---|---|---|---|---|
| | Medium (mOsm) | Bile[a] (mOsm) | Absorbate[b] (mOsm) | Water (μl/g h) | $Na^+$ (μequiv/g h) | "Posm"[c] (nl/g h mOsm) |
| 1 SW | 1,089 | 415 | 830 | 9.5 | 4.4 | 14 |
| 2 SW | 1,932 | 442 | 1,950 | 8.1 | 8.8 | 5.5 |
| 3 SW | 2,730 | 467 | 3,470 | 6.6 | 12.7 | 2.9 |

From Laverty and Skadhauge (2012); modified from Skadhauge (1980)

[a] Bile osmolality is taken to be identical to plasma

[b] Absorbate osmolality was calculated as follows: $Na^+$ absorption rate corrected for accompanying chloride ions and for osmotic coefficient (0.9) divided by the water absorption rate

[c] Posm refers to an overall permeability coefficient, which however is likely dominated by branchial water movement. Posm is calculated from the intestinal water absorption rate and the calculated osmotic gradient (medium—bile), ignoring the low urine flow rates (i.e., water absorption = branchial water loss in the steady state)

However, water absorption, measured using an external volume marker (EDTA), or minimally absorbed solutes ($Mg^{++}$ or $SO_4^{--}$), actually decreased in fish exposed to 2× or 3× seawater (Skadhauge and Lotan 1974; Skadhauge 1980). These data suggest an adaptive reduction in branchial water permeability, which would clearly reduce the energetic cost of drinking hypersaline fluids. Indeed, the calculated values for whole animal osmotic permeability ("Posm"), derived from water absorption rates and the prevailing osmotic gradient, are markedly decreased in 2× and 3× seawater (Table 2).

A number of earlier balance studies had reached the same conclusion, even for seawater adaptation (Oide and Utida 1968; Motais et al. 1969; Shehadeh and Gordon 1969; Isaia and Hirano 1975). While there is reasonably strong, albeit indirect, evidence for permeability changes in hyperosmotic environments, the exact mechanism remains unclear. A number of studies have focused on the aquaporin family of membrane proteins (AQPs). Although generally regarded as ubiquitous cell membrane water channels, the AQP family is diverse in terms of permeabilities (i.e., water, glycerol, and possibly some gasses), cellular localization, and regulation (Verkman 2011). One aquaporin in particular, the aquaglyceroporin AQP3, has been extensively studied in the gills. Several species adapted to seawater, including European and Japanese eels (*Anguilla anguilla*; *Anguilla japonica*), Atlantic salmon (*Salmo salar*), and sea bass (*Dicentrarchus labrax*), had reduced gill mRNA levels for AQP3, relative to freshwater-adapted fish (Cutler and Cramb 2002; Tse et al. 2006; Cutler et al. 2007; Giffard-Mena et al. 2007; Tipsmark et al. 2010). The total gill AQP3 protein levels, as seen with western blotting, also decreased with seawater acclimation in eels (Lignot et al. 2002; Tse et al. 2006) and in both seawater- and hypersaline-acclimated silver sea bream (*Sparus sarba*) (Deane and Woo 2006). However, other studies have found conflicting results, possibly due to species differences (Watanabe et al. 2005; Choi et al. 2013) and changes in cell-specific expression within the gills. Thus, immunohistochemical staining has demonstrated markedly reduced AQP3 protein expression in pavement cells or in non-chloride cells in the basal layers of the gill arch and primary filaments with seawater acclimation, while MRC staining was unchanged in European and Japanese eels and in Mozambique tilapia (Lignot et al. 2002; Watanabe et al. 2005; Tse et al. 2006; Cutler et al. 2007). More recently, studies on the killifish *Fundulus heteroclitus*, which can rapidly adapt to changing salinities, demonstrated an overall decrease in gill AQP3 mRNA during seawater acclimation, but no change in total AQP3 protein levels (Jung et al. 2012). This discrepancy was accounted for by the observation that protein expression in MRC cells actually increased, but decreased in yet another cell type—the pillar cells of the secondary lamellae. The possible role of aquaporins in mediating changes in branchial permeability should be further examined, especially under conditions of extreme hypersalinity. However, it is also clear that such studies will need to account for other aquaporin isoforms, other functions, such as ammonia transport (Cutler et al. 2007), species differences, and the complex cellular organization of the gills. Also of interest is the potential role of extracellular calcium in modulating the water permeability of the gills (Potts and Fleming 1970; Ogasawara and Hirano 1984; Wood and Grosell 2008).

# 3 Chalk It Up! How to Absorb Water From Hypersaline Fluids

The mechanism of intestinal water absorption in marine- and hypersaline-adapted fish is one of solute-linked (NaCl-driven) transport, likely using a variation of the "standing gradient" 3-compartment model where a hyperosmotic condition is created in the lateral space between mucosal epithelial cells (Diamond and Bossert 1967; Skadhuage 1969; Skadhuage 1974; Larsen et al. 2009, Whittamore 2012). However, the actual process depends on several additional features, including pre-dilution of the ingested fluid before it reaches the intestine, a complex array of transporters on the mucosal epithelium, and an exquisite balance of luminal fluid pH. As with other aspects of osmoregulation, there are likely to be important species differences. In addition, there is much to be learned regarding possible segmental specializations along the intestine. Nevertheless, the current knowledge in this field provides a fascinating look at some unique adaptive features.

As noted earlier, a number of species are able to maintain osmotic balance in 2× or 3× seawater environments, through a combination of intestinal absorption of ingested water to replace that lost across permeable surfaces, and a reduction in water loss by permeability changes. Eventually, however, increasing levels of salinity in the ingested fluids will reach a "turning point osmolality" (Skadhauge 1969, 1974) of zero net water absorption due to a maximal gut-absorption capacity (Maetz and Skadhauge 1968; Skadhauge 1969, 1980) and due to the accumulation of non-absorbed solutes, mainly $Mg^{++}$ and $SO^{--}$ ions, which begin to exert an increasingly large osmotic potential (Genz et al. 2011; Grosell 2011; Whittamore 2012). The situation is mitigated somewhat by pre-dilution of ingested fluid as it passes through the esophagus. The properties of this epithelium change with seawater adaptation in eels, to a barrier with relatively low water permeability and high $Na^+$ and $Cl^-$ ion permeability (Kirsch et al. 1975; Hirano and Mayer-Gostan 1976; Nagashima and Ando 1994), thus allowing for the passive removal of as much as 50 % of the NaCl in ingested seawater and a substantial reduction in the osmotic pressure. Active NaCl transport also plays a role (Parmelee and Renfro 1983) and appears to be mediated by parallel sodium–hydrogen and chloride–bicarbonate exchangers on the luminal membrane (Nagashima and Ando 1994; Esbaugh and Grosell 2014). In European flounder (*Platichthys flesus*), an increase in esophageal blood flow was also observed in fish adapted to 100 % seawater versus 10 % seawater, suggesting an additional adaptive process for enhancing epithelial NaCl absorption and desalination in the esophagus by the efficient transport of absorbate to the gills and by providing increased metabolic activity of the epithelium (Parmelee and Renfro 1983).

Intestinal transport of NaCl, driven by $Na^+/K^+$-ATPase (NKA) activity, increases with seawater and hypersaline adaptation (Skadhauge 1969, 1974; see also Table 2). Several studies have shown both increased intestinal gene expression and activity of NKA in seawater- and hypersaline-adapted fish (Colin et al. 1985;

Cutler et al. 2000; Seidelin et al. 2000; Gonzalez et al. 2005; Guffey et al. 2011). One unusual feature of the intestinal absorptive process in such fishes compared to the standard model of vertebrate intestinal absorption is that the absorbate itself may be hyperosmotic (Skadhauge 1974, 1980). Calculated absorbate osmolalities for *A. dispar* (Table 2) indicate that in all conditions the absorbate is hyperosmotic to the body fluids, and in the case of 2× and 3× seawater hyperosmotic to the surrounding medium as well (Skadhauge 1980). Grosell (2006) calculated absorbate osmolalities for a number of other species, based on the observed $Na^+$ and $Cl^-$ uptake rates, along with "missing cation" concentrations, and consistently arrived at values hyperosmotic to both intestinal fluid and plasma. This phenomenon can be reproduced in vitro, but only when the luminal fluid composition is adjusted to mimic in vivo-like conditions of lower NaCl and high $MgSO_4$ concentrations. In gulf toadfish (*Opsanus beta*) intestine, symmetrical, high NaCl solutions supported isosmotic fluid absorption, whereas asymmetric conditions with luminal solutions designed to match the in vivo composition (low NaCl and high $MgSO_4$) generated a hyperosmotic and more acidic absorbate (Grosell and Taylor 2007). The unusual fluid composition clearly affects the nature of the absorptive process, largely influenced by a complex transport process involving luminal secretion of $HCO_3^-$ and $H^+$ addition to the absorbate (see below). This is further supported by in vivo perfusion studies on European flounder that point to a combination of $HCO_3^-$ secretion and $H^+$ ion absorption as the mechanism for both the hyperosmotic and acidic nature of the absorbate (Whittamore et al. 2010).

Figure 2 is a simplified depiction of some of the transport processes mediating solute and fluid absorption in seawater and hypersaline fish. Many of these details are the result of extensive studies on gulf toadfish, including many under hypersaline conditions (for reviews see Wilson et al. 2002; Grosell 2006, 2011; Grosell et al. 2009b; Whittamore 2012). The figure illustrates a mucosal epithelial cell with lumen and basolateral sides labeled and with a tight junction complex (cross-hatched rectangle) separating this cell from the adjacent one. On the luminal side, near the top, are two of the important transporters mediating NaCl uptake at the luminal membrane (identified by common abbreviations seen in the literature). The sodium-chloride (NC) and sodium-potassium-2 chloride cotransporters use a downhill gradient of $Na^+$ to cotransport $Cl^-$ and $K^+$ ions into the cell. Sodium exits the basolateral side via the $Na^+/K^+$-ATPase (NKA), which is localized in this figure along the lateral margins, in keeping with the 3-compartment lateral interspace model for fluid absorption (Diamond and Bossert 1967; Skadhuage 1969; Larsen et al. 2009; Whittamore 2012). Not shown in this figure is an exit pathway for $Cl^-$ ions at the basolateral side, which possibly includes channels or cotransporters (Grosell 2011; Whittamore 2012). Water may be absorbed by either paracellular or transcellular pathways (dashed arrows). An aquaporin 1 homolog has been localized to the luminal membrane of seawater-adapted Japanese eels, indicating a likely mechanism for transcellular water uptake (Aoki et al. 2003).

Also shown on the luminal membrane is a $Cl^-/HCO_3^-$ exchanger (AE), identified as SLC26A6 in the HUGO Gene nomenclature and known to mediate a large fraction of the $Cl^-$ uptake and $HCO_3^-$ secretion mentioned above (Wilson

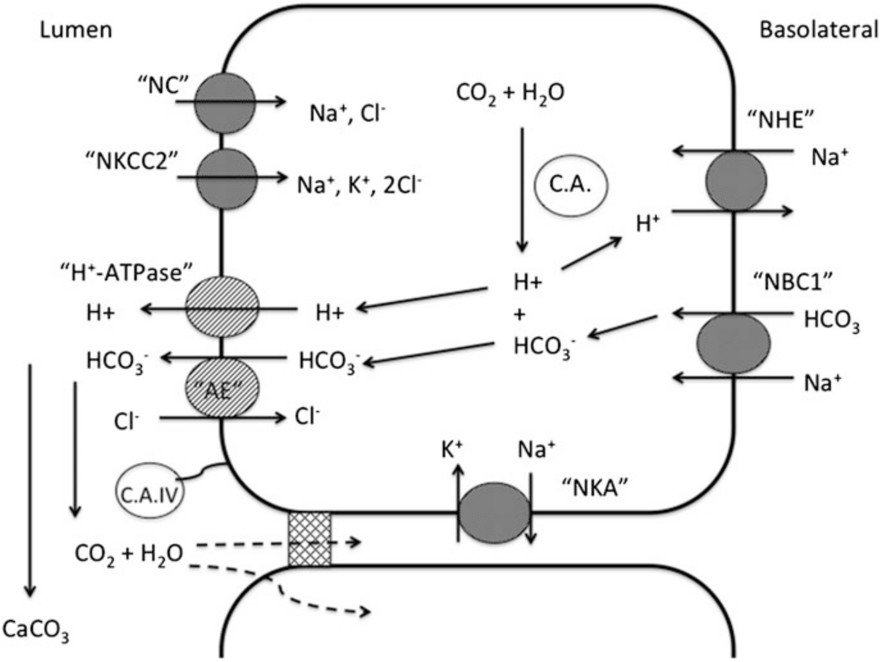


**Fig. 2** Schematic diagram of the major transport processes of the intestinal epithelium of seawater- and hypersaline-acclimated teleosts (adapted from Grosell et al. 2009b). An epithelial cell, with luminal and basolateral sides indicated, is shown along with a tight junction separating this cell from an adjacent cell (hatched rectangle). Major transporters involved in net NaCl and water absorption, and $HCO_3^-$ and $H^+$ luminal secretion are shown (not shown are other putative basolateral elements, including $Cl^-$ and $K^+$ ion channels and a basolateral V-type $H^+$-ATPase). NC: sodium-chloride cotransporter; NKCC: sodium-potassium-2 chloride cotransporter; NHE: sodium-hydrogen exchanger; NBC1: sodium-bicarbonate cotransporter; NKA: sodium-potassium ATPase; C.A.: soluble (cytoplasmic) carbonic anhydrase; $H^+$-ATPase: V-type hydrogen ion ATPase; AE: anion exchanger (SLC26A6); CAIV: membrane-associated carbonic anhydrase IV. The latter three elements ($H^+$-ATPase, SLC26A6, and CAIV) are thought to constitute a "metabolon" of interacting transport components (Grosell 2006; Grosell et al. 2009b). See main text for more details

et al. 2002; Grosell 2006; Kurita et al. 2008; Faggio et al, 2011; Whittamore et al. 2010; Grosell 2011; Whittamore 2012). Bicarbonate ions carried by this exchanger are derived partly from basolateral uptake via a sodium-bicarbonate cotransporter (NBC1), and partly from cytoplasmic hydration of metabolic and recycled $CO_2$, catalyzed by a soluble form of carbonic anhydrase (CA), and subsequent dissociation into $HCO_3^-$ and $H^+$ (Grosell and Taylor 2007).

Luminal anion exchange contributes to fluid absorption from hyperosmotic fluids in several ways. First, $Cl^-$ uptake by this pathway could directly contribute to the solute-coupled fluid absorption. Chloride–bicarbonate exchange also alkalinizes the intestinal lumen, and under these conditions promotes the formation of insoluble $CaCO_3$ ("chalk") and, to a lesser extent, $MgCO_3$. Precipitates of $CaCO_3$, which were observed also in early studies (Smith 1930; Shehadeh and Gordon 1969), have been estimated to lower the luminal osmotic pressure by 70 mOsm or more (Wilson et al. 2002; Whittamore 2012), while at the same time removing part of the secreted $HCO_3^-$ from the luminal fluid. This process is indicated by the longer arrow in the figure. Most of the $H^+$ generated by metabolic hydration of $CO_2$ is transported across the basolateral membrane by a sodium–hydrogen exchanger isoform (NHE) and possibly a vacuolar (V-type) $H^+$-ATPase (Grosell and Taylor 2007; Grosell et al. 2007; Grosell 2011; Whittamore 2012). Although shown on the basal membrane in Figure 2, $H^+$ extrusion along the lateral membrane would also contribute to the acidic and hyperosmotic character of the absorbed fluid, as mentioned earlier. The acid load created by this mechanism thus represents an additional challenge to fish in hypersaline environments (Grosell and Genz 2006; Cooper et al 2010).

A third way by which anion exchange contributes to fluid absorption involves the luminal titration and dehydration of secreted bicarbonate. Figure 2 illustrates the presence of a luminal V-type $H^+$-ATPase, which has been studied extensively in recent years, along with a membrane-associated isoform of carbonic anhydrase (CAIV). It has been proposed that these elements constitute, with SLC26A6, a "metabolon," an effective mechanism for rapidly converting secreted $HCO_3^-$ to $CO_2$, thus further reducing the lumen osmolality (Grosell 2006, 2011; Grosell et al. 2007, 2009a, b). Diffusion of $CO_2$ back into the cytoplasm leads to the regeneration of $HCO_3^-$, mediated by soluble CA. Luminal $H^+$ ion secretion thus reduces the acid load on the fish and promotes fluid absorption by reducing the lumen osmolality. At the same time, it also limits the increase in $HCO_3^-$ concentration in the lumen, thus helping to sustain $Cl^-/HCO_3^-$ exchange (Grosell 2011; Guffey et al. 2011; Whittamore 2012). The overall process of NaCl and water absorption is further augmented by the stimulatory effect of cytoplasmic $HCO_3^-$ ions on a soluble adenylyl cyclase (not shown), which has been shown to regulate the activity of the NKCC cotransporter (Tresguerres et al. 2010).

It has been pointed out that the two processes of $HCO_3^-$ secretion with alkalinization of the luminal fluid favoring $CaCO_3$ precipitation, and luminal $H^+$ secretion favoring $HCO_3^-$ titration, have the potential to partially counteract each other (Grosell et al. 2009b). However, the data seem to suggest that these two processes are additive with regard to reducing lumen osmolality and thus promoting fluid absorption. It has also been proposed that luminal calcium concentrations or other factors could modulate the balance of luminal $H^+$ secretion (Grosell et al. 2009b; Whittamore et al. 2010).

Acclimation to hypersaline conditions upregulates gene expression and activity for many of these transporters, and the effects appear to be specific to some regions of the intestinal tract, at least in some species. In general, it seems that the posterior intestine and rectum become more relevant in adapting to these extreme conditions. Acclimation of gulf toadfish to 50 or 60 ppt hypersalinity markedly increased intestinal $HCO_3^-$ secretion above that of seawater-adapted fish and simultaneously increased whole animal acid excretion, presumably by gills, thus minimizing the impact on acid–base balance (Genz et al. 2008). These results suggest coordinated responses of the hypersaline-associated $HCO_3^-$ secretion process to maintain both osmotic and pH homeostasis. Relative mRNA expression of an isoform of the basolateral sodium-bicarbonate cotransporter (NBC1) in gulf toadfish doubled at 12 and 24 h after transfer from seawater to 60 ppt (Taylor et al. 2010). Cytoplasmic carbonic anhydrase gene expression and activity also increased with the transfer from seawater to 60 ppt in this species (Sattin et al. 2010). The NBC1 response occurred primarily in middle segments of the toadfish intestine, whereas CA was increased in the posterior intestine and rectum as well, suggesting possible regional differences in the source of secreted $HCO_3^-$ during hypersaline adaptation (Grosell 2011).

This idea of regional differences in intestinal responses has received more attention recently. In gulf toadfish, again, transfer from seawater to 60 ppt increased mRNA expression of the V-type $H^+$-ATPase by 20-fold (see Figure 2), but only in the posterior segment of the intestine (Guffey et al. 2011). $H^+$-ATPase activity was also elevated, indicating a specific, functional response in the posterior segment to hypersalinity. The composition of the intestinal fluid changes in vivo as it moves through the intestinal tract, with decreasing $Ca^{++}$ (due to $CaCO_3$ precipitation) and increasing $HCO_3^-$ concentrations, posteriorly. These changes would make conditions less favorable for $Cl^-/HCO_3^-$ exchange as $HCO_3^-$ levels build up, thus impacting fluid absorption. $H^+$ ion secretion in the posterior segment would help by titrating luminal $HCO_3^-$ and recycling the $CO_2$ produced, thus maintaining more favorable conditions for $Cl^-$ and water absorption.

Similarly, segment-specific responses to hypersaline acclimation (versus seawater) were seen in the sea bream (*Sparus aurata*) with large increases in V-type $H^+$-ATPase expression and water absorption rate. However, these changes were seen in the rectum of sea bream, not in the anterior intestine (Gregório et al. 2013).

## 4 Back to the sea: eliminating the ingested salt load

Animals that live in the sea or in close intimacy with the sea have evolved different systems for eliminating excess NaCl from the body, including salt glands and rectal glands. In marine teleosts, the gills become the major mechanism for secreting NaCl. In fact, it has been proposed that ionoregulatory functions of the gills, rather than respiratory function, may have been the driving force for gill evolution (Fu et al. 2010).

The MRC or "chloride" cells are the ionoregulatory cells of the gills. In seawater or hypersaline conditions, these cells actively secrete $Cl^-$ ions by a mechanism similar to that seen in many tissues, including mammalian intestine and airway epithelia (Hirose et al. 2003; Evans et al. 2005; Hwang and Lee 2007; Hwang et al. 2011). $Cl^-$ ions are actively taken up across the basolateral membrane of the MRC by the secretory isoform of sodium-potassium-2 chloride transporter (NKCC1), which in turn relies on the $Na^+/K^+$-ATPase to maintain a favorable inward gradient for sodium. As chloride accumulates in the cell above its electrochemical equilibrium, it can then exit across the apical membrane through a homolog of the cystic fibrosis transmembrane conductance regulator (CFTR). Active chloride transport is accompanied by passive $Na^+$ secretion along an electrical potential gradient, through the paracellular space and across "leaky" tight junctions between the MRC and adjacent cells (Karnaky 1998; Hirose et al. 2003; Evans et al. 2005; Hwang and Lee 2007; Hwang et al. 2011). In order to maintain an osmotic steady state in hypersaline environments, these cells must be able to secrete the entire load of NaCl accumulated from both intestinal absorption and passive influx across body surfaces (primarily gills). Thus, in theory, acclimation to hypersaline conditions might involve adjustments in both transport capacity and ion permeability properties of the gill epithelium. As noted earlier, the gills are also called upon to excrete acid loads derived from the bicarbonate secretory process of the intestine.

Following the original observations of Epstein et al. (1967) of large increases in gill NKA activity in seawater- versus freshwater-adapted *F. heteroclitus*, similar observations have been made on numerous euryhaline species following seawater adaptation (see Evans et al. 2005; Hwang and Lee 2007). For some species, the increased enzymatic activity may be partially attributed to changes in the α-subunit isoform (Richards et al. 2003; Hwang and Lee 2007; Hwang et al. 2011). Changes in NKA activity are paralleled by increased mRNA and protein abundance, although in some species these changes showed a transient response (Tipsmark, et al. 2002; Lin et al. 2004; Scott et al. 2004). Transfer of euryhaline fish to seawater also increases both mRNA and protein expression for gill NKCC and CFTR in several species (Singer et al. 1998; Wilson et al. 2000; Cutler and Cramb 2002; Marshall et al. 2002; Tipsmark et al. 2002, 2004, 2008; Katoh et al. 2003; Wu et al. 2003; Scott et al. 2004; Tse et al. 2006; Hiroi and McCormick 2007; Ouattara et al. 2009; Yang et al. 2011).

In hypersaline environments, the challenges for gill NaCl secretion are even more pronounced. Large, further increases in gill NKA activity have been reported in numerous hypersaline-adapted species, including sea bass (*D. labrax*), sea bream (*S. sarba* and *S. auratus*), pupfish (*Cyprinodon variegatus*), Mozambique tilapia (*O. mossambicus*), sailfin mollies (*P. latipinna*), milkfish (*Chanos chanos*), Senegalese sole (*Solea senegalensis*), and spotted green puffer fish (*Tetraodon nigroviridis*; Karnaky et al. 1976; Kültz et al. 1992; Jensen et al. 1998; Deane and Woo 2004; Gonzalez et al. 2005; Laiz-Carrión et al. 2005; Arjona et al. 2007; Bagherie-Lachidan et al. 2008; Tang et al. 2009). A study of black-chinned tilapia (*Sarotherodon melanotheron*) obtained from different sites in Senegal with a wide range of salinities (3 to 102 ppt) similarly demonstrated a correlation between increasing salinities and NKA activity (Lorin-Nebel et al. 2012).

Changes in gill NKA activity and protein levels can mediate relatively short-term adjustments to seawater or hypersaline adaptation. A number of studies have also shown changes in the numbers, size, and organization of the MRC population, which clearly mediate tolerance of these conditions. In seawater-adapted fish, MRCs are typically found clustered together, along with “accessory” cells, sometimes regarded as a precursor or partially differentiated version of the MRC (Sardet et al. 1979; Hootman and Philpott 1980; Wendelaar Bonga and van der Meij 1989; Sardella et al. 2004; Evans et al. 1999, 2005). These cell clusters form a deep invagination or pit at the apical side and interdigitations along their shared margins (Sardet et al. 1979; Kültz et al. 1995; Karnaky 1998; Uchida et al. 2000; Varsamos et al. 2002; Evans et al. 1999, 2005; Figure 3). The MRCs are characterized by a highly developed basolateral network of membrane infoldings, which provides an amplified membrane surface for $Na^+/K^+$-ATPase transporters (Karnaky 1998; Evans et al. 2005).

In some euryhaline species, rapid changes in branchial $Na^+/K^+$-ATPase activity have been observed that may result from changes in membrane trafficking or subunit isoform switching (Mancera and McCormick 2000; Tipsmark and Madsen 2001). However, in most cases of long-term acclimation to hypersaline conditions, the increased NaCl secretory capacity of the gills results from increases in number or cell size (or both) of the MRC population, along with the multicellular organization described above and increased basolateral infolding (Karnaky et al. 1976; Kültz et al. 1992; Kültz and Onken 1993; Uchida et al. 2000; Varsamos et al. 2002; Laiz-Carrión et al. 2005; Ouattara et al. 2009; Lorin-Nebel et al. 2012). There is considerable evidence that growth hormone/insulin-like growth factor 1 (GH/IGF-1), together with cortisol, mediate the conversion of MRC stem cells into functional MRCs during seawater adaptation (Sakamoto et al. 1993; Wong and Chan 1999; McCormick 2001), although this has not been investigated for hypersaline adaptation. In a study of the hybrid "California" Mozambique tilapia, transition from 35 to 65 ppt salinity significantly increased the number of identified "accessory" MRCs, while also doubling the number of apical pits and apoptotic cells in the gill tissue (Sardella et al. 2004). With even more extreme conditions (up to 95 ppt), the accessory cell number and the apoptotic cell number showed further large increases, while cells identified as mature MRCs decreased. These results could suggest an increased recruitment and turnover of the MRC population, or a transition to an alternative strategy of reducing branchial NaCl permeability in the face of such steep gradients (Sardella et al. 2004). It is worth noting that hypersaline exposure also induces increased mRNA expression of heat shock proteins in the gills of sea bream (Deane et al. 2002; Deane and Woo 2004) and in black-chinned tilapia sampled from the most extreme salinity locations of the Saloum estuary in Senegal (Tine et al. 2010), suggesting a level of salinity that approaches the physiological tolerance and activates stress responses in different species.

As described earlier (Figure 3), the cellular organization of seawater-acclimated gills includes clusters of MRC and accessory cells, with extended cell processes from the latter forming interdigitations along the MRCs (Sardet et al. 1979; Evans et al. 1999, 2005). These processes have been of significant interest in that they represent expanded areas of tight junction interactions within the cell clusters. These tight junctions are in contrast to those between the MRC/accessory cell cluster and the surrounding epithelium of the filaments. Thus, both the ultrastructural complexity and the permeability properties of the MRC/accessory cell junctions suggest increased "leakiness" compared to the rest of the epithelium,

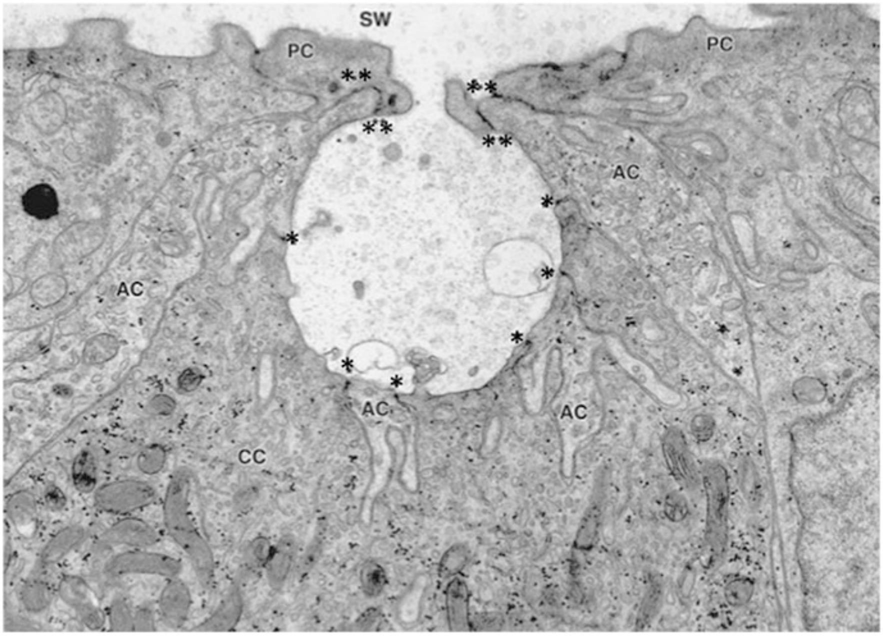


**Fig. 3** Electron micrograph of the apical region of a gill MRC cell cluster from a seawater-acclimated killifish (from Evans et al. 1999). The MRC (previously referred to as a chloride cell—CC) forms a complex with adjacent accessory cells (AC), with a characteristic deep apical pit opening to the external environment (SW). At the boundaries, the cell cluster joins with pavement cells (PC), the likely sites of respiratory gas exchange. In seawater-acclimated fish, accessory cells project interdigitating processes into the MRC; two of these can be seen near the base of the pit in this image. Tight junctions between the MRC and these AC processes are shallow and presumably form low-resistance junctions (*asterisk*), whereas those that form between the MRC and pavement cells are much deeper and "tighter" (*double asterisk*). A similar organization of MRC clusters and tight junctions has been described for hypersaline-acclimated sea bass (Varsamos et al. 2002)

essentially creating small, defined low-resistance pathways within the otherwise low permeability barrier of the gill epithelium (Sardet et al. 1979; Hootman and Philpott 1980; Karnaky 1998; Varsamos et al. 2002; Evans et al. 2005). Sardet et al. (1979), for example, demonstrated single-stranded tight junctional complexes that were permeable to lanthanum within the MRC clusters, but multi-stranded non-penetrable junctions elsewhere in the epithelium. This arrangement provides for a generally "tight" gill epithelium, thus minimizing the passive uptake of salt from the hyperosmotic environment, while still providing a necessary, low-resistance pathway within the MRC/accessory cell clusters, to accommodate paracellular $Na^+$ movement as the counterion for secondary active transport of $Cl^-$ (Sardet et al. 1979; Hootman and Philpott 1980; Karnaky 1998; Evans et al. 2005). It is notable that the organization and junctional structure of hypersaline-acclimated sea bass gills is the same as seawater-adapted fish (Varsamos et al. 2002).

Studies of whole animal transepithelial potentials (TEP) have been performed on several species including eels, flounder, and killifish (Potts and Eddy 1973; House and Maetz 1974; Wood and Grosell 2008, 2009). These measurements are interpreted as diffusion potentials arising from selective ion diffusion across the gill epithelium. In all species, seawater exposure resulted in inside-positive TEP values, consistent with a permeability ratio favoring cation (i.e., $Na^+$) diffusion across the gill epithelium, whereas freshwater exposure resulted in negative TEP values. The positive TEP values in seawater would support extrusion of $Na^+$ ions across the junctions, driven by the secondary active transport of chloride. This pattern can be replicated in killifish exposed to progressive changes in salinity, as would occur with tidal fluctuations normally encountered by this species (Wood and Grosell 2009). Thus, these data support the hypothesis of a cation-selective paracellular pathway in gill epithelium, which may also be capable of relatively rapid (24–48 h) changes in permeability properties (Wood and Grosell 2008).

There is reason to hypothesize (and some evidence to support the idea) that the structural organization and properties of the hypersaline-adapted gill are similar to those of seawater-adapted fish. However, there is also a possibility that a very different adaptive strategy for survival becomes evident with exposure to salinities higher than seawater. Kültz and Onken (1993) used electrophysiological methods to study MRC transport by the operculum epithelium, a surrogate for the gill, from tilapia adapted to a range of salinities. Like the gill, the operculum responds to hypersalinity with a further increase (above seawater) in the number of MRCs. However, chloride transport (measured as the short circuit current, $I_{SC}$) stabilized at salinities above seawater, with no further increase. Moreover, calculated values for both chloride cell conductance and the "leak" conductance decreased at higher salinities (Kültz and Onken 1993). The authors suggest that these opercular changes may be representative of a more widespread, whole body reduction in ion permeability. Thus, in responding to salinity challenges up to seawater levels, fish would increase branchial NaCl secretion to match the increasing load derived from drinking and passive influxes. At higher salinities, however, the strategy would change or be complemented by reduced passive influx, while still maintaining a constant NaCl secretory rate. More work will need to be done to determine whether this is a widespread adaptive mechanism and whether permeability changes also apply to the gill epithelium. Active NaCl secretion by the MRCs requires a passive efflux of $Na^+$ ions through the paracellular tight junctions.

The control of epithelial tight junction permeability, both within the gills and throughout exposed body surfaces, is likely to be critical for hypersaline survival by reducing the passive influx of NaCl. The physiological properties of tight junctions in different tissues and in different environmental circumstances are largely determined by a large family of tight junction proteins known as claudins (Elkouby-Naor and Ben-Yosef 2010). In spotted green puffer fish (*Tetraodon nigroviridis*) exposed to freshwater, seawater, and hypersaline conditions, there was a progressive decrease in mRNA expression of two different claudin-3 gene forms in the gill with increasing salinity, which inversely correlated with increased $Na^+/K^+$-ATPase activity (Bagherie-Lachidan et al. 2008). These same gene forms also showed marked decreases in mRNA expression in kidney, but had increased expression in skin tissue with increasing salinity exposure. In mammalian systems, claudin-3 overexpression induces an increase in the paracellular electrical resistance (Milatz et al. 2010). Thus, the tissue-specific changes seen in claudin-3 gene expression in the puffer fish support the proposal of reduced whole body ion permeability at high salinities (Kültz and Onken 1993), even while gill permeability, possibly confined to the MRC clusters, may be increased in order to provide a paracellular pathway for $Na^+$ leak. Claudin-3 and claudin-4 protein expressions were also seen to decrease with seawater adaptation in tilapia (Tipsmark et al. 2008). It will be of interest to continue further studies on the expression and especially localization of claudins, as well as occludins—another family of tight junction proteins (Chasiotis et al. 2010), under conditions of hypersaline adaptation.

## 5 Conclusions and Outlook

The global diversification of fishes is reflected also in the remarkable success of many species in adapting to extreme environments of all types. In this chapter, we have focused on mechanisms that allow a number of different species to maintain osmotic homeostasis in the face of extreme hypersaline conditions. As in seawater, but with much greater intensity, the challenge is to minimize or offset the loss of water and gain of electrolytes from the external environment. The strategies range from increasing transport capacity in the intestine and gills to changes in branchial water permeability, reduced whole animal electrolyte permeability, organization of branchial secretory cells (MRC complexes), and exquisite regulation of the chemistry and pH of the intestinal contents. No doubt these are not mutually exclusive. It will be important to continue to study additional species and to focus on molecular aspects of these physiological mechanisms. In particular, the roles of tight junctional proteins and of aquaporins in controlling branchial and integument permeabilities are intriguing and should be pursued. Also of interest is the significance of gill MRC-accessory cell interactions and turnover under extreme conditions, and the segmental specialization of transport properties along the intestine. The regulatory mechanisms controlling this plasticity, particularly in species that are subject to varying salinities, are beginning to be addressed at the genomic level (Tine et al. 2008; Whitehead et al. 2013); similar studies of hypersaline-tolerant fish will help to define the role of transcriptional regulation in adaptation to extreme salinity. Finally, it is recognized that hypersaline conditions create a great deal of stress on cells, and the role of stress-protecting mechanisms, such as heat shock proteins, in preventing damage to cellular proteins is an important area to further study. All these areas should provide new insights into the complex mechanisms that allow for adaptation to these conditions.

In a broader context, the role of salinity tolerance as a driver of speciation is being addressed in euryhaline species, particularly in killifish belonging to the genera *Lucania* and *Fundulus* (Fuller et al. 2007; Berdan and Fuller 2012;

Whitehead et al. 2013). For example, *Lucania goodei* and *L. parva* are considered closely related sister species, but with very different salinity tolerances. Evidence indicates that this differential salinity tolerance has led to ecological speciation and a behavioral mechanism of reproductive isolation (Fuller et al. 2007; Berdan and Fuller 2012). In *Fundulus*, the divergence of gene expression patterns in response to osmotic challenge has been observed both between closely related species and between populations of the same species, again providing evidence of salinity tolerance as a force for diversification (Whitehead et al. 2013). A series of recent studies have looked at natural populations of the black-chinned tilapia in West African estuaries (e.g., Saloum estuary in Senegal) with salinities at different sample sites ranging from freshwater to hypersaline (Tine et al. 2008; Ouattara et al. 2009; Lorin-Nebel et al. 2012). Of note is that some upstream sites on the estuary are hypersaline compared to the mouth (i.e., "inverse estuary"), which is thought to be a result of long-term drought conditions and global climate change. As discussed earlier, fish from hypersaline populations exhibited increased number and size of MRCs, as well as increased NKA activity in the gills (Tine et al. 2008; Lorin-Nebel et al. 2012). Such plasticity among these estuarine populations may provide another model system for studying salinity tolerance as a driver of evolution.

## References

Aoki M, Kaneko T, Katoh F, Hasegawa S, Tsutsui N, Aida K (2003) Intestinal water absorption through aquaporin 1 expressed in the apical membrane of mucosal epithelial cells in seawater-adapted Japanese eel. J Exp Biol 206:3495–3505

Arjona FJ, Vargas-Chacoff L, Ruiz-Jarabo I, Martín del Río MP, Mancera JM (2007) Osmoregulatory response of Senegalese sole (*Solea senegalensis*) to changes in environmental salinity. Comp Biochem Physiol A 148:413–421

Bagherie-Lachidan M, Wright SI, Kelly SP (2008) Claudin-3 tight junction proteins in *Tetraodon nigroviridis*: cloning, tissue-specific expression, and a role in hydromineral balance. Am J Physiol 294:R1638–R1647

Berdan EL, Fuller RC (2012) Interspecific divergence of ionoregulatory physiology in killifish: insight into adaptation and speciation. J Zool 287:283–291

Brunet FG, Crollius HR, Paris M, Aury J-M, Gibert P, Jaillon O, Laudet V, Robinson-Rechavi M (2006) Gene loss and evolutionary rates following whole-genome duplication in teleost fishes. Mol Biol Evol 23:1808–1816

Chasiotis H, Wood CM, Kelly SP (2010) Cortisol reduces paracellular permeability and increases occludin abundance in cultured trout gill epithelia. Mol Cell Endocrinol 323:232–238

Choi YJ, Shin HS, Kim NN, Cho SH, Yamamoto Y, Ueda H, Lee J, Choi CY (2013) Expression of aquaporin-3 and -8 mRNAs in the parr and smolt stages of sockeye salmon, *Oncorhynchus nerka*: effects of cortisol treatment and seawater acclimation. Comp Biochem Physiol A 165:228–236

Colin DA, Nonnotte G, Nonnotte L (1985) $Na^+$ transport and enzyme activities in the intestine of the freshwater and sea-water adapted trout (*Salmo gairdneri*). Comp Biochem Physiol 81A:695–698

Cooper CA, Whittamore JM, Wilson RW (2010) $Ca^{2+}$-driven intestinal $HCO_3^-$ secretion and $CaCO_3$ precipitation in the European flounder in vivo: influences on acid-base regulation and blood gas transport. Am J Physiol 298:R870–R876

Cutler CP, Cramb G (2002) Two isoforms of the Na+/K+/2CI(-) cotransporter are expressed in the European eel (*Anguilla anguilla*). Biochim Biophys Acta 1566:92–103

Cutler CP, Brezillon S, Bekir S, Sanders IL, Hazon N, Cramb G (2000) Expression of a duplicate $Na^+$, $K^+$-ATPase ß1 isoform in the European eel (*Anguilla anguilla*). Am J Physiol 279:R222–R229

Cutler CP, Martinez AS, Cramb G (2007) The role of aquaporin 3 in teleost fish. Comp Biochem Physiol 148A:82–91

Day F (1887) British and Irish Salmonidae. Williams and Norgate, London

Deane EE, Woo NYS (2004) Differential gene expression associated with euryhalinity in sea bream (*Sparus sarba*). Am J Physiol 287:R1054–R1063

Deane EE, Woo NYS (2006) Tissue distribution, effects of salinity acclimation, and ontogeny of aquaporin 3 in the marine teleost, silver sea bream (*Sparus sarba*). Marine Biotech 8:663–671

Deane EE, Kelly SP, Luk JCY, Woo NYS (2002) Chronic salinity adaptation modulates hepatic heat shock protein and insulin-like growth factor I expression in black sea bream. Marine Biotech 4:193–205

Diamond JM, Bossert WH (1967) Standing-gradient osmotic flow-a mechanism for coupling of water and solute transport in epithelia. J Gen Physiol 50:2061–2083

Elkouby-Naor L, Ben-Yosef T (2010) Functions of claudin tight junction proteins and their complex interactions in various physiological systems. Int Rev Cell Mol Biol 279:1–32

Epstein FH, Katz AI, Pickford GE (1967) Sodium- and potassium-activated adenosine triphosphatase of gills: role in adaptation of teleosts to salt water. Science 156:1245–1247

Esbaugh AJ, Grosell M (2014) Esophageal desalination is mediated by $Na^+$, $H^+$ exchanger-2 in the gulf toadfish (*Opsanus beta*). Comp Biochem Physiol A171:57–63

Evans DH (2008) Teleost fish osmoregulation: what have we learned since August Krogh, Homer Smith, and Ancel Keys. Am J Physiol 295:R704–R713

Evans DH, Piermarini PM, Potts WTW (1999) Ionic transport in the fish gill epithelium. J Exp Zool 283:641–652

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev 85:97–177

Faggio C, Torre A, Lando G, Sabatino G, Trischitta F (2011) Carbonate precipitates and bicarbonate secretion in the intestine of sea bass, *Dicentrachus labrux*. J Comp Physiol B 181:517–525

Fu C, Wilson JM, Rombough PJ, Brauner CJ (2010) Ions first: $Na^+$ uptake shifts from the skin to the gills before $O_2$ uptake in developing rainbow trout, *Oncorhynchus mykiss*. Proc R Soc B 277:1553–1560

Fuller RC, McGhee KE, Schrader M (2007) Speciation in killifish and the role of salt tolerance. J Evol Biol 20:1962–1975

Genz J, Taylor JR, Grosell M (2008) Effects of salinity on intestinal bicarbonate secretion and compensatory regulation of acid-base balance in *Opsanus beta*. J Exp Biol 211:2327–2335

Genz J, McDonald MD, Grosell M (2011) Concentration of $MgSO_4$ in the intestinal lumen of *Opsanus beta* limits osmoregulation in response to acute hypersalinity stress. Am J Physiol 300:R895–R909

Giffard-Mena I, Boulo V, Aujoulat F, Fowden H, Castille R, Charmantier G, Cramb G (2007) Aquaporin molecular characterization in the sea-bass (*Dicentrarchus labrax*): The effect of salinity on AQP1 and AQP3 expression. Comp Biochem Physiol A 148:430–444

Gonzalez R (2012) The physiology of hyper-salinity tolerance in teleost fish: a review. J Comp Physiol B 182:321–329

Gonzalez RJ, Cooper J, Head D (2005) Physiological responses to hyper-saline waters in sailfin mollies (*Poecilia latipinna*). Comp Biochem Physiol A 142:397–403

Gregório SF, Carvalho ESM, Encarnação S, Wilson JM, Power DM, Canário AVM, Fuentes J (2013) Adaptation to different salinities exposes functional specialization in the intestine of the sea bream (*Sparus aurata* L.). J Exp Biol 216:470–479

Grosell M (2006) Intestinal anion exchange in marine fish osmoregulation. J Exp Biol 209:2813–2827

Grosell M (2011) Intestinal anion exchange in marine teleosts is involved in osmoregulation and contributes to the oceanic inorganic carbon cycle. Acta Physiol 202:421–434

Grosell M, Genz J (2006) Ouabain-sensitive bicarbonate secretion and acid absorption by the marine teleost fish intestine play a role in osmoregulation. Am J Physiol 291:R1145–R1156

Grosell M, Taylor JR (2007) Intestinal anion exchange in teleost water balance. Comp Biochem Physiol A 148:14–22

Grosell M, Gilmour KM, Perry SF (2007) Intestinal carbonic anhydrase, bicarbonate, and proton carriers play a role in the acclimation of rainbow trout to seawater. Am J Physiol 293:R2099–R2111

Grosell M, Genz J, Taylor JR, Perry SF, Gilmour KM (2009a) The involvement of $H^+$-ATPase and carbonic anhydrase in intestinal $HCO_3^-$ secretion in seawater-acclimated rainbow trout. J Exp Biol 212:1940–1948

Grosell M, Mager EM, Williams C, Taylor JR (2009b) High rates of $HCO_3^-$ secretion and $Cl^-$ absorption against adverse gradients in the marine teleost intestine: the involvement of an electrogenic anion exchanger and $H^+$ -pump metabolon? J Exp Biol 212:1684–1696

Guffey S, Esbaugh A, Grosell M (2011) Regulation of apical $H^+$-ATPase activity and intestinal $HCO_3^-$ secretion in marine fish osmoregulation. Am J Physiol 301:R1682–R1691

Hirano T, Mayer-Gostan N (1976) Eel esophagus as an osmoregulatory organ. Proc Natl Acad Sci USA 73:1348–1350

Hiroi J, McCormick SD (2007) Variation in salinity tolerance, gill $Na^+/K^+$-ATPase, $Na^+/K^+/2Cl^-$ cotransporter and mitochondria-rich cell distribution in three salmonids *Salvelinus namaycush*, *Salvelinus fontinalis* and *Salmo salar*. J Exp Biol 210:1015–1024

Hirose S, Kaneko T, Naito N, Takei Y (2003) Molecular biology of major components of chloride cells. Comp Biochem Physiol B 136:593–620

Hootman SR, Philpott CW (1980) Accessory cells in teleoost branchial epithelium. Am J Physiol 238:R199–R206

House CR, Maetz J (1974) On the electrical gradient across the gill of the sea water-adapted eel. Comp Biochem Physiol 47A:917–924

Hwang PP, Lee TH (2007) New insights into fish ion regulation and mitochondrion-rich cells. Comp Biochem Physiol A 148:479–497

Hwang P-P, Lee T-H, Lin L-Y (2011) Ion regulation in fish gills: recent progress in the cellular and molecular mechanisms. Am J Physiol 301:R28–R47

Isaia J, Hirano T (1975) Effect of environmental salinity change on osmotic permeability of the isolated gill of the eel, *Anguilla anguilla* L. J Physiol Paris 70:737–747

Jensen MK, Madsen SS, Kristiansen K (1998) Osmoregulation and salinity effects on the expression and activity of $Na^+$, $K^+$-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). J Exp Zool 282:290–300

Jung D, Sato JD, Shaw JR, Stanton BA (2012) Expression of aquaporin 3 in gills of the Atlantic killifish (*Fundulus heteroclitus*): effects of seawater acclimation. Comp Biochem Physiol A 161:320–326

Karnaky KJ Jr (1998) Osmotic and ionic regulation. In: Evans DH (ed) The physiology of fishes, 2nd edn. CRC, Boca Raton, pp 157–176

Karnaky KJ Jr, Ernst SA, Philpott CW (1976) Teleost chloride cell. I. Response of the pupfish Cyprinodon variegatus gill Na, K-ATPase and chloride cell fine structure to various high salinity environments. J Cell Biol 70:144–156

Katoh F, Hyodo S, Kaneko T (2003) Vacuolar-type proton pump in the basolateral plasma membrane energizes ion uptake in branchial mitochondria-rich cells of killifish *Fundulus heteroclitus*, adapted to low ion environment. J Exp Biol 206:793–803

Kirsch R, Guinier D, Meens R (1975) L'équilibre hydrique de l'Anguille européenne (*Anguilla anguilla* L.). Etude du role de l'oesaphage dans l'utilisation de l'eau de boisson et étude de la perméabilité osmotique branchiale. J Physiol Paris 70:605–626

Kültz D, Onken H (1993) Long-term acclimation of the teleost *Oreochromis mossambicus* to various salinities: two different strategies in mastering hypertonic stress. Marine Biol 117:527–533

Kültz D, Bastrop R, Jürss K, Siebers D (1992) Mitochondria-rich (MR) cells and the activities of the $Na^+/K^+$-ATPase and carbonic anhydrasee in the gill and opercular epithelium of *Oreochromis mossambicus* adapted to various salinities. Comp Biochem Physiol 102B:293–301

Kültz D, Jürss K, Jonas L (1995) Cellular and epithelial adjustments to altered salinity in the gill and opercular epithelium of a cichlid fish (*Oreochromis mossambicus*). Cell Tissue Res 279:65–73

Kurita Y, Nakada T, Kato A, Doi H, Mistry AC, Chang MH, Romero MF, Hirose S (2008) Identification of intestinal bicarbonate transporters involved in formation of carbonate precipitates to stimulate water absorption in marine teleost fish. Am J Physiol 294: R1402–R1412

Laiz-Carrión R, Guerreiro PM, Fuentes J, Canario AVM, Martín del Rio MP, Mancera JM (2005) Branchial osmoregulatory response to salinity in the gilthead sea bream, *Sparus auratus*. J Exp Zool 303A:563–576

Larsen EH, Willumsen NJ, Møjerg N, Sørensen JN (2009) The lateral intercellular space as osmotic coupling compartment in isotonic transport. Acta Physiol 195:171–186

Laverty G, Skadhauge E (2012) Adaptation of teleosts to very high salinity. Comp Biochem Physiol A 163:1–6

Lignot JH, Cutler CP, Hazon N, Cramb G (2002) Immunolocalisation of aquaporin 3 in the gill and the gastrointestinal tract of the European eel *Anguilla anguilla* (L.). J Exp Biol 205:2653–2663

Lin CH, Tsai RS, Lee TH (2004) Expression and distribution of Na, K-ATPase in gill and kidney of the spotted green pufferfish, *Tetraodon nigroviridis*, in response to salinity challenge. Comp Biochem Physiol A 138:287–295

Lorin-Nebel C, Avarre J-C, Faivre N, Wallon S, Charmantier G, Durand J-D (2012) Osmoregulatory strategies in natural populations of the black-chinned tilapia *Sarotherodon melanotheron* exposed to extreme salinities in West African estuaries. J Comp Physiol B 182:771–780

Lotan R, Skadhauge E (1972) Intestinal salt and water transport in a euryhaline teleost, *Aphanius dispar* (Cyprinodontidae). Comp Biochem Physiol 42A:303–310

Maetz J, Skadhauge E (1968) Drinking rates and gill ionic turn-over in relation to external salinities in the eel. Nature 217:371–373

Mancera JM, McCormick SD (2000) Rapid activation of gill $Na^+$, $K^+$-ATPase in the euryhaline teleost *Fundulus heteroclitus*. J Exp Zool 287:263–274

Marshall WS, Grosell M (2005) Ion transport, osmoregulation and acid-base balance. In: Evans DH, Claibourne JB (eds) Physiology of fishes. CRC, Boca Raton, pp 177–230

Marshall WS, Lynch EA, Cozzi RRF (2002) Redistribution of immunofluorescence of CFTR anion channel and NKCC cotransporter in chloride cells during adaptation of the killifish *Fundulus heteroclitus* to sea water. J Exp Biol 205:1265–1273

McCormick SD (2001) Endocrine control of osmoregulation in teleost fish. Am Zool 41:781–794

McDowell RM (2002) The origin of the salmonid fishes: marine, freshwater … or neither? Rev Fish Biol Fish 11:171–179

Milatz S, Krug SM, Rosenthal R, Günzel D, Müller D, Schulzke J-D, Amasheh S, Fromm M (2010) Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. Biochim Biophys Acta 1798:2048–2057

Motais R, Isaia J, Rankin JC, Maetz J (1969) Adaptive changes of the water permeability of the teleostean gill epithelium in relation to external salinity. J Exp Biol 51:529–546

Nagashima K, Ando M (1994) Characterization of esophageal desalination in the seawater eel, *Anguilla japonica*. J Comp Physiol B 164:47–54

Nordlie FG (2006) Physicochemical environments and tolerances of cyprinodontoid fishes found in estuaries and salt marshes of eastern North America. Rev Fish Biol Fish 16:51–106

Ogasawara T, Hirano T (1984) Effects of prolactin and environmental calcium on osmotic water permeability of the gills in the eel, *Anguilla japonica*. Gen Comp Endocrinol 53:315–324

Oide H, Utida S (1968) Changes in intestinal absorption and renal excretion of water during adaptation to seawater in the Japanese eel. Marine Biol 1:172–177

Ouattara N, Bodinier C, Nègre-Sadargues G, D'Cotta H, Messad S, Charmantier G, Panfili J, Baroiller J-F (2009) Changes in gill ionocyte morphology and function following transfer from fresh to hypersaline waters in the tilapia *Sarotherodon melanotheron*. Aquaculture 290:155–164

Parmelee JT, Renfro JL (1983) Esophageal desalination of seawater in flounder: role of active sodium transport. Am J Physiol 245:R888–R893

Potts WTW, Eddy FB (1973) Gill potentials and sodium fluxes in the flounder *Platichthys flesus*. J Comp Physiol 87:29–48

Potts WTW, Fleming WR (1970) The effects of prolactin and divalent ions on the permeability to water of *Fundulus kansae*. J Exp Biol 53:317–327

Potts WT, Foster MA, Rudy PP, Howells GP (1967) Sodium and water balance in the cichlid teleost, *Tilapia mossambica*. J Exp Biol 47:461–470

Richards JG, Semple JW, Bystriansky JS, Schulte PM (2003) $Na^+/K^+$-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. J Exp Biol 206:4475–4486

Sakamoto T, McCormick SD, Hirano T (1993) Osmoregulatory actions of growth hormone and its mode of action in salmonids: a review. Fish Physiol Biochem 11:155–164

Santini F, Harmon LJ, Carnevale G, Alfaro ME (2009) Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. BMC Evol Biol 9:194. doi:10.1186/1471-2148-9-194

Sardella BA, Matey V, Cooper J, Gonzalez RJ, Brauner CJ (2004) Physiological, biochemical and morphological indicators of osmoregulatory stress in "California" Mozambique tilapia (*Oreochromis mossambicus* × *O. urolepis hornorum*) exposed to hypersaline water. J Exp Biol 207:1399–1413

Sardet C, Pisam M, Maetz J (1979) The surface epithelium of teleostean fish gills. Cellular and junctional adaptations of the chloride cell in relation to salt adaptation. J Cell Biol 80:96–117

Sattin G, Mager EM, Beltramini M, Grosell M (2010) Cytosolic carbonic anhydrase in the Gulf toadfish is important for tolerance to hypersalinity. Comp Biochem Physiol A 156:169–175

Scott GR, Richards JG, Forbush B, Isenring P, Schulte PM (2004) Changes in gene expression in gills of the euryhaline killifish *Fundulus heteroclitus* after abrupt salinity transfer. Am J Physiol 287:C300–C309

Seidelin M, Madsen SS, Blenstrup H, Tipsmark CK (2000) Time-course changes in the expression of $Na^+$, $K^+$-ATPase in gills and pyloric caeca of brown trout (*Salmo trutta*) during acclimation to seawater. Physiol Biochem Zool 73:446–453

Shehadeh ZH, Gordon MS (1969) The role of the intestine in salinity adaptation of the rainbow trout, *Salmo gairdneri*. Comp Biochem Physiol 30:397–418

Singer TD, Tucker SJ, Marshall WS, Higgins CF (1998) A divergent CFTR homologue: highly regulated salt transport in the euryhaline teleost *F. heteroclitus*. Am J Physiol 274:C715–C723

Skadhuage E (1969) The mechanism of salt and water absorption in the intestine of the eel (*Anguilla anguilla*) adapted to waters of various salinities. J Physiol 204:135–158

Skadhauge E (1974) Coupling of transmural flows of NaCl and water in the intestine of the eel (*Anguilla anguilla*). J Exp Biol 60:535–546

Skadhauge E (1980) Water transport in the vertebrate intestine. In: Gilles R (ed) Animals and environmental fitness. Physiological and biochemical aspects of adaptation and ecology, vol 1. Pergamon Press, Oxford, pp 79–90

Skadhauge E, Lotan R (1974) Drinking rates and oxygen consumption in the euryhaline teleost *Aphanius dispar* in waters of high salinity. J Exp Biol 60:547–556

Smith HW (1930) The absorption and excretion of water and salts by marine teleosts. Am J Physiol 93:480–505

Tang C-H, Chiu Y-H, Tsai S-C, Lee T-H (2009) Relative changes in the abundance of branchial $Na^+/K^+$-ATPase α-isoform-like proteins in marine euryhaline milkfish (*Chanos chanos*) acclimated to environments of different salinities. J Exp Zool 311A:521–529

Taylor JR, Mager EM, Grosell M (2010) Basolateral NBCe1 plays a rate-limiting role in transepithelial intestinal $HCO_3^-$ secretion, contributing to marine fish osmoregulation. J Exp Biol 213:459–468

Tine M, deLorgeril J, D'Cotta H, Pepey E, Bonhomme F, Baroiller JF, Durand J-D (2008) Transcriptional responses of the black-chinned tilapia *Sarotherodon melanotheron* to salinity extremes. Mar Genomics 1:37–46

Tine M, Bonhomme F, Mckenzie DJ, Durand J-D (2010) Differential expression of the heat shock protein Hsp70 in natural populations of the tilapia, *Sarotherodon melanotheron*, acclimatised to a range of environmental salinities. BMC Ecol 10:11

Tipsmark CK, Madsen SS (2001) Rapid modulation of $Na^+/K^+$-ATPase activity in osmoregulatory tissues of a salmonid fish. J Exp Biol 204:701–709

Tipsmark CK, Madsen SS, Seidelin M, Christensen AS, Cutler CP, Cramb G (2002) Dynamics of $Na^+$, $K^+$, $2Cl^-$ cotransporter and $Na^+$, $K^+$-ATPase expression in the branchial epithelium of brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). J Exp Zool 293:106–118

Tipsmark CK, Madsen SS, Borski RJ (2004) Effect of salinity on expression of branchial ion transporters in striped bass (*Morone saxatilis*). J Exp Zool 301A:979–991

Tipsmark CK, Baltzegar DA, Ozden O, Grubb BJ, Borski RJ (2008) Salinity regulates claudin mRNA and protein expression in the teleost gill. Am J Physiol 294:R1004–R1014

Tipsmark CK, Sørensen KJ, Madsen SS (2010) Aquaporin expression dynamics in osmoregulatory tissues of Atlantic salmon during smoltification and seawater acclimation. J Exp Biol 213:368–379

Tresguerres M, Levin LR, Buck J, Grosell M (2010) Modulation of NaCl absorption by $[HCO_3^-]$ in the marine teleost intestine is mediated by soluble adenylyl cyclase. Am J Physiol 299:R62–R71

Tse WKF, Au DWT, Wong CKC (2006) Characterization of ion channel and transporter mRNA expressions in isolated gill chloride and pavement cells of seawater acclimating eels. Biochem Biophys Res Commun 346:1181–1190

Uchida K, Kaneko T, Miyazaki H, Hasegawa S, Hirano T (2000) Excellent salinity tolerance of Mozambique tilapia (*Oreochromic mossambicus*): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool Sci 17:149–160

Varsamos S, Diaz JP, Charmantier G, Flik G, Blasko C, Connes R (2002) Branchial chloride cells in sea bass (*Dicentrarchus labrax*) adapted to fresh water, seawater, and doubly concentrated seawater. J Exp Zool 293:12–26

Vega GC, Wiens JJ (2012) Why are there so few fish in the sea? Proc R Soc B 279:2323–2329

Verkman AS (2011) Aquaporins at a glance. J Cell Sci 124:2107–2112

Watanabe S, Kaneko T, Aida K (2005) Aquaporin-3 expressed in the basolateral membrane of gill chloride cells in Mozambique tilapia *Oreochromis mossambicus* adapted to freshwater and seawater. J Exp Biol 208:2673–2682

Wendelaar Bonga SE, van der Meij CJM (1989) Degeneration and death, by apoptosis and necrosis of the pavement and chloride cells in the gills of the teleost *Oreochromis mossambicus*. Cell Tissue Res 255:235–243

Whitehead A, Zhang S, Roach JL, Galvez F (2013) Common functional targets of adaptive micro- and macro-evolutionary divergence in killifish. Mol Ecol 22:3780–3796

Whittamore JM (2012) Osmoregulation and epithelial water transport: lessons from the intestine of marine teleost fish. J Comp Physiol B 182:1–39

Whittamore JM, Cooper CA, Wilson RW (2010) $HCO_3^-$ secretion and $CaCO_3$ precipitation play major roles in intestinal water absorption in marine teleost fish in vivo. Am J Physiol 298: R877–R886

Wilson JM, Laurent P, Tufts BL, Benos DJ, Donowitz M, Vogl AW, Randall DJ (2000) NaCl uptake by the branchial epithelium in freshwater teleost fish: an immunological approach to ion-transport protein localization. J Exp Biol 203:2279–2296

Wilson RW, Wilson JM, Grosell M (2002) Intestinal bicarbonate secretion by marine teleost fish—why and how? Biochim Biophys Acta 1566:182–193

Wong CKC, Chan DKO (1999) Chloride cell subtypes in the gill epithelium of Japanese eel *Anguilla japonica*. Am J Physiol 277:R517–R522

Wood CM, Grosell M (2008) A critical analysis of transepithelial potential in intact killifish (*Fundulus heteroclitus*) subjected to acute and chronic changes in salinity. J Comp Physiol B 178:713–727

Wood CM, Grosell M (2009) TEP on the tide in killifish (*Fundulus heteroclitus*): effects of progressively changing salinity and prior acclimation to intermediate or cycling salinity. J Comp Physiol B 179:459–467

Wu YC, Lin LY, Lee TH (2003) $Na^+$, $K^+$, $2Cl^-$ cotransporter: a novel marker for identifying freshwaater- and seawater-type mitochondria-rich cells in gills of the euryhaline tilapia, *Oreochromis mossambicus*. Zool Stud 42:186–192

Yang W-K, Kang C-K, Chen T-Y, Chang W-B, Lee T-H (2011) Salinity-dependent expression of the branchial $Na^+/K^+/2Cl^-$ cotransporter and $Na^+/K^+$-ATPase in the sailfin molly correlates with hypoosmoregulatory endurance. J Comp Physiol B 181:953–964

# Life in the Fast Lane: A Review of Rheophily in Freshwater Fishes

**Nathan K. Lujan and Kevin W. Conway**

**Abstract** Ecological opportunities and physical challenges of fast-water habitats have dramatically shaped the evolution of freshwater fish lineages from a broad diversity of clades globally, often leading to the convergent or parallel evolution of highly similar morphologies. In this chapter, we present a patch dynamics model of how longitudinal shifts in geomorphological and ecological processes from small headwater torrents to large river rapids may differentially affect gene flow among, and evolutionary specialization within, resident rheophilic fish populations. Fast-water habitats offer ecological advantages including predator avoidance and increased foraging efficiency, but require that organisms resist downstream displacement and avoid shifting, crushing substrates. We review the specialized morphological and behavioral characteristics associated with life in fast waters and the taxonomic distribution of these specializations across fishes. We also report results of specific functional studies where available and summarize empirical evolutionary, phylogenetic support for our model and for specific mechanisms or pathways by which rheophilic specializations may arise.

## 1 Introduction

From cold torrential streams high in the Himalayas to large river rapids in the lower Congo and Amazon basins, mesohabitats defined by concentrated high water velocities and rocky substrates are common features of fluvial ecosystems throughout the world. Variously known as torrents, riffles, rapids, chutes, and shoals,

---

N.K. Lujan (✉)
Department of Natural History, Royal Ontario Museum, Toronto, ON, Canada M5S 2C6

Center for Systematic Biology and Evolution, Academy of Natural Sciences of Drexel University, Philadelphia, PA 19103, USA
e-mail: nklujan@gmail.com

K.W. Conway
Biodiversity Research and Teaching Collections and Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA
e-mail: kevin.conway@tamu.edu

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_6

freshwater habitats hosting specialized rheophilic fish assemblages typically have water velocities of at least 40–50 cm/s (Bournaud 1963), but span a wide range of spatial scales, gradients, and maximum water velocities. In high gradient headwaters and montane rivers, particularly those draining young high-elevation mountains like the Andes and Himalayas, torrent zones extend largely uninterrupted for thousands of vertical meters before prevailing channel slopes begin to level out. In medium-sized and mid-elevation rivers where meanders dissipate kinetic energy from the torrent zone, mesohabitats typically coincide with meanders to take on an ordered riffle-run-pool sequence (Keller and Melhorn 1978). In large lowland rivers, average current velocities often exceed those in headwaters (Leopold 1953), but rapids habitats are generally rare, irregularly distributed, and clustered around low-lying, erosion-resistant geological formations.

Organisms that attempt to maintain a fixed position in lotic habitats must avoid or counteract both shear forces (forces created by water flowing in parallel with the channel or substrate) and turbulence (chaotic water movement formed by disruptions to laminar flow). Where streams become constrained by shallow, irregular channel bottoms as in rapids and riffles, both shear forces and turbulence increase (Hoover and Ackerman 2004). A generalized cross section of water velocities over a rounded stone exhibits highest velocities and shear forces within ~5–15 mm of the stone surface along the leading edge and top, with zones of turbulence at the lower upstream and downstream sides of the stone. Despite maximal water velocities near the top of the stone, velocities very close (<5 mm) to the stone surface approach 0 due to frictional drag of the stone on the water in a region known as the boundary layer (Ambühl 1962; Hoover and Ackerman 2004). Larval stages of many insects specialized for life in fast water (e.g., mayflies, stoneflies, psephenid beetles) are small enough or dorsoventrally flattened enough to escape shear stress by living entirely within this boundary layer of very low flow. Many rheophilic fishes also benefit from highly dorsoventrally depressed bodies (see Sect. 4.2), although few if any are small enough to exist entirely within the boundary layer.

Although the thin surface layers of rocks or wood, and spaces within or beneath such objects, can offer refuge from shear forces and the threat of downstream displacement, close association with these substrates also poses a threat to stream organisms when shear stress dislodges the substrates themselves. Indeed, the ever-present threat of both downstream displacement and being injured by shifting substrates makes life in fast-water habitats extremely challenging. Here, we review the distribution of these habitats across the landscape, the gradients in ecological and evolutionary processes that covary with a habitat's longitudinal position, the morphological responses to these processes, and the phylogenetic and functional studies of fast-water fishes and their specializations for life in the extreme. Few species or morphological traits associated with rheophilic habitats have been empirically investigated from a functional, performance, or correlated eco-evolutionary perspective. We therefore consider traits as specialized based largely on theory.

## 2 Taxonomic Distribution of Rheophilic Fishes

Previous comprehensive overviews of rheophilic fishes have focused on fauna inhabiting particular river drainages (Roberts and Stewart 1976; Zuanon 1999; Casatti and Castro 2006) or a circumscribed geological region encompassing multiple drainages (Hora 1930). Regardless of the scale, such studies frequently attempt to divide rheophilic fishes across a number of general categories, ranging from poorly adapted to highly adapted for life in fast-flowing water, based largely on external morphological features. For example, Hora (1930) recognized four general categories of hill-stream fishes in his overview of the Himalayan torrent fauna, ranging from "ordinary" (Hora's category I) to "flattened... provided with well-developed means of attachment" (Hora's category IV). Along similar lines, Roberts and Stewart (1976) divided the fishes collected in their comprehensive survey of the lower Congo rapids into three categories (poorly, moderately, and highly adapted to rapid habitats) and further divided those taxa that they considered to be highly adapted based on whether they were exposed to strong current (their "rheophilic") or avoided such currents (their "hyporheic").

In Appendix Table 1, we have attempted to provide a more global overview of rheophilic fishes than has been attempted previously. Our decision to include a particular taxon is based either on personal observations (for those taxa and regions that we are familiar), on published information contained in larger taxonomic revisions (e.g., Tan 2006; Kottelat 1990), or on faunal overviews for individual river basins (Monsembula Iyaba et al. 2013; Roberts and Stewart 1976; Roberts 1978, 1989a; Kullander et al. 1999; Parenti and Lim 2005; Tan and Kottelat 2009), countries (Chen 1998; Kottelat 2001a, b, 2006; McDowall 1978; Shrestha and Edds 2012; Yue 2000) or larger geographic regions including multiple countries (Talwar and Jhingran 1991; Kottelat et al. 1993; Kottelat and Freyhof 2008; Page and Burr 2011; Skelton 1993). For the sake of simplicity, we focus only on those taxa that would be considered obligate rheophiles, either moderately or highly adapted for life in fast water (sensu Roberts and Stewart 1976). We have included lacustrine or diadromous taxa that may spend only part of their life cycle in fast-flowing water but have excluded marine and estuarine taxa that are reported occasionally from faster water habitats in the lower courses of rivers that exhibit no obvious specializations for life in fast water (e.g., *Dactyloscopus*; Miller and Briggs 1962). Our list, which is likely to be incomplete, contains 419 genera, distributed across 60 families and 16 orders. As one would expect, our list is dominated by teleosts, and otophysans in particular, with 207 genera of Cypriniformes (representing 11 families), 92 genera of Siluriformes (representing 15 families), and 52 genera of Characiformes (representing 11 families). With 37 genera (representing 10 families), the "Perciformes" (sensu Wiley and Johnson 2010) contains the majority of the remaining rheophilic teleosts, most of which belong to one of three families, the Gobiidae (12 genera), the Cichlidae (10 genera), or the Percidae (7 genera). Two genera of lampreys (Petromyzontiformes) represent the only non-teleost taxa that are obligate inhabitants of fast-flowing waters.

# 3 Ecological Processes

## *3.1 Assemblage Composition Model*

River zonation typologies and ecological process models have expanded from static, modular depictions of hydrology and community composition (e.g., Hutchinson 1939; Illies 1961) to contemporary but dynamic linkages between a river's hydrogeomorphology, production sources, and faunal assembly. The recently proposed Riverine Ecosystem Synthesis (RES, Thorp et al. 2006), for example, provides a framework for integrating previous conceptual models (e.g., River Continuum Concept, Flood Pulse Concept, Riverine Productivity Model) into a comprehensive hierarchy of geomorphological processes and metacommunity patch dynamics. Although the original iteration of the RES was explicitly limited to recent ecological timescales (Thorp et al. 2006), a strength of the hierarchical patch dynamics approach is its limitless potential for spatial and temporal expansion. We propose a modified patch dynamics model narrowly focused on the geomorphology of fast-water habitats and community assembly of specialized resident (i.e., non-migratory) fast-water fish assemblages spanning both large, continental drainage basins (e.g., Amazon Basin, Mekong Basin) and timescales long enough to encompass macroevolutionary processes.

Our model (Fig. 1) contrasts high-gradient, high-elevation headwaters, which have low taxonomic and trophic diversity (Lujan et al. 2013) with large lowland rapids hosting species-rich assemblages that are functionally diverse and often narrowly endemic (Roberts and Stewart 1976; Roberts 1978; Zuanon 1999; Camargo et al. 2004). Despite the low $\alpha$-diversity (i.e., local, site-specific species richness) of headwaters, these habitats often exhibit high $\beta$-diversity (i.e., species

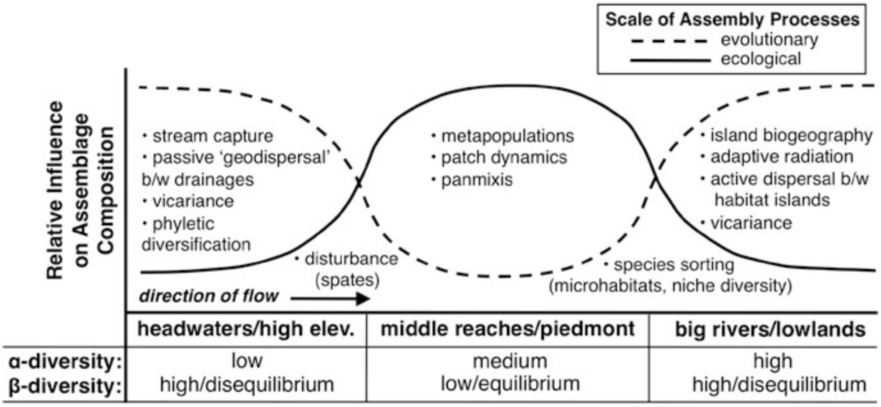


**Fig. 1** A conceptual model describing longitudinal shifts in the evolutionary vs. ecological scale of processes controlling the taxonomic composition of rheophilic fish assemblages from headwaters to large river rapids. See text for further explanation and references supporting specific components of the model

richness summed across sites; Carrara et al. 2012) due to low active dispersal between habitat patches combined with a slow background rate of passive "geodispersal" (i.e., stream capture) and subsequent vicariant speciation (e.g., Waters et al. 2001; Ribeiro 2006). Moreover, headwater populations may be small and have low genetic diversity due to founder effects (Hänfling and Weetman 2006; Barson et al. 2009), increasing rates of genetic drift and vicariant speciation if isolated from larger downstream populations.

Comparative community and habitat diversity data are largely lacking for large, lowland river rapids, but we hypothesize that, as with oceanic islands (Diamond et al. 1976; Losos and Ricklefs 2009), the species richness and functional and habitat diversity of river rapids correlate with their size and their distance from other large rapids. An additional factor unique to rivers is hydraulic energy, which should multiply effects of distance by increasing both the cost of dispersal and the reward of philopatry. Even relatively small distances between rapids with high hydraulic energies may promote isolation, vicariance, and endemic diversification by increasing the universal risk of downstream export should individuals move far from a preferred microhabitat (Markert et al. 2010; Schwarzer et al. 2011). As with both terrestrial islands (Losos and Schluter 2000) and lakes (Wagner et al. 2014), instances and rates of localized diversification (i.e., adaptive radiation) should also scale with rapids size, and vicariant speciation rates among rapids should increase with greater geographic distance and/or hydraulic energy. Also, given spatial variation in localized rates and outcomes of evolutionary specialization and diversification in both headwater and lowland river rapids habitats (i.e., all specialists are not present in all habitats), and the uneven distribution and scale of barriers to dispersal between similar habitat patches, we predict that niche occupancy and assemblage structure across the landscape rarely equilibrate and these disequilibria contribute to greater $\beta$-diversity.

At intermediate elevations and reaches, fish populations are typically more genetically diverse (Hänfling and Weetman 2006; Barson et al. 2009) and fish assemblages are more species rich than in headwaters, but they still lack many taxa and functional components of lowland habitats (Lujan et al. 2013). Fast-water habitats at intermediate elevations consist primarily of riffles periodically interrupted by deeper, slower runs and pools. Relative riffle length and pool depth increase as channel slope decreases (Wohl et al. 1993), suggesting that there is a gradient between upstream zones where habitat patches remain close enough and geomorphologically variable enough over long timescales to facilitate the free exchange of genetic diversity (panmixis), and downstream zones where geographic distances between habitat patches may be great enough to impede such gene flow (Langerhans et al. 2003).

Regular dispersal of individuals among habitat patches in intermediate reaches maintains equilibria of species richness and assemblage composition across similar habitat patches (Stewart et al. 2002; Winemiller et al. 2010). These equilibria may be dynamic if differential reproduction and survival across patches create sources and sinks within a metapopulation, evidence of which has been observed in the genetic structure of European sculpin (*Cottus gobio*; Hänfling and Weetman 2006) and in the community structure of a Taiwanese river following disturbance (Chen et al. 2004). At the upstream interface between torrent and riffle zones, where stream hydrologies remain flashy and species richness begins to increase but patch sizes remain small, the heterogeneous and stochastic distribution of disturbance (largely due to spates) should be an important driver of source–sink patch dynamics. At the downstream interface between the riffle and large river rapids zones, where species and habitat diversity begin to approach maxima but where habitat patches remain regularly distributed, species should sort themselves according to microhabitats in a predictable fashion (e.g., Arrington et al. 2005; Fig. 1).

## *3.2 Drivers of Specialization*

Although our model predicts that over long time periods the composition of specialized fish assemblages in upland headwaters and large, lowland river rapids will be under strong evolutionary control (vicariant speciation in both, adaptive radiation in the latter), local species interactions need to be understood to infer the drivers of evolutionary specialization. Studies of fish behavior in torrential headwaters and large river rapids are scarce, but several studies have examined the effects of water velocity on species interactions in the mesohabitats (i.e., riffle, run, or pool reaches) of the North American piedmont streams. For example, Schlosser (1987) observed that in an ecologically diverse, North American assemblage of 15 species of stream fishes, juveniles and small-bodied adults occurred at higher densities in riffles and raceways than in pools, whereas large-bodied adults preferred pools. A choice experiment demonstrated that preference for shallow, fast-water habitat by small individuals was much greater when large fish were present versus absent, suggesting that an important motivation for fish to occupy fast-water habitat is predator avoidance (Schlosser 1987).

Increases in water velocity also reduce predation on macroinvertebrates by other macroinvertebrates (Meissner et al. 2009; Worischka et al. 2012) and contribute to increased rates of primary production, even when effects of habitat depth on light level are removed (Kevern and Ball 1965; Horner et al. 1990). Therefore, both algae and macroinvertebrates tend to be more abundant in mesohabitats that are fast and shallow versus slow and deep (Kevern and Ball 1965; Brown and Brussock 1991; Buffagni and Comin 2000). Together, these trends suggest that specialization on fast-water habitats may also contribute to increased foraging efficiency and reduced competition among herbivores and invertivores. In an elevational survey of an Andean affluent of the Amazon, Lujan et al. (2013) observed that the first native fish species to occur at high elevations were invertivorous, rheophilic catfishes (Astroblepidae, Trichomycteridae). Comparison of macroinvertebrate density in habitats with fish versus without fish showed that these catfishes contributed to a >50 % reduction in macroinvertebrate abundance. The second fish trophic guild to appear along the elevational gradient was herbivores. Considering the physiological challenges that likely limit elevational progression of aquatic organisms (e.g., oxygen limitation; Verberk et al. 2011), the occurrence first of invertivores and then of herbivores suggests that these are the most ecologically and physiologically advantageous trophic niches offered by such extreme habitats. In addition to refuge from predation and increased foraging efficiency, relatively higher dissolved oxygen concentrations and clean substrates also are benefits afforded to fast-water specialists, although the relative importance of these factors is likely much greater in modern, anthropogenically impacted (e.g., sedimented, eutrophied, hydrologically altered) streams than in naturally functioning ecosystems (Agostinho et al. 1994; Orrego et al. 2009). In summary, there is considerable evidence to suggest that fast-water habitats offer a range of ecological opportunities to fish lineages having either preexisting specializations or the evolutionary or behavioral ability to overcome the physical challenges of occupying these zones.

# 4 Specializations for Rheophily

## *4.1 Body Shape*

Extreme hydrologic forces largely limit the food resources and refuge available in fast-water habitats to species with specializations for resisting downstream displacement while feeding, thus driving the convergent evolution of body shape and mouth orientation (Figs. 2, 3, and 4, Appendix Table 1; Langerhans 2008). Microevolutionary (intraspecific) shifts toward a fusiform body, a higher aspect ratio caudal fin, and respectively upturned or downturned mouths have been associated with occupation of fast-flowing habitats by mid-water and near-bottom dwelling fishes (Langerhans et al. 2003; Langerhans 2008). Macroevolutionary shifts in the body morphology of pelagic rheophiles are harder to generalize, except to say that forked caudal fins and fusiform bodies are common (e.g., *Gila elegans*), but exceptions include compressed and high-bodied rheophilic members of the Cichlidae (e.g., *Retroculus*), Characidae (e.g., *Hemibrycon*), Serrasalmidae (e.g., *Ossubtus*), and Cyprinidae (e.g., *Labeo* or *Schismatorhynchos*).

Among pelagic, rheophilic lineages, repeated trends in mouth orientation are more apparent than trends in body shape. The invertivorous–herbivorous South American characiform family Anostomidae is the most morphologically disparate of these, spanning rheophilic genera with both dorsally directed (e.g., *Sartor*, *Synaptolaemus*) and ventrally directed mouths (e.g., *Hypomasticus*; Sidlauskas and Vari 2008). Most members of the herbivorous, rheophilic family Parodontidae have a ventrally directed mouth (Fig. 2h), and a single herbivorous, rheophilic species in the family Serrasalmidae (*Ossubtus xinguense*) is noteworthy for both its extremely downturned mouth (Fig. 2i) and its narrow endemism to rapids of the lower Xingu (Amazon Basin; Jégu 1992). Other fishes with distinctively downturned mouths include convergent pairs of taxa from South America and Africa: one with curved, tube snouts and the shared ability to generate and sense

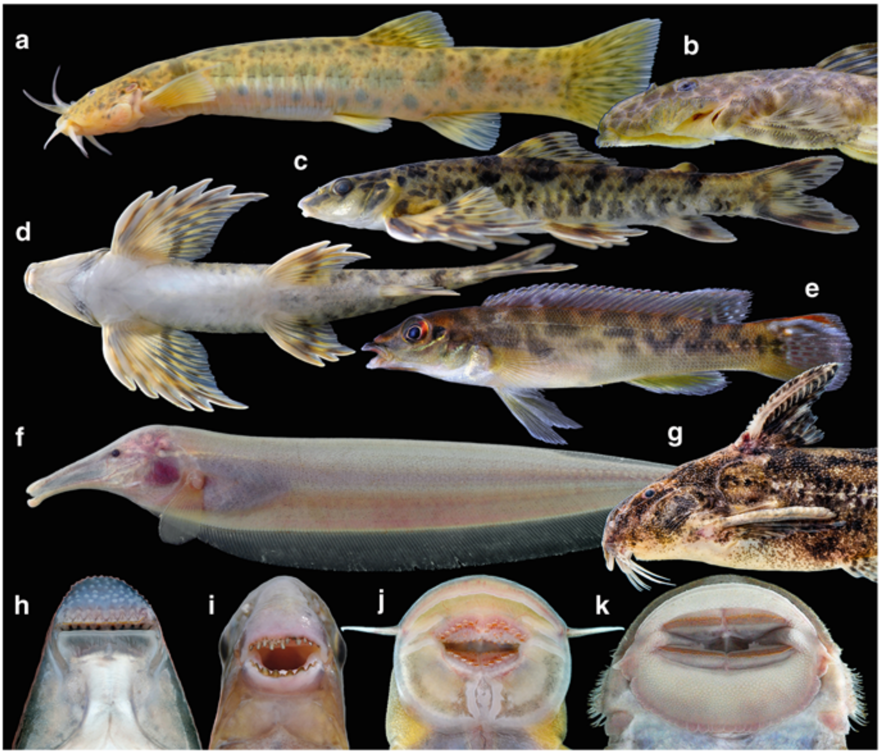


**Fig. 2** Examples of specialized rheophilic fishes from neotropical rivers: (**a**) *Trichomycterus guianensis* (Trichomycteridae; Kuribrong River, Guyana), (**b**) *Lithoxus jantjae* (Loricariidae; Ventuari River, Venezuela), (**c**) lateral and (**d**) ventral views of *Characidium declivirostre* (Crenuchidae; Orinoco River, Venezuela), (**e**) *Teleocichla* n.sp. "preta" (Cichlidae; Xingu River, Brazil), (**f**) *Sternarchorhynchus hagedornae* (Apteronotidae; Inambari River, Peru), (**g**) *Rhynchodoras xingui* (Doradidae; Xingu River, Brazil), (**h**) *Parodon buckleyi* (Parodontidae; Inambari River, Peru), (**i**) *Ossubtus xinguense* (Serrasalmidae; Xingu River, Brazil), (**j**) *Astroblepus* sp. (Astroblepidae; Araza River, Peru), (**k**) *Cordylancistrus platycephalus* (Loricariidae; Santiago River, Ecuador). Photo (**g**) by L. M. Sousa, all others by NKL

electrical fields (*Sternarchorhyncus*, Fig. 2f, and *Campylomormyrus curvirostris*, Fig. 3f), and another with short, ventrally directed mouths (*Rhynchodoras*, Fig. 2g, and *Campylomormyrus alces*, Fig. 3g) to presumably extract invertebrate prey from interstitial spaces in substrates (Marrero and Winemiller 1993). A wide range of pelagic rheophilic and mostly herbivorous genera in the order Cypriniformes also contain species with ventrally oriented mouths. Examples include the North American genus *Campostoma*, the European *Chondrostoma*, and the Asian *Scaphiodonichthys*.

A striking exception to these predominantly herbivorous and invertivorous examples is the evolutionary convergence on a pelagic, large-eyed, upturned-mouth morphology by rheophilic, piscivorous members of the neotropical characiform family Cynodontidae, the Eurasian catfish family Siluridae, and the

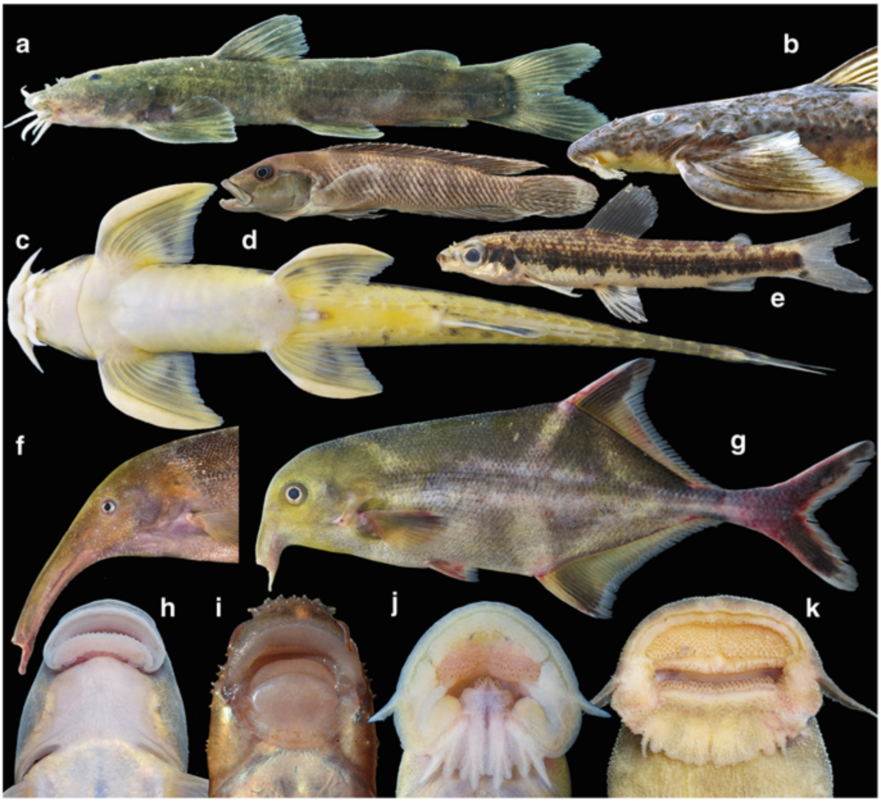


**Fig. 3** Examples of specialized rheophilic fishes from tropical African rivers: (**a**) *Amphilius zairensis* [Amphiliidae; Congo River, Democratic Republic of the Congo (DRC)], (**b**) *Doumea gracila* (Amphiliidae; Lobe River, Cameroon), (**c**) *Phractura fasciata* (Amphiliidae; Congo River, DRC), (**d**) *Lamprologus mocquardi* (Cichlidae; Congo River, DRC), (**e**) *Nannocharax gracilis* (Distichodontidae; Congo River, DRC), (**f**) *Campylomormyrus curvirostris* (Mormyridae; Congo River, DRC), (**g**) *Campylomormyrus alces* (Mormyridae; Congo River, DRC), (**h**) *Labeo* sp. (Cyprinidae; Congo River, DRC), (**i**) *Garra* sp. (Cyprinidae; Congo River, DRC), (**j**) *Chiloglanis congicus* (Mochokidae; Congo River, DRC), (**k**) *Euchilichthys* cf. *royauxi* (Mochokidae; Congo River, DRC). Photos by J. P. Sullivan

paleotropical Cyprinidae. Three cynodontid genera (*Cynodon*, *Hydrolycus*, and *Raphiodon*), one silurid genus (*Belodontichthys*), and two cyprinid genera (*Macrochirichthys* and *Securicula*) all share a preference for large river fast-water habitats, a slender elongate body, large eyes, an enormous upturned gape with many sharp teeth (excluding the toothless cyprinids), and large wing-like pectoral fins to facilitate quick dashes to the surface to capture prey.

Many rheophilic fishes resist dislodgement by moving out of the water column and on or into bottom substrates, driving the selection for strongly dorsoventrally depressed (flattened) or anguiliform (eel-like) body shapes. The advantage to a fish of being small-bodied, benthic, and dorsoventally flattened is derived in part from

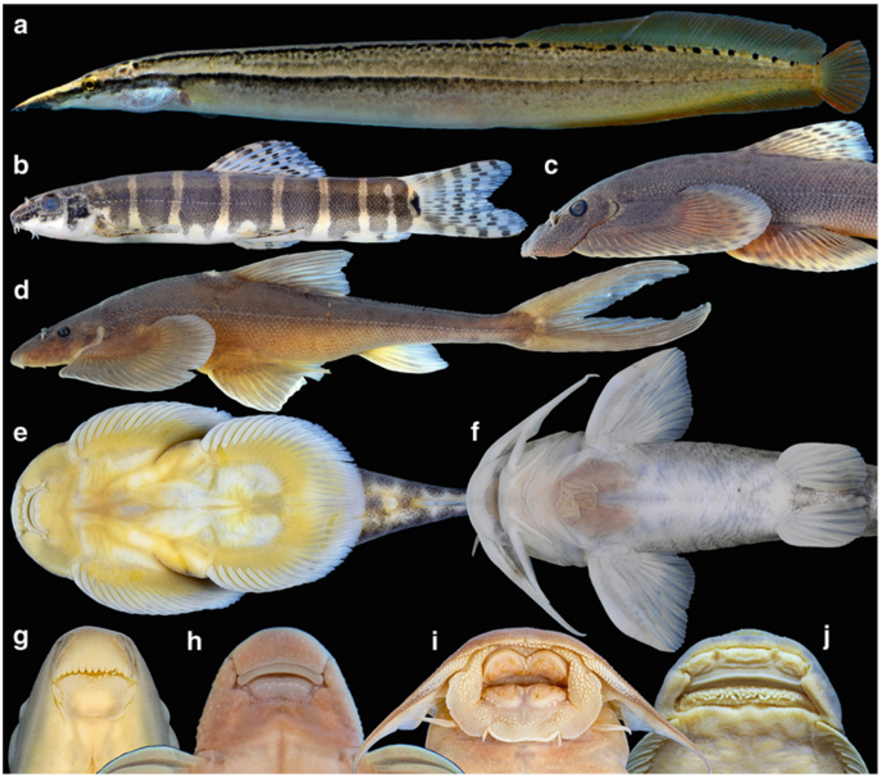


**Fig. 4** Examples of specialized rheophilic fishes from tropical Asian rivers: (**a**) *Macrognathus pavo* (Mastacembelidae; Kyeintali Chaung River, Myanmar), (**b**) *Serpenticobitis zonatus* (Serpenticobitidae; Mekong Drainage, Laos), (**c**) *Sinogastromyzon puliensis* (Balitoridae; Wuxi stream, Taiwan), (**d**) *Jinshaia abbreviata* (Balitoridae; Yangtze River, China), (**e**) *Metahomaloptera omeiensis* (Balitoridae; Yangtze River, China), (**f**) *Glyptothorax platypogon* (Sisoridae; Ci'harang River, Indonesia), (**g**) *Crossocheilus elegans* (Cyprinidae, Kinabatangan River basin, Malaysia), (**h**) *Psilorhynchus pseudecheneis* (Psilorhynchidae; Indrawati River, Nepal), (**i**) *Exostoma* sp. (Sisoridae; Chao Phraya River, Thailand), (**j**) *Gastromyzon introrsus* (Balitoridae; Padas and Labuk rivers, Malaysia). Photo (**a**) by R. Britz, (**i**) by H. H. Ng, (**h**) by KWC, all others by H. H. Tan

its maximized occupancy of the thin boundary layer where water velocities approach zero. Dorsoventral depression also maximizes contact with, and therefore frictional adhesion to, solid substrates on which fish can gain access to algal and invertebrate food resources while minimizing energy expenditure. A hydrodynamic dorsal profile along with specialized skin surface features (e.g., unculi, keeled scales, see *Attachment organs* and *Scales* below) may further enhance adhesion by maximizing down pressure created by current flowing parallel with the substrate while minimizing lift by disrupting laminar surface flow across the skin surface. Striking examples of extremely dorsoventrally depressed rheophilic fishes include members of the Neotropical Loricariidae (e.g., *Lithoxus*, Fig. 2b) and the Asian cypriniform family Balitoridae (e.g., *Sinogastromyzon*, Fig. 3c).

Most benthic species are too large and high-bodied to live entirely within the boundary layer. They are therefore exposed, at least along their dorsum, to some of the fastest flows in a stream. Among benthic, rheophilic fish lineages, hydrodynamic forces have consistently driven selection for a fusiform body with a steep, wedge-shaped head, slender caudal peduncle, and large wing-like paired fins that extend laterally and are steeply canted to deflect flow dorsally (Figs. 2b–d, 3b, c, and 4c–e). This body shape and fin arrangement presents a dorsally rounded and ventrally flat shape that maximizes substrate contact and hydrodynamic down forces while minimizing drag. Moreover, the hydrodynamic effects of this morphology may be behaviorally exaggerated by some species as water velocities increase (Carlson and Lauder 2010).

An alternative approach taken by some non algae-scraping benthic rheophilic species is to largely escape the flow by living and foraging mostly within interstitial spaces between or under rocks and retaining or exaggerating an anguiliform body shape. Elongate, anguiliform body types can be observed among rheophilic members of the neotropical catfish families Heptapteridae (e.g., *Myoglanis aspredinoides*) and Trichomycteridae (e.g., *Trichomycterus*, Fig. 2a), the African catfish family Clariidae (e.g., *Gymnallabes*), the Eurasian loach families Cobitidae (e.g., *Pangio*), Nemacheilidae (e.g., *Aborichthys*), and Gastromyzontidae (e.g., *Glaniopsis*), and the spiny eel family Mastacembellidae (e.g., *Macrognathus*, Fig. 4a).

## 4.2 Attachment Organs

Many benthic rheophiles have specialized "attachment organs" to prevent displacement by adhering directly to substrates. In neotropical rivers, members of the invertivorous catfish family Astroblepidae and invertivorous/herbivorous family Loricariidae, which are sister groups (Sullivan et al. 2006), have the upper and lower lips fused into a ventrally oriented oral disc, which surrounds jaws specialized for scraping substrates (Fig. 2j, k). Analogous oral discs are seen among rheophilic invertivorous–herbivorous members of the African catfish subfamily Chiloglanidinae (Mochokidae, Fig. 3j, k; Roberts and Stewart 1976; Roberts 1989b; Geerinckx and De Kegel 2014) and certain genera of the Asian catfish subfamily Glyptosterninae (Fig. 4i; Thomson and Page 2006). Oral adhesive discs of a different sort are found in herbivorous members of the African and Asian tribe Labeonini of the Cyprinidae (e.g., *Garra*, *Discogobio* and *Placocheilus*, Fig. 3h, i). In these examples, the lower lip is greatly expanded posteriorly to form a large mental pad that is continuous around the edge of the mouth via a well-developed frenum (Fig. 3h, i; Saxena and Chandy 1966; Stiassny and Getahun 2007). The extremities of the mental pad and rostal cap form the outer margin of the oral adhesive disc, the intricacies of which are highly variable both within and between the different labeonin genera in which they are found, making the oral disc important for labeonin classification (Zhang 2005; Stiassny and Getahun 2007) and species identification (e.g., Zhou et al. 2005; Zhang and Zhou 2012). The well-developed oral discs of lampreys also are used to attach directly to the substrate in fast-flowing water, especially during resting periods between bouts of active swimming (Reinhardt et al. 2008).

The lips of a number of rheophilic fishes can also be manipulated to form temporary oral adhesive organs. In members of the South East Asian cypriniform family Gyrinocheilidae, the greatly enlarged and fleshy lips are supported internally by highly flexible chondroid bodies, which enable the lips to evert and form an oral sucker (Benjamin 1986). The waterfall-climbing amphidromous gobies of the genus *Sicyopterus*, which inhabit fast-flowing streams along continental margins and on remote oceanic islands as juveniles and adults, possess a greatly enlarged, fleshy upper lip, which when pushed down onto the substrate also forms a type of oral sucker (Schoenfuss and Blob 2003; Maie et al. 2012; Cullen et al. 2013; see Sect. 4.3 below). Recent functional morphological studies focused on the oral disc of the Neotropical loricarioid genera *Astroblepus* (Geerinckx et al. 2011) and *Pterygoplichthys* (Crop et al. 2013) and the oral sucker of the goby *Sicyopterus* (Cullen et al. 2013) have demonstrated the adhesive capability of these oral structures and, at least among the catfishes, the capacity to feed and respire while also adhering to substrates (Geerinckx et al. 2011; Crop et al. 2013). Scanning electron microscopy studies of the surface of loricariid and cypriniform oral discs have revealed a bewildering diversity of unicellular, keratinized projections or unculi (sensu Roberts 1982) that vary from short rounded knobs in some species to long, digitate or hooked projections in others (Roberts 1982; Benjamin 1986; Pinky et al. 2004; Geerinckx et al. 2011). Roberts (1982) and Pinky et al. (2004) have hypothesized that unculi likely function to both protect the epidermis from abrasion and to increase friction against substrates, thereby enhancing the ability of oral discs to both adhere to substrates and rasp them of epilithic algae.

The South American catfish families Loricariidae and Trichomycteridae both have specialized opercular apparati that allow for clusters of elongate, external teeth (odontodes) to be forcefully everted or shifted from a position flush with the body, to jutting outward from the side of the head (de Pinna 1998; Geerinckx and Adriaens 2006). This mechanism has been coopted for different purposes among the Loricariidae and derived subfamilies of the Trichomycteridae (e.g., territorial defense, parasitic station holding; de Pinna 1998), but basal lineages comprising the subfamily Trichomycterinae (Fig. 2a) are hypothesized to use this mechanism primarily as an attachment organ to assist with benthic station holding and locomotion in fast-water habitats (de Pinna 1998; see Sect. 4.3).

Post-cranial attachment organs also are diverse in benthic rheophilic fishes and include adhesive discs formed through various configurations or modification of the paired fins and a wide variety of unculiferous pads in ostariophysans. The pelvic fins, when well developed, are usually united to form a ventral adhesive disc in members of the Gobiidae, which have been shown to exhibit strong adhesion in the amphidromous sicydiine gobies that have been investigated to date (Blob et al. 2006; Maie et al. 2012). In several genera of the Gastromyzontidae (e.g., *Gastromyzon*, *Hypergastromyzon*, *Neogastromyzon*, and *Sinogastromyzon*), the pelvic fins are united across the midline to form a single cuplike structure (Fig. 4e), which in combination with greatly enlarged pectoral fins is considered to function as a suction disc (Wickler 1971; Tan 2006; De Meyer and Geerinckx 2014), presumably generating negative pressure between the body and substrate by evacuating interstitial water via respiration and preventing the inflow of water via broad and closely adpressed paired fins. A more sophisticated ventral suction disc, formed by elements of the pectoral- and pelvic-fin girdles is found in members of the predominantly marine family Gobiesocidae, which also includes a small number of freshwater members in the genus *Gobiesox* that inhabit short coastal rivers and streams throughout Central America and northern South America (Briggs 1955; Briggs and Miller 1960). Though the adhesive capabilities of freshwater clingfishes have yet to be investigated, a recent investigation of the adhesive capabilities of a large intertidal species of *Gobiesox* revealed an astonishing ability to adhere to irregular surfaces (Wainwright et al. 2013).

Putative friction devices, formed by aggregations of acute, posteriorly directed unculi, are present on the anteroventral surface of the body in several unrelated groups of benthic rheophilic ostariophysans (Roberts 1982). To date, these structures have been investigated only from gross morphological perspectives, with much remaining to be learned about their specific function in different groups. Perhaps the simplest of these putative friction devices are paired-fin pads, located along the ventral surface of the anterior-most paired-fin rays in many benthic rheophilic ostariophysans (Conway et al. 2012a). The narrow paired-fin pads of non-siluriform ostariophysans are externally homogeneous (despite being homoplasious) and are formed by a thickening of the epidermis and/or subdermis combined with an unculiferous superficial layer (Conway et al. 2012a). The largest unculiferous paired-fin pads can be observed among members of the highly rheophilic catfish subfamilies Amphiliinae and Doumeinae in Africa (Amphiliidae, Fig. 3c) and the Glyptosterninae in Asia (Sisoridae, Fig. 4f), associated with the ventral surface of a highly modified pectoral-fin spine (Hora 1922, 1930).

A more extensive friction pad, referred to as the thoracic adhesive organ, is located between the pectoral fins in members of certain sisorid genera (Fig. 4f; e.g., *Glyptothorax*, *Pseudecheneis, Pseudolaguvia, Conta*, and *Caelatoglanis*; Bhatia 1950; Saxena 1961; Sinha et al. 1990; Singh and Agarwal 1993; Das and Nag 2005; Ng and Kottelat 2005; Ng 2006) and formed by a series of longitudinal or oblique folds of skin. The unculi distributed across the surface of the thoracic adhesive organ are typically arranged in regular rows and often exhibit a hooked tip (Das and Nag 2005, 2009; Ng 2006). In several highly benthic rheophilic sisorid taxa (e.g., *Exostoma*, *Myersglanis*, *Oreoglanis*, and *Parachiloglanis*), a thoracic adhesive organ is absent (Ng 2006), yet similar folds of unculiferous skin are present instead along the leading edge of the snout, ventral surface of the highly flattened barbels and the surface of the paired-fin pads (Ng 2006; Conway et al. 2012a; see Sect. 5). Instead of unculi, the entire bodies of loricariid catfishes and the pelvic-fin spines of astroblepid catfishes are covered with small, posteriorly oriented odontodes, which likely serve similar functions to unculi, i.e., abrasion resistance and substrate friction (Alexander 1965; Blake 2006), although a single cave-dwelling astroblepid species is known to have coopted odontodes for a mechanosensory function (see Sect. 4.4, Haspel et al. 2012).

### *4.3 Locomotion*

Fishes adapted for life in high-flow environments tend to share a number of specialized physiological and biomechanical traits related to swimming, including relatively more red muscle, stiffer bodies, higher steady swimming performance, and lower unsteady swimming performance (Langerhans 2008). Several rheophilic species specialized for life in particularly high flow environments have even evolved distinctive modes of locomotion that exploit the traction provided by adhesive organs. The most extreme of these is the "ratcheting" mode that has been observed in the goby *Sicyopterus* and in astroblepid catfishes. *Sicyopterus* are capable of climbing vertical surfaces that are wetted but out of the water column by attachment with the oral and pelvic-fin discs, and by use of repeated extension and retraction of the upper lip to inch forward (Blob et al. 2006; Maie et al. 2012; Cullen et al. 2013). Astroblepid catfishes, on the other hand, have a highly mobile pelvic girdle with long, strap-like *protractor* and *retractor ischii* muscles that can slide the girdle anteroposteriorly along the trunk. In this way, they can maintain station with their oral disc (Fig. 2j), engage irregularities in the substrate with posteriorly directed odontodes on the ventral surface of their pelvic-fin spines, and propel themselves forward via posterior retraction of the pelvic girdle (Crop et al. 2013). Using this method, astroblepid catfish have also been observed climbing vertical wetted surfaces outside the water column (Johnson 1912). In contrast to longitudinal "ratcheting," species in the Neotropical subfamily Trichomycterinae (Trichomycteridae, Fig. 2a) can use external teeth (odontodes) on either side of their head to gain lateral traction and "elbow" their way upstream against fast current (de Pinna 1998).

A third mode of locomotion via surface attachment is the "power burst" used by goby species that lack an oral disc (e.g., *Lentipes*, *Sicydium*) and by some *Characidium* species (Crenuchidae, Fig. 2c, d; Buckup et al. 2000). This involves strong undulations of the body combined with surface detachment and attachment to move forward (Blob et al. 2006; Maie et al. 2012). A fourth form of rheophilic locomotion that also involves substrate interaction is anguiliform movement, in which forward movement is made through serpentine undulations of the body against substrates and/or the water. Anguiliform movement is typically paired with an anguiliform body shape (Fig. 4a) and the regular occupation of interstitial spaces under or between substrates.

## 4.4 Sensation

Rheophiles, like other fishes, have at their disposal four different sensory modalities (vision, mechanosensation, chemosensation, and electrosensation) to navigate and detect predators, competitors, mates, and prey. Although many rheophiles have large eyes and likely good visual acuity, the importance of vision can be highly variable depending on a species' activity cycle (diurnal or nocturnal), their preferred water depth, prevailing water clarity (Muntz 1982), mode of foraging, and the degree to which their nonvisual sensory systems are developed. Loss or reduction of eyes was reported for eight rheophilic species endemic to the rapids of the lower Congo River by Roberts and Stewart (1976), all of which are species that live in or under the substrate, including catfishes and spiny eels with well-developed chemosensory systems. Several cave-dwelling rheophilic species from around the world are also blind (e.g., *Astroblepus riberae*, *Cryptotora thamicola*; Cardona and Guerao 1994; Kottelat 1988, 1998).

Given the importance of flow detection for avoidance and navigation by rheophilic fishes, one might predict that the water velocity of a fish's preferred habitat would correlate with development of the mechanosensory system; however, the only study we are aware of that examined this found no relationship between habitat flow characteristics and the number or distribution of neuromasts across 12 species of European stream fishes (Beckman et al. 2010). Indeed, both the species with the greatest and the least concentrations of neuromasts were considered rheophilic (Beckman et al. 2010). In an apparently exceptional case of coopting dermal teeth (odontodes) for mechanosensation, Haspel et al. (2012) demonstrated that the blind and cave-dwelling naked suckermouth catfish *Astroblepus pholeter* has unusually few neuromasts but is able to obtain flow and substrate information via deflection of modified odontodes distributed around the body (see chapter "Cave Environments").

Otophysan fishes, including all minnows, catfishes, tetras, and South American knifefishes, are abundant and diverse in many freshwater habitats, including rapids (Appendix Table 1). Otophysans are considered hearing specialists because they have an inner ear–swimbladder (i.e., otophysic) connection composed of modified peri- and endolymphatic spaces and the Weberian ossicles (the Weberian apparatus). These modifications are known to improve sensitivity to high frequency sound (Ladich 2000), but some rheophilic otophysans exhibit changes to the Weberian apparatus that contribute to reduced hearing sensitivity. The actinopterygian swimbladder is typically a single, medial, elongate organ that occupies much of the dorsal visceral cavity (Longo et al. 2013). In some benthic rheophilic otophysans (e.g., some catfishes: Amphiliidae, Callichthyidae, Loricariidae, Sisoridae; and loaches: Nemacheilidae, Balitoridae, and Gastromyzontidae), the swimbladder is anteroposteriorly shortened, anteriorly shifted to a position immediately posterior to the cranium, and subdivided into two bilaterally symmetrical lobes.

Moreover, these groups also show varying degrees of skeletal encapsulation of the bladder (see Sect. 4.8). This encapsulation and reduction in swimbladder size is correlated with a reduction in the Weberian ossicles of some groups (e.g., Callichthyidae, Loricariidae), and a reduction in hearing sensitivity (Lechner and Ladich 2008). It has been suggested that in benthic fishes with little or no need for buoyancy, the hydrostatic function of the swimbladder is minimized and the organ is shrunk but not entirely lost because of its remaining acoustic utility (Lechner and Ladich 2008). Many non-otophysan rheophilic fishes have only a vestigial swimbladder (e.g., Cichlidae: *Steatocranus tinanti*; Schulz-Mirbach et al. 2012) or have lost the swimbladder entirely (e.g., Percidae: *Etheostoma* spp.; Evans and Page 2003). Although encapsulation of the swimbladder and modification of the external capsule structure have been hypothesized to have an acoustic function in some otophysans (Weitzman 2003), it seems more likely, given the known decreases in hearing sensitivity associated with swimbladder size reduction (Lechner and Ladich 2008; Schulz-Mirbach et al. 2012), that the capsule functions primarily to structurally reinforce a presumably structurally weak region of the body. A second hypothesis focused on the bilateral bifurcation of the bladders and the sometimes megaphone-like shape of the bladder capsules (e.g., Loricariidae: *Otocinclus*; Weitzman 2003) is that these modifications function to increase a fish's directional sensitivity to sound, although this hypothesis requires further testing (Stewart and Smith 2009).

One uninvestigated yet potential mechanosensory specialization that is seen in many neotropical catfish species from fast and turbid whitewater rivers is the extreme elongation of caudal-fin filaments. Species in several families (e.g., Cetopsidae, Heptapteridae, Loricariidae, and Pimelodidae) produce long caudal filaments either throughout their life or only as juveniles. It has been hypothesized that these caudal filaments may function as a specialized mechanosensory system for the detection of predators approaching from downstream (Lujan and Chamon 2008; K. Winemiller, pers. comm.). This is supported in part by research on marine (e.g., sea robins, *Prionotus* spp.) and lentic freshwater fishes (e.g., gouramis, *Trichogaster trichogaster*) in which distal portions of elongated fin rays have numerous free nerve endings and elicit a tactile response when touched (Kasumyan 2011). This example of elongation contrasts with the apparent trend toward reduction of barbels, another typically elongate sensory structure that is shortened in several clades of rheophilic fishes. For example, within the river loaches of the Eurasian family Nemacheilidae members inhabiting lakes or slow-flowing rivers (e.g., *Yunnanilus* or *Lefua*) typically exhibit longer barbels than those inhabiting the interstitial spaces between rocks in fast-flowing rivers and streams (e.g., *Schistura* or *Turcinoemacheilus*). Likewise, loricariid catfishes from more lentic habitat (e.g., *Pterygoplichthys*) often have much longer barbels than species from fast-water habitats (e.g., *Chaetostoma*).

Species that have enhanced chemosensory and/or electrosensory systems are disproportionately represented in specialized rheophilic fish assemblages. Chemosensory specialists include all catfishes and many cypriniforms, whereas electrosensory specialists include neotropical gymnotiforms (e.g., *Sternarchorhynchus*, Fig. 2f), African mormyrids (e.g., *Campylomormyrus*, Fig. 3f, g), and, to a lesser extent, catfishes in the family Mochokidae (Boyle et al. 2014). In general, there is little evidence of sensory specializations developing as adaptations specifically for rheophily, but rather a prevailing pattern of preadaptation of chemo- and electrosensory specialists for life in fast water (Roberts and Stewart 1976).

## 4.5 Feeding

As noted in Sect. 4.1, many rheophilic fishes have strikingly upturned or downturned mouths. The upturned mouths of rheophilic fishes are typically either a specialization for top-water piscivory (e.g., *Belodontichthys*, *Macrochirichthys,* and *Securicula*) or are associated with the removal of invertebrates and sponges from the sides or undersides of rocks and wood (e.g., *Gnathodolus*, *Sartor*, and *Synaptolaemus*; Zuanon 1999; Sidlauskas and Vari 2008). Downturned mouths are usually specialized for invertivory when narrow (e.g., *Rhynchodoras*, Fig. 2g; *Sternarchorhynchus*, Fig. 2f; *Astroblepus*, Fig. 2j; *Chiloglanis*, Fig. 3j) or epilithic algal scraping when broad (e.g., *Parodon*, Fig. 2h; *Cordylancistrus*, Fig. 2k; *Euchilichthys*, Fig. 3k). Likewise, there is a general trend toward invertivores having relatively few, long, and acute teeth (e.g., *Sartor*, *Leporacanthicus*) and herbivores having smaller, broader, and more numerous teeth (e.g., *Cordylancistrus*, Fig. 2k; *Euchilichthys*, Fig. 3k; Lujan et al. 2012). As noted in Sect. 4.2, oral discs that surround the downturned mouths of algae-scrapers may facilitate feeding by increasing the surface adhesion and scraping friction of both the oral jaws and the surface of the disc (Ono 1980; Benjamin 1986). Oral discs likely also facilitate prey detection when equipped with taste buds (Ono 1980), and invertivore feeding by helping to occlude the cavities in which mobile prey hide, preventing their escape.

The teeth of rheophilic fishes are often highly specialized for particular food items and some of the strangest vertebrate dentitions described to date belong to benthic rheophilic fishes that scrape at substrates. For example, in members of the goby genus *Sicyopterus*, the tiny functional incisor-like teeth of the premaxillae are only the terminal stage in a complicated sequence of tooth development that involves a whorl of 20–40 rows of replacement teeth in various stages of development (Mochizuki and Fukui 1983). Between the basal pedicel and the dentin shaft of each functional tooth in the upper jaw of *Sicyopterus japonicus* is a complex articulation that provides each tooth with a certain degree of mobility, facilitating the scraping of algae from irregular surfaces (Sahara et al. 2013). The ability of teeth to interact with the substrate is taken to the extreme in certain groups of algivorous loricariid catfishes that possess uniquely flexible teeth with a flattened region along the shaft that is composed of soft dentin and unmineralized tissue, which allow the tooth to flex up to 180° between the tooth's rigid base and its hardened, hypermineralized cusp (Geerinckx et al. 2012). Though the majority of rheophilic substrate scrapers are benthic or demersal species with broad downturned mouths, the ayu (*Plecoglossus*) represents an interesting example of a seemingly unspecialized pelagic rheophile that scrapes algae via rapid lateral strikes of the lower jaw against the surface of rocks. The teeth in the lower jaw of the adult ayu are comblike and contrast sharply with the typical conical teeth present in the upper jaw (Uehara and Miyoshi 1993). Instead of scraping at the substrate with specialized teeth, substrate-scraping members of the Cypriniformes utilize highly keratinized jaw sheaths that are typically formed by low block-like unculi (Roberts 1982; Pinky et al. 2004).

## *4.6 Reproduction*

Few if any rheophilic fishes are opportunistic life history strategists (i.e., early maturation, continuous reproduction, small body size, small clutch size, and little parental care), and only a minority are equilibrium strategists (i.e., late maturation, low fecundity, high parental care, and high juvenile survivorship, e.g., cave spawners listed below; Winemiller and Rose 1992). Most fishes, including most rheophiles, are periodic strategists (i.e., late maturation, high fecundity, little parental care, and low juvenile survivorship) and spawn by males and females aligning themselves and synchronously broadcasting gametes for external fertilization. Viviparity (internally fertilizing with live birth) and oviparity (internal fertilizing with egg laying) are rare, if not entirely absent from specialized rheophilic fishes, although viviparous poeciliids (e.g., *Priapella*, *Alfaro*, *Pseudoxiphophorus*; M. Tobler, pers. comm.) are common occupants of lotic habitats throughout Central America, and some oviparous members of the South American catfish family Auchenipteridae (e.g., *Auchenipterus*; Mazzoldi et al. 2007) can also be found in marginal areas near rapids.

One would nonetheless predict that in a fast-flowing stream environment there would be selection for specializations that minimize the risk of gametes being washed downstream before fertilization. Indeed, there are examples among rheophiles of both behavioral and morphological responses to this challenge. Behavioral responses include the synchronous deposition of eggs into cracks in rocks or wood (e.g., *Cyprinella*: Mayden and Simons 2002), the synchronous burrowing of males and females into sand or gravel substrates while spawning (e.g., North American darters of the genera *Etheostoma* and *Nothonotus*: Page and Swofford 1984; Warren et al. 1986; members of the south Asian genus *Opsarichthys*: R. Britz, pers. comm.), and the establishment and defense of sheltered nest cavities in or under rocks or wood (e.g., *Etheostoma*, subgenus *Catanotus*: Page 1980; Loricariidae, tribe Ancistrini: Sabaj et al. 1999). Morphological responses appear to be largely limited to elongation of the male genital papilla, which is seen in rheophilic catfishes of the African families Amphiliidae and Mochokidae (Roberts and Stewart 1976) and is taken to an extreme in the neotropical family Astroblepidae (Buitrago-Suárez and Galvis 1997). Male *Astroblepus* can have a robust genital appendage that extends to over a centimeter in length, and they are exceptional among fishes in having seminal vesicles near their testes, suggesting that the elongate papilla may have an intromittent function and that fertilization may be internal (Buitrago-Suárez and Galvis 1997).

## 4.7 Scales

Many benthic rheophiles exhibit reduced squamation compared to pelagic close relatives. Within monophyletic groups of benthic rheophiles, there is a general correlation between the extent of ventral squamation, particularly between the paired fins (the region of the body in closest contact with the substrate), and exposure to strong currents. For example, in the cypriniform genus *Psilorhynchus*, ventral squamation ranges from relatively complete in those taxa found in riffle habitats at lower elevations to a few poorly ossified, flap-like scales or complete absence of scales in members inhabiting higher elevation mountain torrents (Conway et al. 2012b). Similar trends in ventral squamation are present in the African/Asian cyprinid genus *Garra* (Stiassny and Getahun 2007) and the South American characid genus *Characidium* (Fig. 2d, Lujan et al. 2013). A reduction in the overall size combined with an increase in depth of embedment has been noted in scales covering the head, nape, and ventral surface in benthic rheophilic cichlids (Kullander 1988) and percids (Zorach 1972), and scales covering the body in nemacheiline loaches that inhabit swift currents are typically reported to be small and deeply embedded (e.g., Kottelat 1990; Zhou and Kottelat 2005; Conway 2011). Scales may be completely absent in several groups of benthic rheophiles, either plesiomorphically (e.g., all groups of benthic rheophilic catfishes; Fink and Fink 1981) or uniquely derived (e.g., middle eastern species of *Turcinoemacheilus*, Bănărescu and Nalbant 1964; certain species of *Pangio*, Britz et al. 2012). Reasons for scale reduction in benthic rheophilic lineages are likely numerous. In the absence of functional data, we speculate that scales may simply get in the way of maintaining close contact with the substrate (when on the ventral surface) or may interfere with hydrodynamics (when on the head, nape, or lateral body surfaces). On the other hand, the development of elongate keratinous ridges (or keels) over the surface of anterior body scales in numerous groups of benthic rheophilic cypriniforms and percids (Wiley and Collette 1970) that retain them may function to reduce lift by disrupting laminar water flow over the body (A. Summers, pers. comm.).

## 4.8 Skeleton

In contrast to the trend toward reduction of scales in benthic rheophiles, other parts of the skeleton may be greatly reinforced, either through suturing, fusion, or hyperossification. In addition to being dorsoventrally depressed, the skulls of benthic rheophiles that are exposed to strong currents often exhibit a high degree of suturing between adjacent elements (Sawada 1982; He et al. 1999). In members of several groups of rheophilic catfishes (e.g., Amphiliidae, Aspredinidae, Loricarioidea, and Sisoridae), the vertebral column is greatly reinforced through the anteroposterior expansion of neural arches and spines and interlocking of pre- and postzygapophyses of adjacent centra (He et al. 1999), or in some cases through the interlocking of accessory vertebral processes (De Pinna 1996). Similar reinforcement of the vertebral column is exhibited by highly rheophilic members of the Gastromyzontidae and Balitoridae (Sawada 1982). As described in Sect. 4.4, several groups of rheophilic catfishes (e.g., Amphiliidae, Aspredinidae, Loricarioidea, and Sisoridae; de Pinna 1996) and benthic, rheophilic cypriniforms (e.g., cobitoid loaches, Psilorhynchidae, Gobioninae; Alexander 1964; Sawada 1982; Bird and Hernandez 2007; Conway 2011) have elements of the vertebral column, and in some cases the neurocranium, that are highly modified to form a bony capsule around the swimbladder, and this capsule may serve a primarily structural role.

Ventral to the vertebral column, enlargement of the coracoid and basipterygium of the pectoral- and pelvic-fin girdles is also common in benthic rheophilic ostariophysans (e.g., Loricariidae and Gastromyzontidae; Schaefer 1984; Sawada 1982), as is the strengthening of the connection between these elements of the right and left sides of the body (Chang 1945). Fin rays that frequently encounter the substrate may also be heavily reinforced in benthic rheophiles. Lundberg and Marsh (1976) noted two major trends in the pectoral-fin rays of cypriniform fishes that they associated with the degree of substrate contact, including the simplification of anterior rays and foreshortening of fin ray segments, with highly benthic species exhibiting higher numbers of simple (unbranched) rays and shorter hemitrichial segments than benthopelagic or pelagic species. They concluded that such modifications acted to increase the flexibility, tensile strength, and resistance to buckling of rays that frequently contact the substrate (Lundberg and Marsh 1976). Along similar lines, Taft (2011) documented a number of structural differences along the length of individual lepidotrichia in the pectoral-fin rays of benthic vs. pelagic percomorphs that likely affect flexibility in the former.

## 5 Phylogenetic Patterns

With highly divergent scales of habitat, food-web, and ecomorphological diversity, headwaters and large river rapids are predicted to produce dramatically different phylogeographic and morphological patterns. Clades that are widespread across upland habitats should exhibit allopatric lineage diversity, low overall morphological diversity, and specializations for fast water that are synapomorphic for clades encompassing all rheophiles (i.e., plesiomorphic for clade members). Fish communities in large lowland river rapids should be largely paraphyletic while also exhibiting higher rates of localized adaptive radiation and autapomorphic specializations for life in fast water. Distributions of morphological specializations across phylogenies currently available for rheophilic fishes provide support for these predictions.

Headwater taxa for which at least partially representative species- or subspecies-level phylogenies are available include the Andean Trichomycterinae (Trichomycteridae) and Astroblepidae, and the predominantly South Asian Sisoridae. In studies of intraspecific genetic diversity in the montane species *Trichomycterus areolatus*, Unmack et al. (2009) and Quezada-Romegiall et al. (2010) found high levels of genetic divergence across river basins draining the Chilean Andes, and this molecular divergence corresponded with subtle variation in body shape that may warrant recognition of additional, undescribed species (Pardo 2002). Likewise, Schaefer et al. (2011) observed relatively deep, drainage-specific genetic divergence in species of *Astroblepus* from the Andes of southern Peru. Species in this study differed phenotypically only in subtle characteristics of their morphology (e.g., teeth uni- or bicuspid, presence/absence of adipose-fin membrane), but all shared plesiomorphic specializations for life in fast water, including an adhesive oral disc, pelvic-fin friction pads, a highly mobile pelvic girdle, and a distinctive "ratcheting" form of locomotion (i.e., putative synapomorphies of the Astroblepidae). The highland Andean genus *Chaetostoma* and Guiana Shield genera *Exastilithoxus* and *Neblinichthys* (Loricariidae) reveal a similar pattern of shared specializations for fast water, subtle phenotypic variation, and deep drainage-specific genetic divergence (Lujan et al. 2015).

The pattern among headwater clades is a bit more complex in members of the Asian catfish family Sisoridae, in which the presence/absence and specific morphology of the thoracic adhesive pad show considerable homoplasy, with up to four independent origins based on phylogenetic hypotheses derived from both morphological (de Pinna 1996; Ng 2006) and molecular data (Jiang et al. 2011). Occurrence of the thoracic adhesive pad is generally correlated with occupation of fast-water habitats, although the pad is entirely absent from one of the most extremely rheophilic clades (Glyptosternoidea sensu Peng et al. 2004), being replaced therein by well-developed peripheral friction pads on the underside of the anterior snout margin, barbels, and paired fins. In a molecular phylogenetic study of glyptosternoid biogeography, Guo et al. (2005) attributed much of the lineage diversity within this clade to the breakup of Southeast Asian river drainages following uplift of the Himalayan Mountains. Based on the presence of well-defined patches of unculi across the body of species in the large-bodied, non-rheophilic genus *Bagarius* (Roberts 1983) and a close (possibly sister group; Jiang et al. 2011; Ng 2006) relationship between *Bagarius* and the specialized rheophilic genus *Glyptothorax* (which have a synapomorphic thoracic friction pad; Jiang et al. 2011), de Pinna (1996) hypothesized that the latter genus may have been "preadapted" for the development of a friction pad.

Despite a lack of phylogenetic diversity estimates for whole fish communities in large river rapids, it is clear from species lists for various rapids (e.g., lower Congo River rapids, Africa: Roberts and Stewart 1976; lower Xingu River rapids, South America: Camargo et al. 2004) that these fish communities are assembled from a wide range of clades with broad geographic distributions. Limited phylogenetic hypotheses available for clades inclusive of large river rheophiles support the repeated occurrence of both endemic specialists (e.g., *Ossubtus xinguense*, Ortí et al. 2008) and localized diversification of these specialists within rapids (e.g., *Lamprologus* spp., Schelly and Stiassny 2004; *Steatocranus* spp., Schwarzer et al. 2011; *Teleocichla* spp., Kullander 1988; *Teleogramma depressum*, Markert et al. 2010).

## 6 Conclusions and Outlook

Fast-water habitats in the headwaters and lowland channels of river drainages host diverse and often highly specialized fish assemblages. In addition to promoting endemism and specialization within their boundaries, rapids can play an import role in limiting the distributions of, and gene flow among, large-bodied and/or more lentic species (Lovejoy and De Araújo 2000; Willis et al. 2007; Torrente-Vilara et al. 2011). Unfortunately, humans have historically viewed large river rapids less as evolutionary incubation chambers than as impediments to navigation and opportunities to generate hydroelectric power. In order to both facilitate navigation and produce hydroelectricity, a large shoal on the Tennessee River (Mississippi River drainage) that was long famous for its diversity and abundance of unionid mussels ("Muscle Shoals") was inundated by Wilson Dam in 1922 (Williams et al. 2008). Currently, the large Belo Monte dam complex is under construction on the rapids of the lower Xingu River in Brazil, and the Grand Inga dam complex has been proposed for rapids of the lower Congo River. Moreover, hundreds of smaller dams are already under construction or are being proposed for high elevation rivers draining the Andes (Finer and Jenkins 2012) and Himalayas (Grumbine and Pandit 2013).

As the shortcomings and gaps in this review illustrate, there is much yet to be discovered about the ecology, diversity, and evolutionary specialization of rheophilic fishes. Such studies are needed now more than ever in order to fully understand the threat to biodiversity posed by major human impacts. Some of these studies will require innovative techniques or new technologies. For example, obtaining precise information on microhabitats and associated fish assemblages within the deep and fast-flowing rapids of large rivers might only be possible with the use of submersible video, 3D laser-mapping (e.g., Pizarro et al. 2004), and Doppler velocimetry (e.g., Jackson et al. 2009) technologies. Other studies can be conducted using currently available whole specimens, tissues, and technologies. These include comparative functional studies of morphological features considered to be adaptations for life in fast-flowing water, including many poorly understood specializations mentioned herein (e.g., hydrodynamic function of keratinous ridges on scales and sensory function of elongate caudal-fin filaments). Many aspects of the patch dynamics model presented herein would benefit from more detailed phylogenetic and phylogeographic studies from which interspecific relationships and gene flow between populations may be inferred. To the extent possible using fossil and independent geologic calibrations, it will be important to time-calibrate phylogenies so that correlations between rapids formation and lineage diversification may be examined. Some of these studies are currently under way by ourselves and others, but many await future inquiring minds.

**Acknowledgments** We are particularly grateful to Ralf Britz, Heok Hui Tan, Heok Hee Ng, and John Sullivan for sharing their photographs of rheophilic fishes. We thank Adam Summers, James Albert, Ralf Britz, Maurice Kottelat, Heok Hui Tan, Heok Hee Ng, and Kirk Winemiller for helpful discussions on ideas presented herein, and we acknowledge funding from the US National Science Foundation (OISE-1064578, International Research Fellowship, to NKL; IOS# 1256793 to KWC; the iXingu Project, NSF DEB-1257813).

## References

Agostinho AA, Julio HF, Petrere M (1994) Itaipu reservoir (Brazil): impacts of the impoundment on the fish fauna and fisheries. In: Cowx IG (ed) Rehabilitation of freshwater fisheries. Fishing News Books, Oxford, pp 161–184

Alexander RMN (1964) The structure of the Weberian apparatus in the Siluri. Proc Zool Soc Lond 142:419–440

Alexander RMN (1965) Structure and function in the catfish. J Zool 148:88–152

Ambühl H (1962) Die Besonderheiten der Wasserströmung in physikalischer, chemischer und biologisher Hinsicht. Schweiz Z Hydrol 24:367–382

Arrington DA, Winemiller KO, Layman CA (2005) Community assembly at the patch scale in a species rich tropical river. Oecologia 144:157–167

Bănărescu PM, Nalbant TT (1964) Süßwasserfische der Türkei. 2. Teil Cobitidae. Mitt Hamb Zool Mus Inst 61:159–201

Barson NJ, Cable J, van Oosterhout C (2009) Population genetic analysis of microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source-sink metapopulation structure, founder events and population bottlenecks. J Evol Biol 22:485–497

Beckman M, Erõs T, Schmidtz A, Bleckman H (2010) Number and distribution of superficial neuromasts in twelve common European cypriniform fishes and their relationship to habitat occurrence. Int Rev Hydrobiol 95:273–284

Benjamin M (1986) The oral sucker of *Gyrinocheilus aymonieri* (Teleostei: Cypriniformes). J Zool Lond B 1:211–254

Bhatia B (1950) Adaptive modifications in a hill-stream catfish, *Glyptothorax telchitta* (Hamilton). Proc Nat Inst Sci India 16:271–285

Bird NC, Hernandez PL (2007) Morphological variation in the Weberian apparatus of Cypriniformes. J Morphol 268:739–757

Blake RW (2006) Biomechanics of rheotaxis in six teleost genera. Can J Zool 84:1173–1186

Blob RW, Rai R, Julius ML, Schoenfuss HL (2006) Functional diversity in extreme environments: effects of locomotor style and substrate texture on the waterfall-climbing performance of Hawaiian gobiid fishes. J Zool 268:315–324

Bournaud M (1963) Le courant, facteur écologique et éthologique de la vie aquatique. Hydrobiologia 21:125–165

Boyle, KS, Colleye O, Parmentier E (2014) Sound production to electric discharge: sonic muscle evolution in progress in *Synodontis* spp. catfishes (Mochokidae). Proc R Soc Lond. Ser B, Biol Sci 281:20141197

Briggs JC (1955) A monograph of the clingfishes (Order Xenopterygii). Stanford Ichthyol Bull 6:1–224

Briggs JC, Miller RR (1960) Two new freshwater clingfishes of the genus *Gobiesox* from southern Mexico. Occas Pap Mus Zool Univ Mich 616:1–15

Britz R, Ali A, Raghavan R (2012) *Pangio ammophila*, a new species of eel-loach from Karnataka, southern India (Teleostei: Cypriniformes: Cobitidae). Ichthyol Explor Freshw 23:45–50

Brown AV, Brussock PP (1991) Comparisons of benthic invertebrates between riffles and pools. Hydrobiologia 220:99–108

Buckup PA, Zamprogno C, Vieira F, Teixeira RL (2000) Waterfall climbing in Characidium (Crenuchidae: Characidiinae) from eastern Brazil. Ichthyol Explor Freshw 11:273–278

Buffagni A, Comin E (2000) Secondary production of benthic communities at the habitat scale as a tool to assess ecological integrity in mountain streams. Hydrobiologia 422:183–195

Buitrago-Suárez UA, Galvis G (1997) Description of some accessory structures of the urogenital system in the neotropical family Astroblepidae (Pisces, Siluroidei). Rev Acad Colombiana Cienc Exact Fís Natur 21:347–352

Camargo M, Giarrizzo T, Isaac V (2004) Review of the geographic distribution of fish fauna of the Xingu River Basin, Brazil. Ecotropica 10:123–147

Cardona L, Guerao G (1994) *Astroblepus riberae*, una nueva especie de siluriforme cavernicola del Peru (Osteichthyes: Astroblepidae). Mém Biospéol 21:21–24

Carlson RL, Lauder GV (2010) Living on the bottom: kinematics of benthic station-holding in darter fishes (Percidae: Etheostomatinae). J Morphol 271:25–35

Carrara F, Altermatt F, Rodriguez-Iturbe I, Rinaldo A (2012) Dendritic connectivity controls biodiversity patterns in experimental metacommunities. Proc Natl Acad Sci U S A 109:5761–5766

Casatti L, Castro RMC (2006) Testing the ecomorphological hypothesis in a headwater riffles fish assemblage of the rio São Francisco, southeastern Brazil. Neotrop Ichthyol 4:203–214

Chang HW (1945) Comparative study on the girdles and their adjacent structures in Chinese homalopterid fishes with special reference to the adaptation to torrential stream. Sinensia 16:9–26

Chen YY (ed) (1998) Fauna Sinica. Osteichthyes. Cypriniformes II. Science Press, Beijing

Chen LH, Chu KCM, Chiu YW (2004) Impacts of natural disturbance on fish communities in the Tachia River, Taiwan. Hydrobiologia 522:149–164

Conway KW (2011) Osteology of the South Asian Genus *Psilorhynchus* McClelland, 1839 (Teleostei: Ostariophysi: Psilorhynchidae) with investigation of its phylogenetic relationships within the Order Cypriniformes. Zool J Linn Soc 163:50–154

Conway KW, Lujan NK, Lundberg JG, Mayden RL et al (2012a) Microanatomy of the paired-fin pads of ostariophysan fishes (Teleostei: Ostariophysi). J Morphol 273:1127–1149

Conway KW, Mayden RL, Shrestha J, Edds DR (2012b) Redescription of the Nepalese endemic torrent minnow *Psilorhynchus pseudecheneis* with comments on *P. homaloptera* (Teleostei: Psilorhynchidae). Ichthyol Explor Freshw 23:193–210

Crop WD, Pauwels E, Hoorebeke LV, Geerinckx T (2013) Functional morphology of the Andean climbing catfishes (Astroblepidae, Siluriformes): alternative ways of respiration, adhesion, and locomotion using the mouth. J Morphol 274:1164–1179

Cullen JA, Maie T, Schoenfuss HL, Blob RW (2013) Evolutionary novelty versus exaptation: oral kinematics in feeding versus climbing in the waterfall-climbing Hawaiian goby *Sicyopterus stimpsoni*. PLoS One 8:e53274

Das D, Nag TC (2005) Structure of adhesive organ of the mountain-stream catfish, *Pseudocheneis sulcatus* (Teleostei: Sisoridae). Acta Zool 86:231–237

Das D, Nag TC (2009) Organs of adhesion in some mountain-stream teleosts of India: structure-function relationship. In: Gorb SN (ed) Functional surfaces in biology, vol 2. Springer Science and Business Media, Dordrecht, pp 105–122

De Meyer J, Geerinckx T (2014) Using the whole body as a sucker: combining respiration and feeding with an attached lifestyle in hill stream loaches (Balitoridae, Cypriniformes). J Morphol 275(9):1066–1079

de Pinna MCC (1996) A phylogenetic analysis of the Asian catfish families Sisoridae, Akysidae, and Amblycipitidae, with a hypothesis on the relationships of the Neotropical Aspredinidae (Teleostei, Ostariophysi). Fieldiana Zool 84:1–83 de Pinna MCC (1998) Phylogenetic relationships of neotropical Siluriformes (Teleostei: Ostariophysi): historical overview and synthesis of hypothesis. In: Malabarba LR, Reis RE, Vari RP, Lucena ZM et al (eds) Phylogeny and classification of Neotropical fishes. Edipucrs, Porto Alegre, pp 279–330

Diamond JM, Gilpin ME, Mayr E (1976) Species-distance relation for birds of the Solomon Archipelago, and the paradox of the great speciators. Proc Natl Acad Sci U S A 73:2160–2164

Evans JD, Page LM (2003) Distribution and relative size of the swim bladder in *Percina*, with comparisons to *Etheostoma*, *Crystallaria*, and *Ammocrypta* (Teleostei: Percidae). Environ Biol Fishes 66:61–65

Finer M, Jenkins CN (2012) Proliferation of hydroelectric dams in the Andean Amazon and implications for Andes-Amazon connectivity. PLoS One 7:1–9

Fink SV, Fink WL (1981) Interrelationships of the ostariophysan fishes (Teleostei). Zool J Linn Soc 72:297–353

Geerinckx T, Adriaens D (2006) The erectile cheek-spine apparatus in the bristlenose catfish *Ancistrus* (Loricariidae, Siluriformes), and its relation to the formation of a secondary skull roof. Zoology 109:287–299

Geerinckx T, De Kegel B (2014) Functional and evolutionary anatomy of the African suckermouth catfishes (Siluriformes: Mochokidae): convergent evolution in Afrotropical and Neotropical faunas. J Anat 225(2):197–208

Geerinckx T, Herrel A, Adriaens D (2011) Suckermouth armored catfish resolve the paradox of simultaneous respiration and suction attachment: a kinematic study of *Pterygoplichthys disjunctivus*. J Exp Zool A Ecol Genet Physiol 315:121–131

Geerinckx T, Huysseune A, Boone M, Claeys M et al (2012) Soft dentin results in unique flexible teeth in scraping catfish. Physiol Biochem Zool 85:481–490

Grumbine RE, Pandit MK (2013) Threats from India's Himalaya dams. Science 339:36–37

Guo X, He S, Zhang Y (2005) Phylogeny and biogeography of Chinese sisorid catfishes re-examined using mitochondrial cytochrome *b* and 16S rRNA gene sequences. Mol Phylogenet Evol 35:344–362

Hänfling B, Weetman D (2006) Concordant genetic estimators of migration reveal anthropologically enhanced source-sink population structure in the river sculpin, *Cottus gobio*. Genetics 173:1487–1501

Haspel G, Schwartz A, Streets A, Camacho DE, Soares D (2012) By the teeth of their skin, cavefish find their way. Curr Biol 22:R629–R630

He SP, Gayet M, Meunier FJ (1999) Phylogeny of the Amphiliidae (Teleostei: Siluriformes). Ann Sci Nat 20:117–146

Hoover TM, Ackerman JD (2004) Near-bed hydrodynamic measurements above boulders in shallow torrential streams: implications for stream biota. J Environ Eng Sci 3:365–378

Hora SL (1922) Structural modifications in the fishes of mountain torrents. Rec Indian Mus 24:31–61

Hora SL (1930) Ecology, bionomics and evolution of the torrential fauna, with special reference to organs of attachment. Philos Trans R Soc Lond B 218:171–282

Horner RR, Welch EB, Seeley MR, Jacoby JM (1990) Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. Freshw Biol 24:215–232

Hutchinson GE (1939) Ecological observations on the fishes of Kashmir and Indian Tibet. Ecol Monogr 9:145–182

Illies J (1961) Versuch einer allgemeinen biozönotischen Gliederung der Fließgewässer. Int Rev Hydrobiol 46:205–213

Jackson PR, Oberg KA, Gardiner N, Shelton J (2009) Velocity mapping in the lower Congo River: a first look at the unique bathymetry and hydrodynamics of Bulu Reach, West Central Africa. In: Proceedings of the 6th IAHR symposium on river coastal and estuarine morphodynamics, Santa Fe, Argentina, pp 1007–1014

Jégu M (1992) *Ossubtus xinguense*, nouveaux genre et espéce du Rio Xingu, Amazonie, Brésil (Teleostei: Serrasalmidae). Ichthyol Explor Freshw 3:235–252

Jiang W, Ng HH, Yang J, Chen X (2011) Monophyly and phylogenetic relationships of the catfish genus *Glyptothorax* (Teleostei: Sisoridae) inferred from nuclear and mitochondrial gene sequences. Mol Phylogenet Evol 61:278–289

Johnson RDO (1912) Notes on the habits of a climbing catfish (*Arges marmoratus*) from the Republic of Colombia. Ann NY Acad Sci 22:327–333

Kasumyan AO (2011) Tactile reception and behavior of fish. J Ichthyol 51:1035–1103

Keller EA, Melhorn WN (1978) Rhythmic spacing and origin of pools and riffles. Geol Soc Am Bull 89:723–730

Kevern NR, Ball RC (1965) Primary productivity and energy relationships in artificial streams. Limnol Oceanogr 10:74–87

Kottelat M (1988) Two species of cavefishes from northern Thailand in the genera *Nemacheilus* and *Homaloptera* (Osteichthyes: Homalopteridae). Rec Aust Mus 40:225–231

Kottelat M (1990) Indochinese nemacheilines. A revision of nemacheiline loaches (Pisces: Cypriniformes) of Thailand, Burma, Laos, Cambodia and southern Viet Nam. Verlag Dr. Friedrich Pfeil, München

Kottelat M (1998) *Homaloptera yuwonoi*, a new species of hillstream loach from Borneo, with a new generic name for *H. thamicola* (Teleostei: Balitoridae). Ichthyol Explor Freshw 9:267–272

Kottelat M (2001a) Freshwater fishes of northern Vietnam. A preliminary check-list of the fishes known or expected to occur in northern Vietnam with comments on systematics and nomenclature. Environment and Social Development Unit, East Asia and Pacific Region. The World Bank, Washington, DC

Kottelat M (2001b) Fishes of Laos. WHT Publications, Colombo

Kottelat M (2006) Fishes of Mongolia. A check-list of the fishes known to occur in Mongolia with comments on systematics and nomenclature. The World Bank, Washington, DC

Kottelat M, Freyhof J (2008) Handbook of European freshwater fishes. Kottelat, Freyhof, Cornol, Berlin

Kottelat M, Whitten AJ, Kartikasari SN, Wirjoatmodjo S (1993) Freshwater fishes of Western Indonesia and Sulawesi. Periplus Editions, Hong Kong

Kullander SO (1988) *Teleocichla*, a new genus of South American rheophilic cichlid fishes with six new species (Teleostei: Cichlidae). Copeia 1988:196–230

Kullander SO, Fang F, Delling B, Åhlander E (1999) The fishes of the Kashmir Valley. In: Nyman L (ed) River Jhelum. Kashmir Valley: impacts on the aquatic environment. Swedmar, Göteborg, pp 99–16

Ladich F (2000) Acoustic communication and the evolution of hearing in fishes. Philos Trans R Soc Lond B 355:1285–1288

Langerhans RB (2008) Predictability of phenotypic differentiation across flow regimes in fishes. Integr Comp Biol 48:750–768

Langerhans RB, Layman CA, Langerhans AK, Dewitt TJ (2003) Habitat-associated morphological divergence in two Neotropical fish species. Biol J Linn Soc 80:689–698

Lechner W, Ladich F (2008) Size matters: diversity in swimbladders and Weberian ossicles affects hearing in catfishes. J Exp Biol 211:1681–1689

Leopold LB (1953) Downstream change in velocity in rivers. Am J Sci 251:606–624

Longo S, Riccio M, McCune AR (2013) Homology of lungs and gas bladders: insights from arterial vasculature. J Morphol 274:687–703

Losos JB, Ricklefs RE (2009) Adaptation and diversification on islands. Nature 457:830–836

Losos JB, Schluter D (2000) Analysis of an evolutionary species-area relationship. Nature 408:847–850

Lovejoy NR, De Araújo MLG (2000) Molecular systematics, biogeography and populations structure of Neotropical freshwater needlefishes of the genus *Potamorrhaphis*. Mol Ecol 9:259–268

Lujan NK, Chamon CC (2008) Two new species of Loricariidae (Teleostei: Siluriformes) from main channels of the upper and middle Amazon Basin, with discussion of deep water specialization in loricariids. Ichthyol Explor Freshw 19:271–282

Lujan NK, Winemiller KO, Armbruster JW (2012) Trophic diversity in the evolution and community assembly of loricariid catfishes. BMC Evol Biol 12:124

Lujan NK, Roach KA, Jacobsen D, Winemiller KO et al (2013) Aquatic community structure across an Andes-to-Amazon fluvial gradient. J Biogeogr 40:1715–1728

Lujan NK, Armbruster JW, Lovejoy NR, López-Fernández H (2015) Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. Mol Phylogenet Evol 82:269–288

Lundberg JG, Marsh E (1976) Evolution and functional anatomy of the pectoral fin rays in cyprinoid fishes, with emphasis on the suckers (family Catostomidae). Am Midl Nat 96:332–349

Maie T, Schoenfuss HL, Blob RW (2012) Performance and scaling of a novel locomotor structure: adhesive capacity of climbing gobiid fishes. J Exp Biol 215:3925–3936

Markert JA, Schelly RC, Stiassny MLJ (2010) Genetic isolation and morphological divergence mediated by high-energy rapids in two cichlid genera from the lower Congo rapids. BMC Evol Biol 10:149

Marrero C, Winemiller KO (1993) Tube-snouted gymnotiform and mormyriform fishes: convergence of a specialized foraging mode in teleosts. Environ Biol Fishes 38:299–309

Mayden RL, Simons AM (2002) Crevice spawning behavior in *Dionda dichroma*, with comments on the evolution of spawning modes in North American shiners (Teleostei: Cyprinidae). Rev Fish Biol Fisher 12:327–337

Mazzoldi C, Lorenzi V, Rasotto MB (2007) Variation of male reproductive apparatus in relation to fertilization modlaities in the catfish families Auchenipteridae and Callichthyidae (Teleostei: Siluriformes). J Fish Biol 70:243–256

McDowall RM (1978) New Zealand Freshwater Fishes a guide and natural history. Heinemann Education Books, Auckland

Meissner K, Juntunen A, Malmqvist B, Muotka T (2009) Predator–prey interactions in a variable environment: responses of a caddis larva and its blackfly prey to variations in stream flow. Ann Zool Fenn 46:193–204

Miller RR, Briggs JC (1962) *Dactyloscopus amnis*, a new sand stargazer from rivers of the Pacific slope of southern Mexico. Occas Pap Mus Zool Univ Mich 627:1–11

Mochizuki K, Fukui S (1983) Development and replacement of upper jaw teeth in gobiid fish, *Sicyopterus japonicus*. Jpn J Ichthyol 30:27–36

Monsembula Iyaba RJC, Liyandja T, Stiassny MLJS (2013) Fishes of the N'sele River (Pool Malebo, Congo basin, Central Africa): a list of species collected in the main channel and affluent tributaries, Kinshasa Province, Democractic Republic of Congo. Check List 9:941–956

Muntz WRA (1982) Visual adaptations to different light environments in Amazonian fishes. Rev Can Biol Exp 41:35–46

Ng HH (2006) A phylogenetic analysis of the Asian catfish family sisoridae (Teleostei: Siluriformes), and the evolution of epidermal characters in the group. Dissertation, University of Michigan

Ng HH, Kottelat M (2005) *Caelatoglanis zonatus*, a new genus and species of the Erethistidae (Teleostei: Siluriformes) from Myanmar, with comments on the nomenclature of *Laguvia* and *Hara* species. Ichthyol Explor Freshw 16:13–22

Ono RD (1980) Fine structure and distribution of epidermal projections associated with taste buds on the oral papillae in some loricariid catfishes (Siluroidei: Loricariidae). J Morphol 164:139–159

Orrego R, Adams SM, Barra R, Chiang G et al (2009) Patterns of fish community composition along a river affected by agricultural and urban disturbance in south-central Chile. Hydrobiologia 620:35–46

Ortí G, Sivasunder A, Dietz K, Jégu M (2008) Phylogeny of the Serrasalmidae (Characiformes) based on mitochondrial DNA sequences. Genet Mol Biol 31:343–351

Page LM (1980) The life histories of *Etheostoma olivaceum* and *Etheostoma striatulum*, two species of darters in central Tennessee. Biol Notes Ill Nat Hist Surv 113:1–14

Page LM, Burr BM (2011) Peterson field guide to freshwater fishes of North America North of Mexico, 2nd edn. Houghton Mifflin Harcourt, New York, NY

Page LM, Swofford DL (1984) Morphological correlates of ecological specialization in darters. Environ Biol Fishes 11:139–159

Pardo R (2002) Morphologic differentiation of *Trichomycterus areolatus* Valenciennes 1846 (Pisces: Siluriformes: Trichomycteridae) from Chile. Gayana 66:203–205

Parenti LR, Lim KKP (2005) Fishes of the Rajang Basin, Sarawak, Malaysia. Raff Bull Zool Suppl 13:175–208

Peng Z, He S, Zhang Y (2004) Phylogenetic relationships of glyptosternoid fishes (Siluriformes: Sisoridae) inferred from mitochondrial cytochrome *b* gene sequences. Mol Phylogenet Evol 31:979–987

Pinky MS, Yashpal M, Ojha J, Mittal AK (2004) Occurence of keratinization in the structures associated with lips of a hill stream fish *Garra lamta* (Hamilton) (Cyprinidae, Cypriniformes). J Fish Biol 65:1165–1172

Pizarro O, Uestice R, Singh H (2004) Large area 3D reconstructions from underwater surveys. Oceans 2:678–687

Quezada-Romegiall C, Fuentes M, Véliz D (2010) Comparative population genetics of *Basilichthys microlepidotus* (Atheriniformes: Atherinopsidae) and *Trichomycterus areolatus* (Siluriformes: Trichomycteridae) in north central Chile. Environ Biol Fishes 89:173–186

Reinhardt UG, Eidietis L, Friedle SE, Moser ML (2008) Pacific lamprey climbing behavior. Can J Zool 86:1264–1272

Ribeiro AC (2006) Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. Neotrop Ichthyol 4:225–246

Roberts TR (1978) An ichthyological survey of the Fly River in Papua New Guinea with descriptions of new species. Smithson Contrib Zool 281:1–72

Roberts TR (1982) Unculi (horny projections arising from single cells), an adaptive feature of the epidermis of ostariophysan fishes. Zool Scr 11:55–76

Roberts TR (1983) Revision of the South and Southeast Asian sisorid catfish genus *Bagarius*, with description of a new species from the Mekong. Copeia 1983:435–445

Roberts TR (1989a) The freshwater fishes of western Borneo (Kalimantan Barat, Indonesia). Mem Cal Acad Sci 14:1–210

Roberts TR (1989b) Systematic revision and description of new species of suckermouth catfishes (*Chiloglanis*, Mochokidae) from Cameroun. Proc Cal Acad Sci 46:151–178

Roberts TR, Stewart DJ (1976) An ecological and systematic survey of fishes in the rapids of the Lower Zaire of Congo River. Bull Mus Comp Zool 147:239–317

Sabaj MH, Armbruster JW, Page LM (1999) Spawning in *Ancistrus* (Siluriformes: Loricariidae) with comments on the evolution of snout tentacles as a novel reproductive strategy: larval mimicry. Ichthyol Explor Freshw 10:217–229

Sahara N, Moriyama K, Iida M, Watanabe S (2013) Unique features of pedicellate attachment of the upper jaw teeth in the adult gobiid fish *Sicyopterus japonicus* (Teleostei, Gobiidiae): morphological and structural characteristics and development. J Morphol 274:512–524

Sawada Y (1982) Phylogeny and zoogeography of the superfamily Cobitoidea (Cyprinoidei, Cypriniformes). Mem Fac Fish Hokkaido Univ 28:65–223

Saxena SC (1961) Adhesive apparatus of an Indian hill-stream sisorid fish. Copeia 1961:471–473

Saxena SC, Chandy M (1966) Adhesive apparatus in certain Indian hill stream fishes. J Zool 148:315–340

Schaefer SA (1984) Mechanical strength of the pectoral spine/girdle complex in *Pterygoplichthys* (Loricariidae: Siluroidei). Copeia 1984:1005–1008

Schaefer SA, Chakrabarty P, Geneva AJ, Sabaj Pérez MH (2011) Nucleotide sequence data confirm diagnosis and local endemism of variable morphospecies of Andean astroblepid catfishes (Siluriformes: Astroblepidae). Zool J Linn Soc 162:90–102

Schelly RC, Stiassny MLJ (2004) Revision of the Congo River *Lamprologus* Schilthuis, 1891 (Teleostei: Cichlidae), with descriptions of two new species. Am Mus Novit 3451:1–40

Schlosser IJ (1987) The role of predation in age- and size-related habitat use by stream fishes. Ecology 68:651–659

Schoenfuss HL, Blob RW (2003) Kinematics of waterfall climbing in Hawaiian freshwater fishes (Gobiidae): vertical propulsion at the aquatic–terrestrial interface. J Zool 26:191–205

Schulz-Mirbach T, Metscher BD, Ladich F (2012) Relationships between swim bladder morphology and hearing abilities – a case study on Asian and African cichlids. PLoS One 7:e42292

Schwarzer J, Misof B, Ifuta SN, Schliewen UK (2011) Time and origin of cichlid colonization of the lower Congo Rapids. PLoS One 6:e22380

Shrestha OH, Edds DR (2012) Fishes of Nepal: mapping distributions based on voucher specimens. Emporia State Res Stud 48:14–74

Sidlauskas BL, Vari RP (2008) Phylogenetic relationships within the South American fish family Anostomidae (Teleostei, Ostariophysi, Characiformes). Zool J Linn Soc 154:70–210

Singh N, Agarwal NK (1993) Organs of adhesion in four hillstream fishes, a comparative morphological study. In: Bahuguna HN, Singh HR (eds) Advances in limnology: proceedings of the national symposium on advances in limnology and conservation of endangered fish species, 23–25 October 1989. Narendra Publishing House, New Delhi, pp 311–316

Sinha AK, Singh I, Singh BR (1990) The morphology of the adhesive organ of the sisorid fish, *Glyptothorax pectinopterus*. Jpn J Ichthyol 36:427–431

Skelton P (1993) A complete guide to the fishes of Southern Africa. Struik Publishers, Cape Town

Stewart PC, Smith ME (2009) Conspecific sound localization in *Otocinclus affinis*. Proc Inst Acoust 31:230–234

Stewart DJ, Ibarra M, Barriga-Salazar R (2002) Comparison of deep-river and adjacent sandy-beach fish assemblages in the Napo River Basin, Eastern Ecuador. Copeia 2002:333–343

Stiassny MLJ, Getahun A (2007) An overview of labeonin relationships and the phylogenetic placement of the Afro-Asian genus *Garra* Hamilton, 1922 (Teleostei: Cyprinidae), with the description of five new species of *Garra* from Ethiopia, and a key to all African Species. Zool J Linn Soc 150:41–83

Sullivan JP, Lundberg JG, Hardman M (2006) A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriforms) using rag1 and rag2 nuclear gene sequences. Mol Phylogenet Evol 41:636–662

Taft NK (2011) Functional implications of variation in pectoral fin ray morphology between fishes with different patterns of pectoral fin use. J Morphol 272:1144–1152

Talwar PK, Jhingran AG (1991) Inland fishes of India and adjacent countries. Oxford & IBH Publishing, New Delhi

Tan HH (2006) The Borneo suckers. Revision of the torrent loaches of Borneo (Balitoridae: *Gastromyzon*, *Neogastromyzon*). Natural History Publications (Borneo), Kota Kinabalu

Tan HH, Kottelat M (2009) The fishes of the Batang Hari drainage, Sumatra, with description of six new species. Ichthyol Explor Freshw 20:13–69

Thomson AW, Page LM (2006) Genera of the Asian catfish families Sisoridae and Erethistidae (Teleostei: Siluriformes). Zootaxa 1345:1–96

Thorp JH, Thomas MC, Delong MD (2006) The riverine ecosystem synthesis: biocomplexity in river networks across space and time. River Res Appl 22:123–147

Torrente-Vilara G, Zuanon J, Leprieur F, Oberdorff T et al (2011) Effects of natural rapids and waterfalls on fish assemblage structure in the Madeira River (Amazon Basin). Ecol Freshw Fish 20:588–597

Uehara K, Miyoshi S (1993) Structure of the comblike teeth of the Ayu Sweetfish *Plecoglossus altivelis* (Teleostei: Isospondyli): I. Denticles and tooth attachment. J Morphol 217:229–238

Unmack PJ, Bennin AP, Habit EM, Victoriano PF et al (2009) Impact of ocean barriers, topography, and glaciation on the phylogeography of the catfish *Trichomycterus areolatus* (Teleostei: Trichomycteridae) in Chile. Biol J Linn Soc 97:876–892

Verberk WCEP, Bilton DT, Calosi P, Spicer JI (2011) Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. Ecology 92:1565–1572

Wagner CE, Harmon LJ, Seehausen O (2014) Cichlid species-area relationships are shaped by adaptive radiations that scale with area. Ecol Lett 17:583–592

Wainwright DK, Kleinteich T, Kleinteich A, Gorb SN, Summers AP (2013) Stick tight: suction adhesion on irregular surfaces in the northern clingfish. Biol Lett 9:20130234

Warren ML, Burr BB, Kuhajda B (1986) Aspects of the reproductive biology of *Etheostoma tippecanoe* with comments on egg-burying behavior. Am Midl Nat 116:215–218

Waters JM, Craw D, Youngson JH, Wallis GP et al (2001) Genes meet geology: fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. Evolution 55:1844–1851

Weitzman SH (2003) Hearing in catfishes, especially that of the family Loricariidae. In: Evers HG, Seidel I (eds) Baensch catfish atlas, vol 1. Mergus Verlag GmbH, Melle, pp 31–39

Wickler W (1971) Verhaltensstudien an einem hochspezialisierten Grundfisch, *Gastromyzon borneensis* (Cyprinoidea, Gastromyzonidae). Z Tierpsychol 29:467–480

Wiley ML, Collette BB (1970) Breeding tubercles and contact organs in fishes: their occurrence, structure, and significance. Bull Am Mus Nat Hist 143:143–216

Wiley EO, Johnson GD (2010) A teleost classification based on monophyletic groups. In: Nelson JS, Schultze HP, Wilson MVH (eds) Origin and phylogenetic interrelationships of teleosts. Verlag Dr. Friedrich Pfeil, Munich, pp 123–182

Williams JD, Bogan AE, Garner JT (2008) Freshwater mussels of Alabama and the Mobile Basin in Georgia, Mississippi, and Tennessee. University of Alabama Press, Tuscaloosa

Willis SC, Nunes MS, Montaña CG, Farias IP, Lovejoy NR (2007) Systematics, biogeography, and evolution of the Neotropical peacock basses *Cichla* (Perciformes: Cichlidae). Mol Phylogenet Evol 44:291–307

Winemiller KO, Rose KA (1992) Patterns of life-history diversification in North American fishes: implications for population regulation. Can J Fish Aquat Sci 49:2196–2218

Winemiller KO, Flecker AS, Hoeinghaus DJ (2010) Patch dynamics and environmental heterogeneity in lotic ecosystems. J North Am Benthol Soc 29:84–99

Wohl EE, Vincent KR, Merritts D (1993) Pool and riffle characteristics in relation to channel gradient. Geomorphology 6:99–100

Worischka S, Koepsch C, Hellmann C, Winkelmann C (2012) Habitat overlap between predatory benthic fish and their invertebrate prey in streams: the relative influence of spatial and temporal factors on predation risk. Freshw Biol 57:2247–2261

Yue PQ (ed) (2000) Fauna Sinica. Osteichthyes. Cypriniformes III. Science Press, Beijing

Zhang E (2005) Phylogenetic relationships of labeonine cyprinids of the disc-bearing group (Pisces: Teleostei). Zool Stud 44:130–143

Zhang E, Zhou W (2012) Sinigarra napoense, a new genus and species of labeonin fishes (Teleostei: Cyprinidae) from Guangxi Province, South China. Zootaxa 3586:17–25

Zhou W, Kottelat M (2005) Schistura disparizona, a new species of loach from Salween drainage in Yunnan (Teleostei: Balitoridae). Raffles Bull Zool 13:17–20

Zhou W, Pan XF, Kottelat M (2005) Species of *Garra* and *Discogobio* (Teleostei: Cyprinidae) in Yuanjiang (Upper Red River) drainage of Yunnan Province, China with description of a new species. Zool Stud 44:445–453

Zorach T (1972) Systematics of the percid fishes, *Etheostoma camurum* and *E. chlorobranchium* new species, with a discussion of the subgenus Nothonotus. Copeia 1972:427–447

Zuanon J (1999) História natural da ictiofauna de corredeiras do Rio Xingu, na região de Altamira, Pará. Dissertation, Universidade Estadual de Campinas

# Hydrogen Sulfide-Toxic Habitats

**Rüdiger Riesch, Michael Tobler, and Martin Plath**

**Abstract** Hydrogen sulfide ($H_2S$) is a potent respiratory toxicant that is lethal to most metazoans (including fishes) in micromolar concentrations, as demonstrated by mass kills following environmental spills. Nevertheless, a number of teleosts have adapted to, and thrive in, habitats with high ambient $H_2S$ concentrations as found, for example, at marine hydrothermal vents ("black smokers"), cold seeps, or in freshwater sulfide springs. Livebearing fishes (Poeciliidae) dominate amongst freshwater fishes inhabiting sulfide spring in the New World and are the most studied group of freshwater sulfide-dwellers. In this chapter, we identify targets of directional selection in sulfidic habitats and demonstrate how these affect different levels of biological organization (e.g., cellular functioning and molecular evolution, morphology and organ evolution, whole body performance and eco-physiological traits, life histories). We highlight multifarious selective regimes arising from correlated abiotic stressors (like hypoxia) and altered ecological parameters (like truncated ecological communities and altered predatory regimes). Finally, we discuss the evidence for replicated ecological speciation as a result of independent evolutionary transitions in different lineages of poeciliids into sulfide waters, and we summarize studies examining the question of how local adaptation translates into the emergence of reproductive isolation due to selection against non-adapted individuals migrating between habitat types.

---

R. Riesch (✉)
School of Biological Sciences, Centre for Ecology, Evolution and Behaviour, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK
e-mail: Rudiger.Riesch@rhul.ac.uk

M. Tobler
Division of Biology, Kansas State University, 116 Ackert Hall, Manhattan, KS 66506, USA

M. Plath
College of Animal Science and Technology, Northwest A&F University, Yangling 712100, PR China

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_7

## 1 Hydrogen Sulfide-Rich Waters: Chemical Properties, Occurrence, and Physiological Consequences

Even though alchemists already knew about hydrogen sulfide ($H_2S$) during the middle ages, it was not formally described until 1777 as "Stinkerde" by the German-Swedish chemist C.W. Scheele due to its characteristic rotten-egg smell (Bagarinao 1992). $H_2S$ is a colorless gas that is heavier than air, and can be oxidized by a variety of agents to form sulfur dioxide ($SO_2$), sulfates (e.g., $H_2SO_4$), and elemental sulfur (Reiffenstein et al. 1992). In aqueous solution, $H_2S$ dissociates to $HS^-$ and $S^{2-}$ depending on the pH. While the ratio of $H_2S$ to $HS^-$ at pH 7.0 is roughly 50:50, approximately 91 % of sulfide at pH 6.0 exists in the unionized form, but only approximately 9 % at pH 8.0 (i.e., in seawater; Schoonen and Barnes 1988).

Hydrogen sulfide is a ubiquitous substance in natural environments and is constantly produced in animal cells as a by-product of cysteine catabolism (Stipanuk 2004; Stipanuk and Ueki 2011). Environmental hydrogen sulfide often results from the bacterial breakdown of organic matter in the absence of oxygen and accordingly occurs in anoxic sediments of swamps, salt marshes, tidal creeks, and cold seeps, from which it can diffuse into the water column at sometimes high, albeit often temporally variable, concentrations (Bagarinao 1992). Naturally occurring $H_2S$ is further found in high and sustained concentrations in aquatic environments associated with under-earth oil deposits and geothermal activity (Jannasch and Taylor 1984; Rosales Lagarde 2012). Besides its natural origin in ecosystems, $H_2S$ often also stems from anthropogenic sources like pulp and paper mills, urban sewers, farming, natural gas production, tanneries, or geothermal power plants (e.g., Bagarinao 1992; Reiffenstein et al. 1992).

Due to its lipid solubility, $H_2S$ has the ability to pass through biological membranes and readily invade organismal systems, where it acts as a potent toxicant. Sulfide toxicity results from various effects at the molecular and cellular levels. For example, it binds to the porphyrin ring of hemoglobin and myoglobin, leading to the formation of sulfhemoglobin and sulfmyoglobin, which are non-functional in (blood) oxygen transport (Bagarinao 1992; Grieshaber and Völkel 1998). Sulfide also binds to and inhibits cytochrome *c* oxidase in the respiratory chain of mitochondria, thus hampering ATP production (Cooper and Brown 2008). In freshwater fishes, concentrations as low as 0.4 μM $H_2S$ can lead to reduced growth and survival, necrosis, lower survival of eggs, and higher incidence of offspring deformities (Bagarinao 1992). However, sulfide concentrations of acute toxicity (96-h $LC_{50}$) vary substantially among species (U.S. EPA 2013), as well as with temperature, oxygen content, and pH (e.g., Geiger et al. 2000; Affonso and Rantin 2005). Furthermore, juveniles are usually more negatively affected than adults, and freshwater fishes more than marine species (Bagarinao 1992).

Most organisms are capable of sulfide detoxification to some extent, but this process ceases at various ambient sulfide concentrations, when the inhibition of cytochrome *c* oxidase eventually blocks electron transport through the respiratory chain (Lagoutte et al. 2010; Pfenninger et al. 2014). One enzyme that plays a key role in sulfide homeostasis in most domains of life is sulfide:quinone oxidoreductase (SQR; Theissen et al. 2003; Shahak and Hauska 2008; Marcia et al. 2009). SQR is tied to the mitochondrial respiratory chain and binds sulfide molecules that are subsequently processed by additional enzymes to form thiosulfate, which can be excreted via urine in mammals and likely also in fish (U.S. EPA 2013; Hildebrandt and Grieshaber 2008).

While higher concentrations of $H_2S$ are usually toxic to most metazoans, lower concentrations can also have beneficial effects. In freshwater fishes very low concentrations of $H_2S$ ($<0.4$ μM) can result in enhanced growth and survival (Bagarinao 1992). Recent studies further uncovered a role of $H_2S$ in cell signaling. $H_2S$ can, for example, affect the functioning of ion channels and is involved in the regulation of vasodilation and inflammation (Szabó 2007; Li et al. 2011).

## 2 Fishes in $H_2S$-Rich Environments

In many aquatic environments where $H_2S$ production is related to bacterial decomposition of organic material under anoxic conditions, $H_2S$ concentrations often peak intermittently in particular microhabitats. Accordingly, a wide variety of fish taxa inhabit environments exhibiting temporally restricted occurrence of $H_2S$ (e.g., Bagarinao and Vetter 1989; Geiger et al. 2000; Affonso and Rantin 2005). However, for this chapter, we primarily focus on systems in which $H_2S$ occurs at high and sustained concentrations, such as in cold seeps, hydrothermal vents, and particularly freshwater sulfide springs.

Hydrothermal vents and cold seeps have been the focus of extensive research over the past decades (reviewed by Van Dover 2000; Little and Vrijenhoek 2003). Most organisms associated with them are invertebrates, including tube worms (Siboglinidae), vent clams (Vesicomyidae), vent mussels (Bathymodiolinae), and crustaceans like vent and cold-seep shrimps (Alvinocarididae) and crabs (Bythograeidae). These species have evolved different strategies of dealing with the presence of toxic $H_2S$. Obligatory symbioses with $H_2S$-detoxifying bacteria have been described for several invertebrate taxa (e.g., Cavanaugh et al. 1981; Grieshaber and Völkel 1998; Vrijenhoek et al. 2007; Beinart et al. 2012), while others evolved morphological and structural modifications that exclude $H_2S$ from the body, are able to detoxify sulfide in the body wall, or bind and oxidize sulfide via blood components or in the mitochondria (Reiffenstein et al. 1992; Grieshaber and Völkel 1998). Yet, others evolved sulfide-insensitive cytochrome *c* oxidase or generate ATP from sulfide oxidation (Reiffenstein et al. 1992; Grieshaber and Völkel 1998).

Several fishes have been described from hydrothermal vents and cold seeps, and the family Zoarcidae appears to represent the dominant group among them (e.g., Biscoito et al. 2002; Wolff 2005; Biscoito 2006). Most of these fishes are transient visitors of hydrothermal vents and cold seeps (Biscoito et al. 2002; Wolff 2005;

Biscoito 2006), but more than 20 species of fishes are considered hydrothermal vent and cold seep endemics (Wolff 2005). Among them are, for example, a bythitid (*Bythites hollisi*; Cohen et al. 1990), several zoarcids (Rosenblatt and Cohen 1986; Biscoito et al. 2002), and at least two cynoglossid flatfish species of the genus *Symphurus* (Munroe and Hashimoto 2008; Munroe et al. 2011). The latter two have been described to occur in enormous densities of around 90 individuals/m$^2$ around hydrothermal vents and appear to even rest on surfaces of molten or recently congealed sulfur flows (Tunnicliffe et al. 2010, 2013). As predators, fish play an important role in structuring vent communities (e.g., Micheli et al. 2002; Tunnicliffe et al. 2013) and linking vent communities to the surrounding deep-sea environment by redistributing secondary production (Tunnicliffe et al. 2003). Nonetheless, studying the evolutionary ecology of fishes associated with marine, $H_2S$-rich habitats has largely been hampered by problems associated with assessing, capturing, and maintaining these highly specialized taxa (but see Tunnicliffe et al. 2013).

This contrasts with teleost fishes inhabiting freshwater sulfide springs, the evolutionary ecology of which is better researched due to their accessibility. A list of all known fish populations/species inhabiting freshwater sulfide springs is provided in Table 1. Most known members come from the order of toothcarps (Cyprinodontiformes), in particular from the family of livebearing fishes (Poeciliidae) (Fig. 1), which can be found in a variety of sulfidic freshwater habitats in the New World (Fig. 2a–g). One of the few exceptions is the synbranchid eel *Ophisternon aenigmaticum* that co-occurs with sulfide-adapted *Poecilia mexicana* in some sulfide springs of southern Mexico (Gordon and Rosen 1962; Riesch et al. 2009; Greenway et al. 2014).

Most research on sulfide spring fishes has been conducted on poeciliids, and little is known about the biology of non-poeciliid inhabitants of sulfidic freshwaters (Table 1), so we mainly focus on sulfide-adapted poeciliid fishes in the following sections. Basic information on *Aphanius ginaonis* from a thermal sulfur spring (probably also releasing $H_2S$) in southern Iran can be found in Coad (1980) and Teimori (2013), and no information is available on the biology of *Cyprinodon bobmilleri* from a northern Mexican sulfide spring apart from the species description (Lozano-Vilano and Contreras-Balderas 1999).

Amongst the best-researched groups of $H_2S$-adapted fishes are the mollies in the *Poecilia mexicana* species complex in southern Mexico (e.g., Plath et al. 2007a, 2013; Tobler et al. 2008a, 2011; Riesch et al. 2010c, 2011a). Within a small area of approximately 200 km$^2$ in the federal states of Tabasco and Chiapas, populations in the *P. mexicana* species complex have independently colonized sulfide springs in at least four river drainages (from east to west: Tacotalpa, Puyacatengo, Ixtapangajoya, and Pichucalco; Tobler et al. 2011; Palacios et al. 2013). In two drainages, sulfide spring populations have been described as distinct species: *P. thermalis* in the Ixtapangajoya (Steindachner 1863) and *P. sulphuraria* in the Pichucalco drainage (Alvarez 1948). In the Tacotalpa drainage, adaptation of *P. mexicana* to $H_2S$ is coupled with the occurrence of the species in karstic underground habitats (i.e., caves; see chapter “Cave Environments”). The

**Table 1** Compilation of currently known freshwater fishes occurring in habitats with permanently high $H_2S$

| Family | Species | Site locality of $H_2S$-adapted population(s) | Species characteristics | Reference |
|---|---|---|---|---|
| Cyprinodontidae | *Aphanius ginaonis* | Genow hot sulfuric spring, Hormuzgan drainage, Iran | Egg-laying, sulfur-endemic | Coad (1980), Teimori (2013) |
| | *A. dispar* | Several hot sulfuric springs in the Hormuzgan and Helleh drainages, Iran | Egg-laying | Teimori (2013) |
| | *A. furcatus* | Two hot sulfuric springs in the Hormuzgan drainage, Iran | Egg-laying | Teimori (2013) |
| | *Cyprinodon bobmilleri* | Río San Fernando drainage (Baños de San Ignacio), Nuevo León, México | Egg-laying, sulfide-endemic | Lozano-Vilano and Contreras-Balderas (1999) |
| Poeciliidae | *Acanthophacelus (Poecilia) reticulata* | Poza Azufre, Venezuela | Livebearing | Winemiller et al. (1990), Tobler and Hastings (2011), Riesch et al. (2014) |
| | *Gambusia affinis* | Vendome Well/ Black Sulfur Springs, Oklahoma | Livebearing | Tobler and Hastings (2011), Riesch et al. (2014) |
| | *G. holbrooki* | (1) Green Springs, (2) Newport Springs, and (3) Panacea Mineral Springs, Florida | Livebearing | Riesch et al. (2014) |
| | *G. hubbsi* | Archie's Blue Hole, The Bahamas | Livebearing | Riesch et al. (2014) |
| | *G. eurystoma* | Río Pichucalco (Baños del Azufre), Tabasco, México | Livebearing, sulfide-endemic | Miller (1975), Tobler et al. (2008c), Tobler and Plath (2009a) |

(continued)

**Table 1** (continued)

| Family | Species | Site locality of $H_2S$-adapted population(s) | Species characteristics | Reference |
|---|---|---|---|---|
| | *G. sexradiata* | Río Puyacatengo (Mogote del Puyacatengo), Tabasco, México | Livebearing | Riesch et al. (2010b, 2014) |
| | *Limia sulphurophila* | Balnearios La Zurza, Dominican Republic | Livebearing, sulfide-endemic | Hamilton (2001) |
| | *Poecilia formosa* | Río San Fernando drainage (Baños de San Ignacio), Nuevo León, México | Livebearing | Lozano-Vilano and Contreras-Balderas (1999) |
| | *P. latipinna* | Panacea Mineral Springs, Florida | Livebearing | Riesch et al. (2014) |
| | *P. mexicana* | (1) Río Tacotalpa (El Azufre, Cueva del Azufre)<br>(2) Río Puyacatengo (La Lluvia and Puyacatengo springs), both Tabasco, Mexico | Livebearing | Gordon and Rosen (1962), Tobler et al. (2011), Plath et al. (2013) |
| | *P. thermalis* (*P. sulphuraria*[1]) | (1) Río Pichucalco (Baños del Azufre and La Gloria springs)<br>(2) Río Ixtapangajoya (La Esperanza) | Livebearing, sulfide-endemic | Steindachner (1863), Alvarez (1948), Tobler and Plath (2009b), Tobler et al. (2008c), Palacios et al. (2013) |
| | *Pseudoxiphophorus bimaculatus* | La Gloria springs, Chiapas, México | Livebearing | Greenway and Tobler (unpublished) |
| | *Xiphophorus hellerii* | La Gloria springs, Chiapas, México | Livebearing | Greenway and Tobler (unpublished) |
| Synbranchidae | *Ophisternon aenigmaticum* | Río Tacotalpa (El Azufre, Cueva del Azufre), Tabasco, Mexico | Livebearing | Gordon and Rosen (1962), Riesch et al. (2009), Greenway et al. (2014) |

We also included several species of the genus *Aphanius* even though direct evidence for the presence of $H_2S$ in the inhabited hot sulfuric springs is as yet lacking

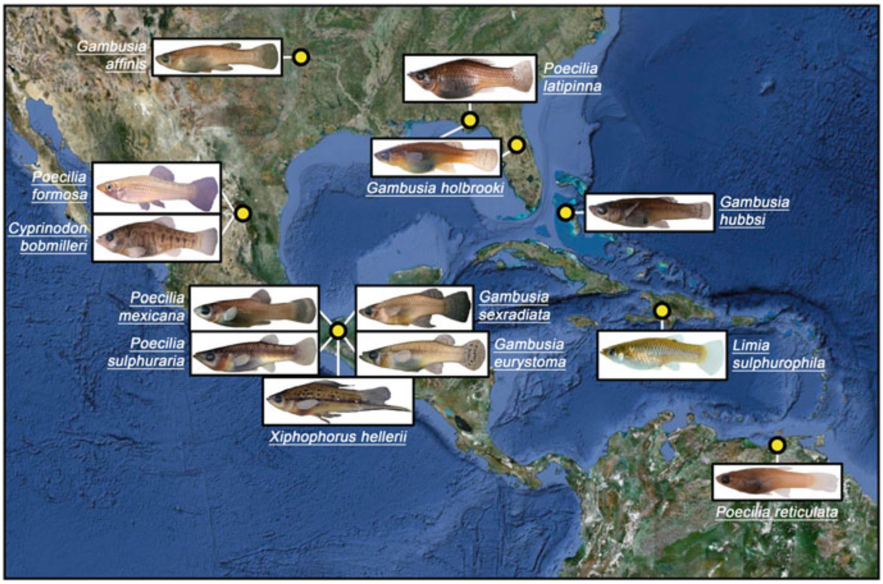


**Fig. 1** Map of representative freshwater fishes occurring in sulfidic habitats (*yellow circles*) in the New World (modified from Riesch et al. 2014)

so-called cave mollies have been described from the Cueva del Azufre, a sulfur cave in which some sulfide springs of the Tacotalpa system are situated (Gordon and Rosen 1962; Plath and Tobler 2010; Tobler and Plath 2011). Another nearby cave (Cueva Luna Azufre) is $H_2S$ free and harbors another form of cave-adapted mollies (Tobler et al. 2008b).

The occurrence of sulfide spring populations in close proximity (often <100 m) to ancestral populations in nonsulfidic environments allows for powerful comparative approaches in studying adaptation to $H_2S$-rich environments. The well-known physiological and environmental effects of $H_2S$ (e.g., Bagarinao 1992; Grieshaber and Völkel 1998), along with the presence of strong divergent selection in sulfidic habitats, also allow formulating specific predictions regarding evolutionary adaptations of fish populations inhabiting them, which we put forth at the beginning of the following subchapters. The comparison of trait divergence in evolutionarily replicated transitions from nonsulfidic to sulfidic habitats further allows disentangling shared (i.e., convergent) and unique (i.e., population-, species-, or genus-specific) patterns of this divergence (see Langerhans and DeWitt 2004; Langerhans and Riesch 2013). Indeed, comparing evolutionary diversification along replicated ecological gradients has been proposed as one of the most powerful approaches to investigate adaptation in natural systems (Elmer and Meyer 2011;

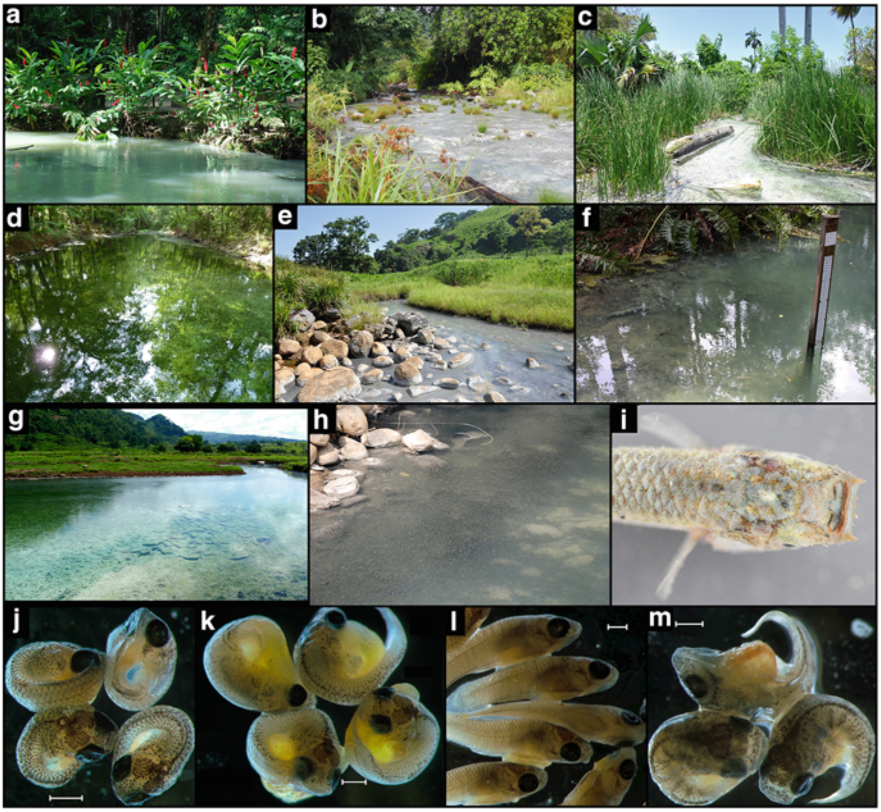


**Fig. 2** (**a**–**g**) Diversity of sulfidic habitats. (**a**) El Azufre, Tabasco, Mexico; (**b**) La Lluvia, Tabasco, Mexico; (**c**) La Zurza, Dominican Republic; (**d**) Newport Springs, Florida, US; (**e**) La Gloria, Chiapas, Mexico; (**f**) Green Springs, Florida, US; (**g**) Baños del Azufre, Tabasco, Mexico; (**h**) Dense school of *P. sulphuraria* at the Baños del Azufre; (**i**) Dorsal view of a sulphur molly (*P. sulphuraria*), note the large head and prominent lip appendages; (**j**–**m**) Late-stage embryos of (**j**) *Gambusia sexradiata* and (**l**) *Poecilia mexicana* from nonsulfidic habitats as well as the sulfur endemics (**k**) *Gambusia eurystoma* and (**m**) *Poecilia sulphuraria*; scale bar = 1 mm. Photos by R Riesch (**d**, **f**, **j**–**m**), M Schulte (**a**, **h**), and M Tobler (**b**, **c**, **e**, **g** and **i**)

Losos 2011; Langerhans and Riesch 2013). Finally, these systems are particularly suited to investigate how evolutionary adaptation may provide environmentally dependent fitness advantages, as traits beneficial in one habitat type can be counter-selected in another (Tobler et al. 2009b; Plath et al. 2013). By the end of this chapter, we will, therefore, scrutinize the evidence that adaptation to sulfidic conditions in poeciliids promotes the emergence of reproductive isolation and drives ecological speciation (Nosil 2012).

## 3 $H_2S$ as a Selective Factor and an Environmental Engineer

The biochemical and physiological consequences of the presence of $H_2S$ themselves undoubtedly represent a strong source of natural selection, especially considering that $H_2S$ concentrations in sulfide springs, on average, exceed 190 µM (with peak concentration reaching over 1,000 µM; Tobler et al. 2006, 2011), concentrations that are acutely toxic for most metazoans (Bagarinao 1992; U.S. EPA 2013). To understand the phenotypic trait divergence between populations from sulfidic and nonsulfidic habitats, however, it is important to note that the two habitat types vary in a suite of environmental characteristics, each of which can potentially also serve as a source of selection and drive trait divergence (reviewed in Tobler and Plath 2011). (1) $H_2S$ is highly reactive at room temperature and spontaneously oxidizes in aqueous solution (Cline and Richards 1969; Chen and Morris 1972). This reaction is biologically relevant because the presence of $H_2S$ leads to and aggravates hypoxia in aquatic systems, resulting in a distinct inverse correlation of $H_2S$ and oxygen concentrations (Bagarinao 1992; Tobler et al. 2006). As oxygen available for respiration is limited and at the same time is required for the oxidative detoxification of $H_2S$, there is trong selection for efficient oxygen acquisition in sulfidic habitats. (2) Although sulfide spring water appears to be mostly of meteoric origin, groundwater interaction with water-soluble mineral sediments causes fundamental alterations of water chemistry. Compared to adjacent, nonsulfidic waters, sulfide springs are typically characterized by increased concentrations of bicarbonate, calcium sulfate, and sodium chloride (Rosales Lagarde 2012), with consequences for fish osmoregulation. Furthermore, sulfide springs appear to have a slightly lower pH (likely due to the presence of sulfuric acid from chemical and bacterial $H_2S$ oxidation) and elevated average temperatures in southern Mexico (Tobler et al. 2006, 2011), while water in sulfidic springs in Florida is actually colder than in the surrounding nonsulfidic habitats (Riesch et al. unpubl. data). (3) Due to their high toxicity, sulfidic springs are characterized by reduced species diversity compared to adjacent nonsulfidic habitats, both in terms of fish and aquatic invertebrate communities (Tobler et al. 2008c; Riesch et al. 2009; Greenway et al. 2014); at the same time, poeciliid fish densities are usually higher in sulfide habitats (Jourdan et al. 2014; Greenway et al. 2014). Hence, selection from piscivorous fishes and interspecific competition is relaxed, while intraspecific competition is intensified. Similarly, the presence of parasites varies along the sulfide-to-nonsulfide gradient (Tobler et al. 2007, 2014b). $H_2S$ also fundamentally alters food webs in aquatic environments, as primary production is shifted from photoautotrophs (like algae) to chemoautotrophs (sulfide oxidizing bacteria; Langecker et al. 1996; Roach et al. 2011). Accordingly, fishes in southern Mexican sulfidic and nonsulfidic habitats differ in trophic resource use, and the diet shifted from algae and detritus to sulfide bacteria, invertebrates, and detritus in the course of sulfide spring colonization (Roach et al. 2011; Tobler 2008, unpublished data).

In summary, understanding the complexity of environmental gradients is pivotal, as multivariate trait divergence likely is not only related to direct effects of $H_2S$, but may also occur in response to a set of correlated selective factors. Moreover, additional differences might exist that could be specific to certain habitats, like differences in the strength and direction of sexual selection (Langerhans and Riesch 2013; see discussion in Riesch et al. 2011a, unpubl. data). Elucidating causal relationships between sources of selection and organismal responses certainly remains a primary challenge in the understanding of sulfide spring fish evolution.

## 4 Behavioral Responses to $H_2S$

The primary behavioral response of fish upon $H_2S$ exposure is aquatic surface respiration (ASR), during which fish exploit the more oxygen-rich topmost layer of the water column for respiration (see Brauner et al. 1995 as an example of ASR in response to $H_2S$-induced hypoxia). This behavior has been widely interpreted as an adaptation facilitating oxygen acquisition in hypoxic environments (see chapter "Low-Oxygen Lifestyles"). In sulfide spring *Poecilia*, individual fish spend a considerable amount of their time budget conducting ASR (Tobler et al. 2009a), and the ability to conduct ASR directly affects short-term survivability in the toxic environments (Plath et al. 2007b). However, performing ASR does not affect the survivability of nonsulfidic populations in sulfidic water, and exposure to $H_2S$ in non-adapted fish often elicits an evasion response, where fish jump out of the water (Tobler et al. 2008c; also see Abel et al. 1987 for a similar response in *Rivulus marmoratus*, a species occurring in intermittently sulfidic habitats). ASR, however, comes at a cost of constraining time available for benthic foraging, as evidenced by behavioral observations in the wild and examining gut fullness (Tobler et al. 2009a). Accordingly, constraints in energy availability (Tobler et al. 2006; Tobler 2008) may be a key driver in the evolution of various traits, ranging from metabolic physiology, energetically demanding behaviors to life-history traits.

*Poecilia* from sulfidic habitats have reduced energetically costly behaviors like male sexual behavior and size-dependent alternative mating tactics (Plath et al. 2003, 2006; Plath 2008), as well as shoaling behavior (Parzefall 1993). In fish from the Tacotalpa drainage, these changes appear to have a genetic basis, as population differences are also seen in common-garden-reared laboratory offspring. In the case of shoaling (the tendency to form loose social aggregations), the trade-off between benefits (predator protection) and costs arising from shoal formation (resource competition) may be shifted toward the cost-side, as evidenced by the fact that experimentally food-deprived *P. mexicana* from nonsulfidic habitats also shoal less (Plath and Schlupp 2008). Interestingly, the observation of dense schools of *P. sulphuraria* and *P. thermalis* is counter to this trend (Fig. 2h), and so it seems that those phylogenetically older sulfide endemics have evolved mechanisms to cope more efficiently with sulfide toxicity (thus reducing the costs of group living). Schooling (involving synchronized swimming and coordinated escape maneuvers) may reduce bird predation especially in the clear spring regions of those sulfide springs, where bird predation rates can be more than 20 times higher than in sulfide-free waters (Riesch et al. 2010a).

Early studies in the Río Tacotalpa system reported a heritable reduction of aggressive behavior in sulfide- and cave-adapted fish (Parzefall 1974, 1979). A more recent investigation found support for this pattern, but the phylogenetically old sulfide-endemic *P. sulphuraria* from the Pichulaco drainage showed no overall reduced aggressiveness, again suggesting derived mechanisms that allow coping with $H_2S$ more effectively (Bierbach et al. 2012). Constraints in energy availability also affect the evolution of mating preferences—an important driver of sexual selection (Andersson 1994): sulfide-adapted females from the Tacotalpa drainage display a stronger preference for males with good nutritional state, probably because the indicator value of male nutritional state increases under extreme environmental conditions (Plath et al. 2005).

## 5 Physiological Adaptations

Progress toward understanding the molecular and physiological basis of $H_2S$ adaptation has been made largely through two major research avenues: (1) Genetic and gene expression studies—sometimes in conjunction with biochemical analyses—have shed light on mechanisms that allow for the maintenance of organismal function in the presence of environmental $H_2S$. (2) Analyses of metabolic rates have uncovered links between life in extreme environments and metabolic physiology.

From a physiological perspective, sulfide spring fishes can theoretically cope with $H_2S$ exposure through minimizing adverse effects of potentially elevated endogenous concentrations or by maintaining low endogenous concentrations despite the constant influx of environmental $H_2S$. The increasing number of known molecular targets of $H_2S$ (Li et al. 2011) allows for testing explicit predictions about physiological adaptations. For example, the cytochrome *c* oxidase complex (COX) in the mitochondrial respiratory chain is one of the primary targets of $H_2S$ toxicity. Indeed, two of three evolutionarily independent sulfide spring lineages in the *P. mexicana* complex have independently evolved COXs that maintain functionality with increasing $H_2S$ concentrations (Pfenninger et al. 2014). Furthermore, gene expression studies have indicated that the gene encoding sulfide:quinone oxidoreductase (see above) is consistently upregulated in natural sulfide spring populations (Kelley and Tobler unpublished data). Common-garden-raised individuals from one population (Tacotalpa) also retain higher constitutive expression of this gene even in the absence of environmental $H_2S$ in the laboratory, which suggests evolutionary divergence in gene regulation between ecotypes (Tobler et al. 2014a). Hence, evidence is mounting that adaptation to $H_2S$ includes both mechanisms that reduce adverse effects of potentially elevated endogenous concentrations and mechanisms that maintain low endogenous concentrations.

Life in sulfidic environments has also been documented to be associated with modifications of energy metabolism. *Poecilia mexicana* in sulfidic habitats of the Tacotalpa drainage have significantly reduced total energy demands during routine metabolism; this reduction in total routine metabolic rates, however, is driven by body size reductions in extreme environments, while mass-specific metabolic rates are not suppressed (Riesch et al. 2011c; Passow and Tobler unpublished data). Reductions of energetic demands have been interpreted to be a consequence of either reduced resource availability (both oxygen and carbon availabilities to fuel metabolism are limited in sulfidic environments) or of elevated maintenance costs related to energetically costly adaptations to $H_2S$ (Riesch et al. 2011c; Passow and Tobler unpublished data; see also discussions in Riesch et al. 2010c, 2011a, b). Common-garden experiments and manipulations of food availability also indicated that sulfide populations have evolved a greater ability to modulate mass-specific routine metabolic rates in response to environmental energy availability [Passow and Tobler unpublished data; but see Riesch et al. (2011c) for a different pattern in cave mollies from the sulfidic Cueva del Azufre]. The increase in the mass-specific metabolic rate reaction norm might be linked to foraging constraints in sulfidic environments, such that individual fish can ramp up metabolism when energy is readily available and idle when energy is scarce. It remains a major open question how estimates of routine metabolic rates are affected by the presence of $H_2S$, and it is pivotal to overcome technical difficulties in measuring oxygen consumption of fish in the presence of $H_2S$, which in itself consumes oxygen in aqueous solution, to isolate the potential effects of $H_2S$ in driving metabolic rate variation in natural populations.

## 6 Morphological Adaptations

Morphological evolution ought to be governed by optimizing the oxygen uptake because respiratory adaptations are a key for surviving in sulfidic environments, where supplementary oxygen is required for sulfide detoxification (Bagarinao 1992; Grieshaber and Völkel 1998), but oxygen availability is limited (Cline and Richards 1969; Chen and Morris 1972). Indeed, sulfide spring populations in the *P. mexicana* complex exhibit a number of morphological modifications that are presumably related to respiratory efficiency. Most conspicuously, sulfide spring populations are characterized by significantly larger heads than the ancestral populations in nonsulfidic habitats (Tobler et al. 2011; Palacios et al. 2013). The increase in head size is directly correlated with an increase in gill surface area (Tobler et al. 2011), which increases oxygen acquisition efficiency in hypoxic waters. Furthermore, increased head size is also likely associated with an increase in buccal volume, which should increase ventilation efficiency. Sulfide populations are also characterized by wider mouths and—at least in some populations—enlarged lower jaws and lips (Palacios et al. 2013). In *P. thermalis* and the Baños del Azufre population of *P. sulphuraria*, lower lips also exhibit lateral lip appendages (Fig. 2i; Tobler and Plath 2008; Riesch et al. 2011b; Palacios et al. 2013). Jaw and lip modifications likely facilitate the skimming of the uppermost water level during ASR (Winemiller 1989), but trophic niche differentiation may also have affected jaw morphology (Tobler 2008). Note that increases in head size have been documented not only for the *P. mexicana* complex but also for a variety of other sulfide spring lineages in other species and genera (Tobler and Hastings 2011; Riesch et al. unpubl. data).

Sulfide spring populations also differ in the relative size of several internal organs from their nonsulfidic ancestors. In southern Mexico, they have a reduced intestinal length reflecting a change from a mostly algi- and detritivorous to a more protein-rich diet (Tobler 2008). In addition, relative liver size is enlarged in most sulfide spring populations and may be related to increased detoxification abilities of $H_2S$ (Tobler unpublished data). Finally, sulfide spring populations consistently exhibit reduced brain sizes both in terms of mass (Tobler unpublished data) and volume (Eifert et al. 2014). Specifically, sulfide spring fish have repeatedly evolved a smaller optic tectum, alluding to reduced visual information processing in sulfidic waters that are often turbid due to colloidal sulfur.

## 7 Life-history Adaptations

Specific predictions for life-history evolution can be derived from a combination of life-history theory and physiological considerations. For example, livebearing should be favored in sulfidic habitats due to the protection of young from $H_2S$ during gestation (physiological homeostasis), aided by the possibility of active choice of less toxic microhabitats by females (e.g., Blackburn 1999 for benefits of livebearing). Not surprisingly, the majority of teleosts in sulfide-toxic freshwater habitats are livebearers (Table 1). Even amongst marine taxa, bythitids and some zoarcids (genus *Zoarces*) are livebearing (Blackburn 2005), although it is highly likely that the zoarcids associated with cold seeps and hydrothermal vents are oviparous like most other zoarcids (Møller and Gravlund 2003). Moreover, since the ratio of sulfide influx to oxidation is crucial for efficient detoxification, larger offspring size equates to a more efficient body surface-to-volume ratio (theory: Powell 1989; empirical evidence for the mussel *Macoma balthica*: Jahn et al. 1997), and thus should enhance $H_2S$ detoxification in neonates. However, as we outlined above, poeciliids in sulfidic springs are essentially resource limited due to increased population densities and their reliance on ASR. Again, drawing on a combination of life-history theory and previous studies (intraspecific competition, theory: Brockelman 1975; Jørgensen et al. 2011; empirical evidence for the guppy: Bashey 2008; low-quality environments, theory: Rollinson and Hutchings 2013a; empirical evidence for Atlantic salmon, *Salmo salar*: Rollinson and Hutchings

2013b), we would again predict large neonates in sulfidic waters. Thus, theory provides clear predictions that poeciliids from sulfidic environments should produce larger offspring than their closest relatives from nonsulfidic waters. Furthermore, if extremophile poeciliids in sulfidic habitats are indeed resource limited, then we would further predict that they should produce fewer offspring per clutch (i.e., reduced fecundity), have fewer energy reserves (i.e., reduced body fat), and be smaller (i.e., reduced standard length and lean weight) than their closest relatives from nonsulfidic habitats.

As predicted, poeciliid fishes of at least nine different species produce significantly larger offspring in sulfidic waters than their closest relatives from nonsulfidic waters (Fig. 2j–m), and this is usually coupled with a reduced fecundity in toxic environments (Riesch et al. 2010b, c, 2014). However, the latter pattern is simply due to the well-established life-history trade-off between offspring size and fecundity (Smith and Fretwell 1974), rather than a result of direct selection on fecundity per se (Riesch et al. 2014). Patterns for the other life-history traits, however, are much more population-, species-, or genus-specific, and do not follow such a strongly convergent pattern across taxa in sulfidic waters. In southern Mexico, for example, female fat content was indeed lower in extremophile *Gambusia* compared to *Gambusia* from nonsulfidic habitats, but the pattern did not hold for extremophile *Poecilia*, for which we found the highest (not the lowest) fat reserves in the sulfide-endemic *P. sulphuraria* (Riesch et al. 2010b). Similarly, male standard length and fat content were reduced in *P. mexicana* from toxic waters, but male lean weight was actually larger compared to *P. mexicana* from nontoxic waters of southern Mexico (Riesch et al. 2011a). Patterns of female standard length and lean weight, on the other hand, followed the predicted trend in extremophile *Poecilia* from southern Mexico (i.e., females in toxic habitats were smaller and weighed less for a given standard length), but were somewhat stochastic in extremophile *Gambusia*, where only the sulfide-endemic *G. eurystoma* followed the predicted trend (Riesch et al. 2010b). This unpredictability of life-history traits other than offspring size and fecundity was further confirmed in a recent study on phenotypic divergence between extremophile and non-extremophile *Gambusia* of five different species from the U.S.A., the Bahamas, and Mexico (Riesch et al. unpubl. data). Standard length was in fact reduced in sulfidic waters in female *G. affinis* from Oklahoma as well as males of the *G. eurystoma*/*G. sexradiata* complex from southern Mexico, while individuals from sulfidic waters were actually larger compared to their counterparts from nonsulfidic waters in male *G. affinis* from Oklahoma, male and female *G. holbrooki* from Florida, females from the *G. eurystoma*/*G. sexradiata* complex from Mexico, as well as male and female *G. hubbsi* from the Bahamas (Riesch et al. unpubl. data). Lean weight and fat content, on the other hand, followed the predicted trend only in *G. affinis* from Oklahoma (female fat content and male lean weight), *G. eurystoma*/*G. sexradiata* (male and female lean weight and fat content), and *G. hubbsi* (male and female lean weight and female fat content), but the trend was reversed in all other cases (Riesch et al. unpubl. data).

All this suggests that toxicity mainly drives the evolution of offspring size and, as a direct result of the trade-off between offspring size and fecundity, indirectly also fecundity in sulfidic waters, while the effects of toxicity on the other life-history traits can often be overridden and could reflect genetic drift, as well as differences in sexual conflict, sexual selection, resource availability, or certain demographic variables like sex ratio, or overall population density (Langerhans and Riesch 2013).

## 8 Does Convergent Adaptation Lead to Replicated Speciation?

While divergence in several important phenotypic traits is largely convergent in replicated sulfide systems, a major question is whether also reproductive isolation evolved repeatedly as a consequence of adaptation to sulfidic environments, and if so, what mechanisms drove the emergence of reproductive isolation. Obviously, $H_2S$ toxicity is a potent driver of speciation that has already left descendants behind that have reached a considerable degree of reproductive isolation: *G. eurystoma*, *P. thermalis*/*sulphuraria*, and *Limia sulphurophila*. For the *P. mexicana* species complex, we have been able to demonstrate that reproductive isolation is the product of a complex interplay between natural and sexual selection (e.g., Tobler et al. 2009b; Plath et al. 2010, 2013; Riesch et al. 2010a). One strong selective force being at least partially responsible for reproductive isolation in this system is the presence or absence of $H_2S$ per se: Reciprocal transplant experiments between adjacent sulfidic and nonsulfidic habitats in three of the four drainages have revealed high mortality of immigrant, nonsulfide-adapted fish in toxic waters, and in one drainage (Río Tacotalpa) also of immigrant, sulfide-adapted fish in nontoxic waters (Tobler et al. 2009b; Plath et al. 2010, 2013). Furthermore, differences in predation regimes between sulfidic and nonsulfidic habitats are likely to contribute to reproductive isolation, because predatory fish are absent from sulfidic waters (Tobler et al. 2006, 2008c), but bird predation rates are more than 20 times higher compared to nonsulfidic waters (Riesch et al. 2010a). Since prey species usually evolve adaptive but costly behaviors that are closely linked to the respective predation regime and level of threat in their environment (Cheney and Seyfarth 1990; Kelley 2008), and poeciliid fishes are known to exhibit interpopulation differences in antipredator responses that probably make them more prone to predation by predators they rarely encounter under natural conditions (e.g., Seghers 1974; Kelley and Magurran 2003; Templeton and Shriner 2004), this is likely to also contribute to reproductive isolation. In agreement with this hypothesis, a recent study on antipredator behavior in the *P. mexicana* species complex uncovered a slight reduction in predator avoidance when exposed to several predatory and non-predatory fish common in nonsulfidic habitats of southern Mexico (Bierbach et al. 2013).

Besides natural selection, sexual selection also plays an important part in establishing and maintaining reproductive isolation in the southern Mexican

*P. mexicana* species complex, because several studies have now uncovered a female preference for their own over foreign (immigrant) males in nontoxic habitats but not vice versa (Tobler et al. 2009b; Plath et al. 2010, 2013). Interestingly, the strength of female preferences for own over immigrant male phenotypes was negatively correlated with the strength of natural selection against migrating males (Plath et al. 2013). Such a pattern can be interpreted as a signal of reinforcement (Kirkpatrick 2001; Servedio 2007), where mating preferences evolve to reduce the production of unfit hybrid offspring. One condition for reinforcement to evolve is reduced hybrid vigor (at least under environmental conditions as encountered in natural habitats), and even though we are as yet lacking the appropriate data, it is tempting to speculate that co-evolving nuclear/mitochondrially encoded (e.g., OXPHOS) gene complexes may play a key role in this context.

If all studies investigating population genetic structure in sulfidic systems are taken into account (e.g., Plath et al. 2007a, 2013; Tobler et al. 2008a, 2009b, 2011; Riesch et al. unpubl. data), then progress toward ecological speciation (i.e., complete reproductive isolation) is highly variable. This is partially reflecting the variation in the strength of divergent selection, and thus highlighting the complexity of selective regimes even in replicated environmental gradients that are seemingly governed by a single, strong selective agent. Accordingly, poeciliids from sulfidic springs are a prime model for (ecological) speciation research, because they span the full spectrum of the speciation continuum between panmixis and full reproductive isolation, allowing elucidating the different mechanisms establishing, driving, and constraining speciation in these systems.

## 9 Conclusions and Outlook

Research on teleost fishes in sulfidic habitats is relatively recent and investigating the specific adaptations allowing fish to survive in the presence of high and sustained levels of $H_2S$ has made great advances over the last few decades. Nonetheless, important questions concerning the evolutionary ecology of teleost fishes in sulfidic habitats remain to be answered. For example, recent studies have begun to look at the interplay of shared (i.e., convergent) and unique responses to diversification in sulfidic habitats (e.g., Riesch et al. 2010b, 2014, submitted; Tobler and Hastings 2011; Tobler et al. 2011; Plath et al. 2013), but identifying the actual selective forces responsible for the various unique responses in different sulfidic habitats has so far been difficult. Furthermore, we are currently lacking a comprehensive understanding of why $H_2S$ led to predictable phenotypic divergence and reproductive isolation only in some but not in other systems (e.g., Plath et al. 2013; Riesch et al. unpubl. data). Given the rapidly improving techniques and steadily decreasing costs for next generation sequencing, studies identifying genomic islands of adaptation to $H_2S$ across drainages within species as well as between species or genera will likely soon be conducted. These will help answer questions like whether or not the same single-nucleotide polymorphisms (SNPs) are found to associate with convergent phenotypic differentiation across systems, or whether there are alternate solutions to the same selective force (e.g., mutation-order speciation; Langerhans and Riesch 2013). Given the widespread occurrence of sulfide spring poeciliids, coupled with independent evolution in those systems, extremophile poeciliids should provide great models to investigate this. Finally, a major gap in our understanding of fish in sulfide springs is how the few egg-laying species cope with $H_2S$ toxicity. More specifically, how do these species ensure proper egg development when clutches are exposed to $H_2S$ and cannot move between microhabitats if $H_2S$ concentrations fluctuate?

**Acknowledgments** We would like to thank the American Livebearers Association, the Erwin Riesch-Stiftung, the Freunde und Förderer der Goethe-Universität Frankfurt, the German Academic Exchange Service (DAAD), the German Ichthyological Society (GfI), the German Research Foundation (DFG), the Herrmann Willkomm-Stiftung, the Human Frontier Science Program (HFSP), the National Geographic Society, the National Science Foundation of the USA (NSF, IOS-1121832), and the Swiss National Science Foundation for financial support over many years. Our research was only possible due to the collaborative support and substantial help rendered by L Arias-Rodriguez and JR Indy (Villahermosa), FJ Garcia-Léon (La Paz), I Schlupp (Oklahoma), as well as a large number of undergraduate, graduate, and postdoctoral researchers that have circled through our labs.

## References

Abel DC, Koenig CC, Davis WP (1987) Emersion in the mangrove forest fish *Rivulus marmoratus*: a unique response to hydrogen sulfide. Environ Biol Fish 18:67–72

Affonso EG, Rantin FT (2005) Respiratory responses of the air-breathing fish *Hoplosternum littorale* to hypoxia and hydrogen sulfide. Comp Biochem Physiol Part C: Toxicol Pharmacol 141:275–280

Alvarez J (1948) Descripción de una nueva especie de *Mollienisia* capturada en Baños del Azufre, Tabasco (Pisces, Poeciliidae). An Esc Nac Cienc Biol 5:275–281

Andersson MB (1994) Sexual selection. Princeton University Press, Princeton, NJ

Bagarinao T (1992) Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquat Toxicol 24:21–62

Bagarinao T, Vetter RD (1989) Sulfide tolerance and detoxification in shallow-water marine fishes. Mar Biol 103:291–302

Bashey F (2008) Competition as a selective mechanism for larger offspring size in guppies. Oikos 117:104–113

Beinart RA, Sanders JG, Faure B, Sylva SP, Lee RW, Becker EL, Gartman A, Luther GW, Seewald JS, Fisher CR, Girguis PR (2012) Evidence for the role of endosymbionts in regional-scale habitat partitioning by hydrothermal vent symbioses. Proc Natl Acad Sci U S A 109: E3241–E3250

Bierbach D, Klein M, Sassmannshausen V, Schlupp I, Riesch R, Parzefall J, Plath M (2012) Divergent evolution of male aggressive behaviour another reproductive isolation barrier in extremophile poeciliid fishes? Int J Evol Biol 2012:148745

Bierbach D, Schulte M, Herrmann N, Zimmer C, Arias-Rodriguez L, Indy JR, Riesch R, Plath M (2013) Predator avoidance in extremophile fish. Life 3:161–180

Biscoito M (2006) Chordata, Chondrichthyes & Osteichthyes. In: Desbruyères D, Segonzac M, Bright M (eds) Handbook of deep-sea hydrothermal vent fauna. Denisia 18:489–490

Biscoito M, Segonzac M, Almeida AJ, Desbruyeres D, Geistdoerfer P, Turnipseed M, Van Dover C (2002) Fishes from the hydrothermal vents and cold seeps – an update. Cah Biol Mar 43:359–362

Blackburn DG (1999) Viviparity and oviparity: evolution and reproductive strategies. In: Knobil TE, Neill JD (eds) Encyclopedia of reproduction. Academic Press, New York, NY, pp 994–1003

Blackburn DG (2005) Evolutionary origins of viviparity in fishes. In: Grier HJ, Uribe MC (eds) Viviparous fishes. New Life Publications, Homestead, FL, pp 287–301

Brauner CJ, Ballantyne CL, Randall DJ, Val AL (1995) Air breathing in the armoured catfish (*Hoplosternum littorale*) as an adaptation to hypoxic, acidic, and hydrogen sulphide rich waters. Can J Zool 73:739–744

Brockelman WY (1975) Competition, the fitness of offspring, and optimal clutch size. Am Nat 109:677–699

Cavanaugh CM, Gardiner SL, Jones ML, Jannasch HW, Waterbury JB (1981) Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. Science 213:340–342

Chen KY, Morris JC (1972) Kinetics of oxidation of aqueous sulfide by oxygen. Environ Sci Technol 6:529–537

Cheney DL, Seyfarth RM (1990) How monkeys see the world. University of Chicago Press, Chicago, IL

Cline JD, Richards FA (1969) Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature and pH. Environ Sci Technol 3:838–843

Coad BW (1980) A re-description of *Aphanius ginaonis* (Holly, 1929) from southern Iran (Osteichthyes: Cyprinodontiformes). J Nat Hist 14:33–40

Cohen DM, Rosenblatt RH, Moser HG (1990) Biology and description of a bythitid fish from deep-sea thermal vents in the tropical eastern Pacific. Deep-Sea Res 37:267–283

Cooper CE, Brown GC (2008) The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. J Bioenerg Biomembr 40:533–539

Eifert C, Farnworth M, Schulz–Mirbach T, Riesch R, Bierbach D, Klaus S, Wurster A, Tobler M, Streit B, Indy JR, Arias–Rodriguez L, Plath M (2014) Brain size variation in extremophile fish: local adaptation vs. phenotypic plasticity. J Zool Lond (online first)

Elmer KR, Meyer A (2011) Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends Ecol Evol 26:298–306

Geiger SP, Torres JJ, Crabtree RE (2000) Air breathing and gill ventilation frequencies in juvenile tarpon, *Megalops atlanticus*: responses to changes in dissolved oxygen, temperature, hydrogen sulfide, and pH. Environ Biol Fishes 59:181–190

Gordon MS, Rosen DE (1962) A cavernicolous form of the poeciliid fish *Poecilia sphenops* from Tabasco, Mexico. Copeia 1962:360–368

Greenway R, Arias-Rodriguez L, Diaz P, Tobler M (2014) Patterns of macroinvertebrate and fish diversity in freshwater sulphide springs. Diversity 6:597–632

Grieshaber MK, Völkel S (1998) Animal adaptations for tolerance and exploitation of poisonous sulfide. Annu Rev Physiol 60:33–53

Hamilton A (2001) Phylogeny of *Limia* (Teleostei: Poeciliidae) based on NADH dehydrogenase subunit 2 sequences. Mol Phylogenet Evol 19:277–289

Hildebrandt TM, Grieshaber MK (2008) Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. FEBS J 275:3352–3361

Jahn A, Janas U, Theede H, Szaniawska A (1997) Significance of body size in sulphide detoxification in the Baltic clam *Macoma balthica* (Bivalvia, Tellinidae) in the Gulf of Gdansk. Mar Ecol Prog Ser 154:175–183

Jannasch HW, Taylor CD (1984) Deep-sea microbiology. Annu Rev Microbiol 38:487–514

Jørgensen C, Auer SK, Reznick DN (2011) A model for optimal offspring size in fish, including live-bearing and parental effects. Am Nat 177:E119–E135

Jourdan J, Bierbach D, Riesch R, Dzienko J, Karau N, Oranth A, Stadler S, Schießl A, Wigh A, Arias-Rodriguez L, Indy JR, Klaus S, Zimmer C, Plath M (2014) Microhabitat use, population densities, and size distributions of sulfur cave-dwelling *Poecilia mexicana*. PeerJ 2:e490

Kelley JL (2008) Assessment of predation risk by prey fishes. In: Magnhagen C, Braithwaite VA, Forsgren E, Kapoor BG (eds) Fish behaviour. Science Publishers, Enfield, NH, pp 269–301

Kelley JL, Magurran AE (2003) Effects of relaxed predation pressure on visual predator recognition in the guppy. Behav Ecol Sociobiol 54:225–232

Kirkpatrick M (2001) Reinforcement during ecological speciation. Proc R Soc Lond B 268:2616

Lagoutte E, Mimoun S, Andriamihaja M, Chaumontet C, Blachier F, Bouillaud F (2010) Oxidation of hydrogen sulfide remains a priority in mammalian cells and causes reverse electron transfer in colonocytes. Biochim Biophys Acta 1797:1500–1511

Langecker TG, Wilkens H, Parzefall J (1996) Studies on the trophic structure of an energy rich Mexican cave (Cueva de las Sardinas) containing sulfurous water. Mem Biospeol 23:121–125

Langerhans RB, DeWitt TJ (2004) Shared and unique features of evolutionary diversification. Am Nat 164:335–349

Langerhans RB, Riesch R (2013) Speciation by selection: a framework for understanding ecology's role in speciation. Curr Zool 59:31–52

Li L, Rose P, Moore PK (2011) Hydrogen sulfide and cell signaling. Annu Rev Pharmacol Toxicol 51:169–187

Little CTS, Vrijenhoek RC (2003) Are hydrothermal vent animals living fossils? Trends Ecol Evol 18:582–588

Losos JB (2011) Convergence, adaptation, and constraint. Evolution 65:1827–1840

Lozano-Vilano ML, Contreras-Balderas S (1999) *Cyprinodon bobmilleri*: new species of pupfish from Nuevo León, México (Pisces: Cyprinodontidae). Copeia 1999:382–387

Marcia M, Ermler U, Peng G, Michel H (2009) The structure of *Aquifex aeolicus* sulfide:quinone oxidoreductase, a basis to understand sulfide detoxification and respiration. Proc Natl Acad Sci U S A 106:9625–9630

Micheli F, Peterson CH, Mullineaux LS et al (2002) Predation structures communities at deep-sea hydrothermal vents. Ecol Monogr 72:365–382

Miller RR (1975) Five new species of Mexican poeciliid fishes of the genera *Poecilia*, *Gambusia*, and *Poeciliopsis*. Occ Pap Mus Zool Univ Mich 672:1–44

Møller PR, Gravlund P (2003) Phylogeny of the eelpout genus *Lycodes* (Pisces, Zoarcidae) as inferred from mitochondrial cytochrome b and 12S rDNA. Mol Phylogenet Evol 26:369–388

Munroe TA, Hashimoto J (2008) A new Western Pacific Tonguefish (Pleuronectiformes: Cynoglossidae): the first Pleuronectiform discovered at active hydrothermal vents. Zootaxa 1839:43–59

Munroe TA, Tyler J, Tunnicliffe V (2011) Description and biological observations on a new species of deepwater symphurine tonguefish (Pleuronectiformes: Cynoglossidae: *Symphurus*) collected at Volcano–19, Tonga Arc, West Pacific Ocean. Zootaxa 3061:53–66

Nosil P (2012) Ecological speciation. Oxford University Press, Oxford

Palacios M, Arias-Rodriguez L, Plath M, Eifert C, Lerp H, Lamboj A, Voelkel G, Tobler M (2013) The rediscovery of a long described species reveals additional complexity in speciation patterns of poeciliid fishes in sulfide springs. PLoS One 8:e71069

Parzefall J (1974) Rückbildung aggressiver Verhaltensweisen bei einer Höhlenform von *Mollienesia sphenops* (Pisces, Poeciliidae). Z Tierpsychol 35:66–84

Parzefall J (1979) Zur Genetik und biologischen Bedeutung des Aggressionsverhaltens von *Poecilia sphenops* (Pisces, Poeciliidae). Z Tierpsychol 50:399–422

Parzefall J (1993) Schooling behaviour in population-hybrids of *Astyanax fasciatus* and *Poecilia mexicana* (Pisces, Characidae and Poeciliidae). In: Schröder H, Bauer J, Schartl M (eds) Trends in ichthyology. Blackwell Science, Oxford, pp 297–303

Pfenninger M, Lerp H, Tobler M, Passow C, Kelley JL, Funke E, Greshake B, Erkoc UK, Berberich T, Plath M (2014) Parallel evolution of *cox*-genes in $H_2S$-tolerant fish as key adaptation to a toxic environment. Nat Commun 5:3873

Plath M (2008) Male mating behavior and costs of sexual harassment for females in cavernicolous and extremophile populations of Atlantic mollies (*Poecilia mexicana*). Behaviour 145:73–98

Plath M, Schlupp I (2008) Parallel evolution leads to reduced shoaling behavior in two cave dwelling populations of Atlantic mollies (*Poecilia mexicana*, Poeciliidae, Teleostei). Environ Biol Fish 82:289–297

Plath M, Tobler M (2010) Subterranean fishes of Mexico (*Poecilia mexicana*, Poeciliidae). In: Trajano E, Bichuette ME, Kapoor BG (eds) The biology of subterranean fishes. Science Publishers, Enfield, NH, pp 283–332

Plath M, Parzefall J, Schlupp I (2003) The role of sexual harassment in cave and surface dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). Behav Ecol Sociobiol 54:303–309

Plath M, Heubel KU, Garcia de Leon FJ, Schlupp I (2005) Cave molly females (*Poecilia mexicana*, Poeciliidae, Teleostei) like well-fed males. Behav Ecol Sociobiol 58:144–151

Plath M, Brümmer A, Parzefall J, Schlupp I (2006) Size–dependent male mating behaviour and sexual harassment in a population of Atlantic mollies (*Poecilia mexicana*) from a sulphur creek. Acta Ethol 9:15–21

Plath M, Hauswaldt JS, Moll K, Tobler M, García de León FJ, Schlupp I, Tiedemann R (2007a) Local adaptation and pronounced genetic differentiation in an extremophile fish, *Poecilia mexicana*, inhabiting a Mexican cave with toxic hydrogen sulphide. Mol Ecol 16:967–976

Plath M, Tobler M, Riesch R, García de León FJ, Giere O, Schlupp I (2007b) Survival in an extreme habitat: the roles of behaviour and energy limitation. Naturwissenschaften 94:991–996

Plath M, Riesch R, Oranth A, Dzienko J, Karau N, Schießl A, Stadler S, Wigh A, Zimmer C, Arias-Rodriguez L, Schlupp I, Tobler M (2010) Complementary effect of natural and sexual selection against immigrants maintains differentiation between locally adapted fish. Naturwissenschaften 97:769–774

Plath M, Pfenninger M, Lerp H, Riesch R, Eschenbrenner C, Slattery PA, Bierbach D, Herrmann N, Schulte M, Arias-Rodriguez L, Indy JR, Passow C, Tobler M (2013) Genetic differentiation and selection against migrants in evolutionarily replicated extreme environments. Evolution 67:2647–2661

Powell E (1989) Oxygen, sulfide and diffusion: why thiobiotic meiofauna must be sulfide-insensitive first-order respirers. J Mar Res 47:887–932

Reiffenstein RJ, Hulbert WC, Roth SH (1992) Toxicology of hydrogen sulfide. Annu Rev Pharmacol Toxicol 32:109–134

Riesch R, Plath M, Schlupp I, Tobler M, Langerhans RB (2014) Colonisation of toxic environments drives predictable life-history evolution in livebearing fishes (Poeciliidae). Ecol Lett 17:65–71

Riesch R, Duwe V, Herrmann N, Padur L, Ramm A, Scharnweber K, Schulte M, Schulz-Mirbach-T, Ziege M, Plath M (2009) Variation along the shy–bold continuum in extremophile fishes (*Poecilia mexicana*, *Poecilia sulphuraria*). Behav Ecol Sociobiol 63:1515–1526

Riesch R, Oranth A, Dzienko J, Karau N, Schießl A, Stadler S, Wigh A, Zimmer C, Arias-Rodriguez L, Schlupp I, Plath M (2010a) Extreme habitats are not refuges: poeciliids suffer from increased aerial predation risk in sulphidic southern Mexican habitats. Biol J Linn Soc 101:417–426

Riesch R, Plath M, García de León F, Schlupp I (2010b) Convergent life-history shifts: toxic environments result in big babies in two clades of poeciliids. Naturwissenschaften 97:133–141

Riesch R, Plath M, Schlupp I (2010c) Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). Ecology 91:1494–1505

Riesch R, Plath M, Schlupp I (2011a) Toxic hydrogen sulphide and dark caves: pronounced male life-history divergence among locally adapted *Poecilia mexicana* (Poeciliidae). J Evol Biol 24:596–606

Riesch R, Schlupp I, Langerhans RB, Plath M (2011b) Shared and unique patterns of embryo development in extremophile poeciliids. PLoS One 6:e27377

Riesch R, Schlupp I, Schleucher E, Hildenbrand P, Köhler A, Arias-Rodriguez L, Plath M (2011c) Reduced starvation resistance and increased metabolic rates in an unusual cave organism: the cave molly (*Poecilia mexicana*, Poeciliidae). Bull Fish Biol 13:41–56

Roach KA, Tobler M, Winemiller KO (2011) Hydrogen sulfide, bacteria, and fish: a unique, subterranean food chain. Ecology 92:2056–2062

Rollinson N, Hutchings JA (2013a) The relationship between offspring size and fitness: integrating theory and empiricism. Ecology 94:315–324

Rollinson N, Hutchings JA (2013b) Environmental quality predicts optimal egg size in the wild. Am Nat 182:76–90

Rosales Lagarde L (2012) Investigations of karst brackish-sulfidic springs and their role in sulfidic springs and their hydrogeology, subsurface water-rock interactions, and speleogenesis at the northern Sierra de Chiapas, Mexico. PhD dissertation, New Mexico Institute of Mining and Technology, Socorro, NM

Rosenblatt RH, Cohen DM (1986) Fishes living in deepsea thermal vents in the tropical eastern Pacific, with descriptions of a new genus and two new species of eelpouts (Zoarcidae). Trans San Diego Soc Nat Hist 21:71–79

Schoonen MAA, Barnes HL (1988) An approximation of the second dissociation constant for $H_2S$. Geochim Cosmochim Acta 52:649–654

Seghers BH (1974) Geographic variation in the responses of guppies (*Poecilia reticulata*) to aerial predators. Oecologia 14:93–98

Servedio MR (2007) Male versus female mate choice: sexual selection and the evolution of species recognition via reinforcement. Evolution 61:2772–2789

Shahak Y, Hauska G (2008) Sulfide oxidation from cyanobacteria to humans: sulfide-quinone oxidoreductase (SQR). In: Hell R, Dahl C, Knaff DB, Leustek T (eds) Advances in photosynthesis and respiration. Springer, Berlin, pp 319–335

Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. Am Nat 108:499–506

Steindachner F (1863) Beiträge zur Kenntniss der Sciaenoiden Brasiliens und der Cyprinodonten Mejicos. Sitzungsberichte der Mathematisch-Naturwissen-schaftlichen Classe der Kaiserlichen Akademie der Wissenschaften 48:162–185

Stipanuk MH (2004) Sulfur amino acid metabolism: pathways for production and removal of homocysteine amd cysteine. Annu Rev Nutr 24:539–577

Stipanuk MH, Ueki I (2011) Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. J Inherit Metab Dis 34:17–32

Szabó C (2007) Hydrogen sulphide and its therapeutic potential. Nat Rev Drug Discov 6:917–935

Teimori A (2013) The evolutionary history and taxonomy of *Aphanius* (Teleostei: Cyprinodontidae) species in Iran and the Persian Gulf region. Doctoral dissertation, Ludwig-Maximilians-University Munich, Munich

Templeton CN, Shriner WM (2004) Multiple selection pressures influence Trinidadian guppy (*Poecilia reticulata*) antipredator behavior. Behav Ecol 15:673–678

Theissen U, Hoffmeister M, Grieshaber M, Martin W (2003) Single eubacterial origin of eukaryotic sulfide:quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during anoxic and sulfidic times. Mol Biol Evol 20:1564–1574

Tobler M (2008) Divergence in trophic ecology characterizes colonization of extreme habitats. Biol J Linn Soc 95:517–528

Tobler M, Hastings L (2011) Convergent patterns of body shape differentiation in four different clades of poeciliid fishes inhabiting sulfide springs. Evol Biol 38:412–421

Tobler M, Plath M (2009a) Threatened fishes of the world: *Gambusia eurystoma* Miller, 1975 (Poeciliidae). Environ Biol Fish 85:251

Tobler M, Plath M (2009b) Threatened fishes of the world: *Poecilia sulphuraria* (Alvarez, 1948) (Poeciliidae). Environ Biol Fish 85:333–334

Tobler M, Plath M (2011) Living in extreme environments. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago, IL, pp 120–127

Tobler M, Schlupp I, Heubel K, Riesch R, García de León FJ, Giere O, Plath M (2006) Life on the edge: hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters. Extremophiles 10:577–585

Tobler M, Schlupp I, García de León FJ, Glaubrecht M, Plath M (2007) Extreme habitats as refuge from parasite infections? Evidence from an extremophile fish. Acta Oecol 31:270–275

Tobler M, DeWitt TJ, Schlupp I, García de León FJ, Herrmann R, Feulner PGD, Tiedemann R, Plath M (2008a) Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. Evolution 62:2643–2659

Tobler M, Riesch R, García de León F, Schlupp I, Plath M (2008b) A new and morphologically distinct population of cavernicolous *Poecilia mexicana* (Poeciliidae: Teleostei). Environ Biol Fish 82:101–108

Tobler M, Riesch R, García de León FJ, Schlupp I, Plath M (2008c) Two endemic and endangered fishes, *Poecilia sulphuraria* (Alvarez, 1948) and *Gambusia eurystoma* Miller, 1975 (Poeciliidae, Teleostei) as only survivors in a small sulphidic habitat. J Fish Biol 72:523–533

Tobler M, Riesch R, Tobler CM, Plath M (2009a) Compensatory behaviour in response to sulphide-induced hypoxia affects time budgets, feeding efficiency, and predation risk. Evol Ecol Res 11:935–948

Tobler M, Riesch R, Tobler CM, Schulz-Mirbach T, Plath M (2009b) Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations. J Evol Biol 22:2298–2304

Tobler M, Palacios M, Chapman LJ, Mitrofanov I, Bierbach D, Plath M, Arias-Rodriguez L, García de León FJ, Mateos M (2011) Evolution in extreme environments: replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. Evolution 65:2213–2228

Tobler M, Henpita C, Bassett B, Kelley JL, Shaw J (2014a) $H_2S$ exposure elicits differential expression of candidate genes in fish from sulfidic and non-sulfidic environments. Comp Biochem Physiol A 175:7–14

Tobler M, Plath M, Riesch R, Schlupp I, Grasse A, Munimanda GK, Setzer C, Penn DJ, Moodley Y (2014b) Selection from parasites favors immunogenetic diversity but not divergence among locally adapted host populations. J Evol Biol 27:960–974

Tunnicliffe V, Barross JA, Gebruk AV, Giere O, Holland ME, Koschinsky A, Reysenbach A-L, Shank TM, Summitt AM (2003) Group report: what are the interactions between biotic processes at vents and physical, chemical, and geological conditions? In: Halbach P, Tunnicliffe V, Hein J (eds) Energy and mass transfer in marine hydrothermal systems. Dahlem University Press, Berlin, pp 250–270

Tunnicliffe V, Koop BF, Tyler J, So S (2010) Flatfish at seamount hydrothermal vents show strong genetic divergence between volcanic arcs. Mar Ecol 31:158–167

Tunnicliffe V, Tyler J, Dower JF (2013) Population ecology of the tonguefish *Symphurus thermophilus* (Pisces; Pleuronectiformes; Cynoglossidae) at sulphur-rich hydrothermal vents on volcanoes of the northern Mariana Arc. Deep Sea Res II Top Stud Oceanogr 92:172–182

U.S. EPA (U.S. Environmental Protection Agency) (2013) ECOTOX database. http://cfpub.epa.gov/ecotox/. Consulted on 23 Sept 2013

Van Dover CL (2000) The ecology of deep-sea hydrothermal vents. Princeton University Press, Princeton, NJ

Vrijenhoek RC, Duhaime M, Jones WJ (2007) Subtype variation among bacterial endosymbionts of tubeworms (Annelida: Siboglinidae) from the Gulf of California. Biol Bull 212:180–184

Winemiller KO (1989) Development of dermal lip protuberances for aquatic surface respiration in South American characid fishes. Copeia 1989:382–390

Winemiller KO, Leslie M, Roche R (1990) Phenotypic variation in male guppies from natural inland populations: an additional test of Haskins' sexual selection/predation hypthesis. Environ Biol Fishes 29:173–191

Wolff T (2005) Composition and endemism of the deep-sea hydrothermal vent fauna. Cah Biol Mar 46:97–104

# Cave Environments

**Matthew L. Niemiller and Daphne Soares**

**Abstract** Caves and other associated subterranean habitats represent some of the most challenging environments on Earth. Despite many significant abiotic and biotic obstacles to overcome, most notably the complete absence of light, several groups of fishes thrive in subterranean habitats. The aim of this chapter is to provide a broad yet thorough review of our current knowledge regarding fish adaptations to one of the most extreme habitats on the planet. We begin our chapter by providing an overview of subterranean habitats, focusing on caves, and the major constraints cavefishes must overcome to live in such environments in complete darkness with limited food resources. We then provide an overview of taxonomic diversity and geographic distribution of cavefishes with a brief discussion of syntopy of cavefishes in subterranean habitats. Next we summarize what is known about the physiological, behavioral, and morphological adaptations that have evolved in cavefishes in response to the primary physicochemical and ecological stressors in subterranean habitats. In particular, we focus on adaptations associated with nonvisual sensory modalities. Finally, we offer a few suggestions for avenues of future research in cavefish adaptation and sensory evolution.

## 1 Subterranean Habitats

Subterranean habitats prevail on the continental mass. In fact, the entirety of air-filled and water-filled cavities beneath the Earth's surface may even be greater than that of surface habitats (Culver and Pipan 2009). For example, 97 % of all unfrozen freshwater is subsurface, while rivers and lakes account for less than 2 %. Subterranean habitats also house a substantial and often underappreciated biodiversity ranging from bacteria and fungi to crustaceans and insects to vertebrates,

---

M.L. Niemiller (✉)
Illinois Natural History Survey, University of Illinois Urbana-Champaign, Champaign, IL 61820, USA
e-mail: cavemander17@gmail.com

D. Soares
Biological Sciences, New Jersey Institute of Technology, Newark, NJ 07102, USA

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_8

such as fishes, salamanders, and bats. It has even been estimated that up to 40 % of the planet's prokaryotic biomass may be underground (Whitman et al. 1998). However, subterranean organisms remain one of the least understood faunas, partly because of the difficulty in exploring and sampling the natural cavities found underground.

Subterranean habitats are generally categorized into one of three main types: interstitial habitats, superficial subterranean habitats, and caves (Culver and Pipan 2009). Interstitial habitats include both shallow aquatic habitats that regularly interchange with surface water, while deep habitats have little or no surface water exchange. Shallow interstitial habitats consist of water-filled cavities between grains of unconsolidated sediments, such as riverbeds, sea and freshwater lake bottoms, and the hyporheic zone—the aquifer immediately below and alongside the channels of rivers and streams. The hyporheic zone represents an ecotone between surface water above and groundwater below (Culver and Pipan 2009). Deep interstitial habitats are permanent groundwater in small cavities or fractures in consolidated rocks, such as basalt. Such aquifers may be slowly recharged by water percolating through strata down from the surface or have no connections whatsoever with the surface.

Superficial subterranean habitats include habitats near the surface, such as perched subsurface aquifers that issue on the surface as seeps (i.e., hypotelminorheic; Mestrov 1962), cracks and shallow tubes in lava (i.e., mesocavern; Howarth 1983), talus slopes, and epikarst—cracks, fissures, and small cavities in the uppermost section of karstified rock (Culver and Pipan 2008, 2009). Interstitial habitats and especially superficial subterranean habitats support a significant fauna with many species highly modified for subterranean life, the fauna of which might have given rise to some species in deeper subterranean habitats (Culver and Pipan 2008). These habitats have been found in recent years to be much more extensive and ecologically important than previously thought (Culver et al. 2006; Culver and Pipan 2008). However, we will focus on the third subterranean habitat, caves, because almost all cavefishes are found in these larger subterranean voids.

Caves are the most well-known subterranean habitats and are defined as natural cavities in solid rock with areas of complete darkness that are larger than a few millimeters in diameter (Culver and Pipan 2009). Most caves are developed in karst landscapes characterized by cracks, fissures, sinkholes, caves, and subterranean drainages formed from the dissolution of soluble rock, including limestones, dolomites, and gypsum (Ford and Williams 2007). Karst accounts for 15 % of the land surface of the Earth in rocks as old as 400 million years (Palmer 2007). In karst regions, rainwater becomes weakly acidic as it interacts with carbon dioxide in the atmosphere and soil. As water percolates into cracks and fractures in bedrock, it slowly dissolves away the rock to create a network of cavities and passages that, over time, can be become quite large. Karst cave systems generally consist of water percolating from the soil–rock interface through epikarst in many small solution fissures and cavities oriented both vertically and horizontally until reaching a cave stream, which often marks the boundary between the vadose (unsaturated zone) above and the phreatic (saturated) zone below (Culver and Pipan 2009). Cave streams can be recharged either from epikarst or by surface streams that sink underground. Ultimately, cave streams will exit at springs that then follow surface hydrological patterns or drain into the phreatic zone at the water table. The phreatic zone often consists of large water-filled cavities that have very slow flow rates and long residence times. It is in cave streams and the phreatic waters where most cavefish are found.

## 2 Subterranean Ecosystems

Subterranean ecosystems develop in rocks or sediments that protect them against surface environmental changes. Therefore, in contrast to most surface ecosystems that are short-lived (rivers, wetlands, or forests), these may persist relatively unchanged for millions of years (Gibert and Deharveng 2002). Animals that live in continually dark caves are faced with unique challenges. Subterranean habitats are characterized by the absence of light, and without vision, novel, and often unusual strategies are needed to navigate as well as locate and identify food, predators, and conspecifics. In addition to complete darkness, several other abiotic factors affect subterranean organisms and influence subterranean ecosystems, such as relatively stable temperature, high humidity, and hydrological characteristics, such as periodic flooding (Culver and Pipan 2009). These factors have likely led to major regressive and constructive evolutionary changes in organisms permanently restricted to subterranean habitats.

Organisms that are obligate residents of subterranean habitats are known as troglobites. These animals are no longer able to survive above ground and have evolved unusual and highly specialized morphological and life history traits, termed troglomorphy (Christiansen 1962). Many independent lineages of troglobites have evolved derived features such as eye loss, pigmentation loss, wing loss, elongation of body appendages, increased life span, decrease in the number of eggs and increase in their size and development time, decrease in respiratory metabolism, loss of water regulatory processes, and various other physiological strategies and augmentation of nonvisual sensory systems (Jeannel 1926; Vandel 1964; Culver 1982; Culver and Pipan 2009; Soares and Niemiller 2013). Subterranean communities are generally divided by the two dominant habitats: terrestrial and aquatic. Terrestrial species that are also cave obligates are referred to as troglobionts or troglobites, while aquatic species are termed stygobionts or stygobites. Subterranean communities are also made from a complex of species at different stages of adaptation to underground environments. These species are traditionally classified as stygoxene or trogloxene (i.e., temporary inhabitants), stygophile or troglophile (i.e., with strong hypogean affinities), or stygobite or troglobite (i.e., obligately subterranean). Only a part of the community (the stygobites and troglobites) possesses the morphological, physiological, and behavioral specializations for underground life. Subterranean ecosystems are heterogeneous environments where species from different ecological and biogeographical origins cohabitate following multiple and successive colonization processes.

Although many species can be found in subterranean habitats that exhibit differing levels of troglomorphy and adaptation, here we will focus on true troglobites, and cavefishes in particular. The continual darkness of caves and other subterranean habitats imposes a sensory constraint that offers a unique opportunity to examine not only how these nonvisual sensory modalities become transformed but also how they coevolve. Aquatic cave organisms such as cavefishes not only survive in perpetual darkness but also negotiate the challenges of an aquatic environment. Variation in water volume and flow can be large in subterranean streams, especially during floods. High flow can wash fish downstream deeper underground or out onto the surface. Farmers in some provinces in China, for example, report cavefishes in their rice paddies every rainy season. Torrential flash floods in Ecuador make the environment extremely hostile to any aquatic subterranean life, and yet a population of cave-dwelling naked sucker-mouthed catfishes (Siluriformes: Astroblepidae) persists (Haspel et al. 2012). Such variation in water level and flow, however, along with more subtle changes in pH and temperature, may be important cues for reproduction during seasonal flood events for many cave animals (Culver and Pipan 2009).

While catastrophic floods have the potential for long-lasting effects on populations of cavefishes and other aquatic cave life, a study on cave populations of the Atlantic molly (*Poecilia mexicana*) revealed very limited individual dislocation among ecologically different habitat types (characterized by differences in light and hydrogen sulfide levels) after a 50-year flood event in the Cueva del Azufre system (Plath et al. 2010). However, there was evidence for the loss of small-scale genetic structuring within habitats. In this system, it appears as if natural selection is so strong between habitat types that even catastrophic events, such as extreme floods, do not lead to a breakdown of genetic differentiation among populations.

Trophic factors may act as significant selective pressures for the evolution of behavior and sensory adaptations in subterranean organisms. Because of the absence of light, there is no photosynthesis in caves, and chemosynthesis rarely supplies much trophic support (Culver and Pipan 2009). Consequently, many cave systems are resource-limited. With few exceptions, cave communities rely on external organic matter typically in the form of dissolved and particulate organic carbon transferred from the surface hydrologically (Simon and Benfield 2001) and the active movement of animals that fall or wander into caves and die to drive cave ecosystems. In addition, the regular movement of animals into and out of caves, such as colonial bats that produce guano piles, may be particularly important in several otherwise energy-limited cave systems. These allochthonous resources often drive the community dynamics in subterranean ecosystems (Hüppop 2000; Huntman et al. 2011), which typically have few trophic levels. Several cavefishes are top predators in the subterranean ecosystems that they inhabit (e.g., amblyopsid cavefishes), although the trophic positions of many cavefish species have not been well-studied. In general, little is known about the abiotic characteristics of caves inhibited by most cavefishes. The few ecological studies have only recorded basic measures, such as pH and water temperature, and only two have conducted time-series studies characterizing cavefish habitats (Trajano 1991, 1997). To our knowledge, none have incorporated these data into studies of sensory neurobiology.

The exceptional richness in endemics is directly related to the fragmentation of the subterranean habitats, which fosters evolutionary drift in isolated populations. The long-term persistence and relative stability of subterranean environments compared to epigean ones allow this process to take place. Subterranean populations are actually clusters of numerous metapopulations that may evolve into species with time. Endemicity tends to be much higher in subterranean than epigean habitats for the lineages that have colonized both habitats in the same geographical area. The higher proportion of geographically isolated taxa, relicts of ancient faunas, in subterranean (as compared with epigean) regional fauna, has long been recognized by biospeleologists (Jeannel 1926). Relicts have been identified in most groups that have colonized subterranean habitats, except in the wet tropics and in areas affected by quaternary glaciations (Juberthie and Decu 1994, 1998; Howarth 1987; Holsinger 1993).

## 3 Systematic and Geographic Distribution

Colonization of and adaptation to subterranean habitats have evolved many times in ray-finned fishes (class Actinopterygii). Over 300 species of fishes have been reported to live in caves and associated subterranean habitats (Proudlove 2006, 2010). However, about 165 described species representing 10 orders and 21 families live exclusively in freshwater subterranean habitats and exhibit some degree of troglomorphy (Fig. 1; Table 1). At least another 19 undescribed species have been noted in the literature. The greatest biodiversity is in the orders Cypriniformes (carps, minnows, and loaches; 82 species) and Siluriformes (catfishes; 49 species), which account for 78 % of all described cavefishes. Interestingly, 81 % of all species are otophysans. At the family level, the cypriniform families Cyprinidae (minnows; 41 species) and Nemacheilidae (loaches; 38 sp.) and the siluriform families Trichomycteridae (pencil catfishes; 16 species) and Heptapteridae (three-barbeled catfishes; 14 species) are the most diverse. Only the families Bythitidae (viviparous brotulas), Gobiidae (gobies), and Eleotridae (sleeper gobies) have invaded subterranean habitats from the sea rather than freshwater (Proudlove 2010).

Subterranean fishes are known from every continent but Antarctica and Europe (Fig. 1; Table 2). The highest cavefish diversity occurs in the karst of Southeast Asia (5 orders, 10 families and 94 species). Over 58 % (55 species) of all described cavefishes in Asia are known from China, where new species are being described every year. Eleven species are known from the Asian subcontinent of India. However, hotspots of cavefish diversity do not necessarily correspond to regions of great biodiversity of other stygobionts (Trajano 2001; Soares and Niemiller

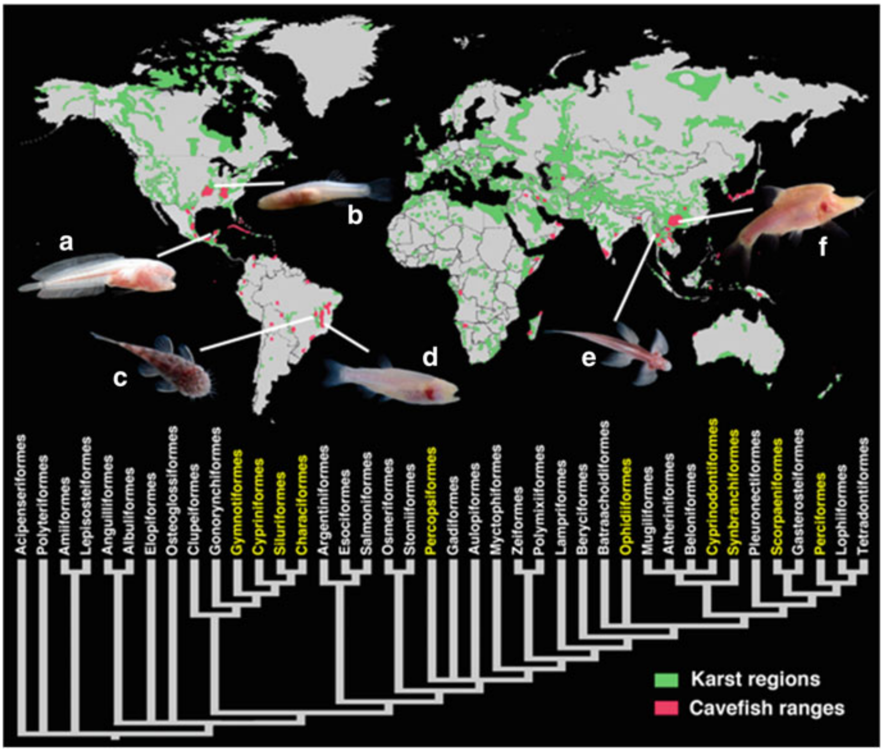


**Fig. 1** Phylogenetic diversity and geographic distribution of cavefishes (from Soares and Niemiller 2013). Cavefishes are known from ten orders (in *yellow*) and every continent but Europe and Antarctica: (**a**) *Typhliasina pearsei* (Ophidiiformes: Bythitidae) from Mexico; (**b**) *Troglichthys rosae* (Percopsiformes: Amblyopsidae) from the United States; (**c**) *Ancistrus cryptophthalmus* (Siluriformes: Loricariidae) from Brazil; (**d**) *Stygichthys typhlops* (Characiformes *incertae sedis*) from Brazil; (**e**) *Cryptotora thamicola* (Cypriniformes: Balitoridae) from Thailand; and (**f**) *Sinocyclocheilus furcodorsalis* (Cypriniformes: Cyprinidae) from China. Karst regions were modified from Williams and Ford (2006) and the phylogeny is based on Li et al. (2008). Photographs by Thomas M. Iliffe (**a**) and Dante B. Fenolio (**b**–**f**)

2013). Thirty-three species are known from South America, with the greatest diversity in Brazil (20 species). In North America, 25 cavefish species are known, including 10 and 9 species in Mexico and the United States, respectively.

## 4 Syntopy of Cavefish Species

It has been estimated that up to 40 % of all cave obligate fishes coexist with at least one other stygobiotic species (Trajano 2001). Over half of known cases of syntopy correspond to species of the same family, with the majority of cases occurring in Southeast Asia and South America. One cave in Guangxi Zhuang Autonomous

**Table 1** Taxonomic diversity of obligate subterranean fishes

| Order/family | Genera | Species (undescribed) |
|---|---|---|
| Order Characiformes | 2 | 2 |
| Characidae | 2 | 2 |
| Order Cypriniformes | 26 | 82 |
| Balitoridae | 1 | 1 |
| Cobitidae | 1 | 2 |
| Cyprinidae | 14 | 41 |
| Nemacheilidae | 10 | 38 |
| Order Siluriformes | 19 | 49 (8) |
| Astroblepidae | 1 | 2 |
| Callichthyidae | 1 | 1 |
| Clariidae | 3 | 5 |
| Heptapteridae | 4 | 14 (3) |
| Ictaluridae | 3 | 4 |
| Loricariidae | 1 | 3 |
| Siluridae | 2 | 4 (1) |
| Trichomycteridae | 4 | 16 (4) |
| Order Gymnotiformes | 1 | 1 |
| Sternopygidae | 1 | 1 |
| Order Percopsiformes | 4 | 6 (8) |
| Amblyopsidae | 4 | 6 (8) |
| Order Ophidiiformes | 4 | 8 (1) |
| Bythitidae | 4 | 8 (1) |
| Order Cyprinodontiformes | 1 | 1 |
| Poeciliidae | 1 | 1 |
| Order Synbranchiformes | 2 | 5 |
| Synbranchidae | 2 | 5 |
| Order Scorpaeniformes | 1 | 1 (1) |
| Cottidae | 1 | 1 (1) |
| Order Perciformes | 7 | 12 (1) |
| Eleotridae | 4 | 8 (1) |
| Gobiidae | 3 | 4 |
| Total | 67 | 167 (19) |

Region, China, harbors four stygobiotic species, including *Sinocyclocheilus macrophthalmus*, *Protocobitis typhlops*, *Oreonectes translucens*, and another undescribed species of *Oreonectes* (Zhao et al. 2011; Fenolio et al. 2013).

The mechanisms behind multiple cavefish subterranean communities have not been well investigated, although it has generally been thought that subterranean ecosystems cannot support more than one level of predators (Mohr and Poulson 1966; Gibert and Deharveng 2002). Therefore, partitioning of diet and habitat may allow coexistence of multiple cavefish species with minimal competition (Trajano 2001). For example, habitat partitioning has been posited to explain the coexistence

**Table 2** Taxonomic diversity of obligate subterranean fishes by continent and country around the world

| Continent/country | Orders | Families | Genera | Species |
|---|---|---|---|---|
| Africa | 3 | 4 | 7 | 9 |
| DRC | 1 | 1 | 1 | 1 |
| Madagascar | 1 | 2 | 2 | 4 |
| Nambia | 1 | 1 | 1 | 1 |
| Somalia | 2 | 2 | 3 | 3 |
| Asia | 5 | 10 | 31 | 94 |
| China | 1 | 3 | 9 | 55 |
| India | 3 | 4 | 5 | 11 |
| Indonesia | 2 | 2 | 2 | 2 |
| Iran | 1 | 2 | 2 | 2 |
| Iraq | 1 | 1 | 2 | 2 |
| Japan | 1 | 1 | 1 | 2 |
| Laos | 1 | 2 | 3 | 3 |
| Malaysia | 1 | 1 | 1 | 1 |
| Oman | 1 | 1 | 1 | 2 |
| Philippines | 1 | 1 | 1 | 1 |
| Thailand | 2 | 4 | 6 | 9 |
| Turkmenia | 1 | 1 | 1 | 1 |
| Vietnam | 2 | 2 | 3 | 3 |
| Australia | 2 | 2 | 3 | 5 |
| Australia | 2 | 2 | 2 | 3 |
| Papua New Guinea | 1 | 1 | 1 | 2 |
| North America | 7 | 8 | 14 | 25 |
| Bahamas | 1 | 1 | 1 | 2 |
| Belize | 1 | 1 | 1 | 1 |
| Cuba | 1 | 1 | 1 | 3 |
| Mexico | 5 | 6 | 6 | 10 |
| United States | 3 | 3 | 7 | 9 |
| South America | 3 | 7 | 13 | 33 |
| Argentina | 1 | 1 | 1 | 1 |
| Bolivia | 1 | 2 | 2 | 2 |
| Brazil | 3 | 6 | 11 | 20 |
| Columbia | 1 | 1 | 1 | 4 |
| Ecuador | 1 | 1 | 1 | 1 |
| Peru | 1 | 1 | 1 | 1 |
| Trinidad | 1 | 1 | 1 | 1 |
| Venezuela | 1 | 3 | 3 | 3 |
| Total | 10 | 21 | 67 | 167[a] |

[a] *Ogilbia galapagosensis* (Ophidiformes, Bythitidae) is known from the Galapagos Islands

of *Schistura oedipus* and *Cryptotora thamicola* in Mae Lana Cave in Northwestern Thailand (Trajano 2001). *Schistura oedipus* occurs in slow-flowing reaches of the base-level cave stream, whereas *C. thamicola* is found in fast-flowing waters of waterfalls in tributaries of the main cave stream. Two species of amblyopsid cavefishes occur in the Mammoth Cave system of Kentucky. However, their distributions have been explained by competitive interactions, whereby *Amblyopsis spelaea* inhabits deeper waters in base-level streams, while *Typhlichthys subterraneus* occurs in the master drains of vertical shafts that are tributaries to base-level streams (Woods and Inger 1957; Poulson 1992; Niemiller and Poulson 2010). It is unclear whether *Amblyopsis* is precluded from upstream reaches because of its larger size or because of aggressive dominance by *Typhlichthys*. Alternatively, *Typhlichthys* may be precluded from downstream habitats because of possible decreases in food supply or competitive dominance by *Amblyopsis*.

# 5 Subterranean Colonization and Speciation

Speciation in subterranean fauna has often been explained by two contrasting hypotheses. The traditional view is that most cave organisms are relictual and isolated, whereby speciation is achieved when populations become stranded underground as surface conditions become inhospitable and slowly evolve troglomorphic features. This hypothesis has been particularly prevalent for cave fauna in temperate regions and has been termed the climate-relict hypothesis (Holsinger 1988, 2000; Peck and Finston 1993). Under the climate-relict hypothesis, surface ancestors adapted to cool, moist habitats retreat into subterranean habitats in response to climatic fluctuations. Caves serve as refuges in this model. As surface conditions become inhospitable, extirpation of surface populations facilitates allopatric speciation. In contrast, the adaptive-shift hypothesis posits that surface ancestors preadapted to subterranean life exploited new subterranean niches with reduced competition and quickly evolved in sympatry or parapatry with related surface populations (Howarth 1973, 1981; Holsinger 2000; Niemiller et al. 2008).

Under the adaptive-shift hypothesis, divergent natural selection between surface and cave habitats is required to overcome the homogenizing effects of gene flow. Speciation eventually results if the underlying genetic changes lead to reproductive isolation. This scenario is known as ecological speciation (Schluter 2000; Nosil 2012). Adaptive shifts may or may not also be involved under a climate-relict scenario, where divergence between surface and cave populations may involve strictly neutral changes, adaptive changes that may or may not contribute to reproductive isolation, or changes involving regressive morphologies that result from loss-of-function mutations that are maladaptive on the surface but selectively neutral in subterranean habitats. Therefore, the key distinction between the climate-relict and adaptive-shift hypotheses is whether divergence occurs in the absence (allopatry) or presence (parapatry or sympatry) of gene flow (Niemiller et al. 2008).

Among cavefishes, evidence is accumulating in support of both hypotheses from recent molecular studies. Consistent with the climate-relict hypothesis, the origin of the Somalian cave cyprinid *Phreatichthys andruzii* is estimated to be the end of the Pliocene (2.6–1.4 Mya) during a period of extreme aridity resulting in the progressive disappearance of many surface river systems (Colli et al. 2009). In the same study, the cave cyprinid *Garra barreimiae*, which occurs in the Arabian Emirates and Oman, diverged from surface populations during the Pleistocene, perhaps during two periods of aridity in Eastern Africa between 1.8 and 0.8 Mya (Colli et al. 2009).

Climatic changes, particularly during the late Pliocene and Pleistocene, have been posited historically to be drivers of speciation in amblyopsid cavefishes of eastern North America (Woods and Inger 1957; Swofford 1982; Barr and Holsinger 1985; Willis and Brown 1985; reviewed in Niemiller and Poulson 2010). Although the specifics for the hypotheses about the origin of each amblyopsid cave lineage differ, all implicate Pleistocene glaciation cycles as the primary factor behind subterranean invasion and subsequent extinction of ancestral surface populations. Recent molecular studies suggest that the major lineages within Amblyopsidae originated well before the Pleistocene (Niemiller et al. 2013a, c), with the most recent common ancestor of troglobiotic lineages dating to the Miocene (10.3 Mya). However, several lines of evidence support at least three independent colonization events into subterranean habitats and evolution of troglomorphic forms that occurred much more recently, perhaps in the late Pliocene or Pleistocene (Niemiller et al. 2013a). First, different cave lineages do not share loss-of-function (LOF) mutations in the eye photoreceptor gene *rhodopsin*. Shared LOF mutations would be expected if all cave amblyopsids were derived from a single ancestor that colonized caves well before the Pleistocene. Second, eye histological data support independent colonization events, as cave lineages differ in the number and degree of eye structures that are degenerate or have been lost (Eigenmann 1909; Niemiller and Poulson 2010). Third, geological, hydrological, and biogeographical evidence suggests that long-distance dispersal via subterranean corridors is highly unlikely (reviewed in Niemiller and Poulson 2010). Consequently, cave amblyopsids have limited opportunities for dispersal, resulting in high genetic differentiation among populations, even at local scales. Genetic variation throughout the range of *Typhlichthys* is strongly associated with hydrological drainage boundaries, with divergence among lineages within the genus largely occurring during the late Pliocene and Pleistocene (Niemiller et al. 2012). Populations of *Amblyopsis* north and south of the Ohio River in Indiana and Kentucky, USA, are genetically distinct dating to the formation of the modern Ohio River during the Pleistocene ca. 0.8 Mya (Niemiller et al. 2013c). Although it is unclear whether many cave lineages were already primarily subterranean before dramatic climatic changes during the late Pliocene and Pleistocene, climatic and geological processes during this time period have had significant effects on the partitioning of genetic variation and evolution of cave amblyopsids. Regardless, morphological differences also exist between these genetic lineages, and populations north of the Ohio River are now recognized as a distinct species, *Amblyopsis hoosieri* (Chakrabarty et al. 2014).

Speciation of the blind characid *Astyanax mexicanus* has been well-studied from several perspectives. However, speciation in *Astyanax* appears quite complex with at least two subterranean invasions by ancestral surface forms occurring over the past several years, far older than previously thought (see review by Gross 2012). Divergent selective pressures between dissimilar surface and cave habitats have driven phenotypic evolution in a variety of morphological, physiological, and behavioral traits, such as vision, pigmentation, metabolism, mechanosensory sensitivity, feeding behavior, and sleep patterns, among others (e.g., Protas et al. 2006, 2007; Gross et al. 2009; Duboue et al. 2011; Yoshizawa et al. 2012; Borowsky and Cohen 2013). Despite substantial ecologically driven phenotypic differences between cave and surface forms, blind cavefish and their eyed surface relatives are interfertile, which has facilitated a wealth of studies on the genetic and developmental basis of cave adaptation in the laboratory (Jeffery 2001, 2008, 2009; Borowsky and Wilkens 2002; Borowsky 2010). Population genetic analyses suggest that gene flow also occurs or has occurred in the recent past between cave and surface populations in nature (Bradic et al. 2012; but see contrasting results of Strecker et al. 2012). However, the two forms maintain their respective phenotypic differences in the wild, strongly implicating an ecological speciation scenario consistent with the predictions of the adaptive-shift scenario.

Another example consistent with the adaptive-shift model is the evolution of cave forms of the poeciliid *Poecilia mexicana* from Cueva del Azufre and Cueva Luna Azufre, Tabasco, southern Mexico. This species is currently undergoing ecological speciation between adjacent surface and cave populations, as well as populations inhabiting highly sulfidic habitats (Riesch et al. 2011). Gene flow is low from cave into surface habitats, with no evidence for gene flow in the opposite direction into cave habitats (Tobler et al. 2009; Plath et al. 2010). Reproductive isolation between surface and cave populations has been attributed to predation (Tobler 2009), sexual selection, and hydrogen sulfide-induced toxicity on males (Tobler et al. 2009), and reproductive failure of surface females and higher rates of stress-related infections in darkness (Riesch et al. 2011). These studies provide experimental evidence for divergent selection through both biotic and abiotic factors in the development of reproductive isolation in ecological speciation and the evolution of cave organisms.

## 6 Loss of Vision and Pigmentation

### *6.1 Eye Degeneration*

Although appearing eyeless, most adult cavefishes actually have small degenerate eyes buried deep within their orbits. The molecular and cellular mechanisms of eye degeneration have been studied most extensively in *Astyanax mexicanus* and we will focus our brief discussion on this species. For an in depth review of eye regression, see the review by Protas and Jeffery (2012). Different *Astyanax* cavefish populations show varying degrees of eye degeneration: Curva, Los Sabinos, and Pachón have smaller vestigial eyes than Rio Subterráneo or Molino cavefish (Wilkens 1988, 2007; Wilkens and Strecker 2003). Small eye primordia are formed in embryos, but subsequently arrest, degenerate, and sink into the orbits (Cahn 1958; Yamamoto et al. 2004). Compared with surface fish, the cavefish's embryonic lens is relatively small, and the optic cup is missing its ventral sector. Later in development, precursor cells do not differentiate in the cavefish lens, which becomes progressively smaller and vestigial during development (Wilkens 1988; Langecker et al. 1993; Soares et al. 2004; Strickler et al. 2007). The arrest in cavefish eye growth is likely a consequence of the dysfunction of a crucial regulatory component(s) in the developing eye. Previous studies suggest that lens apoptosis and dysfunction is caused by expanded sonic hedgehog (*shh*) gene expression along the embryonic midline (Yamamoto and Jeffery 2000; Yamamoto et al. 2004). Genetic studies have found 8–12 quantitative trail loci involved in eye size reduction in *Astyanax* (Wilkens 1988; Protas et al. 2007, 2008). However, none of these QTLs are linked to *shh*, suggesting that upstream modulators of the SHH signaling system or other genetic factors may be important in eye degeneration (Protas and Jeffery 2012).

Loss of eyes could have had direct adaptive importance in lowering the energetic cost of maintaining eyes in an environment where they lack utility or could be selected indirectly through the pleiotropic need to expand other sensory systems. It is also possible that neutral effects can contribute to the process by which eyes were lost in the cave populations of *Astyanax*. A recent paper by Rohner et al. (2013) suggests that the expression of the protein HSP90 (heat shock protein 90) masks variation in genes, such as those governing the size of the *Astyanax* eye. Expression of the *hsp90α* gene is specifically activated in the lens of Pachón, Chica, and Tinaja *Astyanax* cavefish embryos just prior to apoptosis. Pharmacological inhibition of HSP90 function suppresses lens apoptosis and to some extent rescues lens differentiation, suggesting that HSP90 in the lens may be a proapoptotic factor. Thus, cavefish lens apoptosis may be controlled by the activation of proapoptotic factors and suppression of antiapoptotic factors (Hooven et al. 2004; Rohner et al. 2013).

## *6.2 Pigmentation*

The reduction or complete loss of melanin pigmentation has evolved in many cavefish lineages. Melanin protects animals from the damaging effects of ultraviolet light and also plays important roles in vision, defense, and immunity. In surface habitats, albino and depigmented individuals likely exhibit reduced fitness. However, selection for pigmentation is relaxed in aphotic environments, such as caves. It remains unclear what evolutionary mechanisms are responsible for albinism and depigmentation and whether benefits are conferred for loss of melanin pigmentation in subterranean organisms (Bilandzija et al. 2013).

Quantitative trait loci (QTL) analyses have revealed that reduction in melanophores in *Astyanax* is a complex genetic trait controlled by a large number of genes (Jeffery 2009). Pigmentation regression has at least three primary causes in *Astyanax* cavefish. First, the mostly albino Pachón, Molino, and Micos *Astyanax* cavefish forms have accumulated different loss-of-function mutations in the *Oca2* gene, causing defective melanin synthesis during the first step of the melanin synthesis pathway (Protas et al. 2006; Gross and Wilkens 2013). In particular, these *Oca2* mutations are large exon deletions in three cavefish populations. In the Japanese population, the mutation is presumably in a regulatory region (Protas et al. 2006; Gross and Wilkens 2013). Second, some cavefish populations, including some of those with albinism, have also accumulated different mutations in the melanocortin-1 receptor (*Mc1r*) gene causing a reduction in the number of melanophores and melanin pigmentation due to defective MSHα signaling (Gross et al. 2009). The *Oca2* and *Mc1r* genes are probably epistatic in *Astyanax*, as they are in humans (King et al. 2003), but it is impossible to tell which of the two genes mutated first in the cave populations where they coexist. Third, mutations in additional genes that remain to be identified also may contribute to the reduction of melanophores.

Because cave and surface forms of *Astyanax* readily interbreed and produce viable offspring, they have become model systems for examining the roles of selection and neutral mechanisms of pigmentation regression. QTLs corresponding to reduction in melanophores have positive and negative polarities, suggesting that genetic drift may govern this trait (Jeffery 2009). However, the evolutionary cause of albinism through loss of function of *Oca2* may be selection. The interruption of melanin synthesis during its initial step in several different *Astyanax* populations as well as in other cavefishes from Brazil (namely, *Stygichthys*, *Ancistrus*, and *Rhamdiopsis*; Felice et al. 2008) implicates convergence and a role for selection. It may be beneficial to knockout melanin synthesis as early as possible in the synthesis pathway to conserve energy. However, it is unlikely that melanin is very costly to produce. An alternative hypothesis is that albinism confers evolutionary benefits through pleiotropy. A recent study by Bilandzija et al. (2013) provided the first evidence that interruption of melanin synthesis offers potential benefits in *Astyanax* cavefish (Pachón). The initial substrate in the melanin synthesis pathway, L-tyrosine, is also used in the catecholamine (CAT) synthesis pathway. Interrupting melanin synthesis at its first step results in excess L-tyrosine, dopamine, and norepinephrine in pre-feeding larvae and adult brains of cavefish, relative to surface fish. Dopamine and norepinephrine are produced by the CAT pathway, which control feeding and sleep. In particular, CAT norepinephrine has been implicated in decreased sleep of *Astyanax* cavefish (Duboue et al. 2011). These results suggest that blocking melanin synthesis through mutations in *Oca2* may enhance the CAT pathway and the resulting behavioral and physiological changes associated with cave adaptation.

# 7 Physiological and Life History Adaptations

## 7.1 Circadian and Circannian Rhythms

The circadian clock is synchronized with the day–night cycle primarily by light. The conservation of circadian rhythms appears to be a general phenomenon in cave-dwelling and other subterranean fishes. The endogenous rhythms in swimming activity (Erckens and Weber 1976; Cordiner and Morgan 1991; Pati 2001; Zafar and Morgan 1992; Espinasa and Jeffery 2006) and melatonin synthesis (Wilkens et al. 1993) have been reported in different cavefish species. The persistence of circadian rhythms in the cave environment may have physiological roles unrelated to day/night cycles, such as the synchronization by feeding. Cavallari et al. (2011) examined the Somalian cavefish *Phreatichthys andruzzii* and found that this fish possesses a clock that is not only entrained by periodic food availability but also has a long infradian period, lacks temperature compensation. However, the clock is no longer entrainable by light. Some amblyopsid species also cannot be entrained to light–dark cycles (Poulson and Jegla 1969; Poulson and White 1969). This has been viewed as evidence that circadian rhythms are not maintained by selection in subterranean habitats (Poulson and White 1969). With regard to circannian rhythms, several cavefishes appear to exhibit yearly cycles in reproductive activity (e.g., Poulson 1963; Iliffe 1993; Trajano 2001). However, nonseasonal reproduction has been suggested for *Typhlogarra widdowsoni* and *Caecocypris basimi* (Banister and Bunni 1980), and possibly the catfish *P. kronei* (Trajano 1991).

## 7.2 Fecundity, Egg Size, and Other Reproductive Traits

Although data on reproduction are limited for many cavefish species, it has been generally hypothesized that cave species tend to produce fewer but larger eggs than their surface relatives. Larger eggs contain more yolk and yield larger larvae that may have greater starvation tolerance, greater swimming ability to catch prey and avoid predators, and a larger gape to accommodate a wider range of prey sizes (Niemiller and Poulson 2010). This trend is clearly identifiable in amblyopsids (Fig. 2), in which cave species produce 50–80 % fewer eggs than surface relatives that are also significant larger. For example, eggs range from 1.9 mm to 2.3 mm diameter in three cave amblyopsids (*Amblyopsis*, *Troglichthys,* and *Typhlichthys*), whereas egg size in the surface *Chologaster* typically ranges from 0.9 mm to 1.2 mm (but up to 1.9 mm) (Niemiller and Poulson 2010). A similar trend has been observed in *Astyanax* (Wilkens 1988), *Ancistrus cryptophthalmus* (Trajano 2001), *Horaglanis krishnai* (Mercy et al. 1982), and *Poecilia mexicana* (Riesch et al. 2010). Several additional reproductive traits are known to differ as well between cave and surface relatives, such as reduced reproductive rates and increased investment in offspring (Culver 2005). For example, Poulson (1963) estimated at least a 50 % reduction in the proportion of females breeding at any given time in populations of cave amblyopsids versus surface amblyopsids. Cave amblyopsids also showed up to a 500 % increase in the number of broods produced, as a result of increased longevity (see Sect. 7.3). Female surface-dwelling *Chologaster cornuta* breed only once in their lifetimes, whereas female cave-dwelling *Amblyopsis spelaea* may breed up to five times (Poulson 1963; Niemiller and Poulson 2010). Finally, there may be a trend of increased parental care in cave amblyopsids. *Amblyopsis spelaea* and *A. hoosieri* females from some populations brood eggs in their branchial cavities (Eigenmann 1909; Woods and Inger 1957; Poulson 1963; Niemiller and Poulson 2010). The jugular position of the genital papilla in adults and the attachment of the gill membrane to the isthmus in all amblyopsid species suggest that all amblyopsids may brood eggs in their branchial cavities. However, *A. spelaea* and *A. hoosieri* are the only species known to exhibit such behavior. Estimates of total egg volume based on average egg and clutch sizes for other amblyopsid species exceed the brachial volume (Niemiller and Poulson 2010). It remains unknown if other amblyopsids and other cavefish species exhibit such parental care.

## *7.3 Growth, Development, and Longevity*

Several cavefishes exhibit reduced growth rates, increased age to sexual maturity, and increased longevity compared to surface relatives. One of the best-studied groups is the amblyopsid cavefishes, which show a reduction in growth rates and development and an increase in longevity with increasing cave adaptation (Poulson 1963; Niemiller and Poulson 2010). Maximum life spans may approach 20 years in some amblyopsid cave species (Poulson 2001; Niemiller and Poulson 2010). Several other cavefishes are known to have considerable life spans, at least under laboratory conditions, such as *Astyanax* (10+ years; Trajano 2001), *Pimelodella kronei* (18 years; Trajano 2001), and *Phreatichthys andruzzii* (23+ years; Trajano 2001). These interrelated traits may represent an adaptive response to low food supplies in subterranean habitats (Hüppop 2000). Coupled with the transition from semelparity to iteroparity, these adaptations likely increase the chances for population persistence over time, as a population is less likely to be extirpated during periods of extreme food shortages that result in little to no recruitment (Hüppop 2000; Niemiller and Poulson 2010).

## *7.4 Starvation Resistance and Metabolism*

Because of the absence of light and photosynthesis, most cave ecosystems are characterized by few trophic levels. Cavefishes are often top predators in these ecosystems where they are considered generalist carnivores, opportunistically

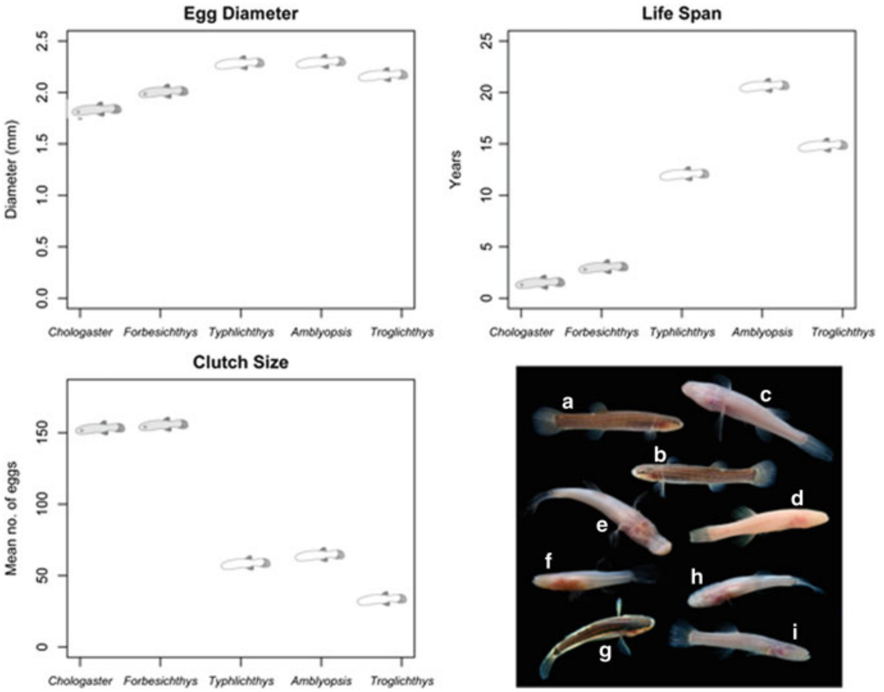


**Fig. 2** Several life history traits, such as egg size, clutch size, and life span show trends associated with the degree of cave adaptation in amblyopsid cavefishes. Egg size and life span show an increasing trend from surface-dwelling *Chologaster* to facultative cave-dwelling *Forbesichthys* to obligate cave-dwelling *Typhlichthys*, *Amblyopsis*, and *Troglichthys*. Clutch size decreases with increasing cave adaptation. Data from Niemiller and Poulson (2010). The *lower right panel* is a composite of amblyopsid diversity, including three surface species and six stygobiotic species: (**a**) *Forbesichthys agassizii*, (**b**) *F. papilliferus*, (**c**) *Amblyopsis spelaea*, (**d**) *A. hoosieri*, (**e**) *Speoplatyrhinus poulsoni*, (**f**) *Troglichthys rosae*, (**g**) *Chologaster cornuta*, (**h**) *Typhlichthys eigenmanni*, and (**i**) *Typhlichthys subterraneus*. Photographs by Dante B. Fenolio (all but **d**) and Matthew L. Niemiller (**d**)

feeding on aquatic invertebrates (reviewed in Trajano 2001). Many cavefishes, such as amblyopsids, feed almost exclusively on live prey, whereas others may opportunistically scavenge (e.g., *Pimelodella kronei*). Such differences in feeding strategies may be reflected in the nonvisual sensory adaptations employed by various cavefishes (see Sect. 8). Some species have more unusual diets. *Poecilia mexicana* has a diet comprised primarily of chironomid larvae and other invertebrates and also bacteria in the chemoautotrophic cave ecosystem in which it lives (Langecker et al. 1996; Tobler 2008).

Food scarcity is a major problem in many subterranean habitats and is demonstrated by a few studies that found a high proportion of cavefish stomachs that were empty or contained very few prey items (Trajano 2001; Tobler 2008; Niemiller and Poulson 2010). However, feeding is one of the least understood aspects of cavefish biology (Trajano 2001). In addition to nonvisual sensory and behavioral adaptations for detecting prey and increasing feeding efficiency in complete darkness (see Sect. 8), cavefishes have adopted several other strategies to cope with limited food resources.

Starvation resistance can be accomplished by four primary mechanisms (Hüppop 2000), acting alone or in concert, in cavefishes: (1) reduction in activity and metabolism; (2) consumption of large amounts of food during single to infrequent feeding events; (3) storage of increased amounts of fat; and (4) reduction of the digestive absorptive epithelia or reduction of gut length. Poulson (1963) found an increasing ability to cope with food scarcity in amblyopsid cavefishes. The surface-dwelling *Chologaster cornuta* expired after just 45 days, whereas cave-dwelling *Troglichthys rosae* is known to survive up to 20 months without food (Eigenmann 1909; Niemiller and Poulson 2010). Decreases in metabolic rate and activity compared to surface relatives have been observed in several cavefishes (Poulson 1963; Hüppop 1986; Trajano 2001; Niemiller and Poulson 2010). The general conclusion of these studies is that cave species have lower metabolic rates compared to epigean species. It is hypothesized that this reduction may be a result of alterations in food availability (Hüppop 1986) or ambient dissolved oxygen (in crayfishes: Caine 1978; Gannon et al. 1999). Many cavefish species deposit large quantities of fat throughout their bodies. For example, at least four species of heptapterine cavefishes accumulate fat along the base of the adipose and anal fins (Weber 1996; Weber and Wilkens 1998; Trajano 2001), but this does not occur in their surface relatives. Cave *Astyanax* has an improved ability to store fat compared with surface fish and often builds up enormous reserves in captivity (Wilkens 1988).

## *7.5 Hypoxia*

Cavefishes living in phreatic habitats, habitats that experience periodic drought, habitats with large amounts of organic matter subsequently broken down by bacteria, or those that experience other unusual conditions must cope with low levels of dissolved oxygen (DO) (reviewed in Trajano 2001). For example, Trajano and Bockmann (2000) measured DO values as low as 0.7 mg $l^{-1}$ in phreatic habitats of Brazil. Several other cavefishes are known to inhabit phreatic waters with low DO levels, such as *Barbopsis devecchi* and *Phreatichthys andruzzii* in Somalia (Ercolini et al. 1982) and *Satan eurystomus* and *Trogloglanis patternsoni* in the Edwards Aquifer of Texas, USA (Longley and Karney 1979). Trajano (2001) hypothesized that reduced metabolic rates, which have been interpreted as an adaptation to low food supplies (Poulson 1963), may also aid in low DO tolerance. Subterranean populations of *Indoreonectes evezardi* are known to exhibit air-gulping behavior when faced with hypoxic conditions in stagnant pools during the dry seasons (Biswas et al. 1990). For a general overview over fishes in hypoxic environments, please see chapter “Low-Oxygen Lifestyles”.

### 7.6 Depth Tolerance

Cavefishes have been found at varying depths in cave aquifers, as well as in noncave aquifers, caves flooded by impoundments, and at great depths in lakes. Tolerance to depth appears to be highly variable (reviewed in Trajano 2001). Many species are known from depths <30 m. However, the cave catfishes *Satan eurystomus* and *Trogloglanis pattersoni* are known from wells as deep as 610 m. These species have a reduced swim bladder, reduced muscles, and reduced ossification, presumably to cope with high hydrostatic pressure (Langecker and Longley 1993; Trajano 2001).

## 8 Nonvisual Sensory Adaptations

In subterranean habitats, the lack of light is the most obvious sensory constraint. Cavefishes are immediately recognizable for their regressive evolutionary changes in response to aphotic conditions, namely degeneration of eyes and reduction in pigmentation. However, as we discuss below, there are also several constructive adaptations that have evolved in various cavefishes, most notably an increase mechanosensation.

### 8.1 Mechanoreception

As visual inputs deteriorate, the importance of mechanosensory information undoubtedly increases. Cavefishes have evolved a variety of solutions for increasing their sense of touch. Not only can they have longer appendages (such as fins and barbels), often they have hypertrophied lateral lines, but also have novel adaptations for mechanosensation. Cavefishes may use elongated fins, particularly pectoral fins, and barbels to touch obstacles and other objects (Fig. 3), and they use this tactile information along with hydrodynamic imaging to sense their environment (Windsor et al. 2008). The mechanosensory lateral line system is comprised of hair cell receptors (neuromasts) that respond to relative motion between the body and the water surrounding it, and this system is in some respects akin to a sense of distant touch. With the lateral line, however, the presence of objects is not determined by direct contact; instead, it is mediated by the water disturbances these objects create, described as touch at a distance (Dijkgraaf 1962). The morphology of neuromasts can be indicative of life history and environmental constraints. Two generalized types of neuromasts exist: superficial neuromasts, which are found on the surface of the body, and canal neuromasts, which are located in ossified canals or grooves. Neuromast type and morphology are widely believed to have evolved in response to varying levels of background flow or noise in the environment (Coombs et al. 1988). Fish generally possess both lateral-line types and a trend has been observed whereby fishes with a higher proportion of surface neuromasts tend to be found in low-noise environments such as quiet waters, whereas fishes exhibiting a higher proportion of canal neuromasts inhabit "noisy" environments such as rapidly running streams (Dijkgraaf 1962). Within surface neuromasts, there is a range of anatomical features that exposes the hair cells to the water flow. On one end of this range are papillae neuromasts that have likely moved away from the boundary layer near the body and are highly sensitive to subtle water disruptions in quiet waters. On the other end of this continuum, neuromasts in pits can be less sensitive to signal and noise. Cavefishes can have specializations in their lateral line in the number of neuromasts present on their bodies as well as the type of neuromasts. The Pachon cave form of *Astyanax* not only has many more neuromasts than its surface counterparts (Montgomery et al. 2001; Windsor et al. 2008; Sharma et al. 2009; Yoshizawa et al. 2010) but also has evolved an adaptive behavior to enhance the mechanosensory sensitivity. Pachon *Astyanax* has evolved a form of active sensing in which it uses a kick-and-glide swimming style to generate a relatively stable, dipole-like flow signal during the glide phase of the swimming cycle (Sharma et al. 2009; Patton et al. 2010). With this strategy, *Astyanax* uses the distortions of nearby obstacles in the self-generated flow field to sense those obstacles and spends more time gliding when investigating a new object (Patton et al. 2010). Another example comes from cave amblyopsids. Cave species of amblyopsids also have papillae neuromasts that are larger and that have longer cupulae than their surface relatives, which allows the detection of moving prey at a greater distance than their surface relatives (Poulson 1963; Niemiller and Poulson 2010). Another unusual mechanosensory strategy used by cavefishes is the use of skin teeth to create hydrodynamic images. Haspel et al. (2012) demonstrated that the cave loricarioid catfish *Astroblepus pholeter* has adapted not only to complete darkness but also to life in fast, turbulent water. This species has unusually few neuromasts but shows a hypertrophy of mechanosensory skin denticles along the dorsal surface of the head and trunk (Fig. 3). These fish are endemic to a single cave that contains a fast outgoing stream, which often becomes torrential after heavy rains. The direction of water flow is detected by deflection of the denticles, and the proximity to the substrate can be detected by changes in fluid velocity. This is the first report of a mechanosensory role of denticles and of a non-neuromast mechanosensory adaptation to subterranean habitats in fishes.

## *8.2 Hearing*

Because of strong selection to develop and enhance nonvisual sensory modalities, there is the potential for enhanced hearing ability in cavefishes. Improved hearing abilities in subterranean habitats may be adaptive for several reasons, such as integrating with other nonvisual senses to detect prey, predators, or conspecifics. Many cave systems inhabited by cavefishes are prone to dramatic changes in water

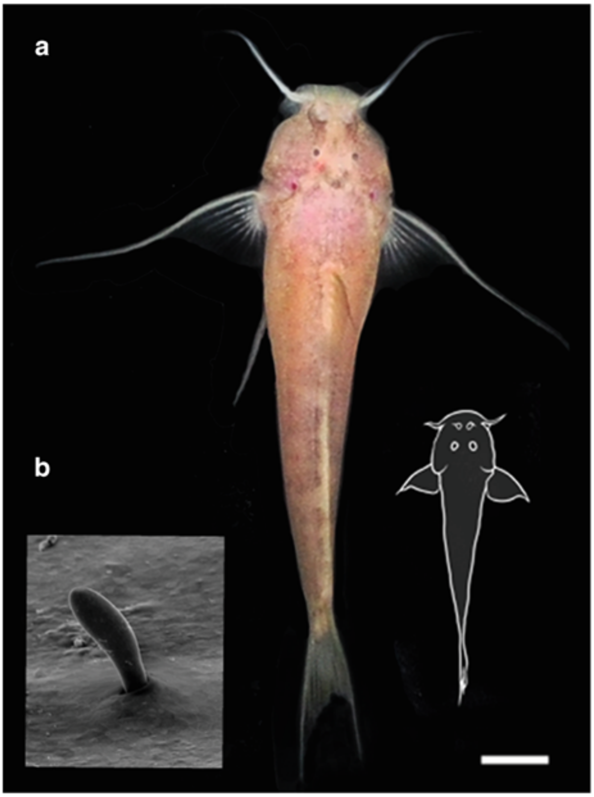


**Fig. 3** Many cavefishes exhibit hypertrophy of mechanosensory modalities. (**a**) *Astroblepus pholeter* has longer barbs and fins than those of the related surface species (the insert diagram is of *A. mancoi*; the scale bar represents 1 cm for the cavefish and 2 cm for the surface species); (**b**) Scanning electron micrograph of a single mechanosensory denticle (MSD: based on Haspel et al. 2012)

level and flow associated with flood events. Hearing sensitivity and range and acoustic communication have received little attention in cavefishes, limited to just three groups. Popper (1970) found no differences in hearing sensitivities between cave and surface forms of *Astyanax*. Both forms had thresholds comparable to other otophysan fishes with a minimum threshold at 1,000 Hz. More recently, Schulz-Mirbach et al. (2008) found pronounced morphological differences between the otoliths of the lab-reared cave and surface ecotypes of the Atlantic molly (*Poecilia mexicana*). In addition to contour differences, the saccule of the cave ecotype has a deep sulcus that is lacking in the surface ecotype. In subsequent studies, Schulz-Mirbach et al. (2010, 2011) tested for morphological differences of all three inner ear otoliths (saccule, utricle, and lagena), their respective sensory epithelia (maculae), and for differences in hearing sensitivities between cave and surface ecotypes. Differences in shape of all three otoliths were found between ecotypes. The otoliths of the lagena and utricle were heavier in the cave ecotype. However, hearing sensitivity was similar between the two ecotypes with greatest sensitivities between 200 and 300 Hz, as measured by auditory evoked potentials (AEPs). In addition, the authors found no evidence for intraspecific acoustic communication in both cave and surface ecotypes. Consequently, differences in otolith morphology between ecotypes may reflect metabolic differences but with minimal influence on hearing sensitivity or acoustic behavior.

The only other study to date reporting differences in hearing abilities between related cave and surface fishes is Niemiller et al. (2013b), who compared hearing ranges using AEPs among three amblyopsid cavefishes: surface-dwelling *Forbesichthys agassizii* and cave-dwelling *Typhlichthys subterraneus* and *Amblyopsis spelaea*. All three species exhibited similar hearing sensitivities $\leq$800 Hz, consistent with previous studies in other cavefishes (i.e., Popper 1970 and Schulz-Mirbach et al. 2010). Surprisingly, the two cave species were unable to hear above 800 Hz, whereas surface-dwelling *F. agassizii* showed a response up to 2 kHz, the maximum frequency tested. Differences in saccular hair cell densities were also noted. *Typhlichthys subterraneus* and *A. spelaea* had significantly lower hair cell densities compared to surface *F. agassizii*. The reduction in hair cell density suggests peripheral involvement in high-frequency hearing loss in the cave species. Loss of high-frequency hearing in *Typhlichthys* and *Amblyopsis* represents the first report of regressive evolution of hearing in a subterranean organism. In addition to testing hearing ability, Niemiller et al. (2013b) characterized aquatic environmental sound profiles in cave and surface habitats inhabited by each amblyopsid cavefish species and the surface *F. agassizii*. Audio recordings from native cave habitats revealed that riffles in cave streams and water droplets dripping from the ceiling create loud high-frequency background noise generally above 800 Hz. The authors hypothesized that cave amblyopsids may have lost hearing at high frequencies in response to living in loud acoustic cave environments. However, it remains to be determined what factors contribute most to high background noise levels in aquatic cave habitats and the evolutionary mechanisms (i.e., neutral loss or selection) behind auditory regression.

Although vocalizations may play an important role in agonistic and mating behaviors in many fishes, acoustic communication has largely been unexplored in cavefishes. Acoustic communication could be important in the darkness of caves, but the only study to investigate acoustic communication in cavefishes found no evidence for the production of species-specific sounds in lab-reared cave-dwelling *P. mexicana* (Schulz-Mirbach et al. 2010). However, surface mollies also did not produce sounds. The authors hypothesized that enhancement of the cephalic lateral line system may compensate for the lack of visual communication in caves, but noted that cavefish might produce sounds in their natural habitats. The possibility of acoustic communication in other cavefishes remains to be examined.

## 8.3 Chemoreception

Chemosensation plays important roles in many aspects of fish biology, including feeding, orientation, conspecific recognition, reproduction, and escape from predators. This modality may be particularly important to the detection of limited food resources and the location of potential mates during the breeding season in the complete darkness of caves. A large number of cavefishes belong to families that have prominent chemosensation, such as catfishes, but locating a chemical source at a distance is not trivial in water, since the chemical plume can often be turbulent. Solubility rather than volatility is the key chemical property for chemicals in water, olfactory organs must be adapted for chemosensation in water rather than air, and the physical properties of water make durable scent marking more difficult. Gustation is important for the location and identification of food and can be a very highly developed sense in some teleost lineages, such as cyprinids. Taste buds are the receptor organs of gustation, are bulbous in shape, and consist of receptor, supporting, and basal cells. Taste buds are primarily distributed on the epithelia of the lips, mouth, pharynx, and gill arches and around the head, but some species, such as catfishes, also have concentrations of taste buds located on well-developed barbels or even distributed all over their bodies and fins. In *Astyanax*, the gustatory system is expanded in cave morphs and is largely evident in an increase in taste bud density along the lips, pharynx, and ventral surface of the head, often with a five- to sevenfold increase in the number of taste buds (Schemmel 1967; Varatharasan et al. 2009; Yamamoto et al. 2009). In addition, taste buds contain more receptor cells, are innervated by more axon profiles, and have an accelerated rate of development relative to those in the surface morph (Boudriot and Reutter 2001; Varatharasan et al. 2009). Cave *Astyanax* uses a different feeding posture from the surface morph's to better facilitate foraging on the bottom of cave pools (Schemmel 1980; Hüppop 1987); the surface form prefers food items on the surface of the water. Constructive changes in jaw size and taste buds may be genetically linked to eye degeneration (Protas et al. 2008; Yamamoto et al. 2009).

Olfaction is accomplished through the binding of odorants to olfactory receptors in the olfactory epithelium, which is often highly folded as lamellae and arranged in rosettes located within paired olfactory sacs. Several cavefishes show increases in the number of lamellae and in the overall surface area of their olfactory epithelium relative to their surface relatives (Jeffery 2008; Niemiller and Poulson 2010). In addition, cavefishes often have larger olfactory bulbs in the brain (Jeffery 2008; Niemiller and Poulson 2010). In cave *Astyanax*, the region of the brain that processes chemoreception in the telencephalon is enlarged by 40 % relative to the surface form (Peters et al. 1993). Recently, Bibliowicz et al. (2013) showed behaviorally that there is a correlation between the absence of eyes and an increased attraction to food extract. In addition, eyeless troglomorphic *Astyanax* possessed significantly larger naris size than their eyed, nontroglomorphic counterparts. In another study of the well-documented cave population of the molly *Poecilia mexicana* (Cyprinodontiformes: Poeciliidae), cave-dwelling individuals also exhibit increased numbers of taste buds on the dorsal surface of the head (Parzefall 2001). In addition to food detection, this may be an adaptation to improve communication between males and females during pre-copulatory mating behavior (Plath et al. 2003). Most amblyopsid cavefishes show little reaction to chemical stimuli of living or dead prey that are found in their environments in a laboratory setting but will regularly react to mechanosensory stimuli (Niemiller and Poulson 2010). However, at least one species (*Typhlichthys eigenmanni*) is attracted to exudates of both conspecifics and prey (Aumiller and Noltie 2003). The cave cyprinid *Phreatichthys andruzzii* (Cypriniformes: Cyprinidae) shows a negative response to chemical cues of unfamiliar conspecifics, which may be related to potential territoriality and the existence of distinct social groups in this species (Berti and Zorn 2001). A more intense repulsion is exhibited to heterospecific cues, possibly representing an antipredator response. Studies have shown that *P. andruzzii* can distinguish different spatial regions through the perception of endogenous and exogenous odors (Paglianti et al. 2006; Ceccolini et al. 2010). The recognition of a fish's own odor or other chemical cues in the environment is likely to be adaptive in caves, because the risk of repeatedly exploring the same volume of water is minimized, which allows the fish to invest more time and energy for other functions, such as reproduction, in an energy-limited environment (Ceccolini et al. 2010).

## *8.4 Electroreception*

Although electroreception has evolved in several clades of fishes whereby some species detect faint electric fields created by other aquatic organisms (passive electroreception) or even generate their own electric fields using ampullary organs (active electroreception), none of the few cavefishes studied to date are known to employ this unique sensory modality (Weber 1995). However, a few clades with electroreception also contain subterranean cavefish species, including the orders Siluriformes (catfishes) and Gymnotiformes (South American knifefishes). Electroreception is particularly useful for navigation, orientation, and foraging in low-light conditions, such as in deep, murky water, or for species that are active at night, and could be a preadaptation to living in caves. One enticing possibility for electrolocation in a cavefish is the knife fish *Eigenmannia vicentespelaea* from Brazil. Triques (1996) based the description of *E. vicentespelaea* on two specimens collected in 1978 in São Vicente I Cave in Goias, Brazil, but no behavioral or electrophysiological experiments have been done up to date, although their ecology has been examined by Bichuette and Trajano (2006).

## *8.5 Magnetoreception*

Several species of fishes are known to orient using Earth's magnetic fields (Lohmann and Johnsen 2000; Wiltschko and Wiltschko 2005), but no studies to date have demonstrated whether cavefishes can self-orient to magnetic fields. However, the aquatic European cave salamander (*Proteus anguinus*) can detect magnetic fields (Schlegel et al. 2009), raising the possibility that some cavefishes may also be able to exploit magnetoreception to navigate within subterranean habitats.

## 9 Conclusion and Outlook

Cavefishes have evolved an integrated suite of behavioral, morphological, and physiological adaptations to cope with environmental stresses and to exploit the often-limited resources in their subterranean habitats. The close similarity of several cave adaptations among diverse fish taxa around the world indicates that cave adaptation may be a general process resulting from similar selective pressures. Most notable are the degeneration of eyes, reduction in pigmentation, enhancement of mechanosensory abilities, slower metabolism, increased longevity, and often the development of more elongate fins, a larger, more slender body form. Unfortunately, many researchers have concentrated their efforts in explaining the conspicuous regressive characters displayed by cavefishes (as well as other troglobionts) rather than developing a deeper understanding of the cave environment and how its inhabitants interact and take advantage of the limited resources. In addition, our understanding of sensory biology, and ecology of cavefishes is largely limited to just a handful of species, particularly the characid *Astyanax*, the poeciliid *Poecilia mexicana*, and amblyopsid cavefishes. *Astyanax* has been an invaluable model in our knowledge of cavefish behavior, ecology, and evolution, but several important questions remain. Have different cavefish lineages and species evolved similar if not the same adaptive strategies to nonvisual sensory specialization, or are sensory modalities in cavefishes governed by subtle variation in abiotic conditions in subterranean habitats?

Another important question is determining whether particular cave adaptations are constrained by ancestry? To address this question, comparative studies of different cavefish lineages are needed to better understand the evolution of cave adaptation. A limiting factor at the present time is that the phylogenetic relationships of many cavefish groups are unknown or have not been elucidated with confidence. An accurate phylogeny is necessary to understand the number of subterranean colonization events within a cavefish clade and to provide a phylogenetic backbone for comparative analyses. Continued advances in molecular genetics, particularly next-generation sequencing technologies, have made generating genetic data feasible for all nonmodel taxa, such as cavefishes. Several molecular phylogenies resolving relationships among cavefishes and their surface relatives have been generated in recent years, for example, for the genus *Lucifuga* of Cuba (Garcia-Machado et al. 2011), amblyopsids of North America (Niemiller et al. 2013a,c), the genus *Astyanax* of Mexico (Strecker et al. 2012), and the genus *Sinocyclocheilus* of China (Liang et al. 2011). The independent derivation of cavefishes also offers a unique opportunity to understand the contributions of stochastic and deterministic forces that shape the evolution of stygobionts. In particular, several clades are comprised of several closely related species of cavefishes that vary in degree of troglomorphy (i.e., cave adaptation), such as cyprinids of Southeast Asia (the genera *Sinocyclocheilus* and *Triplophysa* in particular). Such clades are especially well suited to study the evolutionary responses to novel environments.

## References

Aumiller SR, Noltie DB (2003) Chemoreceptive responses of the southern cavefish *Typhlichthys subterraneus* Girard, 1860 (Pisces, Amblyopsidae) to conspecifics and prey. Subterranean Biology 1:79–92

Banister KE, Bunni MK (1980) A new blind cyprinid fish from Iraq. Bull Br Mus Nat Hist (Zool) 38:151–158

Barr TC, Holsinger JR (1985) Speciation in cave faunas. Annu Rev Ecol Systemat 16:313–337

Berti R, Zorn L (2001) Locomotory responses of the cave cyprinid *Phreatichthys andruzzii* to chemical signals from conspecifics and related species: new findings. Environ Biol Fishes 62:107–114

Bibliowicz J, Alie A, Espinasa L, Yoshizawa M, Blin M, Hinaux H, Legendre L, Pere S, Reaux S (2013) Differences in chemosensory response between eyed and eyeless *Astyanax mexicanus* of the Rio Subterráneo cave. EvoDevo 4:25

Bichuette MA, Trajano E (2006) Morphology and distribution of the cave knifefish *Eigenmannia vicentespelaea* Triques, 1996 (Gymnotiformes: Sternopygidae) from central Brazil, with an expanded diagnosis and comments on subterranean evolution. Neotropical Ichthyology 4:99–105

Bilandzija H, Ma L, Parkhurst A, Jeffery WR (2013) A potential benefit of albinism in *Astyanax* cavefish: downregulation of the oca2 gene increases tyrosine and catecholamine levels as an alternative to melanin synthesis. PLoS One 8:e80823

Biswas J, Pati AK, Pradhan RK (1990) Circadian and circannual rhythms in air gulping behavior of cave fish. J Interdiscipl Cycle Res 21:257–268

Borowsky R (2010) The evolutionary genetics of cave fishes: convergence, adaptation and pleiotropy. In: Trajano E, Bichuette ME, Kapoor BG (eds) Biology of subterranean fishes. Science Publishers, Enfield, NH, pp 141–168

Borowsky R, Cohen D (2013) Genomic consequences of ecological speciation in *Astyanax* cavefish. PLoS One 8:e79903

Borowsky R, Wilkens H (2002) Mapping a cavefish genome: polygenic systems and regressive evolution. J Hered 93:19–21

Boudriot F, Reutter K (2001) Ultrastructure of the taste buds in the blind cave fish *Astyanax jordani* ("*Anoptichthys*") and the sighted river fish *Astyanax mexicanus* (Teleostei, Characidae). J Comp Neurol 434:428–444

Bradic M, Beerli P, Garcia-de Leon FJ, Esquivel-Bobadilla S, Borowsky RL (2012) Gene flow and population structure in the Mexican blind cavefish complex (*Astyanax mexicanus*). BMC Evol Biol 12:9

Cahn PH (1958) Comparative optic development in *Astyanax mexicanus* and two of its blind cave derivatives. Bull Am Mus Nat Hist 115:75–112

Caine EA (1978) Comparative ecology of epigean and hypogean crayfish (Crustacea: Cambaridae) from northwestern Florida. Am Midl Nat 99:315–329

Cavallari N, Frigato E, Vallone D, Fröhlich N, Lopez-Olmeda JF, Foà A, Berti R, Sánchez-Vázquez FJ, Bertolucci C, Foulkes NS (2011) A blind circadian clock in cavefish reveals that opsins mediate peripheral clock photoreception. PLoS Biol 9:e1001142

Ceccolini F, Paglianti A, Streitenberger C, Berti R (2010) Can chemical cues act as landmarks in the orientation of the cave fish *Phreatichthys andruzzii*? Can J Zool 88:884–888

Chakrabarty P, Prejean JA, Niemiller ML (2014) The Hoosier cavefish, a new and endangered species (Amblyopsidae, *Amblyopsis*) from the caves of southern Indiana. ZooKeys 412:41–57

Christiansen KA (1962) Proposition pour la classification des animaux cavernicoles. Speluna 2:75–78

Colli L, Pagalianti A, Berti R, Gandolfi G, Tagliavini J (2009) Molecular phylogeny of the blind cavefish *Phreatichthys andruzzii* and *Garra barreimiae* within the family Cyprinidae. Environ Biol Fishes 84:95–107

Coombs S, Janssen J, Webb J (1988) Diversity of lateral line systems: evolutionary and functional considerations. In: Atema J, Fay RR, Popper AN, Tavolga WN (eds) Sensory biology of aquatic animals. Springer, New York, NY, pp 553–593

Cordiner S, Morgan E (1991) Endogenous locomotor activity patterns in the blind Mexican cave fish *Astyanax mexicanus*. J Interdiscipl Cycle Res 22:103

Culver DC (1982) Cave life. Harvard University Press, Cambridge, MA

Culver DC (2005) Life history evolution. In: Culver DC, White WB (eds) Encyclopedia of caves. Elsevier, London, pp 346–349

Culver DC, Pipan T (2008) Superficial subterranean habitats–gateway to the subterranean realm? Cave Karst Sci 35:5–12

Culver DC, Pipan T (2009) The biology of caves and other subterranean habitats. Oxford University Press, Oxford

Culver DC, Pipan T, Gottstein S (2006) Hypotelminorheic – a unique freshwater habitat. Subterranean Biology 4:1–8

Dijkgraaf S (1962) The functioning and significance of the lateral line organs. Biol Rev 38:51–105

Duboue ER, Keene AC, Borowsky RL (2011) Evolutionary convergence on sleep loss in cavefish populations. Curr Biol 21:671–676

Eigenmann CH (1909) Cave vertebrates of America, vol 104. Carnegie Institute of Washington, Washington, DC, pp 1–241

Erckens W, Weber F (1976) Rudiments of an ability for time measurement in the cavernicolous fish *Anoptichthys jordani* Hubbs and Innes (Pisces Characidae). Experientia 32:1297–1299

Ercolini A, Berti R, Chelazzi L, Messana G (1982) Researches on the phreatobic fishes of Somalia: achievements and prospects. Monit Zool Ital Suppl 17:219–241

Espinasa L, Jeffery WR (2006) Conservation of retinal circadian rhythms during cavefish eye degeneration. Evol Dev 8:16–22

Felice V, Visconti MA, Trajano E (2008) Mechanisms of pigmentation loss in subterranean fishes. Neurotrop Ichthyol 6:657–662

Fenolio DB, Zhao Y, Niemiller ML, Stout JF (2013) In-situ observations of seven enigmatic cave loaches and one cave barbel from Guangxi, China, with notes on conservation status. Speleobiology Notes 5:19–33

Ford DC, Williams PW (2007) Karst hydrogeology and geomorphology. Wiley, West Sussex

Gannon AT, Demarco VG, Morris T, Wheatly MG, Kao Y (1999) Oxygen uptake, critical oxygen tension, and available oxygen for three species of cave crayfishes. J Crustacean Biol 19:235–243

Garcia-Machado E, Hernandez D, Garcia-Debras A, Chevalier-Monteagudo P, Metcalfe C, Bernatchez L, Casane D (2011) Molecular phylogeny and phylogeography of the Cuban cave-fishes of the genus *Lucifuga*: evidence for cryptic allopatric diversity. Mol Phlyogenet Evol 61:470–483

Gibert J, Deharveng L (2002) Subterranean ecosystems: a truncated functional biodiversity. Bioscience 52:473–481

Gross JB (2012) The complex origin of *Astyanax* cavefish. BMC Evol Biol 12:105

Gross JB, Wilkens H (2013) Albinism in phylogenetically and geographically distinct populations of *Astyanax* cavefish arises through the same loss-of-function *Oca2* allele. Heredity 111:122–130

Gross JB, Borowsky R, Tabin CJ (2009) A novel role for *MC1R* in the parallel evolution of depigmentation in independent populations of the cavefish *Astyanax mexicanus*. PLoS Genet 5: e1000326

Haspel G, Schwartz A, Streets A, Camacho DE, Soares D (2012) By the teeth of their skin, cavefish find their way. Curr Biol 22:R629–R630

Holsinger JR (1988) Troglobites: the evolution of cave-dwelling organisms. Am Sci 76:747–760

Holsinger JR (1993) Biodiversity of subterranean amphipod crustaceans: global patterns and zoogeographic implications. J Nat Hist 27:821–835

Holsinger JR (2000) Ecological derivation, colonization, and speciation. In: Wilkens H, Culver DC, Humphreys WF (eds) Ecosystems of the world, vol 30. Elsevier, Oxford, pp 399–415

Hooven TA, Yamamoto Y, Jeffery WR (2004) Blind cavefish and heat shock protein chaperones: a novel role for hsp90α in lens apoptosis. Int J Dev Biol 48:731–738

Howarth FG (1973) The cavernicolous fauna of Hawaiian lava tubes. I. Introduction. Pac Insect 15:139–151

Howarth FG (1981) Non-relictual terrestrial troglobites in the tropic Hawaiian caves. Proceedings of the 8th international congress of speleology (Bowling Green, Kentucky) 2:539–541

Howarth FG (1983) Ecology of cave arthropods. Annu Rev Ecol Syst 28:365–389

Howarth FG (1987) The evolution of non-relictual tropical troglobites. Int J Speleol 16:1–16

Huntman BM, Venarsky MP, Benstead JP, Huryn AD (2011) Effects of organic matter availability on the life history and production of a top vertebrate predator (Plethodontidae: *Gyrinophilus palleucus*) in two cave streams. Freshw Biol 56:1746–1760

Hüppop K (1986) The role of metabolism in the evolution of cave animals. Natl Speleol Soc Bull 47:136–146

Hüppop K (1987) Food finding ability in cave fish (*Astyanax fasciatus*). Int J Speleol 16:59–66

Hüppop K (2000) How do cave animals cope with the food scarcity in caves? In: Wilkens H, Culver DC, Humphreys WF (eds) Subterranean ecosystems. Elsevier, Amsterdam, Netherlands, pp 159–188

Iliffe TM (1993) Fauna troglobia acu'atica de la Peninsula de Yucatan. In: Salazar-Vallejo SI, Gonzalez NE (eds) Biodiversidad Marina y Costera de Mexico. Com. Nal. Biodiversidad Y CIQRO, Mexico, pp 673–686

Jeannel R (1926) Faune cavernicole de la France avec une etude des conditions d'existence dans le domaine souterrain. Lechevalier, Paris. 334 pp

Jeffery WR (2001) Cavefish as a model system in evolutionary developmental biology. Dev Biol 231:1–12

Jeffery WR (2008) Emerging model systems in evo-devo: cavefish and microevolution of development. Evol Dev 10:265–272

Jeffery WR (2009) Regressive evolution in *Astyanax* cavefish. Annu Rev Genet 43:25–47

Juberthie C, Decu V (1994) Structure et diversite du domaine souterrain; particularites des habitats et adaptations des especes. In: Juberthie C, Decu V (eds) Encyclopaedia Biospeologica, Tome 1. Societe Internationale de Biospeologie, Moulis, France, pp 5–22

Juberthie C, Decu V (1998) Encyclopedia Biospeologica, Tome 2. Societe Internationale de Biospeologie, Moulis, France

King RA, Willaert RK, Schmidt RM, Pietsch J, Savage S (2003) *MC1R* mutations modify the class phenotypes of oculocutaneous albinism type 2 (OCA2). Am J Hum Genet 73:638–645

Langecker TG, Longley G (1993) Morphological adaptations of the Texas blind catfishes *Trogloglanis pattersoni* and *Satan eurystomus* (Siluriformes: Ictaluridae) to their underground environment. Copeia 1993:976–986

Langecker TG, Schmale H, Wilkens H (1993) Transcription of the opsin gene in degenerate eyes of cave dwelling *Astyanax fasciatus* (Teleostei, Characidae) and its conspecific ancestor during early ontogeny. Cell Tissue Res 273:183–192

Langecker TG, Wilkens H, Parzefall J (1996) Studies on the trophic structure of an energy-rich Mexican cave (Cueva de las Sardinas) containing sulfurous water. Memoires de Biospeologie 23:121–125

Li C, Lu G, Orti G (2008) Optimal data partitioning and a test case for ray-finned fishes (Actinopterygii) based on ten nuclear loci. Syst Biol 57:519–539

Liang XF, Cao L, Zhang CG (2011) Molecular phylogeny of the *Sinocyclocheilus* (Cypriniformes: Cyprinidae) fishes in northwest part of Guangxi, China. Environ Biol Fishes 92:371–379

Lohmann KJ, Johnsen S (2000) The neurobiology of magnetoreception in vertebrate animals. Trends Neurosci 23:153–159

Longley G, Karney H (1979) Status of *Trogloglanis pattersoni* Eigenmann, the toothless blindcat and status of *Satan eurystomus* Hubbs and Bailey, the widemouth blindcat. Endangered

Species Report 5 (Part 1), Special Publication, U.S. Fish and Wildlife Service, Albuquerque. 54 pp

Mercy TVA, Padmanabhan KG, Pillai NK (1982) Morphological studies of the oocytes of the blind catfish *Horaglanis krishnai* Menon. Zool Anz 209:211–223

Mestrov M (1962) Un nouveau milieu aquatique souterrain: le biotype hypotelminorheique, vol 254. Compte Rendus Academie des Sciences, Paris, pp 2677–2679

Mohr CE, Poulson TL (1966) The life of the cave. McGraw-Hill, New York, NY

Montgomery JC, Coombs S, Baker CF (2001) The mechanosensory lateral line system of the hypogean form of *Astyanax fasciatus*. Environ Biol Fishes 62:87–96

Niemiller ML, Poulson TL (2010) Studies of the Amblyopsidae: past, present, and future. In: Trajano E, Bichuette ME, Kapoor BG (eds) The biology of subterranean fishes. Science Publishers, Enfield, NH, pp 169–280

Niemiller ML, Fitzpatrick BM, Miller BT (2008) Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene genealogies. Mol Ecol 17:2258–2275

Niemiller ML, Near TJ, Fitzpatrick BM (2012) Delimiting species using multilocus data: diagnosing cryptic diversity in the southern cavefish *Typhlichthys subterraneus* (Teleostei: Amblyopsidae). Evolution 66:846–866

Niemiller ML, Fitzpatrick BM, Shah P, Schmitz L, Near TJ (2013a) Evidence for repeated loss of selective constraint in rhodopsin of amblyopsid cavefishes (Teleostei: Amblyopsidae). Evolution 67:732–748

Niemiller ML, Higgs DM, Soares D (2013b) Evidence for hearing loss in amblyopsid cavefishes. Biol Lett 9:20130104

Niemiller ML, McCandless JR, Reynolds RG, Caddle J, Tilquist CR, Near TJ, Pearson WD, Fitzpatrick BM (2013c) Effects of climatic and geological processes during the Pleistocene on the evolutionary history of the northern cavefish, *Amblyopsis spelaea* (Teleostei: Amblyopsidae). Evolution 67:1011–1025

Nosil P (2012) Ecological speciation. Oxford University Press, Oxford

Paglianti A, Messana G, Cianfanelli A, Berti R (2006) Is the perception of their own odour effective in orienting the exploratory activity of cave fishes? Can J Zool 84:871–876

Palmer AN (2007) Cave geology. Cave Books, Dayton, OH

Parzefall J (2001) A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico. Environ Biol Fishes 62:263–275

Pati AK (2001) Temporal organization in locomotor activity of the hypogean loach, *Nemacheilus evezardi*, and its epigean ancestor. Environ Biol Fishes 62:119–129

Patton P, Windsor S, Coombs S (2010) Active wall following by Mexican blind cavefish (*Astyanax mexicanus*). J Comp Physiol A 196:853–867

Peck SB, Finston TL (1993) Galapagos Islands troglobites: the questions of tropical troglobites, parapatric distributions with the eyed-sister-species, and their origin by parapatric speciation. Memoires de Biospeologie 20:19–37

Peters N, Schacht V, Schmidt W, Wilkens H (1993) Gehirnproportionen und Ausprägungsgrad der Sinnesorgane von *Astyanax mexicanus* (Pisces, Characinidae): Ein Vergleich zwischen dem Flußfisch und seinen Höhlenderivaten «Anoptichthys». J Zool Syst Evol Res 31:144–159

Plath M, Parzelfall J, Schlupp I (2003) The role of sexual harassment in cave– and surface–dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). Behav Ecol Sociobiol 54:303–309

Plath M, Hermann B, Schroder C, Riesch R, Tobler M, Garcia de Leon FJ, Schlupp I, Tiedemann R (2010) Locally adapted fish populations maintain small-scale genetic differentiation despite perturbation by a catastrophic flood event. BMC Evol Biol 10:256

Popper AN (1970) Auditory capacities of the Mexican blind cave fish (*Astyanax jordani*) and its eyed ancestor (*Astyanax mexicanus*). Anim Behav 18:552–562

Poulson TL (1963) Cave adaptation in amblyopsid fishes. Am Midl Nat 70:257–290

Poulson TL (1992) The mammoth cave ecosystem. In: Camacho A (ed) The natural history of biospeleology. Museo Nacional de Ciencias Naturales, Madrid, pp 569–611

Poulson TL (2001) Morphological and physiological correlates of evolutionary reduction of metabolic rate among amblyopsid cave fishes. Environ Biol Fishes 62:239–249

Poulson TL, Jegla TC (1969) Circadian rhythms in cave animals. Actes of the 4th international congress of speleology, Ljubljana, Yugoslavia, 4–5:193–195

Poulson TL, White WB (1969) The cave environment. Science 165:971–981

Protas ME, Hersey C, Kochanek D, Zhou Y, Wilkens H, Jeffery WR, Zon LI, Borowsky R, Tabin CJ (2006) Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. Nat Genet 38:107–111

Protas M, Conrad M, Gross JB, Tabin C, Borowsky R (2007) Regressive evolution in the Mexican tetra, *Astyanax mexicanus*. Curr Biol 17:452–454

Protas M, Tabansky I, Conrad M, Gross JB, Vidal O, Tabin CJ, Borowsky R (2008) Multi-trait evolution in a cave fish, *Astyanax mexicanus*. Evol Dev 10:196–209

Protas M, Jeffery WR (2012) Evolution and development in cave animals: from fish to crustaceans. WIREs Dev Biol 1:823–845.

Proudlove GS (2006) Subterranean fishes of the world: an account of the subterranean (hypogean) fishes described to 2003 with a bibliography 1541–2004. International Society for Subterranean Biology, Moulis, France, p 300

Proudlove GS (2010) Biodiversity and distribution of the subterranean fishes of the world. In: Trajano E, Bichuette ME, Kapoor BG (eds) The biology of subterranean fishes. Science Publishers, Enfield, NH, pp 41–63

Riesch R, Plath M, Schlupp I (2010) Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). Ecology 91:1494–1505

Riesch R, Plath M, Schlupp I (2011) Speciation in caves: experimental evidence that permanent darkness promotes reproductive isolation. Biol Lett 7:909–912

Rohner R, Jarosz DF, Kowalko JE, Yoshizawa M, Jeffery WR, Borowsky RB, Lindquist S, Tabin CJ (2013) Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish. Science 342:1372–1375

Schemmel C (1967) Vergleichende Untersuchungen an den Hautsinnesorganen ober-and unterirdisch lebender *Astyanax*-Foramen. Zeitschrift für Morphologie der Tiere 61:255–316

Schemmel C (1980) Studies on the genetics of feeding behaviour in the cave fish *Astyanax mexicanus f. anoptichthys*: an example of apparent monfactorial inheritance by polygenes. Zeitschrift für Tierpsycholologie 53:9–22

Schlegel PA, Steinfartz S, Bulog B (2009) Non-visual sensory physiology and magnetic orientation in the blind cave salamander, *Proteus anguinus* (and some other cave-dwelling urodele species): review and new results on light-sensitivity and non-visual orientation in subterranean urodeles (Amphibia). Anim Biol 59:351–384

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, Oxford

Schulz-Mirbach T, Stransky C, Schlickeisen J, Reichenbacher B (2008) Differences in otolith morphologies between surface- and cave-dwelling populations of *Poecilia mexicana* (Teleostei, Poeciliidae) reflect adaptations to life in an extreme habitat. Evol Ecol Res 10:537–558

Schulz-Mirbach T, Ladich F, Riesch R, Plath M (2010) Otolith morphology and hearing abilities in cave and surface-dwelling ecotypes of the Atlantic molly, *Poecilia mexicana*, (Teleostei: Poeciliidae). Hear Res 267:137–148

Schulz-Mirbach T, Heß M, Plath M (2011) Inner ear morphology in the Atlantic Molly *Poecilia mexicana*—first detailed microanatomical study of the inner ear of a Cyprinodontiform species. PLoS One 6(11):e27734.

Sharma S, Coombs S, Patton P, Burt de Perera T (2009) The function of wallfollowing behaviors in the Mexican blind cavefish and a sighted relative, the Mexican tetra (*Astyanax*). J Comp Physiol A 195:225–240

Simon KS, Benfield EF (2001) Leaf and wood breakdown in cave streams. J N Am Benthol Soc 20:550–563

Soares D, Niemiller ML (2013) Sensory adaptations of fishes to subterranean environments. Bioscience 63:274–283

Soares D, Yamamoto Y, Strickler AG, Jeffery WR (2004) The lens has a specific influence on optic nerve and tectum development in the blind cavefish *Astyanax*. Dev Neurosci 26:308–317

Strecker U, Hausdorf B, Wilkens H (2012) Parallel speciation in *Astyanax* cave fish (Teleostei) in northern Mexico. Mol Phylogenet Evol 62:62–70

Strickler AG, Yamamoto Y, Jeffery WR (2007) The lens controls cell survival in the retina: evidence from the blind cavefish *Astyanax*. Dev Biol 311:512–523

Swofford DL (1982) Genetic variability, population differentiation, and biochemical relationships in the family Amblyopsidae. Master's Thesis, Eastern Kentucky University, Richmond, KY

Tobler M (2008) Divergence in trophic ecology characterizes colonization of extreme habitats. Biol J Linn Soc 95:517–528

Tobler M (2009) Does a predatory insect contribute to the divergence between cave- and surface-adapted fish populations? Biol Lett 5:506–509

Tobler M, Riesch R, Tobler CM, Schulz-Mirbach T, Plath M (2009) Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations. J Evol Biol 22:2298–2304

Trajano E (1991) Population ecology of *Pimelodella kronei*, troglobitic catfish from Southeastern Brazil (Siluriformes, Pimelodiae). Environ Biol Fishes 30:407–421

Trajano E (1997) Population ecology of *Trichomycterus itacarambiensis*, a cave catfish from eastern Brazil (Siluriformes, Trichomycteridae). Environ Biol Fishes 50:357–369

Trajano E (2001) Ecology of subterranean fishes: an overview. Environ Biol Fishes 62:133–160

Trajano E, Bockmann FA (2000) Ecology and behavior of a new cave catfish of the genus *Taunayia* from northeastern Brazil (Siluriformes, Heptapterinae). Ichthyol Expl Freshw 11:207–216

Triques ML (1996) *Eigenmannia vicentespelaea*, a new species of cave dwelling electrogenic Neotropical fish (Ostariophysi: Gymnotiformes: Sternopygidae). Revue Française de Aquariologie 23:1–4

Vandel A (1964) Biospeologie: la biologie des animaux cavernicoles. Gauthier-Villars, Paris

Varatharasan N, Crol RP, Franz-Odenall T (2009) Taste bud development and patterning in sighted and blind morphs of *Astyanax mexicanus*. Dev Dyn 238:3056–3064

Weber A (1995) The lateral line system of epigean and cave dwelling catfishes of the genus *Rhamdia* (Pimelodidae, Teleostei) in Mexico. Memoires de Biospeologie 22:215–225

Weber A (1996) Cave dwelling catfish populations of the genus *Rhamdia* (Pimelodidae, Siluroidei, Teleostei) in Mexico. Memoires de Biospeologie 23:73–85

Weber A, Wilkens H (1998) *Rhamdia macuspanensis*: a new species of troglobitic pimelodid catfish (Siluriformes: Pimelodidae) from a cave in Tabasco, Mexico. Copeia 1998:998–1004

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci U S A 95:6578–6583

Wilkens H (1988) Evolution and genetics of epigean and cave *Astyanax fasciatus* (Characidae, Pisces). Evol Biol 23:271–367

Wilkens H (2007) Regressive evolution: ontogeny and genetics of cavefish eye rudimentation. Biol J Linn Soc 92:287–296

Wilkens H, Strecker U (2003) Convergent evolution of the cavefish *Astyanax* (Charcidae, Teleostei): genetic evidence from reduced eye-size and pigmentation. Biol J Linn Soc 80:545–554

Wilkens H, Langecker TG, Olcese J (1993) Circadian rhythms of melatonin synthesis in the pineal organ of cave-dwelling *Astyanax fasciatus* (Teleostei: Characiformes). Memoires de Biospeleologie 20:279–282

Williams PW, Ford DC (2006) Global distribution of carbonate rocks. Zeitschrift fur Geomorphologie 147(Suppl):1–2

Willis LD, Brown AV (1985) Distribution and habitat requirements of the Ozark cavefish, *Amblyopsis rosae*. Am Midl Nat 114:311–317

Wiltschko W, Wiltschko R (2005) Magnetic orientation and magnetoreception in birds and other animals. J Comp Physiol A 191:675–693

Windsor SP, Tan D, Montgomery JC (2008) Swimming kinematics and hydrodynamic imaging in the blind Mexican cave fish (*Astyanax fasciatus*). J Exp Biol 211:2950–2959

Woods LP, Inger RF (1957) The cave, spring, and swamp fishes of the family Amblyopsidae of central and eastern United States. Am Midl Nat 58:232–256

Yamamoto Y, Jeffery WR (2000) Central role for the lens in cave fish eye degeneration. Science 289:631–633

Yamamoto Y, Stock DW, Jeffery WR (2004) Hedgehog signalling controls eye degeneration in blind cavefish. Nature 431:844–847

Yamamoto Y, Byerly MS, Jackson WR, Jeffery WR (2009) Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cavefish evolution. Dev Biol 330:200–211

Yoshizawa M, Goricki S, Soares D, Jeffery WR (2010) Evolution of a behavioral shift mediated by superficial neuromasts helps cavefish find food in darkness. Curr Biol 20:1631–1636

Yoshizawa M, Yamamoto Y, O-Quin KE, Jeffery WR (2012) Evolution of an adaptive behavior and its sensory receptors promotes eye regression in blind cavefish. BMC Biol 10:108

Zafar NP, Morgan E (1992) Feeding entrains an endogenous rhythm of swimming activity in the blind Mexican cave fish. J Interdiscipl Cycle Res 23:165–166

Zhao YH, Gozlan RE, Zhang CG (2011) Out of sight out of mind: current knowledge of Chinese cave fishes. J Fish Biol 79:1545–1562

# Pickled Fish Anyone?

## The Physiological Ecology of Fish from Naturally Acidic Waters

**Jay A. Nelson**

**Abstract** Naturally acidic freshwaters are found worldwide, yet the fishes of these habitats have been infrequently studied. Systems that formed during the Holocene tend to be depauperate of fishes, yet older, tropical acidic waters are among the most diverse fish habitats. Occupants of naturally acidic waters come from a diverse array of piscine taxa, but geographic location appears to be more important than taxon in driving final fish assemblage structure. Based on studies from relatively rapid exposure, environmental acid is predicted to challenge physiological regulation of many systems across multiple life-history stages of fish. The study of how fish deal with acidity in nature began almost a century ago, and flourished in the last third of the twentieth century as concerns over anthropogenic acidification of freshwaters grew, but has recently diminished, possibly because concerns over anthropogenic ocean acidification have taken priority. Relative to comparison species and conspecific populations, animals indigenous to acidic habitats have less disruption of monovalent ion balance under acid exposure, bind $Ca^{++}$ more tightly at their gills or have $Ca^{++}$-insensitive monovalent ion efflux, exhibit greater blood oxygen transport capacity, and have exercise metabolism and performance metrics that are less affected by low pH. With the exception of ion regulation, these conclusions are based on only a handful of studies; many other potentially interesting facets of fish biology have yet to be investigated. Considering the continued acidification of freshwater and marine environments by man, further study of how fishes adapted to natural acidity will certainly bear fruit.

## 1 Naturally Acidic Waters of the Planet and Their Fishes

Between 1 and 3 % of the earth's surface can be classified as naturally acidic (Clymo 1984). These systems can be found in recently deglaciated regions across the temperate globe, throughout the tropics, and in regions where geological deposits or volcanism releases acid equivalents to the ecosystem. Interestingly,

---

J.A. Nelson (✉)
Department of Biological Sciences, Towson University, Towson, MD 21252-0001, USA
e-mail: jnelson@towson.edu

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_9

the freshwater components of these systems can be depauperate of fish fauna compared to nearby circumneutral systems (Rahel and Magnuson 1983), or they can be among the most speciose freshwater habitats on the planet (Chao 2001). Naturally acidic waters are most commonly formed through a combination of a drainage basin with either weathering-resistant bedrock or little erosion potential, resulting in very ion-poor water with minimal buffering capacity into which natural organic matter (NOM) is released by biotic processes. Incomplete oxidation of both plant and animal debris generates compounds with acidic functional groups such as humic, fulvic, and tannic acids, among others. Release of these compounds into poorly buffered water will lower the pH and, because this complex mixture of compounds also absorbs light across the visible spectrum, will generally darken the waters causing them to often be referred to as "black waters." "Dystrophic" is a limnological term also often applied to these waters. The fishes from these systems tend to be poorly studied; the few studies come primarily from systems of Holocene origin in North America, Eurasia, Southeast Asia, and New Zealand as well as the much older Rio Negro Basin of South America. Among the temperate Holocene systems, the abundance of hydrogen ion appears to be restricting species diversity in the northern hemisphere (Amarasinghea and Welcomme 2002), but less so in New Zealand (Collier et al. 1990). In Southeast Asia, the black water systems are also of Holocene origin (Wüst et al. 2007), but are characterized by only slightly lower species diversity (Beamish et al. 2003). Considering the incredible species diversity of the older Rio Negro system (over 1,000 fish species, approximately three times the diversity of all of Europe) might lead one to assume that long-term natural acidity promotes speciation, but taken in the context of the overall fish species richness of the neotropics, this assumption would be premature. Certainly, other abiotic factors such as oxygen and temperature will also contribute to the ultimate assemblage found in any of these naturally acidic systems.

There can be little generalization concerning which fish taxa will form the piscine community of naturally acidic waters. In north temperate systems, the protacanthopterygian umbrids tend to be the most acid tolerant and occupy these systems, often as the only fish species present (Rahel and Magnuson 1983; Dederen et al. 1986; Fig. 1). The next most acid-tolerant fishes in north temperate systems tend to be advanced teleosts (percids and centrarchids; Rahel and Magnuson 1983; Rask and Virtanen 1986; Gonzalez and Dunson 1989b; Fig. 1). In New Zealand, the most tolerant fishes are also protacanthopterygian, but here it is galaxids found in the black waters. Among the other acid-tolerant fishes in New Zealand are a representative of a very ancient lineage, the jawless fishes (Geotriidae), a family of quite modern teleost (Eleotridae), and then a couple of representatives of the ancestral teleost family Anguillidae (Collier et al. 1990; Fig. 1). Likewise, for tropical systems, one cannot generalize as to which fish taxa will be found in acidic black waters. The occupants of the Rio Negro and its black water tributaries range from amongst the few freshwater elasmobranchs (Potamotrygonidae) through ancient teleosts (Osteoglossidae) up to very modern teleosts such as cichlids, with representatives from many intermediate stages of teleostean evolution. As for all Neotropical waters, the major ostariophysan lineages, characiforms, siluriforms,

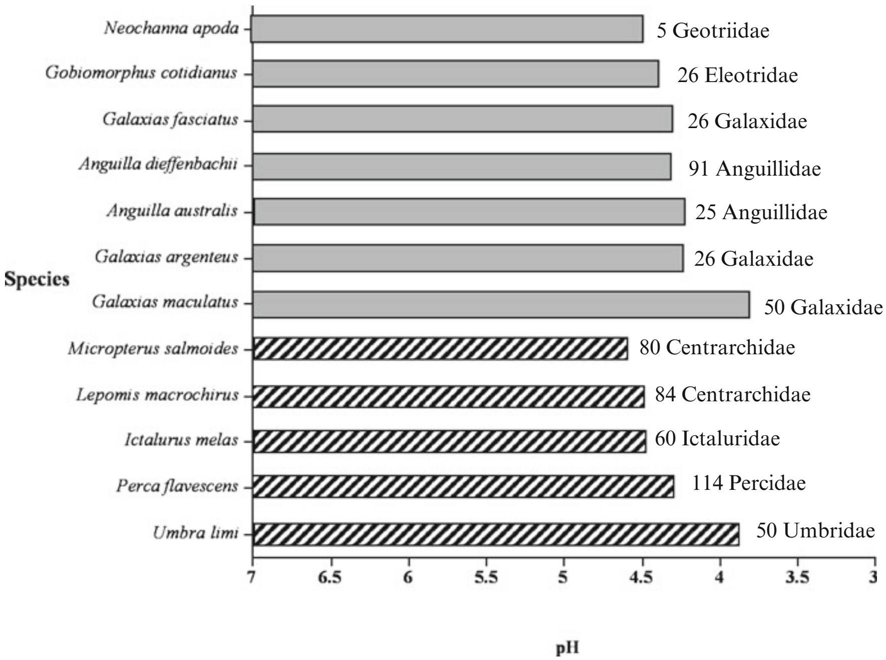


**Fig. 1** Taxonomic composition of fish assemblages from naturally acidic waters in Wisconsin (USA) and New Zealand. The five most acid-tolerant fish species from Wisconsin (*hatched bars*; Rahel and Magnuson 1983) and the seven most acid-tolerant fish from New Zealand (*solid bars*; Collier et al. 1990) plotted by their pH range of occurrence. The number to the right of each bar is the number of water bodies the range was based upon followed by the family of the fish

and gymnotiforms, are well represented amongst the 1,000+ species of the Rio Negro watershed (Goulding et al. 1988). Interestingly, a group not present in the Neotropics and generally thought to be acid-intolerant from laboratory and north temperate studies, the cyprinids, make up a significant portion of the fish assemblages in naturally acidic waters on the Malay peninsula (Beamish et al. 2003) and are found in the most acid waters of Japan (Ikuta et al. 2000). Besides determining whether an animal was present as a naturally acidic system formed, taxon does not appear to be that important in determining final fish assemblage structure. Other biotic factors such as coincident species or whether a given lineage evolved air breathing could be just as important as a species' ability to tolerate acid.

## 2 Why Excess Hydrogen Ion in the Water Is Challenging for Fish

Of all commonly encountered ions, organisms are generally least tolerant of internal changes in [$H^+$] (commonly expressed as the pH, or negative logarithm of [$H^+$]), so it follows that water-breathing organisms that must have thin epithelia in contact with the water for gas exchange would be sensitive to excess [$H^+$] (acid or low pH waters) in their environment. The reason that $H^+$ is such a problem compared with other monovalent cations is that there are no electrons shielding the center of positive charge, in this case a naked proton, from the surrounding environment. This concentrated positive charge has a powerful destabilizing effect on polar biological molecules like proteins that depend upon weak charges to maintain the higher order structure they need to function.

The discovery of cultural acidification of freshwaters in many parts of the world in the latter half of the twentieth century, coincident with faunal changes correlating with acidification, particularly the decline of many economically important fish species, led to a voluminous literature on how environmental $H^+$ influences fish biology (Reviewed by Wood and McDonald 1982; Reid 1995; Kwong et al. 2014). Most of this work progressed with model system, aquaculture, or aquarium species that had not experienced low pH waters in their recent evolutionary history (i.e., acid-naïve fish). This work has taught us much about the immediate and trans-generational effects of low pH waters on fish species and populations, but little about how fish respond to acidic waters on evolutionary timescales. Water-breathing freshwater fish are hyperosmotic to their environment so that they must constantly import ions from their food and environment to compensate for diffusive ion losses, primarily from the gills that must have a large diffusive capacity to function as a respiratory organ (see Dymowska et al. 2012 for a recent review). Acid-naïve fish exposed to large environmental concentrations of $H^+$ generally suffer from increased mortality, net losses of essential monovalent ions such as $Na^+$, $Cl^-$, $HCO_3^-$, and $K^+$, are acidotic, and occasionally asphyxiate (Wood and McDonald 1982; Wood 2001; Kwong et al. 2014; Fig. 2). Asphyxiation is thought to be from excess mucus production increasing the diffusional distance for branchial oxygen uptake and is generally seen only at extremely low pH values or when ($Al^{+3}$) is involved (Reid 1995). Chronic effects of acid exposure can include reduced growth, metabolic alterations, gill damage, reduced skeletal ossification, behavioral abnormalities, endocrine malfunction, immune system compromise, and sensory deprivation (LeDuc et al. 2013; Kwong et al. 2014; Fig. 2). Moreover, the effects of acid exposure are sensitive to a number of environmental covariates such as [$Ca^{++}$], [$Na^+$], and [$Cl^-$], total conductivity, $pO_2$, $pCO_2$, temperature, and the presence of various heavy metals (Wood and McDonald 1982). Of these, environmental [$Ca^{++}$] has been singled out as the most important environmental covariate in determining fish survival in acidic fresh waters, whereas aluminum ($Al^{+3}$) was found to be the most significant metal ion with synergistic toxicity (Wood and McDonald 1982). The most important site of toxicity in adult fish is the fish gill, where inhibition of ion-transport proteins in gill ionocytes and disruption of the integrity of intercellular junctions are frequently reported lesions (Reid 1995; Wood 2001; Kwong et al. 2014; Fig. 2). Investigations into acid precipitation and fish kills generally found that fish were extirpated from waters at pH levels substantially higher than were distressful to adult fish in the laboratory. There are many potential explanations for this finding. Two of the most important turned out to be: 1) that aluminum was commonly released into the waters of acidifying watersheds and had a complex synergistic toxicity that varied with pH level and dissolved organic molecules, and 2) that for most species, individuals were more vulnerable to reduced environmental pH during reproductive life-history stages (Sayer et al. 1993). Aluminum toxicity at low pH is a complex function of oxidation state, pH, environmental [$Ca^{++}$], and dissolved organic carbon, but since it is generally a concern for fish in anthropogenically acidified waters only (Collier et al. 1990), this synergistic toxicity with low pH will not be considered further here.

A multitude of studies employing many species have reported failure of monovalent ion homeostasis in adult fish exposed to acid water (Wood and McDonald 1982; Kwong et al. 2014). Although a general finding, there remains uncertainty as to some of the mechanisms. Increased environmental [$H^+$] is generally associated with an inhibition of both branchial $Na^+$ and $Cl^-$ uptake from the water. Furthermore, acidic water increases branchial permeability to $Na^+$, $Cl^-$, and $K^+$, causing increased losses of these ions from the fish according to their electrochemical gradients. The combination of reduced monovalent ion uptake from the

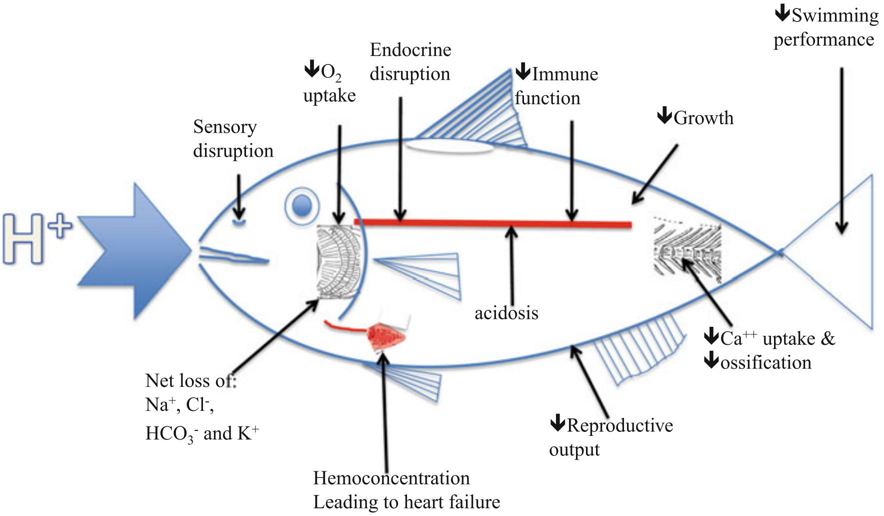


**Fig. 2** Loci of documented physiological disruption by $H^+$ in fishes. A specific physiological lesion induced by low pH is listed with an *arrow* pointing to the primary place in the fish where the lesion is manifested. See text for details

environment, coupled with exacerbated losses of these ions, makes reduced plasma monovalent ion concentrations a hallmark symptom of acid exposure, but the magnitude of the reduction and the environmental pH at which it occurs vary strongly by species (McDonald et al. 1991). Environmental uptake of $Ca^{++}$ is also limited by low pH exposure and $Ca^{++}$ efflux accelerated, which can have significant physiological effects when the animal is not obtaining dietary $Ca^{++}$ (Nelson 1982). The important role of environmental $[Ca^{++}]$ in determining $H^+$ toxicity became apparent from a number of studies showing that $H^+$ displaced $[Ca^{++}]$ from the gill muco-epithelial layer where $Ca^{++}$ has the presumptive role of stabilizing tight junctions thereby limiting the diffusive loss of both $Na^+$ and $Cl^-$ (Reid 1995; Kwong et al. 2014). This effect was generally dependent upon the environmental $[Ca^{++}]$, so that ionoregulatory failure was much more pronounced in softer water at a given pH (Wood and McDonald 1982). In very hard water, acidification can have negligible ionoregulatory effects, and instead acidosis becomes the primary lesion (McDonald et al. 1980), although increased renal excretion of acid equivalents can somewhat compensate for the acidosis (McDonald and Wood 1981). A number of investigators have examined gill morphology in fishes exposed to low pH or acclimating to low pH, but when the confounding effects of $Al^{+++}$ are eliminated, there does not appear to be consistent histological changes associated with $H^+$ exposure unless it is severe and acute (Reid 1995). Milligan and Wood (1982) present a model for how reductions in monovalent plasma ion concentrations might lead to circulatory failure and death, but neurobiological disruptions could be just as relevant when low pH exposure is in situ (e.g., LeDuc et al. 2013) and interactions with unaffected species relevant or when eggs and larval fishes fail to mature (Chulakasem et al. 1989).

Disruptions to fish reproduction at low pH have been reported for all stages of the reproductive process from gametogenesis and spawning through hatching to egg and fry survival (reviewed in Sayer et al. 1993); for most species, one of these early life-history stages is the most vulnerable to low pH and determines the lowest pH at which the species will be found in nature. The absolute lowest pH tolerated by fish is extremely variable among species and populations; just within North American fish species, there is a three order of magnitude difference in the $[H^+]$ of their distribution (Rahel and Magnuson 1983).

The global acidification of the oceans from cultural $CO_2$ production has sparked a recent uptick in research on $H^+$ effects on marine fishes (Branch et al. 2013). Because acidification is by $CO_2$, whether a given effect is from $H^+$ per se or hypercarbia cannot be teased out. Because of the high buffering capacity of marine waters, the pH changes due to cultural $CO_2$ accumulation are small; nevertheless, they have been linked to sensory, learning, and behavioral deficits (Branch et al. 2013), but nothing like the mass mortalities and fish extirpations of acidified freshwaters have been reported for marine systems. Mirroring the interest in fish from naturally acidic freshwaters that emanated from cultural acidification of freshwaters (see below), some investigators are now studying naturally hypercarbic areas of the ocean to predict organismal and ecosystem consequences of ocean acidification (e.g., Hall-Spencer et al. 2008). Despite this interest in $H^+$ effects on fishes stemming from anthropogenic acidification, surprisingly little is still known about fishes from the numerous naturally acidic systems on the planet.

## 3 Fish from Naturally Acidic Waters

Naturally acidic freshwaters account for up to 3 % of the world's freshwater ecosystems (Clymo 1984). Considering this abundance of acidic habitats, there is a surprising dearth of information on hydrogen ions as a selective agent in nature. The presence of fish species endemic to ancient acidic environments (Goulding et al. 1988) and the presence of species and populations in recently acidified waters that are more acid tolerant than conspecifics from more neutral waters (Rahel and Magnuson 1983; Turnpenny et al. 1987) implicate high environmental $[H^+]$ as an agent of acclimatization and natural selection in fish. The fauna of waters acidified in the late Holocene tend to be depauperate of fishes (Rahel and Magnuson 1983; Turnpenny et al. 1987), whereas more established naturally acidic systems (e.g., Rio Negro, Brazil) can have endemic black water species and are among the most diverse fish faunas on earth (Goulding et al. 1988). This seems to indicate that selection for life in soft, acidic waters could lead to speciation, but there is, as of yet, no direct evidence for this. To this author's knowledge, there has not been a comprehensive comparison of species diversity between Neotropical black water systems and closely matched circumneutral ones. Holocene-aged naturally acidic waters of Southeast Asia show reduced species diversity compared to nearby less acidic waters (Beamish et al. 2003).

## 4 History of Research on Fish from Naturally Acidic Waters

Minna Jewell initiated the scientific investigation of fish adapting to naturally acidic waters (Jewell 1922; Jewell and Brown; 1924; Brown and Jewell 1926). She first suggested that $H^+$ could be important in structuring aquatic communities; this idea has been confirmed in recent years. Along with Brown (Brown and Jewell 1926), she carried out the first test of the hypothesis that fish from naturally acidic lakes might have adapted or acclimatized to their environment. They conducted a series of reciprocal transfers between fish from a naturally acidic lake and a nearby circumneutral lake. Because several species from circumneutral waters survived 40 days of enclosure in the naturally acidic lake in equal numbers to those enclosed in the circumneutral lake, the authors concluded that direct effects of $H^+$ on adult fish did not limit fish use of the naturally acidic habitats of northern Michigan (USA). Their experiments were conducted mid-summer when pH levels are highest in these systems (primarily due to photosynthetic $CO_2$ removal from the water and lack of precipitation flushing organic acids from adjacent peat lands), and we now know that eggs and larvae are generally the most vulnerable life-history stages to the effects of low environmental pH (Chulakasem et al. 1989; Sayer et al. 1993). Had Brown and Jewell (1926) worked on larval or juvenile stages in the spring, they likely would have uncovered signs of adaptation or acclimation in these initial experiments. Interestingly, Brown and Jewell (1926) also conducted habitat choice experiments that showed animals from acidic lakes to actually prefer lower pH water. Jewell went on to show with Jobes (Jobes and Jewell 1927) that the alkali reserve (plasma pH with cells and all components of the $CO_2$ equilibrium removed) in brown bullhead (*Ictalurus nebulosus*) was not different between fish from naturally acidic and circumneutral lakes. Meanwhile, other groups were investigating the respiratory characteristics of animals from the Amazon basin, including those from acidic black water habitats. For example, Willmer (1934) found an inverse correlation between the magnitude of the Bohr shift (i.e., a decrease in hemoglobin's binding affinity for oxygen as a function of increasing $pCO_2$ or $[H^+]$) of the fishes' hemoglobin and their perceived environmental $CO_2/H^+$ exposure. This was an entirely logical finding, because a large $CO_2$ Bohr effect in high $[CO_2]$ water could potentiate $O_2$ loading at the gill, but this appears to be the first documented evidence for blood chemistry differences in fish based upon their history of acid exposure.

The great depression and the Second World War seem to have deflected the early tropical fish physiologists (e.g., Keys, Willmer, Carter, and others) from expanding their studies, which surely would have uncovered more interesting findings in fish from tropical black waters. Wiebe et al. (1934), studying the interaction between low pH and lethal oxygen concentration for several temperate freshwater species, made several interesting findings. Their laboratory findings of interspecific differences in how the lethal oxygen concentration for a fish changed as environmental pH changed would fairly accurately predict later published field distributions of fish as a function of pH (Rahel and Magnuson 1983). Wiebe et al. (1934) also clearly showed that the mechanism of fish death at low pH could differ based upon oxygen levels: asphyxiation at low $[O_2]$ and other causes (see above) when $O_2$ was plentiful.

Interest in fish from naturally acidic waters was renewed in the latter half of the twentieth century with the identification of anthropogenic acidification as the culprit in declining fisheries in many parts of the world. Dunson and coworkers pioneered the approach of using animals from naturally acidic waters to gain insight into the effects of acid water on fish. In a paper largely focusing on the decreased blood pH and body $Na^+$ loss of acid-naïve brook trout (*sic*) (*Salvelinus fontinalis*) exposed to low pH, Packer and Dunson (1970) also showed that brook trout from acidic streams had normal total body $[Na^+]$ when exposed to the same low pH. This lab quickly went on to show that laboratory strains of brook trout did not survive in naturally acidic waters where other brook trout strains were resident (Dunson and Martin 1973), and to demonstrate substantial interstrain differences in acid tolerance among brook trout strains from Pennsylvania, USA (Robinson et al. 1976). Dunson's lab also branched out to study fishes from naturally acidic tropical waters at this time, showing substantial tolerance to pHs <4.0 in several characiforms native to the Neotropical black water Rio Negro. The acid resident fishes still lost substantial body $Na^+$ at these ultra-low pHs, but less than species native to circumneutral waters (Dunson et al. 1977).

As scientific interest in cultural acidification grew, a number of labs started attempting to produce acid-tolerant strains of fish either through long-term acclimation protocols or selective breeding for acid tolerance. These attempts produced mixed but mostly negative results. For example, Swarts et al. (1978) artificially selected brook trout for acid tolerance, but did not produce an F1 generation that was any more tolerant than the parents. Daye (1980), Lloyd and Jordan (1964), and Trojnar (1977) also recorded early attempts at producing acid-tolerant fishes that were not successful. However, Gjedrem (1980) was able to show heritable genetic variation in acid tolerance of brown trout (*Salmo trutta*), and by acclimating brown trout to pH 6.0 for 6 weeks, McWilliams (1980) was able to reduce $Na^+$ loss in brown trout at pH 4.0 from the usual 1 % of the total body pool per hour to 0.6 %. It is difficult to ascertain whether there was physiological compensation involved in this response, since the animals had lost 25 % of their body $Na^+$ during acclimation, thereby reducing the animal–environment gradient. McWilliams (1982) later went on to demonstrate that both the acid-tolerant European perch (*Perca fluviatilis*) and acid-acclimated brown trout had lower rates of sodium loss than acid-naïve salmonids. McWilliams (1983) was able to provide a partial mechanistic explanation for this result by showing that $Ca^{++}$ (an important determinant of membrane permeability to monovalent cations) was lost from the surface of acid-acclimated brown trout gills slower in acid water than it was from acid-naïve trout gills. The acid-acclimated brown trout also had significantly reduced plasma levels of both $Na^+$ and $Cl^-$, which would again reduce the animal-environment gradient. In contrast, Swarts et al. (1978) showed no acclimation response to low pH in brook trout; animals exposed to sub-lethal low pH even tended to be less tolerant of lethal pH exposure than conspecifics held in more alkaline waters. Gjedrem and Rosseland (2012) summarize the current state of knowledge concerning genetic adaptation to anthropogenic acidification. While studies have detected considerable variation and significant heritability in the acid tolerance of salmonids, there is still no consistent finding of fish from culturally acidified rivers being more tolerant of low pH exposure (Schom 1985; Staurnes et al. 1995; Rosseland et al. 2001). Gjedrem and Rosseland (2012) conclude that the rate of cultural acidification occurred too fast in the late twentieth century for evolution by natural selection to produce more tolerant species or strains (see also chapter "Evolutionary Toxicology: Population Adaptation in Response to Anthropogenic Pollution" for a general review of population adaptation in response to anthropogenic pollution). Considering that most of these fish populations were also under considerable fishing pressure and exposed to other environmental pollutants (e.g., aluminum), future evolution by natural selection to an anthropogenically acidified world should not be ruled out.

Concern over the impacts of anthropocentric acidification in the late 1970s fueled growth in the number of labs initiating studies into the biology of species and populations from naturally acidic systems in temperate regions, generally concentrating on electrolyte handling. Magnuson's group at Wisconsin-Madison (USA) found that yellow perch (*Perca flavescens*) from an acid lake was more tolerant of acute depressions of environmental pH and had lower body [$Na^+$] than conspecifics from circumneutral waters (Rahel 1983). The perch from circumneutral waters survived acid challenge better after 3 weeks of acclimation to milder acidity, yet the large, significant gap in acid tolerance between these populations and those from naturally acidic waters remained (Rahel 1983). Similarly, Canfield et al. (1985) found reduced plasma osmolarity and [$Na^+$] in largemouth bass (*Micropterus salmoides*) populations from naturally acidic lakes in Florida (USA), and McWilliams (1982) reported lower plasma [$Na^+$] and [$Cl^-$] in brown trout native to ~pH 5.5 water. Rask and Virtanen (1986) also reported lower plasma electrolytes in European perch from acid lakes but normal total body osmolarity. However, Vinogradov and Komov (1985) and Lyons (1982) did not find any differences in plasma [$Na^+$] or plasma [$Na^+$] loss rates upon acid exposure in European and yellow perch, respectively, from naturally acidic environments. Nelson and Mitchell (1992) sampled the same populations as Lyons (1982) and Rahel (1983) and using more sophisticated techniques did find significantly lower plasma [$Cl^-$] but not [$Na^+$] in yellow perch from a naturally acidic lake when sampled at a pH corresponding to their natural environment. The general finding of lower plasma electrolyte levels in fish from naturally acidic waters paralleled the acclimation results of McWilliams (1980), but uncertainty remained as to whether this is a physiological compensation or just a consequence of being in acid water. Several authors (e.g., Rask and Virtanen 1986; Gonzalez and Dunson 1987) reported lower electrolyte loss rates when fish from naturally acidic waters were exposed to even lower pH levels, which hints at a physiological response. Aquatic osmoregulators that move between different salinity habitats tend to osmoconform to some degree by carrying a lower plasma [ion] in freshwater than in brackish or saltwater (Nelson et al. 1996). Fish from dilute, often acidic freshwaters are known to have even lower salt levels in their blood than comparison fish from "typical" fresh waters (Mangnum et al. 1978; McWilliams 1982; Gonzalez and Dunson 1987, 1989a; Hōbe 1987). The ability to tolerate low plasma ion levels in dilute, acidic water is saving energy that would otherwise be used for osmoregulation and would thus appear to be a regulatory response.

The emphasis on curbing air pollution as a way to combat anthropogenic acidification seemed to diminish interest and/or research funding for fish from naturally acidic waters in the late 1980s. Thus, except for several ambitious expeditions to the black waters of the Rio Negro to study primarily fish ionoregulation and follow-up studies by several of the participants, only sporadic information about fish from naturally acidic waters has appeared since then.

## 5 Characteristics of Fish from Naturally Acidic Waters

Relative to comparison species and conspecific populations, animals indigenous to acidic habitats have been reported to (1) be more tolerant of low pH levels (Dunson et al. 1977; Rahel 1983; Dederen et al. 1986; Wilson et al. 1999; Gonzalez et al. 1998); (2) have less disruption of monovalent ion balance during acid exposure (McWilliams 1982; Gonzalez and Dunson 1987, 1989a; Wilson et al. 1999; Gonzalez et al. 1998, 2002); (3) exhibit greater blood oxygen transport capacity (Rask and Virtanen 1986; Nelson et al. 1988; Wood et al. 1998); (4) have exercise metabolism and performance that are less affected by low pH (Holeton and Stevens 1978; Nelson 1989, 1990); and (5) bind $Ca^{++}$ more tightly at their gills (McWilliams 1983; Gonzalez and Dunson 1989b; Gonzalez et al. 1998) or have $Ca^{++}$ insensitive monovalent ion efflux (Gonzalez et al. 1998). Effective acclimatization or adaptation eventually leading to ecological speciation in naturally acidic waters probably involves many more physiological characters than these few that have been found to vary between animals native to acidic habitats and comparison animals.

## 6 Increased $H^+$ Tolerance of Fish from Naturally Acidic Waters

Populations of fish from several species isolated from naturally acidic waters have been shown to survive acute low pH challenges better than conspecifics from more neutral waters: Brook charr (*Salvelinus fontinalis*; Dunson and Martin 1973), Yellow perch (*Perca flavescens*; Rahel 1983), and European perch (*Perca fluviatilis*; Rask 1984). Gonzalez and Dunson (1989a) also found a centrarchid that occupies naturally acidic waters of North America (the banded sunfish, *Enneacanthus obesus*) to be more acid tolerant and to grow better at low pH than congeners from more neutral pH range waters. Other claims of greater acid tolerance for animals from naturally acidic waters were generally made solely on the basis of low pH tolerance or by comparison with other unrelated species.

## 7 Ion Regulation in Fish from Naturally Acidic Waters

Most laboratories studying fish from naturally acidic waters have focused on their ionoregulatory capabilities, almost exclusively at the whole animal or gill level. This is presumably because ionoregulatory failure is the most cited mechanism of mortality in acid-naïve fish exposed to low pH and because naturally acidic waters tend to be ion poor. Far more is known about the $Na^+$, and to a lesser degree $Cl^-$, gill epithelial transport kinetics of fish from naturally acidic waters than any other physiological factor. This research has shown that relative maintenance of monovalent ion balance at low pH tends to be a characteristic of fish from naturally acidic waters, but that this is accomplished through multiple means, leaving many fish from naturally acidic waters with monovalent ion flux characteristics indistinguishable from those of less tolerant species. This lack of convergence in mechanisms of monovalent ion regulation suggests that it is probably not a primary locus of adaptation to naturally acidic waters. As early as the late 1980s, studies on fish from naturally acidic waters were not making a strong case for $Na^+$ flux kinetics to be under strong selection. Comparisons of an acid-tolerant species (yellow perch, Percidae) with two taxonomically disparate and acid-intolerant species (rainbow trout, Salmonidae, and common shiner, Cyprinidae) suggested that a high-affinity $Na^+$ uptake mechanism that was weakly affected by low pH might be an important requirement for acid tolerance (Freda and McDonald 1988). Yet, comparisons of an even more acid-tolerant centrarchid (*E. obesus*) with less tolerant sunfishes (Gonzalez and Dunson 1987, 1989a) showed that *E. obesus* had a low affinity $Na^+$ uptake mechanism that was substantially inhibited at pH levels where the species is found in nature. Sodium efflux fared better, as both acid-tolerant yellow perch and banded sunfish had relatively low rates of $Na^+$ efflux that were less sensitive to environmental pH and [$Ca^{++}$] than comparison animals (Freda and McDonald 1988; Gonzalez and Dunson 1987, 1989a, b). Wilson et al. (1999) compared the physiological response of a Neotropical callichthyid that does not occur in black water with those of two Neotropical characiforms, one a transient black water resident and the other a facultative black water resident, while undergoing a gradual reduction of their environmental water pH to 3.5. They found mortality and the magnitude of ion losses to correlate with each species' tendency to occupy black water and concluded that successful inhabitation of acid waters involves being able to avoid ionic disturbance. In these experiments, the "black water resident" characiform (tambaquí, *Colossoma macropomum*) suffered a net loss of both $Na^+$ and $Cl^-$ at a relatively modest pH level of 5.0 and suffered significant declines in plasma concentration of both of these ions at a pH of 3.0, slightly lower than the lowest natural pH they are likely to see in the Amazon region (3.5) (Wood et al. 1998; Wilson et al. 1999). The "black water-resident" tambaquí for these experiments was obtained from aquaculture operations but was reported to have been occasionally exposed to acidic water (Wood et al. 1998). The source of the fish may prove important as three other characiforms captured in the Rio Negro and measured in Rio Negro water had much lower rates of monovalent ion loss at low pH (Gonzalez et al. 1998), which suggests that other organic substances in the "*cocktail du jour*" that are acidic black waters may be influencing monovalent ion balance. Matsuo and Val (2007) also found that humic compounds ameliorated low pH-induced $Na^+$ and $Ca^{++}$ losses in a black water resident characiform, and Glover et al. (2012) found a significantly greater $Na^+$ uptake in a galaxid population from acidic, humic water when compared with conspecifics from neutral water, when both were measured in humic water. Interestingly, Gonzalez et al. (1998) found that ion fluxes in Rio Negro fishes were also insensitive to environmental [$Ca^{++}$] in the range of 10–100 μM. Gonzalez and colleagues were able to obtain more sophisticated flux measurements on some of the most acid-tolerant Neotropical species from black waters by obtaining them from aquarium dealers in North America (e.g., Gonzalez and Preest 1999; Gonzalez and Wilson 2001). Some of these fish exhibited branchial permeabilities on par with acid-intolerant species, whereas others had very unremarkable $Na^+$ uptake kinetics. Some of these species did exhibit monovalent transport kinetics fairly independent of pH while others again demonstrated flux characteristics that were insensitive to environmental $[Ca^{++}]$. Wood et al. (2002) captured rays of the genus *Potamotrygon* from the Rio Negro and used them to make the only known ion regulation measurements on freshwater elasmobranchs. The authors not only reported substantial monovalent ion losses at pH 4.0 that were somewhat ameliorated when the fluxes were measured in natural black water but also confirmed the conclusion from teleost fishes that extreme monovalent ion regulatory characteristics are not requisite for successful exploitation of naturally acidic waters. Because most of these comparative studies of ion regulation between fishes were made across great taxonomic divides without regard for phylogeny, they offer little information about how the ionoregulatory characteristics changed as species started to adapt to, or evolve in, naturally acidic waters. The vast differences in $Na^+$ influx parameters reported in Table 9.4 by Gonzalez et al. (2005) for fishes captured from the acidic Rio Negro, as well as the vast differences in branchial permeabilities reported across studies of acid-tolerant fishes, would suggest that monovalent ion balance is not limiting the exploitation of acidic habitats. Matsuo and Val (2002) also support this conclusion with an experimental design more suited to inferring evolution of monovalent ion regulation being part of the adaptive suite required to occupy naturally acidic waters. They compared monovalent cation fluxes of two *Corydoras* (Callichthyidae) species, only one of which occupies acidic black water habitats in the Amazon. In this study, the native black water species suffered a greater loss of $Na^+$ at low pH than did the neutral water species, although the acidophilic species did demonstrate $[Ca^{++}]$ insensitive $Na^+$ flux and less $K^+$ loss at low pH than the comparison species (Matsuo and Val 2002). These interspecific comparative studies all suggest that despite monovalent ion regulation being a primary lesion when acid-naïve fish encounter low pH waters, it does not appear to be a determining factor in the successful long-term colonization of acidic waters.

In contrast, several of the intraspecific comparative studies (e.g., McWilliams 1982; Rahel 1983; Rask and Virtanen 1986) and comparisons of an acid-tolerant North American centrarchid with one or two other centrarchids (Gonzalez and Dunson 1987, 1989a) have suggested that reducing net monovalent ion loss may be an important part of acclimatizing or adapting to naturally acidic temperate freshwaters. Kaneko et al. (1999) compared the response of a Japanese cyprinid (*Tribolodon hakonensis*) from a naturally acidic lake with hatchery-raised conspecifics (controls) when both were rapidly changed from neutral to pH 3.5 water. The control fish suffered a 35 % loss of plasma $[Na^+]$ and 33 % mortality within 24 h, whereas the acid-adapted fish lost only 12 % of their plasma $[Na^+]$ and had no mortality over 48 h. Glover et al. (2012) also showed minor improvements to $Na^+$ uptake kinetics in a galaxid population captured from naturally acidic waters.

A few studies using intraspecific comparisons of fish from naturally acidic waters conflict with these results. Lyons (1982) and Vinogradov and Komov (1985) showed no differences in monovalent ion loss between fish from naturally acidic waters and conspecifics from neutral waters upon acid exposure. Furthermore, Nelson and Mitchell (1992) compared plasma ion levels of yellow perch populations from a naturally acidic lake with conspecifics from neutral lakes after 16 h of swimming in acid water and found no difference between them. These latter results support the conclusion from the interspecific comparative studies (reviewed in Gonzalez et al. 2005) that adjustments to monovalent ion regulatory parameters are not a key part of acclimating or adapting to naturally acidic waters.

One problem with drawing inference from these comparative ionic balance studies is that the potential contribution of dietary ions is generally not included, a factor that may be key in nature. Even well-fed laboratory fishes, living in unchallenging ionic waters, extract much of their monovalent ion content from the diet (Bucking and Wood 2006). The role of dietary monovalent ions in preserving ion balance in dilute, naturally acidic waters is unstudied, but certainly merits attention. Wood et al. (2002) inferred as much in their study of *Potamotrygon* sp. from the Rio Negro, because their measured $Na^+$ and $Cl^-$ efflux rates for this species far outstripped rates of uptake even under mildly acidic conditions. Nelson (1988) abandoned environmental $Ca^{++}$ flux studies with perch from naturally acidic lakes when he discovered that the $[Ca^{++}]$ of their gut contents exceeded that of comparison conspecifics from circumneutral lakes, and developing oocytes had the same $[Ca^{++}]$ between lake types. Whether $Ca^{++}$ obtained from the diet or liberated from bone can substitute for environmental $Ca^{++}$ in stabilizing gill tight junctions has not been studied, but may account for the reported calcium independence of monovalent ion permeabilities in some of the reported species and experiments.

## 8 Blood Oxygen Transport

Wiebe et al. (1934) showed that the lethal oxygen levels for a number of freshwater fish species increased exponentially as environmental pH was lowered. Because of the potential for low environmental pH to lower the pH in the erythrocytes of the lamellar capillaries and shift the hemoglobin–oxygen affinity curve toward deoxyhemoglobin (Bohr and Root shifts) reducing blood oxygen uptake at the gills, many investigators have looked for this effect or compensatory responses in blood oxygen transport variables in acidified water (reviewed in Wood and McDonald 1982). Unfortunately, this large body of work has been inconclusive; while rapid exposure to extremely low pH has reproducibly been shown to asphyxiate fishes, possibly through the induction of excess mucus production, the evidence for disruptions of oxygen transport at milder, but still challenging, pH levels is equivocal (Wood and McDonald 1982). Similarly, changes to blood oxygen transport variables such as blood [hemoglobin], hematocrit, and erythrocyte number in fish exposed to acid water are quite variable across studies (reviewed in Wood and McDonald 1982). Many of the changes reported could not be separated from cell swelling expected from plasma ion losses or adrenergic activation of erythrocytes (Milligan and Wood 1982). If oxygen loading is being compromised in the gills of fish from low pH waters, we would expect to see some compensation for this in fish spending thousands to millions of generations in naturally acidic systems. Indeed, Rask and Virtanen (1986) found that European perch from a naturally acidic lake had a higher hematocrit than conspecifics from a circumneutral lake; even more interesting was that the acidic lake perch increased their hematocrit in response to reductions in pH, without changes in osmolality, whereas the neutral lake perch did not (Rask and Virtanen 1986). Nelson et al. (1988) found blood sampled from yellow perch immediately after capture from a naturally acidic lake to have significantly higher [hemoglobin], hematocrit, and mean cell [hemoglobin] than blood from conspecifics in neutral lakes. Since hemoglobin is also the principal blood buffer in fishes, these results do not necessarily accrue from difficulties with oxygen transport. In contrast, McWilliams (1982) found no increase in [hemoglobin] but greater hematocrit and red blood cell number in brown trout from a hatchery with acidic water when compared with conspecifics from a neutral water hatchery, but these were concomitant with significantly lower plasma [ion] in the acid hatchery fish. Reports on blood chemistry of acid-tolerant fishes without comparison conspecific animals range from extremely high hematocrits in an air breathing mudminnow (*Umbra pygmaea*; Dederen et al. 1986) to extremely low hematocrits and [hemoglobin] in the tambaquí (Wood et al. 1998). In both studies, blood oxygen variables were relatively unaffected by reductions in pH, although the tambaquí registered increases in erythrocyte [adenylate] which would facilitate hemoglobin unloading of oxygen in the tissues and likely signals the lack of any impairment of branchial oxygen loading at low pH.

## 9 Blood Acid–Base Status

There is virtually no information on blood acid–base regulation of fish from naturally acidic waters. Nelson and Mitchell (1992) found that yellow perch from a naturally acidic lake carried a higher plasma pH, [$HCO_3^-$], and base excess than comparison conspecifics from two circumneutral lakes in neutral water. Base excess is an indicator of the metabolic acid or base added to the blood and is an approximation of the "Δ strong ion difference" (Stewart 1981; Nelson and Mitchell 1992). The "strong ion difference" theory posits that pH and [$HCO_3^-$] are secondary variables in a solution that respond to changes in the concentrations of primary mineral ion variables (Stewart 1981). Upon exposure to acid water while swimming at 3 cm $s^{-1}$, the acid-lake perch showed increases in both [$HCO_3^-$] and base excess. In contrast, the two acid-naïve populations registered decreases in plasma [$HCO_3^-$] and base excess while plasma pH was constant across all animals and treatments. The higher average hemoglobin concentration in yellow perch from a naturally acidic lake (Nelson et al. 1988) could account for some of the increase in plasma [$HCO_3^-$] found by Nelson and Mitchell (1992), but higher bicarbonate and base excess after 16 h of mild exercise and acid exposure more likely accrued from differences in acid equivalent production, storage, excretion, or gill permeability. Muscle buffering capacity is the same across these yellow perch populations (Nelson and Magnuson 1987), so differential storage in the large white muscle mass is not involved; however, differential storage of $H^+$ equivalents in bone is possible. The higher [$HCO_3^-$] and base excess in perch from naturally acidic lakes could also be a consequence of lower plasma chloride levels. The constraint of electrical neutrality dictates that changes of any plasma anion will result in equimolar changes of other anionic species in the opposite direction, or cationic species in the same direction. Thus, by one view, the lower $Cl^-$ levels "generated" the additional $HCO_3^-$ by increasing the "strong ion difference" (Stewart 1981). Ikuta et al. (2000) report on populations of a cyprinid (*Tribolodon hakonensis*) that occupy acidic lakes in Japan and have enlarged gill ionocytes with a high V-ATPase activity, suggestive of enhanced $H^+$ excretory capacity. A population of this same species had a much less severe plasma acidosis than conspecifics from neutral waters when both were exposed to pH 3.5 (Kaneko et al. 1999). In addition, Wood et al. (1998) showed minimal changes in blood pH and [$HCO_3^-$] in the facultative black water resident tambaquí upon exposure to gradual pH reductions. Certainly, these few studies are indicative of successful acid/base regulation in fish from naturally acidic waters exposed to acid water but are insufficient to form any conclusions as to what, if any, modifications of this regulatory system were necessary to occupy these habitats. Understanding whether changes in the acid/base regulatory system are integral to acclimatization, adaptation, or even speciation in acidic habitats will require more comprehensive evolutionary physiological studies of acid/base regulation under environmentally realistic conditions such as swimming in acid waters.

## 10 Swimming Performance

Although naturally acidic freshwaters present several regulatory challenges to aquatic water-breathing vertebrates, Darwinian fitness in these environments will be determined by more than the ability to regulate ions or maintain acid/base balance. Little is known about how fish populations or species indigenous to acidic water compensate for the additional challenges inherent from essential life activities such as swimming or reproduction.

Adopting the terminology of Fry (1971), swimming activity in fish is controlled by factors such as temperature (Beamish 1978) and morphology (Webb 1975), but is limited by factors such as oxygen, carbon dioxide, pollutants, and disease (Fry 1971; Beamish 1978). Presumably, hydrogen ions are also a limiting factor for most fish, but this has not been adequately tested. The results from acid-naïve fishes generally show reduced endurance swimming ability, but not until the pH levels are fairly extreme. For example, Waiwood and Beamish (1978) found no effect on critical swimming speed ($U_{crit}$) when the pH was reduced to 6.0 in rainbow trout, but West and Garside (1986) found a significant depression of $U_{crit}$ at pH 5.0–6.0. Graham and Wood (1981) found a linear decrease in $U_{crit}$ as pH was progressively depressed below pH 6.0 in the same species. Fish from naturally acidic waters have been shown to have their swimming performance less diminished by acid water, but this result is context dependent: Nelson (1989) reanalyzed the data reported by Holeton and Stevens (1978) to show that *Triportheus angulatus* (Characiformes) acclimated to white water and swimming in black water had reduced critical swimming speeds, whereas fish acclimated to black water and swimming in black water had $U_{crit}$ values similar to controls. This was similar to the result Nelson (1989) obtained for a perch population isolated for 2,000–3,000 generations in a naturally acidic lake in northern Wisconsin (USA). These animals did not have higher absolute swimming speeds in acid water, but their swimming performance was less affected by soft, acid water than it was in conspecifics from circumneutral waters. Swimming in dilute, acid water greatly increased the range and coefficient of variation of critical swimming speeds, meaning that isolated individuals were capable of performing at the same level in acid water, yet many other individuals had their swimming ability impaired by acid water (Nelson 1989).

## 11 Metabolism

There is very little information about metabolic divergence along environmental hydrogen ion gradients at the population or species levels in fish. Nelson and Magnuson (1992) compared the metabolic storage patterns of yellow perch from one naturally acidic lake with two conspecific comparison populations across a single calendar year. They found no metabolic evidence of environmental acid influence. Acid-stressed fish were predicted to have higher liver [glycogen] and lower muscle [glycogen] (Dheer et al. 1987), yet the perch from the acid lake had significantly higher muscle [glycogen] and significantly lower liver [glycogen] than the perch populations from circumneutral waters (Nelson and Magnuson 1992). Furthermore, Nelson and Magnuson (1992) found the acid-lake perch to have normal condition factors and significantly higher visceral and hepatic lipid levels as well. Overall, these results were consistent with preferential use of metabolic pathways that would produce less endogenous $H^+$, but were also consistent with a shifting life-history strategy not concordant with density-driven changes alone (see below).

Nelson (1990) also examined metabolic profiles of yellow perch populations, one from a naturally acidic lake, after exercise in acidic or neutral water. Acid-lake perch generally had significantly less muscle metabolic disturbance after exercise to behavioral exhaustion when compared with conspecifics from neutral lakes swimming in neutral water. This phenomenon was demonstrable via muscle concentrations of lactate, adenosine tri-phosphate, creatine phosphate, adenosine mono-phosphate, and inosine mono-phosphate and suggested that fish that pushed adenylate depletion and glycolysis to extremes had been selected against in this population. The pH of the water had no effect on muscle metabolite accumulation during exercise or recovery in acid-lake perch. In contrast, acid-naïve perch had dramatic differences in muscle metabolite concentrations when swimming to behavioral exhaustion in different pH waters. This disruption of normal muscle metabolism was only evident at behavioral exhaustion; metabolite levels were similar during recovery from exercise, regardless of environmental pH. The acid-naïve fish were given 16 h to acclimate to the low pH water before the tests, so whatever physiological adjustments allowed the acid-lake perch to not suffer the same disruption in muscle metabolism as the acid-naïve perch were not rapidly inducible (Nelson 1990). The author also inferred from metabolite concentrations the apparent relative activities of metabolic pathways that are either proton producing or consuming. When there were noticeable differences between the acid-lake and circumneutral lake perch populations, the acid-lake perch demonstrated greater proton economy for four out of five metabolic pathways. While these results suggest that high concentrations of environmental $H^+$ may drive changes in fish metabolism, there is clearly room for more research in this area.

## 12 General Biology

Considering the prevalence of naturally acidic habitats on earth (Clymo 1984), other than their ionoregulatory characteristics, we know surprisingly little about the fishes that occupy them. Since early life-history stages are generally the most vulnerable to acidic water (Sayer et al. 1993), much of the acclimatization to low pH is likely occurring during development, and adaptive changes are likely to affect early developmental stages as well. Nelson (1988) observed massive egg mortality in yellow perch from a naturally acidic lake in a year when nearby populations from circumneutral lakes were hatching successfully. This would imply that mortality selection for acid tolerance is ongoing in yellow perch in this Holocene acidic ecosystem. How successful reproduction is in the many tropical fishes endemic to acidic waters is unknown to this author. While studies of fish reproduction in low-ionic-strength low-pH waters (e.g., Chulakasem et al. 1989) point to severe reproductive challenges for tropical black water fishes, these fishes seem to thrive, and water chemistry-induced selection on reproductive characters may be driving life-history changes. Nelson and Magnuson (1992) found greater reproductive investment in female perch from naturally acidic lakes; this result coupled with lower growth rates, but normal condition factors and greater visceral lipid stores suggested a shift toward earlier and greater reproductive investment in fish from acid waters. Some fish may even be using their level of acid tolerance as a way to take refuge from less acid-tolerant predaceous fishes (Olsson et al. 2006).

## 13 Conclusions and Outlook

The overwhelming conclusion from this review is that fish from naturally acidic systems merit far more study. Today, we cannot even generalize as to whether acidic habitats promote or restrict piscine diversity nor do we understand what the biological characteristics are that allow some species to thrive in acidic habitats. Considering the fact that we find jawless lampreys and ancestral teleosts among the fauna of naturally acidic systems and that the earth has experienced prior massive acidification events (e.g., Permian-Triassic boundary, 250 MYA; Ogden and Sleep 2012) within the evolutionary history of today's fishes, we would expect the ability to occupy acidic habitats to be pleisiomorphic. This may explain why we find so many diverse fish taxa in extant acidic ecosystems, but the question then becomes, why have so many fishes lost the ability to thrive in acid waters? Minna Jewell (1922) postulated almost a century ago that because of the physiological challenges posed by high environmental [$H^+$], species and populations occupying naturally acidic waters were likely to be special in some way. Today, interest in animals from acidic ocean seeps is surging as scientists seek to predict organismal and ecosystem consequences of human-generated ocean acidification, yet we still know little about how fishes deal with the more extreme acidity they encounter in naturally acidic freshwaters. Understanding how fish acclimatize to, adapt, or even speciate in acidic habitats will not only enrich our knowledge of freshwater fish evolution but may also give us a window into understanding how fish will respond to further anthropogenic acidification of freshwaters and oceans.

## References

Amarasinghea US, Welcomme RA (2002) An analysis of fish species richness in natural lakes. Environ Biol Fish 65:327–339

Beamish FWH (1978) Swimming capacity. In: Hoar WS, Randall DJ (eds) Fish physiology, vol 7. Academic Press, New York, NY, pp 101–187

Beamish FWH, Beamish RB, Lim SLH (2003) Fish assemblages and habitat in a Malaysian blackwater peat swamp. Environ Biol Fish 68:1–13

Branch TA, DeJoseph BM, Ray LJ, Wagner CA (2013) Impacts of ocean acidification on marine seafood. Trends Ecol Evol 28(3):178–186

Brown HW, Jewell ME (1926) Further studies on the fishes of an acid lake. Trans Am Microsc Soc 45:20–34

Bucking C, Wood CM (2006) Gastrointestinal processing of $Na^+$, $Cl^-$, and $K^+$ during digestion: implications for homeostatic balance in freshwater rainbow trout. Am J Physiol Regul Integr Comp Physiol 291:R1764–R1772

Canfield DE, Maceina MJ, Nordlie FG, Shireman JV (1985) Plasma osmotic and electrolyte concentrations of largemouth bass from some acidic Florida lakes. Trans Am Fish Soc 114:423–429

Chao NL (2001) The fishery, diversity, and conservation of ornamental fishes in the Rio Negro Basin, Brazil: a review of Project Piaba (1989–99). In: Chao NL, Petry P, Prang G, Sonneschien L, Tlusty M (eds) Conservation and management of ornamental fish resources of the Rio Negro Basin, Amazonia, Brazil – Project Piaba. Ed. Universidade do Amazonas, Manaus, pp 161–204

Chulakasem W, Nelson JA, Magnuson JJ (1989) Separation of low pH and low ion effects on mortality during development of medaka *Oryzias latipes*. Can J Zool 67:2158–2168

Clymo RS (1984) *Sphagnum* dominated peat bog: a naturally acidic ecosystem. Phil Trans R Soc Lond B 305:229–240

Collier KJ, Ball OJ, Graesser AK, Main MR, Winterbourn MJ (1990) Do organic and anthropogenic acidity have similar effects on aquatic fauna? Oikos 59:33–38

Daye PG (1980) Attempts to acclimate embryos and alevins of Atlantic salmon *Salmo salar*, and rainbow trout *Salmo gairdneri* to low pH. Can J Fish Aquat Sci 37:1035–1038

Dederen LHT, Levven RSEW, Wendelaar Bonga SE, Oyen FGF (1986) Biology of the acid-tolerant fish species *Umbra oygmaea*. J Fish Biol 28:307–326

Dheer JMS, Dheer TR, Mahajan CL (1987) Haematological and haematopoetic response to acid stress in an air-breathing freshwater fish, *Channa punctatus* (Bloch). J Fish Biol 30:577–588

Dunson WA, Martin R (1973) Survival of brook trout in a bog derived acidity gradient. Ecology 54:1370–1376

Dunson WA, Swarts F, Silversti M (1977) Exceptional tolerance to low pH of some tropical black water fish. J Exp Zool 201:157–162

Dymowska AK, Hwang PP, Goss GG (2012) Structure and function of ionocytes in the freshwater fish gill. Respir Physiol Neurobiol 184:282–292

Freda J, McDonald DG (1988) Physiological correlates of interspecific variation in acid tolerance in fish. J Exp Biol 136:243–258

Fry FEJ (1971) The effects of environmental factors on the physiology of fish. In: Hoar WS, Randall DJ (eds) Fish physiology, vol 6. Academic Press, New York, NY, pp 1–98

Gjedrem T (1980) Genetic variation in acid tolerance in brown trout. In: Drablos D, Tollan A (eds) Ecological impact of acid precipitation. SNSF project As-NLH, Norway, p 308

Gjedrem T, Rosseland BO (2012) Genetic variation for tolerance to acidic water in salmonids. J Fish Biol 80:1–14

Glover CN, Donovan KA, Hill JV (2012) Is the habitation of acidic-water sanctuaries by galaxiid fish facilitated by natural organic matter modification of sodium metabolism? Physiol Biochem Zool 85(5):460–469

Gonzalez RJ, Dunson WA (1987) Adaptations of sodium balance to low pH in a sunfish (*Enneacanthus obesus*) from naturally acidic waters. J Comp Physiol B 157:555–566

Gonzalez RJ, Dunson WA (1989a) Acclimation of sodium regulation to low pH and the role of calcium in the acid-tolerant sunfish *Enneacanthus obesus*. Physiol Zool 62:977–992

Gonzalez RJ, Dunson WA (1989b) Differences in low pH tolerance among closely related sunfish of the genus *Enneacanthus*. Environ Biol Fish 26:303–310

Gonzalez RJ, Preest M (1999) Mechanisms for exceptional tolerance of ion-poor, acidic waters in the neon tetra (*Paracheirodon innesi*). Physiol Biochem Zool 72:156–163

Gonzalez RJ, Wilson RW (2001) Patterns of ion regulation in acidophilic fish native to the ion-poor, acidic Rio Negro. J Fish Biol 58:1680–1690

Gonzalez RJ, Wood CM, Wilson RW, Patrick M, Bergman H, Narahara A, Val AL (1998) Effects of water pH and $Ca^{++}$ concentration on ion balance in fish of the Rio Negro, Amazon. Physiol Zool 71:15–22

Gonzalez RJ, Wood CM, Wilson RW, Patrick ML, Val AL (2002) Diverse strategies of ion regulation in fish collected from the Rio Negro. Physiol Biochem Zool 75:37–47

Gonzalez RJ, Wilson RW, Wood CM (2005) Ionoregulation in tropical fishes from ion-poor, acidic blackwaters. In: Randall DJ, Val AL, Almeida-Val VMF (eds) Physiology of tropical fishes – fish physiology, vol 21. Academic Press, Jordan Hill, pp 397–442

Goulding M, Carvalho ML, Ferreira EJG (1988) Rio Negro: rich life in poor water – Amazonian diversity and foodchain ecology as seen through fish communities. SPB Academic Publishing, The Hague

Graham MS, Wood CM (1981) Toxicity of environmental acid to the rainbow trout: interactions of water hardness, acid type, and exercise. Can J Zool 59:1518–1526

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia M (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96–99

Hōbe H (1987) Sulphate entry into soft-water fish (*Salmo gairdneri*, *Catostomus commersoni*) during low ambient pH exposure. J Exp Biol 133:87–109

Holeton GF, Stevens ED (1978) Swimming energetics of an Amazonian characin in ‘black’ and ‘white’ water. Can J Zool 56:983–987

Ikuta K, Yada T, Kitamura S, Ito F, Yamaguchi M, Nishimura T, Kaneko T, Nagae M, Ishimatsu A, Iwata M (2000) Recent studies on the effects of acidification on fish in Japan. Glob Environ Res 4:79–87

Jewell ME (1922) The fauna of an acid stream. Ecology 3:22–28

Jewell ME, Brown HW (1924) The fishes of an acid lake. Trans Am Microsc Soc 43:77–84

Jobes FW, Jewell ME (1927) Studies on the alkali reserve of the blood of *Ameriuras nebulosus* from acid and basic waters. Trans Am Microsc Soc 46:175–186

Kaneko T, Hasegawa S, Uchida K, Ogasawara T, Oyagi A, Hirano T (1999) Acid tolerance of Japanese dace (a cyprinid teleost) in Lake Osorezan, a remarkable acid lake. Zool Sci 16:871–877

Kwong RWM, Kumai Y, Perry SF (2014) The physiology of fish at low pH: the zebrafish as a model system. J Exp Biol 217:651–662

Leduc AOHC, Munday PL, Brown GE, Ferrari MCO (2013) Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. Phil Trans R Soc B 368 (1627):1471–1493

Lloyd R, Jordan DHM (1964) Some factors affecting the resistance of rainbow trout (*Salmo gairdneri*) Richardson to acid waters. Int J Air Water Pollut 8:393–403

Lyons J (1982) Effects of lethal acidity on plasma sodium concentrations in yellow perch (*Perca flavescens*) from a naturally acidic and a naturally alkaline lake. Comp Biochem Physiol 73A (3):437–440

Mangnum CP, Haswell MS, Johansen K, Towle DW (1978) Inorganic ions and pH in the body fluids of Amazon animals. Can J Zool 56:907–916

Matsuo AYO, Val AL (2002) Low pH and calcium effects on net $Na^+$ and $K^+$ fluxes in two catfish species from the Amazon River (*Corydoras*: Callichthyidae). Braz J Med Biol Res 35(3):361–367

Matsuo AYO, Val AL (2007) Acclimation to humic substances prevents whole body sodium loss and stimulates branchial calcium uptake capacity in cardinal tetras *Paracheirodon axelrodi* (Schultz) subjected to extremely low pH. J Fish Biol 70:989–1000

McDonald DG, Wood CM (1981) Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. J Exp Biol 93:101–118

McDonald DG, Hōbe H, Wood CM (1980) The influence of calcium on the physiological responses of the rainbow trout *Salmo gairdneri* to low environmental pH. J Exp Biol 88:109–131

McDonald DG, Freda J, Cavdek V, Gonzalez RJ, Zia S (1991) Interspecific differences in gill morphology of freshwater fish in relation to tolerance of low-pH environments. Physiol Zool 64:124–144

McWilliams PG (1980) Acclimation to an acid medium in the brown trout *Salmo trutta*. J Exp Biol 88:269–280

McWilliams PG (1982) A comparison of physiological characteristics in normal and acid exposed populations of the brown trout *Salmo trutta*. Comp Biochem Physiol A 72(3):515–522

McWilliams PG (1983) An investigation of the loss of bound calcium from the gills of the brown trout *Salmo trutta* in acid media. Comp Biochem Physiol A 74:107–116

Milligan CL, Wood CM (1982) Disturbances in hematology, fluid volume distribution, and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. J Exp Biol 99:397–415

Nelson JA (1982) Physiological observations on developing rainbow trout *Salmo gairdneri* (Richardson) exposed to low pH and varied calcium ion concentrations. J Fish Biol 20:352–372

Nelson JA (1988) Physiological compensation to the environment in a population of perch from a naturally acidic lake. PhD Thesis, University of Wisconsin-Madison, 302 pp

Nelson JA (1989) Critical swimming speeds of yellow perch *Perca flavescens*: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. J Exp Biol 145:239–254

Nelson JA (1990) Muscle metabolite response to exercise and recovery in yellow perch: comparison of populations from naturally acidic and neutral waters. Physiol Zool 63:886–908

Nelson JA, Magnuson JJ (1987) Seasonal, reproductive, and nutritional influences on the white-muscle buffering capacity of yellow perch (*Perca flavescens*). Fish Physiol Biochem 3:7–16

Nelson JA, Magnuson JJ (1992) Metabolic stores of yellow perch (*Perca flavescens*): comparison of a population from an acidic, dystrophic lake with one from circumneutral waters. Can J Fish Aquat Sci 49:2474–2482

Nelson JA, Mitchell GS (1992) Blood chemistry response to acid exposure in yellow perch *Perca flavescens*: comparison of populations from naturally acidic and neutral environments. Physiol Zool 65:493–514

Nelson JA, Magnuson JJ, Chulakasem W (1988) Blood oxygen capacity differences in yellow perch (*Perca flavescens*) from northern Wisconsin lakes differing in pH. Can J Fish Aquat Sci 45:1699–1704

Nelson JA, Tang Y, Boutilier RG (1996) The effects of salinity change on the exercise performance of two Atlantic cod (*Gadus morhua*) populations inhabiting different environments. J Exp Biol 199:1295–1309

Ogden DE, Sleep H (2012) Explosive eruption of coal and basalt and the end-Permian mass extinction. Proc Natl Acad Sci U S A 109:59–62

Olsson K, Stenrotha P, Nyström P, Holmqvist N, McIntosh AR, Winterbourn MJ (2006) Does natural acidity mediate interactions between introduced brown trout, native fish, crayfish and other invertebrates in West Coast New Zealand streams? Biol Cons 130:255–267

Packer RK, Dunson WA (1970) Effects of low environmental pH on blood pH and sodium balance of brook trout. J Exp Zool 174:65–72

Rahel FJ (1983) Population differences in acid tolerance between yellow perch (*Perca flavescens*) from naturally acidic and alkaline lakes. Can J Zool 61(1):147–152

Rahel FJ, Magnuson JJ (1983) Low pH and the absence of fish species in naturally acidic Wisconsin lakes: inferences for cultural acidification. Can J Fish Aquat Sci 40(1):3–9

Rask M (1984) The effect of low pH on perch, *Perca fluviatilis* L. II. The effect of acid stress on different development stages of perch. Ann Zool Fennici 21:9–13

Rask M, Virtanen E (1986) Responses of perch *Perca fluviatilis* from an acidic and a neutral lake to acid water. Water Air Soil Pollut 30:537–543

Reid SD (1995) Adaptation to and effects of acid water on the fish gill. In: Hochachka PW, Mommsen TP (eds) Biochemistry and molecular biology of fishes, vol 5, Environmental and ecological biochemistry. Elsevier, Amsterdam, pp 213–227

Robinson GD, Dunson WA, Wright JE, Mamolito GE (1976) Differences in low pH tolerance among strains of brook trout (*Salvelinus fontinalis*). J Fish Biol 8:5–17

Rosseland BO, Kroglund F, Staurnes M, Hindar K, Kvellestad A (2001) Tolerance to acid water among strains and life stages of Atlantic salmon (*Salmo salar* L.). Water Air Soil Pollut 130:899–904

Sayer MDJ, Reader JP, Dalziel TRK (1993) Freshwater acidification: effects on the early life stages of fish. Rev Fish Biol Fisher 3:95–132

Schom CB (1985) Genetic control of resistance to low pH in Atlantic salmon at the family and stock level. In: Wells PG, Addison RF (eds) Proceedings of the tenth annual aquatic toxicity workshop. Can Tech Rep Fisher Aquat Sci. pp 135–148

Staurnes M, Kroglund F, Rosseland BO (1995) Water quality requirement of Atlantic salmon (*Salmo salar*) in water undergoing acidification or liming in Norway. Water Air Soil Pollut 85:347–352

Stewart PA (1981) How to understand acid-base: a quantitative acid-base primer for biology and medicine. Elsevier, New York, NY, 186 pp

Swarts FA, Dunson WA, Wright JE (1978) Genetic and environmental factors involved in increased resistance of Brook trout to sulfuric acid solutions and mine acid polluted waters. Trans Am Fish Soc 107:651–677

Trojnar JR (1977) Egg hatchability and tolerance of brook trout (*Salvelinus fontinalis*) fry at low pH. J Fish Res Board Can 34:574–579

Turnpenny AWH, Sadler K, Aston RJ, Milner AGP, Lynam S (1987) The fish populations of some streams in Wales and northern England in relation to acidity and associated factors. J Fish Biol 31:415–434

Vinogradov GA, Komov VT (1985) Ion regulation in the perch, *Perca fluviatilis*, in connection with the problem of acidification of water bodies. Voprosy Ikhtiologi 1:137–144

Waiwood KG, Beamish FWH (1978) Effects of copper, pH, and hardness on the critical swimming speed of rainbow trout (*Salmo gairdneri*). Water Res 12:611–619

Webb PW (1975) Hydrodynamics and energetics of fish propulsion. Bull Fish Res Bd Can. 190 pp

West TG, Garside ET (1986) Effects of total aluminum on swimming performance of rainbow trout (*Salmo gairdneri*) in acidified fresh water. Int Union Physiol Sci Banff Satel Symp Abstr. p 60

Wiebe AH, McGayock AM, Fuller AC, Markus HC (1934) The abi1ity of fresh-water fish to extract oxygen at different hydrogen ion concentrations. Physiol Zool 7:435–448

Willmer EW (1934) Some observations on the respiration of certain tropical fresh-water fish. J Exp Biol 11:283–306

Wilson RW, Wood CM, Gonzalez R, Patrick ML, Bergman HL, Narahara A, Val AL (1999) Ion and acid–base balance in three species of Amazonian fish during gradual acidification of extremely soft water. Physiol Biochem Zool 72:277–285

Wood CM (2001) Toxic responses of the gill. In: Schlenk D, Benson WH (eds) Target organ toxicity in marine and freshwater teleosts, vol 1, Organs. Taylor & Francis, Boca Raton, FL, pp 1–89

Wood CM, McDonald DG (1982) Physiological mechanisms of acid toxicity to fish. In: Haines T, Johnson R (eds) Acid rain/fisheries. American Fisheries Society, Bethesda, MD, pp 197–225

Wood CM, Wilson RW, Gonzalez RJ, Patrick ML, Bergman HL, Narahara A, Val AL (1998) Responses of an Amazonian teleost, the tambaquí (*Colossoma macropomum*) to low pH in extremely soft water. Physiol Zool 71:658–670

Wood CM, Matsuo AYO, Gonzalez RJ, Wilson RW, Patrick ML, Val AL (2002) Mechanisms of ion transport in *Potamotrygon*, a stenohaline freshwater elasmobranch native to the ion-poor blackwaters of the Rio Negro. J Exp Biol 205:3039–3054

Wüst R, Rieley J, Page S, van der Kaars S, Wei-Ming M, Jacobsen G, Smith A (2007) Peatland evolution in Southeast Asia during the last 35,000 cal years: implications for evaluating their carbon storage potential. Proceedings of the International Symposium and Workshop on Tropical Peatland, Yogyakarta, 27–29 August, pp 25–40

# Temporary Environments

**Matej Polačik and Jason E. Podrabsky**

**Abstract** Temporary aquatic habitats are common in arid and semiarid ecosystems across the globe. They are an especially harsh and challenging environment for aquatic organisms, and this has resulted in the evolution of only a few successful lineages of fishes that can permanently inhabit and exploit these resources. The most successful group of fishes are the annual killifishes found in Africa and South America that survive the drying of their aquatic habitat as embryos. A few osmeriform fishes and lungfishes experience similar conditions, but instead survive through estivation of the adult form. In this chapter, we review the life history, ecology, and evolutionary biology of these unique and successful fishes that have managed to survive in habitats unavailable to other fishes.

## 1 Introduction

By definition, fish are aquatic animals, and therefore, one can hardly imagine their survival without water. Evolution has shaped nearly every aspect of a teleost fish's biology for life in water. For example, when immersed in water, fish are elegant and efficient swimmers, and their gills are an incredibly efficient organ for gas exchange, nitrogen excretion, and osmoregulation. However, on land most fish are clumsy and inefficient at locomotion, and their gills are almost useless for any purpose. There are, however, many fishes that can tolerate limited water availability or the complete drying of their environment for relatively long periods of time. Temporary aquatic environments are common across almost all terrestrial ecosystems with seasonally changing patterns of precipitation and/or evaporation. These habitats include floodplain pools that may be semi-permanent and closely associated with permanent waters, dry riverbeds that periodically become torrential

---

M. Polačik (✉)
Institute of Vertebrate Biology, v.v.i., Academy of Sciences of the Czech Republic, Květná 8,
60365 Brno, Czech Republic
e-mail: polacik@ivb.cz

J.E. Podrabsky
Department of Biology, Portland State University, P.O. Box 751, Portland, OR 97207, USA
e-mail: jpod@pdx.edu

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_10

rivers, or small rainwater pools that may be completely isolated from permanent waters and may exist for only a few weeks out of the year. In fact, some species of fish have evolved to persist in and even require the periodic drying of their seasonal habitat. The most successful of these fish can be found in three main groups: the annual killifish, lungfishes, and osmeriform fishes of Australia and New Zealand. In this chapter, we briefly survey the types of temporary aquatic habitats inhabited by fish and then focus primarily on fishes specifically adapted to survive and persist in isolated temporary pools.

## 2 Temporary Rivers

Temporary rivers and streams are common in arid and semiarid environments, such as those found across southern Africa, and the Arabian Peninsula. In southern Africa these rivers are fed by rainwater falling in mountains many miles away. Flooding of these rivers connects long distances of riverbed with permanent waters both up and downstream. Many fishes are known to move into ephemeral rivers during floods, and some regularly use the temporary environments as breeding areas or refuge environments for their offspring. In the seasonal Motloutse River in Botswana, a sand tributary of the Limpopo River, five species of fish were collected in temporary pools left in the riverbed following several seasonal floods (van der Waal 1997). These species (*Barbus trimaculatus*, *B. paludinosus*, *Labeo rosae*, *Clarias gariepinus*, and *Oreochromis mossambicus*) are all species known to exploit marginal habitats such as floodplain pools of permanent rivers (van der Waal 1997). From the sampling of several temporary pools in the Mzingwane River, Zimbabwe (another dry tributary of the Limpopo), 28 species of fish common to the Limpopo River were reported representing 13 genera (Minshull 2008). Use of these habitats can be of great benefit to the fish and their offspring due to decreased competition for food and habitat resources that these temporary habitats provide. However, these gains are accompanied by a significant risk of stranding. The fish that end up in these rivers as they dry are not adapted for long-term survival without water and the ephemeral river quickly becomes a death trap, if they are not able to follow the receding floods back to permanent waters. In many cases the fish eventually perish due to dehydration or predation (Minshull 2008). However, a few species of fish have been reported to survive in ephemeral rivers with sandy bottoms by burrowing several meters below the surface to follow the receding water table (Donnelly 1978; Bruton 1979; van der Waal 1997; Minshull 2008). It is unclear how long these fish can survive, and the possibility of true estivation or dormancy in these fishes remains questionable (Bruton 1979). The ecological significance of stranded fish buried in the sand has received very little attention in the mainstream scientific literature, and certainly deserves greater attention considering the rapid rates of desertification and flow reduction in rivers across the arid regions of the world (Descroix et al. 2009; Vicuna et al. 2013; Wedderburn et al. 2014).

## 3 Temporary Pools

In contrast to typical fish environments, such as larger lakes and rivers, temporary pools are normally much more space limited. Quite often they are no more than just puddles emerging after extensive seasonal rainfall. Temporary pools are typically associated with river floodplains, where fine mud deposits in terrain depressions form a layer with low permeability to water (Watters 2009). Groundwater does not typically contribute to their filling, and thus patterns of precipitation and subsequent evaporation determine the presence of water. There is never any permanent and only rarely a short-term connection to larger bodies of water (rivers or lakes). These habitats are therefore highly isolated and adjacent pools merge together only following exceptionally large flood events (Bartáková et al. 2013). Dormant stages of some annual killifish species require a specific pond substrate—dark, clay-rich, vertisol-related soils on alluvial deposits, clearly distinguishable for otherwise more common red-brown colored oxisols (Watters 2009). The presence of this fine, swelling and cracking clay makes the bottom of the pools hard and compact when dry. The characteristics of the substrate lead to alkaline conditions in typical African temporary pools (Watters 2009), whereas South American pools can be alkaline in some areas and acidic in others due to the presence of humic compounds (Podrabsky et al. 1998; Errea and Danulat 2001).

Temporary habitats suitable for long-term persistence of fish share two common geographical traits. First, there is a predictable pattern to their filling with water and desiccation. Seasonal cycles have allowed the evolution of effective ecological adaptations to cope with this type of extreme environment. The rainfall pattern may be unimodal, showing a single peak in annual precipitation, or it may have several precipitation maxima [e.g., “long” and “short” rains in Tanzania, Kenya, and Somalia (Kabanda and Jury 1999; Watters 2009; Cook and Vizy 2013)]. Second, the climate must be mild enough to prevent the water from substantial freezing if the habitats occur in colder areas. In contrast to rivers and larger lakes, small temporary pools provide limited temperature stability and protection against environmental temperature fluctuations. Therefore, fish that have evolved to exist exclusively in temporary pools are geographically mainly restricted to tropical and subtropical regions with distinct wet and dry seasons such as those in Africa and South America. Sometimes the dry and wet phase of a temporary pool is determined not by distinct patterns of precipitation, but rather by seasonal differences in temperature and evaporation rate. This appears to be the case for temporary pools occurring in milder climates in South America (Uruguay, southern Brazil, and northern Argentina) where rainfall patterns are not highly seasonal (Errea and Danulat 2001). In some of these areas, winter temperatures limit evaporation, and the amount of rainfall is sufficient to maintain the small pools, which may sometimes even freeze. In contrast, evaporation rates are substantial during summer, quickly drying the pools.

## 4 Fish That Exploit Temporary Aquatic Habitats

Annual killifish, lungfish, and a few osmeriform fishes are the only fish that seem well adapted to life in temporary waters. However, there is a relatively large and taxonomically diverse group of teleost fishes that sometimes occur in temporary habitats, although they can only survive the dry phases by retreat to permanent waters. The species that facultatively occupy temporary ecosystems typically have one or more of the following traits: (1) specific adaptations such as the ability to breathe air or burrow, (2) high physiological tolerance, or (3) a tendency to live in marginal areas of permanent waters from where they colonize temporary pools during floods.

Catfish are the most common group of fish that are partially adapted to life in temporary pools. Catfish from the Asian genus *Heteropneustes* (Heteropneustidae) have a pair of tubular cavities ("lungs") extending from the gill chambers posteriorly. They enable respiration in a moist aerial environment (Saha et al. 2001). Catfish of the genus *Clarias* (Clariidae) native to Africa and Asia are pioneering fish during seasonal floods (Merron 1989) that sometimes co-occur with annual killifish and lungfish in temporary habitats (Reichard et al. 2009). They have a modified branchial apparatus enabling direct gas exchange with atmospheric air (Singh and Hughes 1971). *Clarias* spp. can survive several weeks in hot, moist mud, although they cannot dig a firm burrow and persist in dry mud like lungfish. Further, their pectoral fins, reinforced by a bony spine, contribute to a wiggling, snake-like form of locomotion that allows them to migrate terrestrially from a desiccating habitat to a wet one or hide from bird predation. Last but not least, *Clarias* spp. have a unique behavioral adaptation. They can prolong the duration of their temporary pool by deepening it further and thus delaying its complete desiccation (van der Waal 1998). Both *Clarias* spp. and *Heteropneustes* spp. can modify their metabolism and excrete urea instead of ammonium to detoxify nitrogenous waste when exposed to dry conditions (Saha et al. 2001).

Labyrinth fish (Anabantoidei) occur in Africa and Asia. They are characterized by an accessory breathing organ, the arborescent organ, a tree-like projection of the first gill arch that extends into the upper portions of the gill chamber. This structure is highly folded and covered with a vascularized layer of skin to maximize the area for gas exchange with the air (Hughes and Munshi 1973; Tay et al. 2006). Species of the genus *Anabas* can persist in moist mud, and they are famous for their mass migrations on dry land during which they may cover several hundred meters. They use spiny gill covers to obtain purchase for forward movement, which is attained by tail twitching (Davenport and Abdul Matin 1990).

Cichlids are typical inhabitants of large water bodies such as rivers and lakes (e.g., Joyce et al. 2011). This is true also for some hardy African cichlids of the tribe Tilapini, with no particular adaptations for survival in temporary pools. However, their general hardiness makes some species frequent occupants of temporary habitats (Reichard et al. 2009; Barson and Nhiwatiwa 2010; White et al. 2012). Some tilapiine cichlids are known for their extreme tolerance to high temperatures (up to 37 °C, Chung 1985). They are also extremely successful omnivores, consuming detritus, plant, and animal matter (e.g., Carrasco et al. 2012). Temporary pools that are occasionally connected to permanent waters by floods represent a suitable nursery habitat, and juvenile tilapiines migrate there (Siziba et al. 2011). Some species are even reported to survive substantial periods in the moist substrate after the water dries out (Minshull 2008), which certainly contributes to their frequent occurrence in temporary habitats.

A common characteristic for an otherwise taxonomically diverse group of small facultative temporary pool fish is their preference for marginal/edge parts of permanent waters. They lack specific adaptations for temporary environments, but their small size makes them prone to be carried away by floods, and they become stranded in temporary pools when floods recede. These fishes are typically characterized by an opportunistic life history strategy that can be beneficial in unpredictable environments (Winemiller and Rose 1992). They mature early and continuously produce small batches of offspring (e.g., livebearers of the family Poeciliidae) and thus populations are able to rebound quickly from local disturbances and rapidly recolonize open habitats (Escalera-Vazquez and Zambrano 2010).

Non-annual killifish are close relatives of annual killifish. They occur in South America (former genus *Rivulus* Poey 1860*,* currently undergoing substantial revision, e.g., Costa 2001), Africa (genera *Aplocheilichthys*, *Aphyosemion* sensu lato, *Epiplatys* sensu lato), and southern Asia (genus *Aplocheilus*). The embryonic phase of their life history does not include diapause, although their embryos do possess some ability to survive aerial exposures. Some species are considered facultative annual killifish exhibiting characteristics that are midway between annual and non-annual killifish. They live in habitats that sometimes dry to moist mud but may also retain water throughout the whole year. The eggs can develop in water but are also capable of persisting several months in damp substrate. An example of a facultative annual killifish is the West African genus *Fundulopanchax* (e.g., Collier 2010; Huntley 2004; Suffia 2004).

In temporary pools of the Old World the huge family of cyprinid fish is represented by a number of small barbs (*Barbus* spp. sensu lato) with unclear taxonomy. They migrate out of the riverbed for reproduction during the rainy season (Minshull 2008). In the New World cyprinids are replaced by characids (e.g., genera *Astyanax*, *Cheirodon*), livebearing poeciliids (genera *Gambusia, Heterandria*, *Poecilia*), and anablepids (genus *Jenynsia*; Errea and Danulat 2001; Escalera-Vazquez and Zambrano 2010).

## 5 Fish Specifically Adapted to Temporary Pools

Fish that are obligate inhabitants of temporary pools and have adapted to the seasonal availability of their aquatic habitat have evolved two main life-history strategies. Both of these strategies rely on metabolic dormancy for survival of dry periods, but differ in the life stage in which dormancy is achieved. The first strategy, survival of drought as adult fish, appears to have evolved in the osmeriform fishes of Australia and New Zealand (Pusey 1989, 1990), and in the sarcopterygian lungfishes (Smith 1939; Fishman et al. 1992). In contrast, the annual killifishes (order Cyprinodontiformes) have evolved to survive dry seasons exclusively as drought-tolerant embryos (Wourms 1972a, b, c; Wildekamp 2004; Watters 2009).

## *5.1 Lungfish*

Although this book focuses on ray-finned fishes (Teleostei) and lungfishes belong to a phylogenetically different class of lobe-finned fishes, their strong ecological linkage to temporary environments warrants their inclusion. The six extant species occur in Africa, Australia, and South America (Graham 1997). Lungfish are characterized by slow maturation, long life span, large size (up to 2 m), relatively low fecundity, and parental care during reproduction (Bemis et al. 1987; Graham 1997). All lungfishes are obligate air breathers. Their life span is long enough that they can survive through several rainy and dry seasons (Greenwood 1986).

The most basal lungfish is the Australian lungfish, *Neoceratodus forsteri* Krefft 1870 (Ceratodontidae), which lives in a small geographic area within two river basins in eastern Australia. It has fleshy fins and large scales and reaches a length of 1.5 m. Its air-breathing capability is limited compared to other lungfishes (Kemp 1986; Kind et al. 2002). *Neoceratodus forsteri* does not estivate and cannot bury itself into the bottom substrate to survive longer periods without water (Kemp 1986). Its single lung, which is less specialized for air breathing than the paired lungs of American and African lungfishes, helps it to survive through periods of aquatic hypoxia rather than habitat desiccation (Graham 1997).

Four lungfish species (*Protopterus aethiopicus* Heckel 1851, *P. amphibius* Peters 1844, *P. annectens* Owen 1839, and *P. dolloi* Boulenger 1900) occur throughout Africa (Graham 1997). Their general appearance is similar to Australian lungfish, but they have small scales and filamentous paired fins. Their paired lungs are well adapted for aerial respiration in the swamps and temporary habitats where they frequently occur (DeLaney et al. 1974, 1977). When their habitat dries, they estivate by digging a vertical burrow into the substrate of their habitat and enclosing themselves in a cocoon of dry mud and mucus, leaving only a small opening at the mouth of the fish (Greenwood 1986). Estivation is associated with a major depression of metabolism (Smith 1931; Janssens 1964; DeLaney et al. 1974) and a shift in nitrogenous waste production from ammonia to urea (Janssens and Cohen 1968; Forster and Goldstein 1966). Refilling of their habitat with water causes rather rapid termination of estivation. Soon after water is available, male *Protopterus* spp. dig and guard tubular nests used for the brooding of embryos and larvae. The nest hole is guarded by male fish, and the male may help to oxygenate the water by agitation while embryos are developing (Greenwood 1986). Young fish grow very rapidly so that they are able to make their own cocoon at the end of first rainy season (Johnels and Svensson 1954; Greenwood 1986). Similarly to *N. forsteri*, *Protopterus* spp. are generalist predators feeding on mollusks, crustaceans, insects, and fish (Curry-Lindahl 1956; Mlewa and Green 2004).

The South American lungfish, *Lepidosiren paradoxa* Fitzinger 1987, occurs throughout the Amazon and Paraná River basins. Its lifestyle and morphology are similar to that of *Protopterus* spp., with the exception that it does not form an estivation cocoon (da Silva et al. 2008). Nest guarding males develop vascular respiratory structures on their paired fins, which increase aquatic gas exchange capacity and allow them to continuously guard the eggs without frequent air breathing (Carter and Beadle 1930; Sawaya et al. 1981; Fonseca de Almeida-Val et al. 2011).

Despite their occurrence in temporary environments, all lungfish embryos absolutely require liquid water for proper development and cannot withstand prolonged aerial incubation. In fact, as described above, all lungfish species breed while water is available and may even invest a great deal of time and energy into nest guarding to aid in the successful development of their offspring.

## *5.2 Annual Killifish*

Annual killifish survive dry seasons exclusively as drought-resistant, diapausing embryos buried in the bottom substrate of temporary pools (Wourms 1972a, c). They are characterized by fast maturation (Errea and Danulat 2001; Blažek et al. 2013), short life spans (Valdesalici and Cellerino 2003; Terzibasi et al. 2008), small size (up to 10 cm, Wildekamp 2004), high reproductive investment (Podrabsky 1999; Blažek et al. 2013), and no parental care (Wildekamp 2004). At the onset of the rainy season, when the pool is filled with water, a cohort of killifish hatches and grows very fast, reaching sexual maturity within weeks. After maturation they spawn on a daily basis until their habitat dries out. Unlike lungfish, adult killifish invariably die when the water in the temporary pond evaporates.

Annual killifish occur in pools associated with savannah regions of Africa (family Nothobranchiidae) and pampa regions of South America (family Rivulidae; Fig. 1). African annual killifish are represented by only three genera: East African *Nothobranchius* (ca 70 species), West African *Pronotobranchius* containing just four described species (Valdesalici 2013), and the monotypic *Fundulosoma thierryi* Ahl 1924. (Knaack 1981; Wildekamp 1983a, 2004). In *Nothobranchius* species, the affiliation to the single genus is reflected in relative uniformity in morphology and size (Wildekamp 2004). In contrast, South American annuals belong to 28 genera (Costa 2001, 2014) and several hundred species (Reis et al. 2003). They are morphologically more diverse and inhabit a greater variety of habitats in tropical, subtropical, and temperate climate zones. Annual rivulids include both small, slender-bodied genera (e.g., *Cynopoecilus*) and relatively large species such as

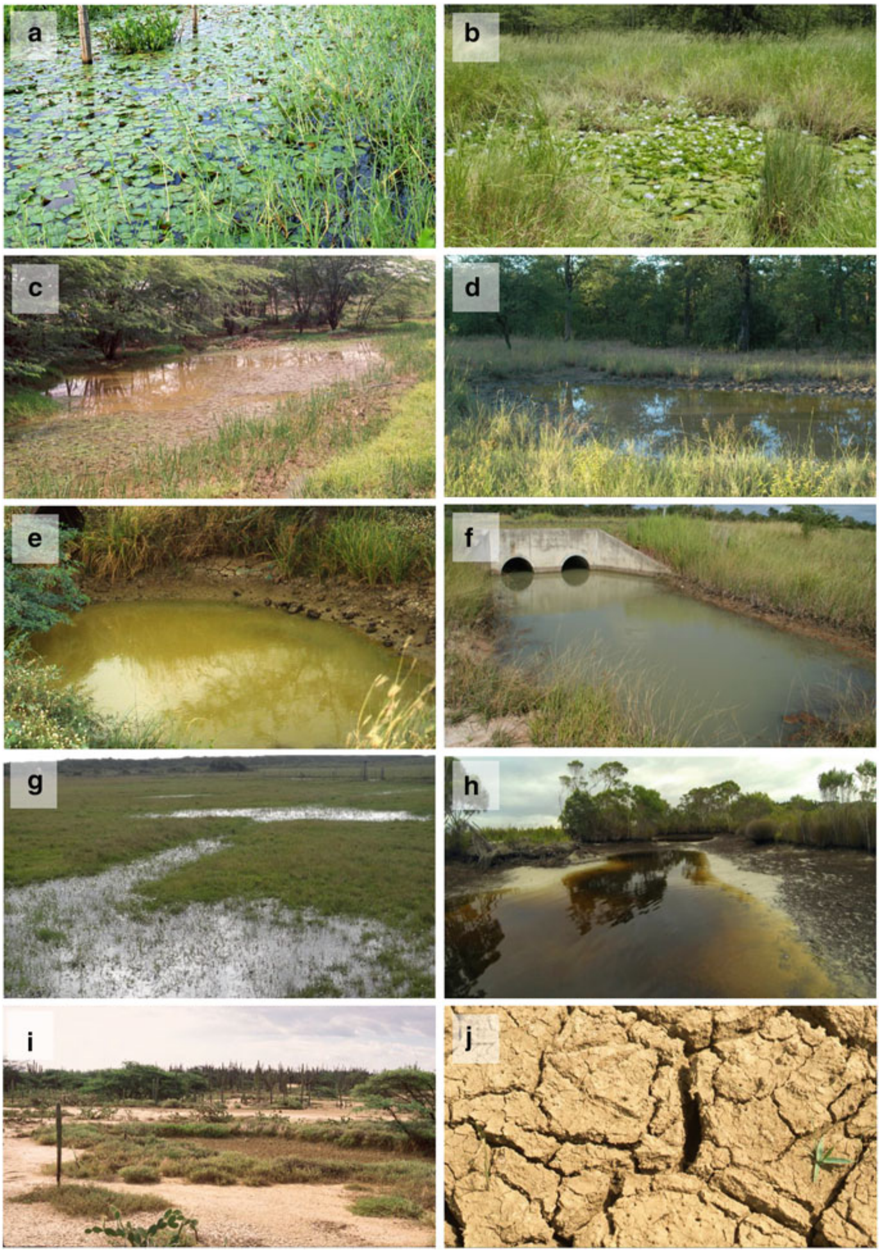


**Fig. 1** Examples of ephemeral ponds inhabited by fish in South America (**a**, **c**, **e**, **g**, **i**, **j**), Africa (**b**, **d**, **f**), and Australia (**h**). Ephemeral ponds are highly variable, but remarkably similar habitats can be found in tropical South American (**a**, **c**, **e**) and African (**b**, **d**, **f**) annual killifish pools (Photos by JE Podrabsky, O Sedláček and R Blažek). Pampean annual killifish pools (**g**) from temperate regions of South America are often nothing more than flooded fields (Photo by M Reichard). Many ephemeral pools have high levels of dissolved organic carbon (such as humic acids) derived from decomposing plants that turn the water a clear *black* or *brown* as illustrated by the example of *Lepidogalaxias salamandroides* habitat from southwestern Australia (**h**, photo by D Morgan) and

some *Austrolebias* or *Austrofundulus*, which can reach maximum size of 10 cm in length and 18 g in mass (Podrabsky 1999).

The life cycle of annual killifish consists of two stages, each highly adapted for either the dry or wet phase of their habitat. During dry seasons, annual killifish populations persist in the form of drought-resistant embryos encased in the dry muddy bottom of temporary pools (Fig. 1i, j). These embryos have been reported to survive for years in the complete absence of water (Scheel 1990). The developmental period in annual killifish is greatly extended by both an intrinsically slow developmental rate compared to embryos of a similar size in other teleost lineages (Parenti 1981), and the occurrence of embryonic diapause (Wourms 1972a, c). Diapause in annual killifish is associated with a cessation of active development and a profound metabolic depression (Levels et al. 1986; Podrabsky and Hand 1999). Embryos of annual killifish may enter diapause at one or all of three stages of diapause, termed diapause I, II, and III (Fig. 2; Wourms 1972a, c). Diapause I occurs early in development prior to formation of the embryonic axis, diapause II occurs midway through development after somitogenesis but prior to the major phases of organogenesis, and diapause III occurs in the fully formed larvae that is ready to hatch (Wourms 1972a, c). There appears to be considerable variation between lineages in terms of which diapause stages are observed under laboratory conditions (Murphy and Collier 1997), although this subject has not been carefully evaluated for a large number of species. Variation in the occurrence of each diapause stage can be highly variable within a single species (Markofsky and Matias 1977a, b; Markofsky et al. 1979; Podrabsky et al. 2010), and in fact within a single clutch of eggs from an individual female (Podrabsky et al. 2010). In addition, under laboratory conditions there is a wide range of variation in the duration of embryonic diapause between individual embryos. Because the first precipitation of any rainy season may not be substantial enough to form a body of water, there is likely a time lag between the initial rains and the hatching of the fish (Polačik et al. 2011). Watters (2009) suggested that increased moisture in the soil stimulates the embryos to complete development leading to fully developed embryo within 5–14 days. Alternately, hatching of diapause III embryos into a pool could be cued by hypoxia of the newly inundated soils, which may take several days to develop. After hatching, the fish can grow extremely fast, adding nearly 25 % to their size each day, and some *Nothobranchius* species can reach sexual maturity within just 17 days (Blažek et al. 2013). In the vast majority of species (especially in *Nothobranchius*), there is pronounced sexual dichromatism with strikingly colored males and relatively dull females (Fig. 3; e.g., Wildekamp 2004). Annual killifish are generally very prolific reproducers. Adult fish spawn each day in multiple

**Fig. 1** (continued) by a llanos pool (**a**) from the Orinoco basin in Venezuela (Photo by JE Podrabsky). Dry ephemeral ponds in Africa and South America are all characterized by a fine clay soil that forms large cracks when dried as illustrated by these images of dry ponds (**i**, **j**) in the Maracaibo basin of Venezuela (Photos by JE Podrabsky). Embryos of annual killifish survive encased in this dried mud

**Fig. 2** The three possible stages of diapause in embryos of annual killifish. Diapause I (*left panel*) may occur early in development prior to the formation of the embryonic axis during the dispersion and reaggregation phases of development. Diapause II (*center panel*) occurs about mid-way through development after completion of neurulation and somitogenesis, but prior to the major phases or organogenesis. Diapause III (*right panel*) occurs in the late pre-hatching embryo (Photos by JE Podrabsky and CL Riggs)

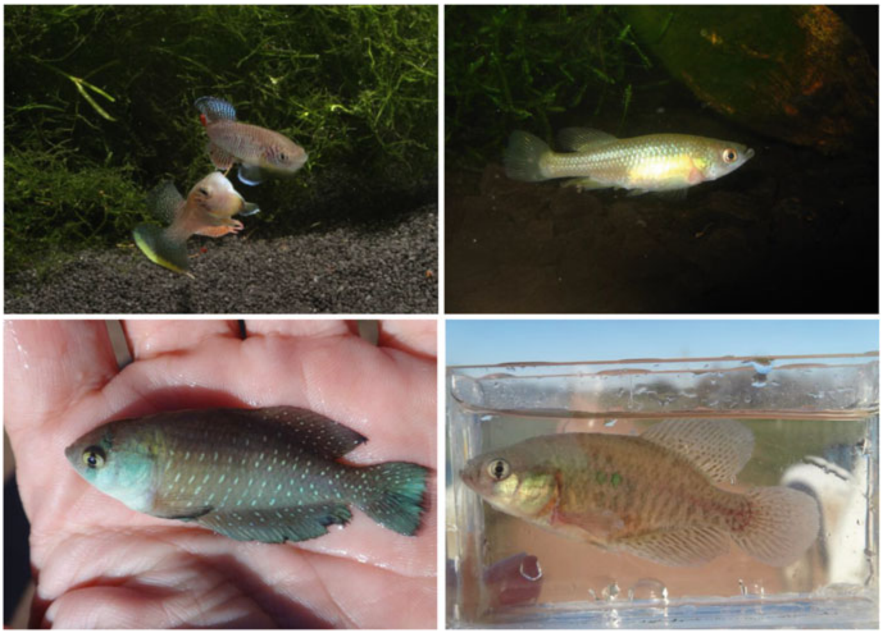


**Fig. 3** Examples of annual killifish behavior and coloration. Fighting African *Nothobranchius furzeri* males (*top*, *left*) and a *N. furzeri* female (*top*, *right*). South American *Austrolebias belotti* male (*bottom*, *left*) and female (*bottom*, *right*) (Photos by R Blažek and M Reichard)

spawning acts (Polačik and Reichard 2011). Depending on the species, body size, and nutrition, the number of eggs laid per day varies from several dozens to several hundreds (Podrabsky 1999; Graf et al. 2010; Blažek et al. 2013). Life span is normally limited by the duration of the pool in African and some tropical American annual killifish (e.g., genus *Austrofundulus*), which may be from several weeks to several months (Errea and Danulat 2001; Terzibasi-Tozzini et al. 2013). However, their intrinsic longevity is short as well and even in captivity their life span is typically less than a year in most *Nothobranchius* (Valdesalici and Cellerino 2003; Terzibasi-Tozzini et al. 2013; Polačik et al. 2014a). Tropical South American annual fish appear to live longer, with maximal ages around 1.5 years in captivity for *Austrofundulus limnaeus* (Podrabsky 1999). The distribution of rainfall in these tropical regions fills the temporary pools during the warm seasons and so the fish are adapted to high water temperatures (Podrabsky et al. 1998; Reichard et al. 2009). In contrast, annual killifish from temperate South America are “winter” species (e.g., genus *Austrolebias*), and intolerant to high temperatures. Their populations typically collapse in spring when the water temperature increases, and this happens long before their habitats desiccate (Errea and Danulat 2001).

Overall, South American and African annual killifish share a striking similarity in almost all aspects of their biology, physiology, and development. This suite of shared characters has been coined by some as the “annual” life history strategy (Karsten et al. 2008). Surprisingly, molecular evidence suggests that the complex suite of characters associated with annual killifish arose independently in Africa and South America from a common ancestor (Murphy and Collier 1997). In fact, it is likely that the ability to persist in temporary environments arose multiple times within South America as well (Hrbek and Larson 1999). Given that these lineages evolved through convergence, they are a remarkable example of the power of selective pressure on life history evolution.

### *5.3 Osmeriform Fishes*

Several osmeriform fish species capable of long-term survival in temporary waters occur on the southwestern and southeastern coast of Australia, Tasmania, New Zealand, and Chile (Galeotti et al. 2010). Although little is known about their biology, available information suggests that they combine both lungfish and annual killifish strategies. For example, *Lepidogalaxias salamandroides* Mees 1961 (Lepidogalaxiidae), endemic to temporary swamps on the southwestern coast of Australia (Fig. 1h), is a small, short-lived species with an apparent maximum life span of 1–2 years (Pusey 1990). Its reproduction is semelparous, and it appears that adults may die shortly after spawning (Pusey 1990). Fertilization is internal with males depositing sperm during copulation using a modified scale sheath (Pusey and Stewart 1989). Very little is known of the early life history of this species. Eggs are likely deposited individually onto vegetation as this behavior was observed at least once in the laboratory (Gill and Morgan 1999). Embryos hatch precociously after 24 days at ambient temperatures. Young *L. salamandroides* grow relatively fast and either reach reproductive maturity and spawn during one rainy season, or estivate through the dry season to reproduce during the following rainy season (Pusey 1990; Allen and Berra 1989). Very little is known about the physiology of estivation in this species, and survival under estivation-inducing conditions in the laboratory was quite low (Pusey 1986, 1989). However, field observations of dry ponds suggest that *L. salamandroides* and a co-occurring galaxid fish, *Galaxiella nigrostriata,* can survive, presumably via estivation, for several months (Allen and Berra 1989; Berra and Allen 1989). Small *G. nigrostriata* have been suggested to estivate in crayfish burrows or inside hollow rushes, roots, and logs (Galeotti et al. 2010). In contrast to *L. salamandroides*, *G. nigrostriata* is an iteroparous species with the spawning season lasting for several weeks. Still, it is a short-lived fish with a life span of about 1 year (Morgan et al. 1998).

## 6 Temporary Pools as a Free Ecological Niche

Temporary pools represent a harsh environment for fish. They are typically space limited, isolated, and prone to stochastic disturbances and weather extremes. So why would fish evolve adaptations for long-term persistence in temporary habitats? Specialization for this marginal niche may offer fishes an ecological opportunity to escape the influence of factors playing crucial roles in the ecology of more typical fish habitats, such as lakes and rivers.

### *6.1 Competition*

In temporary pools, fish escape much of the interspecific competition common in typical fish habitats, where multiple species co-occur. Ephemeral habitats are rich in food resources as they represent favorable environment for many invertebrates, such as crustaceans, insects, and mollusks, which are important prey for fish (e.g., Meintjes 1996; Laufer et al. 2009; Goncalves et al. 2011; Polačik and Reichard 2010). In addition, temporary pools are often populated by a single or just a few fish species (e.g., Podrabsky et al. 1998; Valdesalici 2007; Watters 2009; Reichard 2010). Even if several species occur in sympatry, their trophic niches are typically segregated. For example, lungfish often co-occur with annual killifish in Africa, but their usage of food resources is different. Due to their size and jaw morphology, lungfish consume mainly coarse prey such as mollusks and large crustaceans (Curry-Lindahl 1956; Daffalla et al. 1985; Greenwood 1986), whereas small killifish feed on zooplankton and insect larvae (Goncalves et al. 2011; Polačik and Reichard 2010). In *Nothobranchius* spp., co-occurrence of up to three species with very similar size in a single pool is quite common (Reichard et al. 2009). Despite their similar morphology and close phylogenetic relationships, these sympatric species are characterized by separate trophic niches. Although there is considerable qualitative dietary overlap, three coexisting *Nothobranchius* spp. consume available prey in significantly different proportions, especially when prey diversity is high (Polačik and Reichard 2010; Polačik et al. 2014b). In South American species trophic niche segregation, further facilitated by variations in body size and shape

(Laufer et al. 2009; Arim et al. 2010), is also obvious. Competition for food may be even more intense since the biomass and abundance of annual killifish within a pool may be occasionally exceeded by the biomass and abundance of non-annuals (mainly small characids and livebearers). On the other hand, non-annuals may serve as a food source for larger annual killifish species (e.g., Lanés et al. 2014a, b), leading to a reduction in competition with other sympatric annual killifish species for small invertebrate prey and thus contributing to the segregation of trophic niches among the annuals.

## *6.2 Predation*

Predation pressure on temporary pool fish is lower when compared to typical fish environments. Although there are no rigorous data, relaxed predation is supported by field observations, anecdotal information, and logical synthesis. The dry season represents a safe period for dormant killifish eggs or estivating lungfish from piscine and avian predation. Further, temporary habitats are highly stochastic, with variable availability and diversity of potential food (Meintjes 1996; Jocqué et al. 2007). Such an unpredictable environment favors a generalist feeding strategy in fish and specialized piscivorous fish species are therefore absent in the community (Reichard et al. 2009; Goncalves et al. 2011). Although lungfish may rarely consume a killifish (M. Polačik, unpublished data), and some killifish species occasionally prey on juvenile lungfish (Polačik and Reichard 2010) or smaller killifish (Laufer et al. 2009), fish piscivory is of reduced importance to the population ecology of fishes in temporary pools. Predatory insects likely also have negligible impacts on fish populations in temporary pools. Although co-occurrence of predatory hemipterans, odonatan and coleopteran larvae, and fish is common (Laufer et al. 2009; Polačik and Reichard 2010; Goncalves et al. 2011), predation by invertebrates on fish is likely to be limited to juvenile or bottom-strayed individuals, whereas impact on free swimming adults should be minor.

Predation on temporary pool fish by birds is a poorly explored topic. Several lines of evidence suggest that bird predation may play a significant role in temporary pools. Existence of a trematode parasite parasitizing African *Nothobranchius* with a definite host in piscivorous birds (V. Michálková, pers. comm.) suggests an ecological connection between fish in temporary pools and piscivorous birds. Further, experiments in a seminatural regime showed that a black-crowned night heron (*Nycticorax nycticorax*) selectively preyed on more conspicuous killifish males (Haas 1976), and female-biased sex ratios highly prevail in wild killifish populations (Reichard et al. 2009; Lanés et al. 2014a, b). Alternatively, uneven sex ratios may be caused by frequent male–male aggressive interactions (Haas 1976; Genade et al. 2005; Polačik and Reichard 2011) that sometimes lead to the death of one of the rivals (Genade 2005; M. Polačik unpublished data). The effect of bird predation on temporary pool fish may vary regionally. For example, piscivorous birds were rarely seen in proximity of fish-occupied temporary pools during our fieldwork in southeastern Africa and northern South America. Further, pools that were nearly desiccated but full of live, easily accessible fish are occasionally encountered (M. Polačik, J.E. Podrabsky, personal observations). This suggests that, if present, avian predation pressure is not constant. Local bird species may show little interest to forage in temporary habitats, potentially caused by costs linked to the stochastic occurrence of fish in the mosaic of temporary pools (low try-to-success ratio). In addition, association of many temporary pools with river floodplains means that the birds can preferably exploit nearby rivers as a more reliable source of food. On the contrary, many piscivorous bird species were seen foraging in temporary pools in southern South America (Uruguay and southern Brazil) and field experience (M. Loureiro, pers. comm.) points to the significance of bird predation in this area.

## 7 Temporary Pools as an Ecosystem Requiring Specific Adaptations

Despite diminished competition and predation, temporary pools represent a harsh and extreme environment for fish. In order to exploit this ecosystem, temporary pool fish had to evolve a range of costly adaptations.

### *7.1 Seasonality*

Seasonality is the main feature of any temporary pool ecosystem. It is an enormous challenge, because it requires adaptations to both an aquatic and terrestrial existence, two highly divergent niches. Without the option for escape from a drying pool through emigration (some fish species actually can, but most pools are too isolated for this to be of any practical significance), the capability to switch from the water phase to a dormant, drought-resistant form is of paramount importance. Further, it places rather absolute time constraints on each of the two phases. This is inextricably linked to costs in population growth and life history trade-offs. During the dormant phase reproduction, growth, and development are completely halted, thus forcing individual and population level growth and reproduction into the limited time of the rainy season. Stability of lungfish populations, the density of which is generally low (Reichard et al. 2009), is achieved by adult longevity, and the ability of these fish to survive and persist through many dry and rainy seasons. For lungfish, seasonal time limitations for populations are mainly defined by the time required for their offspring to reach a quantitative threshold of minimum estivation size (about 50 mm, Johnels and Svensson 1954) with enough energetic reserves to survive prolonged estivation (Janssens 1964; DeLaney et al. 1974;

Bemis et al. 1987). The same appears to be true for populations of *L. salamandroides* in Australia (Allen and Berra 1989; Berra and Allen 1989). For annual killifish, individuals must reach the qualitative threshold stage of sexual maturity within the wet phase of the pool in order to produce the next generation through the production of embryos. In some regions, the wet phase may only last a couple of weeks (Terzibasi-Tozzini et al. 2013). Growth and sexual maturation are therefore extremely rapid. For example, *N. kadleci* living in southern Mozambique where rainfall is scarce can mature within only 17 days after hatching (Blažek et al. 2013). Similar rates of maturation are also likely for young *A. limnaeus* based on field observations, but this has yet to be systematically confirmed (J.E. Podrabsky, unpublished data).

The ability of annual killifish embryos to survive the dry season is due to specific adaptations of the embryos for survival during aerial incubation. Embryos cope with desiccating environments by reducing long-term evaporative water loss to extremely low levels (Podrabsky et al. 2001). While this strategy preserves the osmotic environment within the embryo itself, it also likely imposes hypoxia on the embryos due to the low levels of gas exchange. Thus, dehydrated embryos may be physiologically anoxic and/or hypercapnic. It is possible that dehydration tolerance and the extreme anoxia tolerance (see below) exhibited by annual killifish embryos co-evolved in some manner.

## *7.2 Stochasticity*

Despite the seasonally predictable nature of rainfall patterns in areas that support temporary pools, there are significant temporally and spatially stochastic components to the existence of temporary pool ecosystems. Pool inundation is to a large extent unpredictable, as it is dependent on seasonal variations in precipitation that are naturally variable in severity, time, and location (e.g., Camberlin and Philippon 2002). The suitable window for reproduction, therefore, fluctuates both in terms of date of emergence of the pool and the extent of its duration. The fact that lungfish survive through both seasons as adults/sub-adults requires a plastic physiological response to the actual conditions. Triggered by lack of ambient water and food supply, lungfish make a burrow and undergo a cascade of physiological changes (e.g., body torpor, anuria, and decreased oxygen consumption) within a short time. This state is rapidly reversible (DeLaney et al. 1974; Fishman et al. 1992).

In contrast, annual killifish can only survive the dry season as embryos that must be ready to hatch shortly after the onset of rainfall. This group of fish has therefore evolved a whole range of adaptations for coping with environmental stochasticity. Partially unpredictable temporary habitats differ from more stable water environments by providing few reliable cues that are useful predictors of future conditions. In other words, the adaptive value of any particular biological trait is very uncertain. Therefore annual killifish are extremely plastic with broad reaction norms and huge intrapopulation variability in pre- and post-hatching development, growth rate, size, and life span (Podrabsky et al. 2010; Blažek et al. 2013; Polačik et al. 2014a).

Synchronous development of the entire embryo bank might have catastrophic consequences for a population if a false rain only triggered hatching but did not establish a pool. Therefore annual killifish have adopted an extreme strategy of developmental bet-hedging, which ensures that some portion of the embryos produced by previous generations are always ready to hatch, while other embryos contribute to an "embryo bank" in case a cohort of fish fails to reproduce. The ability to arrest development at three different developmental stages and the high degree of variation in the length of each diapause can lead to a wide range of effective developmental rates in even a single clutch of embryos. Wourms (1972c) first described this phenomenon as the "multiplier effect" and suggested it as a form of bet-hedging in the face of environmental uncertainty. This type of variation in developmental rates is likely critical for prolonged survival of populations in a single location in the face of sometimes unpredictable rainfall events sufficient to fill the ponds. For example, the eggs of some *Nothobranchius* species may develop to the pre-hatching stage in as short as 15 days or as long as several years (Blažek et al. 2013; M. Polačik unpublished data). Development in both *Nothobranchius* and *Austrofundulus* is asynchronous even within the progeny of the same parents and within the same clutch (Podrabsky et al. 2010; Polačik et al. 2014a).

Bet-hedging may be even manifested during post-hatching development. Some individuals grow and mature faster than others, and huge variance in size is a rule, at least in captivity. Recently, Polačik et al. (2014a) showed that there are alternative life history strategies linked to the length of embryonic development. This is of particular importance in regions where rainfall is so erratic that some pools may fill and dry multiple times during a single rainy season, allowing for the possibility of multiple generations in a single year (Terzibasi-Tozzini et al. 2013). If the previous generation of fish managed to spawn, a second generation of fish may hatch from rapidly developing "escape" embryos that do not arrest in diapause II (Podrabsky et al. 2010). Since the secondary pool can only emerge later in the rainy season, its expected duration is inevitably shorter than the duration of a standard pool that does not desiccate. The predictably shorter persistence of the secondary pool represents an evolutionary pressure which resulted in a life history trade-off between fast developing "escape" embryos and slow developing embryos that enter into diapause II and delay hatching until the next rainy season. In *Austrofundulus* there is evidence that embryos developing along these two alternative developmental pathways hatch with differing aerobic and anaerobic capacities (Chennault and Podrabsky 2010). In *N. furzeri*, individuals originating from eggs with fast development were reported to grow more rapidly and mature earlier than the individuals hatched from long diapausing eggs. On the other hand, as a trade-off, fast-growing fish reached smaller adult body size and had a shorter life span (Polačik et al. 2014a).

The degree of environmental stochasticity is also reflected in the sexual behavior of annual killifish. Although there is abundant potential for mate choice cues (e.g., male size and coloration), mate choice appears to be relaxed in annual killifish, at least in species from geographical regions with a high variation in the timing of pool formation and duration. Because of the extraordinary dynamics of the ecosystem, parents appear to have few environmental cues that can reliably signal the characteristics of the habitat into which their offspring will hatch. Over a longer period, potential channelization of preferences for a specific trait could lead to a mismatch between the trait and the actual environment. Therefore relaxed mate choice is likely adaptive as it is a form of bet-hedging which helps spread risk. It is further enforced by some reproductive specifics, e.g., multiple mating events each day and male coercion (Polačik and Reichard 2011). Studies on African *Nothobranchius* spp. revealed that within a species, females mate quite indiscriminately (Polačik and Reichard 2009; Reichard and Polačik 2010) and a receptive female will spawn with any male making physical contact (Haas 1976). Females can even accept heterospecific partners (Polačik and Reichard 2011). Rather than in female choice, striking male coloration and different body shape may play an important role in male–male interactions. In fact, *Nothobranchius* males present their colors more conspicuously during a characteristic lateral fighting display (Fig. 3) than when approaching a female. Further, large male fins are useful in generating a strong blow of water, which is more likely to be perceived by the rival during the display. It is possible that the evolution of sexual dimorphism in *Nothobranchius* may have been promoted by intrasexual rather than intersexual selection. More consistent female preferences for certain male phenotypes and, in contrast to *Nothobranchius*, elaborate male displays were reported in the South American genus *Austrolebias* (Garcia et al. 2008; Passos et al. 2013), suggesting a higher importance for sexual selection in species with relatively slower maturation rates and from presumably more predictable environments (Laufer et al. 2009; da Fonseca et al. 2013).

### *7.3 Variability*

Temporary pools are extremely variable ecosystems. They vary in size, depth, extent of aquatic vegetation, and food availability. However, this is not necessarily true for all temporary pools, as temperate Pampean pools in southern Brazil and Uruguay (Fig. 1g) are relatively uniformly represented by flat, flooded grassland with maximum depths of up to 40 cm. This uniformity is in stark contrast to the diverse tropical or subtropical African and South American habitats that can range from large deep pools to small and shallow pools, some of which are nothing more than hoof prints in a flooded field (Podrabsky et al. 1998; Reichard et al. 2009).

Due to their small size and limnetic character, temporary pools are intensively affected by temporal fluctuations in environmental parameters that can have profound effects on organismal physiology. Thus, temporary pool fish have evolved wide physiological tolerances to cope with their ever-changing environment. Thriving populations of annual killifish were found in pools with temperatures ranging from 6 °C (temperate South America, Errea and Danulat 2001) to 39 °C (tropical

South America and Africa, Podrabsky et al. 1998; Reichard et al. 2009). Pool temperatures may also change by over 20 °C on a daily basis (Podrabsky et al. 1998; Podrabsky and Somero 2003; Reichard et al. 2009). In deeper, turbid pools, water temperatures may become stratified (Podrabsky et al. 1998), with cooler parts close to the bottom acting as potential temperature refugia (Williams 1985). Seasonal shifts in temperature minima and maxima can exceed 25 °C (R. Blažek, pers. comm.). In addition, rapid drops in water temperature may be caused by torrential rainfall. Embryos of annual killifish can survive a wide range of temperatures from near freezing to temperatures over 40 °C (Matias and Markofsky 1978; J.E. Podrabsky, unpublished data). Adult *A. limnaeus* can survive continuous daily temperature cycling from 20 °C to 37 °C indefinitely and appear to mount unique gene expression changes in response to fluctuating versus constant temperature extremes (Podrabsky and Somero 2003). However, at least under laboratory conditions, temperature cycling is associated with a reduction in reproductive output (Podrabsky et al. 2008). Thus, it is likely that temperature refugia and thermal partitioning of habitat between the sexes may play an important role in the ecology of wild annual killifish populations.

Oxygen partial pressures vary widely in temporary pond habitats, driven to levels of supersaturation by photosynthetic activity during the day, and extreme hypoxia and maybe even anoxia by respiration at night (Williams 1985; Podrabsky et al. 1998; see chapter "Low-Oxygen Lifestyles"). Further, the inundated soils of temporary pools quickly become anoxic (Podrabsky et al. 1998). Embryos of *A. limnaeus* are the most anoxia-tolerant vertebrates yet described and can survive for several months in the complete absence of oxygen at 25 °C (Podrabsky et al. 2007, 2012). In addition, these embryos can develop normally to hatching even under extreme hypoxia ($PO_2$ of 2.1 kPa at 25 °C, Anderson and Podrabsky 2014). Tolerance of low oxygen likely applies to other annual killifish embryos as well, although systematic evaluations have not yet been conducted. As adults, *A. limnaeus* can survive for several hours of anoxia (Podrabsky et al. 2012), and for extended periods in severe hypoxia (J.E. Podrabsky, unpublished data). Other environmental variables are known to vary substantially on a daily and seasonal basis in temporary pools. These include relatively large fluctuations in pH, incident light and radiation, salt concentration, and salt composition (Podrabsky et al. 1998).

## 8 Pool Isolation and Colonization of New Habitats

Temporary pools are often isolated ecosystems, because terrestrial habitats form a strong barrier to fish dispersal. Despite the high tolerances of environmental variations and extremes, temporary pool fish are much more susceptible to local extinction compared to fish from permanent environments (Sheaves and Johnston 2008). Thus, mechanisms of dispersal and gene flow are critical for the long-term survival of populations. In accordance with their different life history strategies,

**Fig. 4** Examples of flooding events that could lead to dispersal of adult annual killifish. Flooded coastal desert in Venezuela (*left*) and African savannah (*right*) (Photos by JE Podrabsky and M Reichard)

annual killifish and lungfish also use different ways of spreading to colonize new habitats.

In addition to temporary pools, lungfishes are found in large, permanent- and semipermanent-water bodies. They are associated with river systems and actively spread within the respective river basins (Greenwood 1986; Mlewa et al. 2005). They can also traverse short distances between adjacent pools through terrestrial environments due to their capability for snake-like locomotion on land and ability to breathe air (e.g., Laurin 2010).

In contrast to lungfish, annual killifish only occur in small water bodies and their drought-resistant eggs were long believed to be the main means of dispersal (Bartáková et al. 2013). In theory, eggs can be caught in plumage of birds or fur of mammals and travel long distances. However, genetic sampling of multiple *N. furzeri* populations in Mozambique recently revealed that dispersal occurs in a step-by-step manner and is not directly associated with river routes. In other words, rather than through eggs the species appears to spread via adult fish that colonize new adjacent habitats during occasional major flood events when water covers large areas of the savannah and coastal desert habitats (Fig. 4; Bartáková et al. 2013; J. E. Podrabsky pers. obs.).

## 9 Significance of Temporary Pool Fish for Humans

Fish associated with periodical habitats are unique. Their extreme characteristics have prompted interest in many branches of scientific research. Due to their peculiar development that includes diapausing stages, they gained the attention of embryologists soon in the 1970s, and the interest is ongoing (Wourms 1972a, b, c; Lesseps et al. 1975; Markofsky and Matias 1977a, b; Matias and Markofsky 1978; Lesseps et al. 1979; Markofsky et al. 1979; Matias 1982, 1983, 1984; Carter and Wourms 1991; Podrabsky and Hand 1999; Carey et al. 2012; Podrabsky et al. 2012). In contrast to traditional fish models in toxicology, like guppies or zebrafish (e.g., Khudoley 1984), annual killifish need not be maintained in a continuous culture and stored eggs can be hatched when necessary. Therefore they were evaluated for use in toxicity screening tests with good results (Van der Hoeven et al. 1982; Shedd et al. 1999).

The extremely short intrinsic life span in some *Nothobranchius* species (Valdesalici and Cellerino 2003) also prompted intensive use of this genus in aging research. Limited longevity enabled testing the effects of particular substances on the length of life span (e.g., resveratrol from red wine: Valenzano et al. 2006; Genade and Lang 2013). The processes of senescence are being studied at molecular (e.g., Valenzano et al. 2011; Baumgart et al. 2012; Kirschner et al. 2012), histological (e.g., Di Cicco et al. 2011), organismal (e.g., Liu et al. 2012), population (e.g., Graf et al. 2010), and interpopulation (e.g., Terzibasi-Tozzini et al. 2013) levels.

Annual killifish recently gained their importance as models to tackle general questions of ecology and evolution. They have been used to study mechanisms of coexistence of closely related species (Polačik and Reichard 2010; Polačik et al. 2014b), sexual selection (Garcia et al. 2008; Polačik and Reichard 2009, 2011; Passos et al. 2013), genetic structuring of populations (Dorn et al. 2011; Bartáková et al. 2013), and life history evolution (Polačik et al. 2014a).

In addition to scientific use, temporary pool fish have the potential to influence human life in more direct ways. African lungfish are locally important for fisheries (e.g., Mlewa and Green 2004). Catfishes (*Clarias* spp.) and *tilapias* (mainly *Oreochromis niloticus*) cichlids occur in and tolerate temporary environments due to their general hardiness. Their fast growth and suitable quality as food fish have facilitated their use in aquaculture and they are the most important aquaculture species in warm climate countries nowadays (e.g., Nyina-Wamwiza et al. 2007; Almeida et al. 2013; Sadeu et al. 2013). An African “fingerpond” aquaculture project is largely based on the use of *tilapias*. This EU-funded project educates local riparian communities around Lake Victoria on the technology of sustainable, low effort fish production utilizing organic waste. Ponds are dug from the landward edge of wetlands and extend like fingers into the swamp. The ponds are stocked with fish through natural flooding in the rainy season. As the waters recede, the trapped fish are cultured using manure, crop, and household wastes to fertilize the ponds and feed the fish. The fast growing *tilapias* are harvested several months later (e.g., Kaggwa et al. 2005).

Annual killifish can consume large quantities of mosquito larvae and they have long been considered to be a potential biological control tool in malaria-affected regions (Wildekamp 1983b; Matias and Adrias 2010). However, other authors have argued that the introductions to new areas may have negative impacts because of nonnative status of killifish species and would be largely unsuccessful due to their specific soil requirements—i.e., annual killifish are already present in areas with suitable conditions (Reichard et al. 2010). Finally, many annual killifish species are collected and bred by a large international community of hobbyists. The fish are popular for their unique lifestyle and typical gorgeous male coloration.

Wild-derived strains with a known site of origin are highly valued and subjects of particular interest from keepers, many of which are intensely interested in the conservation of these unique fish (e.g., Neumann 2008).

## 10 Conclusions and Outlook

Fish adapted to temporary environments (including non-teleosts) represent a very unique evolutionary trajectory among the great diversity of teleost fish. Their unique adaptations and ecology make them valuable models for many areas of scientific research, and offer opportunities for studies that would be impossible or extremely difficult in species from permanent aquatic environments.

Perhaps the most intriguing feature of their biology is their ability to survive the drying of their habitat and the associated forms of metabolic dormancy that are associated with this key feature of their life history (e.g., DeLaney et al. 1974; Podrabsky et al. 2007; da Silva et al. 2008). A better understanding of the physiology of dormancy (e.g., cell cycle and metabolic regulatory processes) may help in identifying novel therapies for human diseases such as stroke, heart attack, and cancer (Podrabsky and Culpepper 2012). Recent work has suggested that maternal provisioning is involved in controlling the developmental trajectory of annual killifish embryos (Podrabsky et al. 2010), offering the opportunity to study how information about parental environment and/or condition may be passed on to their offspring. Future research should focus on the molecular mechanisms that support the ability to enter into a reversible state of metabolic arrest, and to tolerate environmental extremes.

The naturally short life span of some annual killifish species is another focal area for scientific research (Genade et al. 2005). Aging processes at the organismal and histological levels have already been well described (e.g., Terzibasi et al. 2008; Di Cicco et al. 2011) and studies focused on the molecular level are ongoing (e.g., D'Angelo et al. 2014). Future efforts will likely focus on finding functional linkages between particular genes and longevity (Kirschner et al. 2012) and identifying substances that influence life span (Valenzano et al. 2006).

While a wealth of knowledge is accumulating on the physiology and development of annual killifish in the laboratory, very little is known of their biology in the wild (e.g., Reichard et al. 2009). In fact, the situation is analogous to another notoriously well-known laboratory fish model that suffers from a lack of knowledge of their natural biology, the zebrafish *Danio rerio* Hamilton 1822 (Spence et al. 2006). The work covered in this chapter reveals that the basic aspects of annual killifish ecology have only recently been published (e.g., Errea and Danulat 2001; Laufer et al. 2009; Reichard et al. 2009; Polačik and Reichard 2010), and there are many questions that remain to be addressed and studied in the wild. For example, the ecology and physiology of annual killifish diapause are unknown under natural conditions. Specifically, it is unclear when transitions between the various diapause stages might occur and what cues may control entrance or exit from these diapause stages under natural conditions (Watters 2009). We do not know how long fish populations may persist in dry ponds, or what conditions they actually face when they are encased in dried mud. Further, the role of predation on the fish (an important ecological and evolutionary factor) is poorly explored in temporary pools. Similarly, despite intensive study under artificial conditions, no study has addressed the aging process in wild fish, which may differ from laboratory observations.

While our knowledge of the ecological and physiological aspects of wild annual killifish is sparse, the situation is even worse when considering what is known about estivation in galaxiid fishes. Very little is known about the details of their reproduction and survival through the dry season, and this promises to be a rich area for future research (Galeotti et al. 2010).

Better understanding of the fish's natural life cycles is particularly pressing as wetlands including temporary environments are amongst the most endangered ecosystems worldwide (e.g., Shine and de Klemm 1999) while being crucial for maintenance of biodiversity and local climatic and hydrological regimes (e.g., Brooks 2009). In some countries the seasonal water bodies have direct significance as water supplies for cattle and humans (e.g., da Silva et al. 2011). Particularly within the context of ongoing climate change, a significant share of future research should be aimed to help understand and conserve biodiversity in these unique temporary aquatic habitats.

**Acknowledgments** MP was financially supported by CSF project P505/11/P646, and JEP was supported by NIH grant R01 HL095454 and NSF grant IOS1354549.

## References

Allen GR, Berra TM (1989) Life history aspects of the west Australian salamanderfish, *Lepidogalaxias salamandroides* Mees. Rec W Aust Mus 14:253–267

Almeida DB, da Costa MAP, Bassini LN, Calabuig CIP, Moreira CGA, Rodrigues MDN, Perez HJ, Tavares RA, Varela AS, Moreira HLM (2013) Reproductive performance in female strains of Nile tilapia, *Oreochromis niloticus*. Aquacult Int 21:1291–1300

Anderson S, Podrabsky J (2014) The effects of hypoxia and temperature on metabolic aspects of embryonic development in the annual killifish *Austrofundulus limnaeus*. J Comp Physiol B 184:355–370

Arim M, Abades SR, Laufer G, Loureiro M, Marquet PA (2010) Food web structure and body size: trophic position and resource acquisition. Oikos 119:147–153

Barson M, Nhiwatiwa T (2010) Influence of drought and flooding on the colonisation of floodplain pans by riverine fish in the Zimbabwean lowveld. Afr J Aquat Sci 35:205–208

Bartáková V, Reichard M, Janko K, Polačik M, Blažek R, Reichwald K, Cellerino A, Bryja J (2013) Strong population genetic structuring in an annual fish, *Nothobranchius furzeri*, suggests multiple savannah refugia in southern Mozambique. BMC Evol Biol 13:196

Baumgart M, Groth M, Priebe S, Appelt J, Guthke R, Platzer M, Cellerino A (2012) Age-dependent regulation of tumor-related microRNAs in the brain of the annual fish *Nothobranchius furzeri*. Mech Ageing Dev 133:226–233

Bemis WE, Burggren WW, Kemp NE (1987) The biology and evolution of lungfishes. Alan Liss, New York, NY

Berra TM, Allen GR (1989) Burrowing, emergence, behavior, and functional morphology of the australian salamanderfish, *Lepidogalaxias salamandroides*. Fisheries 14:2–10

Blažek R, Polačik M, Reichard M (2013) Rapid growth, early maturation and short generation time in African annual fishes. EvoDevo 4:24

Brooks RT (2009) Potential impacts of global climate change on the hydrology and ecology of ephemeral freshwater systems of the forests of the northeastern United States. Clim Change 95:469–483

Bruton M (1979) The survival of habitat desiccation by air breathing clariid catfishes. Environ Biol Fishes 4:273–280

Camberlin P, Philippon N (2002) The east African March–May rainy season: associated atmospheric dynamics and predictability over the 1968–97 period. J Climate 15:1002–1019

Carey HV, Martin SL, Horowitz BA, Yan L, Bailey SM, Podrabsky JE, Storz JF, Ortiz RM, Wong RP, Lathrop DA (2012) Elucidating Nature's solutions to heart, lung, and blood diseases and sleep disorders. Circ Res 110:915–921

Carrasco NK, Perissinotto R, Nel HA (2012) Diet of selected fish species in the freshwater-deprived St Lucia Estuary, South Africa, assessed using stable isotopes. Mar Biol Res 8:701–714

Carter G, Beadle L (1930) Notes on the habits and development of *Lepidosiren paradoxa*. J Linn Soc Lond Zool 37:197–203

Carter CA, Wourms JP (1991) Cell behavior during early development in the South American annual fishes of the genus *Cynolebias*. J Morphol 210:247–266

Chennault T, Podrabsky JE (2010) Aerobic and anaerobic capacities differ in embryos of the annual killifish *Austrofundulus limnaeus* that develop on alternate developmental trajectories. J Exp Zool A 313:587–596

Chung KS (1985) Adaptability of *Oreochromis mossambicus* to temperature changes. Acta Cient Venez 36:180–190

Collier GE (2010) The genus *Fundulopanchax* – taxonomic history and molecular phylogeny. J Am Killifish Assoc 43:3–26

Cook K, Vizy E (2013) Projected changes in east African rainy seasons. J Climate 26:5931–5948

Costa WJEM (2001) Phylogenetic position and taxonomic status of *Anablepsoides, Atlantirivulus, Cynodonichthys, Laimosemion* and *Melanorivulus* (Cyprinodontiformes: Rivulidae). Ichthyol Explor Freshwat 22:233–249

Costa WJEM (2014) A new genus of miniature cynolebiasine from the Atlantic Forest and alternative biogeographical explanations for seasonal killifish distribution patterns in South America (Cyprinodontiformes: Rivulidae). Vertebr Zool 64:23–33

Curry-Lindahl K (1956) On the ecology, feeding behaviour and territoriality of the African lungfish, African lungfish, *Protopterus aethiopicus* Heckel. Arkiv for Zoologi 9:479–497

D'Angelo L, De Girolamo P, Lucini C, Terzibasi-Tozzini E, Baumgart M, Castaldo L, Cellerino A (2014) Brain-derived neurotrophic factor: mRNA expression and protein distribution in the brain of the teleost *Nothobranchius furzeri*. J Comp Neurol 522:1004–1030

da Fonseca AP, Volcan MV, Sampaio LA, Romano LA, Robaldo RB (2013) Growth of critically endangered fish annual fish *Austrolebias wolterstorffi* (Cyprinodontiformes: Rivulidae) at different temperatures. Neotrop Ichthyol 11:837–844

da Silva G, Giusti H, Sanchez A, Carmo J, Glass M (2008) Aestivation in the South American lungfish *Lepidosiren paradoxa*: effects on cardiovascular function, blood gases, osmolality and leptin levels. Respir Physiol Neurobiol 164:380–385

da Silva FR, Gibbs JP, Rossa-Feres DD (2011) Breeding habitat and landscape correlates of frog diversity and abundance in a tropical agricultural landscape. Wetlands 31:1079–1087

Daffalla A, Elias E, Amin M (1985) The lungfish *Protopterus annectans* (Owen) as a biocontrol agent against schistosome vector snails. J Trop Med Hyg 88:131–134

Davenport J, Abdul Matin AKM (1990) Terrestrial locomotion in the climbing perch, *Anabas testudineus* (Bloch) (Anabantidea, Pisces). J Fish Biol 37:175–184

DeLaney RG, Lahiri S, Fishman AP (1974) Aestivation of the African lungfish *Protopterus aethiopicus*: cardiovascular and respiratory functions. J Exp Biol 61:111–128

DeLaney RG, Lahiri S, Hamilton R, Fishman AP (1977) Acid-base balance and plasma composition in the aestivating lungfish (*Protopterus*). Am J Physiol 232:R10–R17

Descroix L, Mahé G, Lebel T, Favreau G, Galle S, Gautier E, Olivry JC, Albergel J, Amogu O, Cappelaere B, Dessouassi R, Diedhiou A, Le Breton E, Mamadou I, Sighomnou D (2009) Spatio-temporal variability of hydrological regimes around the boundaries between Sahelian and Sudanian areas of West Africa: a synthesis. J Hydrol 375:90–102

Di Cicco E, Tozzini ET, Rossi G, Cellerino A (2011) The short-lived annual fish *Nothobranchius furzeri* shows a typical teleost aging process reinforced by high incidence of age-dependent neoplasias. Exp Gerontol 46:249–256

Donnelly B (1978) Evidence of fish survival during habitat desiccation in Rhodesia. J Limnol Soc South Afr 4:75–76

Dorn A, Ng'oma E, Janko K, Reichwald K, Polacik M, Platzer M, Cellerino A, Reichard M (2011) Phylogeny, genetic variability and colour polymorphism of an emerging animal model: the short-lived annual *Nothobranchius* fishes from southern Mozambique. Mol Phylogenet Evol 61:739–749

Errea A, Danulat E (2001) Growth of the annual fish, Cynolebias viarius (Cyprinodontiformes), in the natural habitat compared to laboratory conditions. Environ Biol Fishes 61:261–268

Escalera-Vazquez LH, Zambrano L (2010) The effect of seasonal variation in abiotic factors on fish community structure in temporary and permanent pools in a tropical wetland. Freshw Biol 55:2557–2569

Fishman AP, Galante RJ, Winokur A, Pack AI (1992) Estivation in the African Lungfish. Proc Am Philos Soc 136(1):61–72

Fonseca de Almeida-Val VM, Nozawa SR, Lopes NP, Rocha Aride PH, Socorro Mesquita-Saad L, de Nazarre Paula da Silva M, Honda RT, Ferreira-Nozawa MS, Val AL (2011) Biology of the South American lungfish *Lepidosiren paradoxa*. In: Jorgensen JM, Joss J (eds) The biology of lungfishes. Science Publishers, Enfield

Forster RP, Goldstein L (1966) Urea synthesis in the lungfish: relative importance of purine and ornithine cycle pathways. Science 153:1650–1652

Galeotti DM, McCullough CD, Lund MA (2010) Black stripe minnow *Galaxiella nigrostriata* (Shipway 1953) (Pisces: Galaxiidae), a review and discussion. J R Soc West Aust 93:13–20

Garcia D, Loureiro M, Tassino B (2008) Reproductive behavior in the annual fish *Austrolebias reicherti* Loureiro and Garcia 2004 (Cyprinodontiformes: Rivulidae). Neotropical Ichthyology 6:243–248

Genade T (2005) Laboratory manual for culturing *N. furzeri*. See http://www.nothobranchius.info/pdfs/lab_protocols_1.pdf

Genade T, Lang DM (2013) Resveratrol extends lifespan and preserves glia but not neurons of the *Nothobranchius guentheri* optic tectum. Exp Gerontol 48:202–212

Genade T, Benedetti M, Terzibasi E, Roncaglia P, Valenzano DR, Cattaneo A et al (2005) Annual fishes of the genus *Nothobranchius* as a model system for aging research. Aging Cell 4:223–233

Gill HS, Morgan DL (1999) Larval development of the salamanderfish, *Lepidogalaxias salamandroides* Mees (Lepidogalaxiidae). Copeia 1999:219–224

Goncalves C, Souza U, Volcan M (2011) The opportunistic feeding and reproduction strategies of the annual fish *Cynopoecilus melanotaenia* (Cyprinodontiformes: Rivulidae) inhabiting ephemeral habitats in southern Brazil. Neurotrop Ichthyol 9:191–200

Graf M, Cellerino A, Englert C (2010) Gender separation increases somatic growth in females but does not affect lifespan in *Nothobranchius furzeri*. PLoS One 5:e11958

Graham J (1997) Air-breathing fishes: evolution, diversity, and adaptation. Academic Press, San Diego, CA

Greenwood PH (1986) The natural history of African lungfishes. J Morphol 190:163–179

Haas R (1976) Sexual selection in *Nothobranchius guentheri* (Pisces-Cyprinodontidae). Evolution 30:614–622

Hrbek T, Larson A (1999) The evolution of diapause in the killifish family Rivulidae (Atherinomorpha, Cyprinodontiformes): a molecular phylogenetic and biogeographic perspective. Evolution 53:1200–1216

Hughes GM, Munshi JSD (1973) Fine structure of the respiratory organs of the Climbing perch, *Anabas testudineus* (Pisces: Anabantidae). J Zool 170:201–225

Huntley W (2004) Peat moss – pros and cons as a spawning media. J Am Killifish Assoc 37:173–176

Janssens PA (1964) The metabolism of the aestivating African lungfish. Comp Biochem Physiol 11:105–117

Janssens PA, Cohen PP (1968) Nitrogen metabolism in the African Lungfish. Comp Biochem Physiol 24:879–886

Jocqué M, Riddoch BJ, Brendonck L (2007) Successional phases and species replacements in freshwater rock pools: towards a biological definition of ephemeral systems. Freshw Biol 52:1734–1744

Johnels AG, Svensson GSO (1954) On the biology of *Protopterus annectens* (Owen). Arkiv Zoologi 7:131–164

Joyce DA, Lunt DH, Genner MJ, Turner GF, Bills R, Seehausen O (2011) Repeated colonization and hybridization in Lake Malawi cichlids. Curr Biol 21:R108–R109

Kabanda T, Jury M (1999) Inter-annual variability of short rains over northern Tanzania. Climate Res 13:231–241

Kaggwa RC, Kansiime F, Denny P, Van Dam PP (2005) A preliminary assessment of the aquaculture potential of two wetlands located in the northern shores of Lake Victoria, Uganda. In: Vymazal J (ed) Natural and constructed wetlands: nutrients, metals and management (conference proceedings). Backhuys Publishers, Leiden, Netherlands pp 350–368

Karsten KB, Andriamandimbiarisoa LN, Fox SF, Raxworthy CJ (2008) A unique life history among tetrapods: an annual chameleon living mostly as an egg. Proc Natl Acad Sci U S A 105:8980–8984

Kemp A (1986) The biology of the Australian lungfish, *Neoceratodus forsteri* (Krefft 1870). J Morphol 190(S1):181–198

Khudoley VV (1984) Use of aquarium fish, *Danio rerio* and *Poecilia reticulata*, as test species for evaluation of nitrosamine carcinogenicity. Natl Cancer Inst Monogr 65:65–70

Kind P, Grigg G, Booth D (2002) Physiological responses to prolonged aquatic hypoxia in the Queensland lungfish *Neoceratodus forsteri*. Respir Physiol Neurobiol 132:179–190

Kirschner J, Weber D, Neuschl C, Franke A, Bottger M, Zielke L, Powalsky E, Groth M, Shagin D, Petzold A, Hartmann N, Englert C, Brockmann GA, Platzer M, Cellerino A, Reichwald K (2012) Mapping of quantitative trait loci controlling lifespan in the short-lived fish *Nothobranchius furzeri*- a new vertebrate model for age research. Aging Cell 11:252–261

Knaack K (1981) *Fundulosoma thierryi* the sole representative of the genus. Tropical Fish Hobbyist 29:59–66

Lanés LEK, Keppeler FW, Maltchik L (2014a) Abundance variations and life history traits of two sympatric species of Neotropical annual fish (Cyprinodontiformes: Rivulidae) in temporary ponds of southern Brazil. J Nat Hist 48:1871–1988

Lanés LEK, Goncalves AC, Volcan MV (2014b) Discovery of endangered annual killifish *Austrolebias cheradophilus* (Aplocheiloidei: Rivulidae) in Brazil, with comments on habitat, population structure and conservation status. Neurotrop Ichthyol 12:117–124

Laufer G, Arim M, Loureiro M, Pineiro-Guerra JM, Clavijio-Baquet S, Fagundex C (2009) Diet of four annual killifishes: an intra and interspecific comparison. Neurotrop Ichthyol 7:77–86

Laurin M (2010) How vertebrates left the water. University of California Press, Berkeley, CA

Lesseps RJ, Geurts van Kessel AHM, Denuce JM (1975) Cell patterns and cell movements during early development of an annual fish, *Nothobranchius neumanni*. J Exp Zool 193:137–146

Lesseps R, Hall M, Murnane MB (1979) Contact inhibition of cell movement in living embryos of an annual fish, *Nothobranchius korthausae*: its role in the switch from persistent to random cell movement. J Exp Zool 207:459–470

Levels PJ, Gubbels REMB, Denuce JM (1986) Oxygen consumption during embryonic development of the annual fish *Nothobranchius korthausae* with special reference to diapause. Comp Biochem Physiol 84A:767–770

Liu CS, Wang X, Feng WR, Li GR, Su F, Zhang SC (2012) Differential expression of aging biomarkers at different life stages of the annual fish *Nothobranchius guentheri*. Biogerontology 13:501–510

Markofsky J, Matias JR (1977a) Effects of light: dark cycles and temperature on embryonic diapause in the East African annual fish, *Nothobranchius guentheri*. Chronobiologia 4:130–131

Markofsky J, Matias JR (1977b) The effects of temperature and season of collection on the onset and duration of diapause in embryos of the annual fish *Nothobranchius guentheri*. J Exp Zool 202:49–56

Markofsky J, Matias JR, Inglima K, Vogelman JH, Orentreich N (1979) The variable effects of ambient and artificial light: dark cycles on embryonic diapause in a laboratory population of the annual fish *Nothobranchius guentheri*. J Exp Zool 83:203–215

Matias JR (1982) Embryonic diapause in annual fishes: evaporative water loss and survival. Experientia 38:1315–1317

Matias JR (1983) The effect of exposure to gaseous ammonia on the duration of diapause II in the embryos of the annual fish, *Nothobranchius guentheri*. Experientia 39:1148–1150

Matias JR (1984) The stage-dependent resistance of the chorion to external chemical damage and its relationship to embryonic diapause in the annual fish, *Nothobranchius guentheri*. Experientia 40:753–754

Matias JR, Adrias AQ (2010) The use of annual killifish in the biocontrol of the aquatic stages of mosquitoes in temporary bodies of fresh water; a potential new tool in vector control. Parasites Vectors 46:3

Matias JR, Markofsky J (1978) The survival of embryos of the annual fish *Nothobranchius guentheri* exposed to temperature extremes and the subsequent effects on embryonic diapause. J Exp Zool 204:219–228

Meintjes S (1996) Seasonal changes in the invertebrate community of small shallow ephemeral pans at Bain's Vlei, South Africa. Hydrobiologia 317:51–64

Merron GS (1989) The annual Okavango catfish run. Afr Wildl 43:302–305

Minshull J (2008) Dry season fish survival in isolated pools and within sand-beds in the Mzingwane River, Zimbabwe. Afr J Aquat Sci 33:95–98

Mlewa CM, Green JM (2004) Biology of the marbled lungfish, *Protopterus aethiopicus* Heckel, in Lake Baringo, Kenya. Afr J Ecol 42:338–345

Mlewa CHM, Green JM, Simms A (2005) Movement and habitat use by the marbled lungfish *Protopterus aethiopicus* Heckel 1851 in Lake Baringo, Kenya. Hydrobiologia 537:229–238

Morgan DL, Gill HS, Potter IC (1998) Distribution, identification and biology of freshwater fishes in south-western Australia. Rec West Aust Mus (Supp 56):1–97

Murphy WJ, Collier GE (1997) A molecular phylogeny for Aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): the role of vicariance and the origins of annualism. Mol Biol Evol 14:790–799

Neumann W (2008) Prachtgrundkärpflinge. Deutsche Killifisch Gemeinschaft

Nyina-Wamwiza L, Wathelet B, Kestemont P (2007) Potential of local agricultural by-products for the rearing of African catfish *Clarias gariepinus* in Rwanda: effects on growth, feed utilization and body composition. Aquacult Res 38:206–214

Parenti LR (1981) A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bull Am Mus Nat Hist 168:335–557

Passos C, Tassino B, Loureiro M, Rosenthal GG (2013) Intra- and intersexual selection on male body size in the annual killifish *Austrolebias charrua*. Behav Process 96:20–26

Podrabsky JE (1999) Husbandry of the annual killifish *Austrofundulus limnaeus* with special emphasis on the collection and rearing of embryos. Environ Biol Fishes 54:421–431

Podrabsky JE, Culpepper KM (2012) Cell cycle regulation during development and dormancy in embryos of the annual killifish *Austrofundulus limnaeus*. Cell Cycle 11:1697–1704

Podrabsky JE, Hand SC (1999) The bioenergetics of embryonic diapause in an annual killifish, *Austrofundulus limnaeus*. J Exp Biol 202:2567–2580

Podrabsky JE, Somero GN (2003) Changes in gene expression associated associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. J Exp Biol 207:2237–2254

Podrabsky JE, Hrbek T, Hand SC (1998) Physical and chemical characteristics of ephemeral pond habitats in the Maracaibo basin and Llanos region of Venezuela. Hydrobiologia 362:67–78

Podrabsky JE, Carpenter JF, Hand SC (2001) Survival of water stress in annual fish embryos: dehydration avoidance and egg envelope amyloid fibers. Am J Physiol 280:R123–R131

Podrabsky JE, Lopez JP, Fan TWM, Higashi R, Somero GN (2007) Extreme anoxia tolerance in embryos of the annual killifish *Austrofundulus limnaeus*: insights from a metabolomics analysis. J Exp Biol 210:2253–2266

Podrabsky JE, Clelen D, Crawshaw LI (2008) Temperature preference and reproductive fitness of the annual killifish *Austrofundulus limnaeus* exposed to constant and fluctuating temperatures. J Comp Physiol A 194:385–393

Podrabsky JE, Garrett IDF, Kohl ZF (2010) Alternative developmental pathways associated with diapause regulated by temperature and maternal influences in embryos of the annual killifish *Austrofundulus limnaeus*. J Exp Biol 213:3280–3288

Podrabsky JE, Riggs CL, Duerr JM (2012) Anoxia tolerance during vertebrate development – insights from studies on the annual killifish *Austrofundulus limnaeus*. In: Padilla P (ed) Anoxia. InTech, Rijeka, pp 3–24

Polačik M, Reichard M (2009) Indirect fitness benefits are not related to male dominance in a killifish. Behav Ecol Sociobiol 63:1427–1435

Polačik M, Reichard M (2010) Diet overlap among three sympatric African annual killifish species (*Nothobranchius* spp.) from Mozambique. J Fish Biol 77:754–768

Polačik M, Reichard M (2011) Asymmetric reproductive isolation between two sympatric annual killifish with extremely short lifespans. PLoS One 6:e22684

Polačik M, Donner M, Reichard M (2011) Age structure of annual *Nothobranchius* fishes in Mozambique: is there a hatching synchrony? J Fish Biol 78:796–809

Polačik M, Blažek R, Řežucha R, Vrtílek M, Terzibasi Tozzini E, Reichard M (2014a) Alternative intrapopulation life history strategies and their trade-offs in an African annual fish. J Evol Biol 27:854–865

Polačik M, Harrod C, Blažek R, Reichard M (2014b) Trophic niche partitioning in communities of African annual fish: evidence from stable isotopes. Hydrobiologia 721:99–106

Pusey BJ (1986) The effect of starvation on oxygen consumption and nitrogen excretion in *Lepidogalaxias salamandroides* (Mees). J Comp Physiol B 156:701–705

Pusey BJ (1989) Aestivation in the teleost fish *Lepidogalaxias salamandroides* (Mees). Comp Biochem Physiol 92A:137–138

Pusey B (1990) Seasonality, aestivation and the life history of the salamanderfish *Lepidogalaxias salamandroides* (Pisces: Lepidogalaxiidae). Environ Biol Fishes 29:15–26

Pusey B, Stewart T (1989) Internal fertilization in *Lepidogalaxias salamandroides* Mees (Pisces: Lepidogalaxiidae). Zool J Linn Soc 97:69–79

Reichard M (2010) *Nothobranchius kadleci* (Cyprinodontiformes: Nothobranchiidae), a new species of annual killifish from central Mozambique. Zootaxa 2332:49–60

Reichard M, Polačik M (2010) Reproductive isolating barriers between colour-differentiated populations of an African annual killifish, *Nothobranchius korthausae* (Cyprinodontiformes). Biol J Linn Soc 100:62–72

Reichard M, Polačik M, Sedláček O (2009) Distribution, colour polymorphism and habitat use of the African killifish, *Nothobranchius furzeri*, the vertebrate with the shortest lifespan. J Fish Biol 74:198–212

Reichard M, Watters BR, Wildekamp RH, Sonnenberg R, Nagy B, Polačik M, Valdesalici S, Cellerino A, Cooper BJ, Hengstler H, Rosenstock J, Sainthouse I (2010) Potential negative impact and low effectiveness in the use of African annual killifish in the biocontrol of aquatic mosquito larvae in temporary water bodies. Parasites Vectors 3:89

Reis RE, Ferraris CJ, Kullander SO (2003) Checklist of the freshwater fishes of South and Central America. EDIPUCRS Porto Alegre, 729 pp

Sadeu CB, Mikolasek O, Pouomogne V, Eyango MT (2013) The use of wild catfish (*Clarias* spp.) in combination with Nile tilapia (*Oreochromis niloticus* L.) in Western Cameroon: technical performances, interests, and limitations. J Appl Aquacult 25:359–368

Saha N, Das L, Dutta S, Goswami UC (2001) Role of ureogenesis in the mud-dwelled Singhi catfish (*Heteropneustes fossilis*) under condition of water shortage. Comp Biochem Physiol A Mol Integr Physiol 128:137–146

Sawaya P, Mimura I, Mimura OM (1981) Gills and lungs of *Lepidosiren paradoxa* Pisces Dipnoi histophysiology. Boletim de Fisiologia Animal 5:107–120

Scheel JJ (1990) Atlas of killifishes of the old world. T.F.H. Publications, Neptune City, NJ

Sheaves M, Johnston R (2008) Influence of marine and freshwater connectivity on the dynamics of subtropical estuarine wetland fish metapopulations. Mar Ecol Prog Ser 357:225–243

Shedd TR, Widder MW, Toussaint MW, Sunkel MC, Hull E (1999) Evaluation of the annual killifish *Nothobranchius guentheri* as a tool for rapid acute toxicity screening. Environ Toxicol Chem 18:2258–2261

Shine C, de Klemm C (1999) Wetlands, water and the law. Using law to advance wetland conservation and wise use. IUCN, Gland, Switzerland, 330 pp

Singh BN, Hughes GM (1971) Respiration of an air-breathing catfish *Clarias batrachus* (Linn.). J Exp Biol 55:421–434

Siziba N, Chimbari MJ, Mosepele K, Masundire H (2011) Spatial and temporal variations in densities of small fishes across different temporary floodplain types of the lower Okavango Delta, Botswana. Afr J Aquat Sci 36:309–320

Smith HW (1931) Metabolism of the lung-fish, *Protopterus aethiopicus*. J Biol Chem 138:97–130

Smith HW (1939) The lungfish. Nat Hist 44:224–225

Spence R, Fatema MK, Reichard M, Huq KA, Wahab MA, Ahmed ZF, Smith C (2006) The distribution and habitat preferences of the zebrafish in Bangladesh. J Fish Biol 69:1435–1448

Suffia D (2004) Breeding and husbandry – *Fundulopanchax puerzli*. J Am Killifish Assoc 37:150–152

Tay YL, Loong AM, Hiong KC, Lee SJ, Tng YYM, Wee NLJ, Lee SML, Wong WP, Chew SF, Wilson JM, Ip YK (2006) Active ammonia transport and excretory nitrogen metabolism in the climbing perch, *Anabas testudineus*, during 4 days of emersion or 10 minutes of forced exercise on land. J Exp Biol 209:4475–4489

Terzibasi E, Valenzano DR, Benedetti M, Roncaglia P, Cattaneo A, Domenici L, Cellerino A (2008) Large differences in aging phenotype between strains of the short-lived annual fish *Nothobranchius furzeri*. PLoS One 3:e3866

Terzibasi-Tozzini E, Dorn A, Ng'oma E, Polačik M, Blažek R, Reichwald K, Petzold A, Watters B, Reichard M, Cellerino A (2013) Parallel evolution of senescence in annual fishes in response to extrinsic mortality, BMC. Evol Biol 13:77

Valdesalici S (2007) A new species of the genus *Nothobranchius* (Cyprinodontiformes: Nothobranchiidae) from the coastal area of northeastern Mozambique. Zootaxa 1587:61–68

Valdesalici S (2013) *Pronothobranchius chirioi* n. sp. a new annual killifish species from the Niger River drainage, with redescriptions of *P. kiyawensis, P. gambiensis* and *P. seymouri* (Cyprinodontiformes: Nothobranchiidae). Killi-Data Series 2013:21–41

Valdesalici S, Cellerino A (2003) Extremely short lifespan in the annual fish *Nothobranchius furzeri*. Proc Roy Soc Lond Ser B Biol Sci 270:S189–S191

Valenzano DR, Terzibasi E, Genade T, Cattaneo A, Domenici L, Cellerino A (2006) Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr Biol 16:296–300

Valenzano DR, Sharp S, Brunet A (2011) Transposon-mediated transgenesis in the short-lived African Killifish *Nothobranchius furzeri*, a vertebrate model for aging. Genes Genom Genet 7:531–538

van der Hoeven JC, Bruggeman IM, Alink GM, Koeman JH (1982) The killifish *Nothobranchius rachowi*, a new animal in genetic toxicology. Mutat Res 97:35–42

van der Waal B (1997) Some observations on the fish life in a seasonal sand river. South Afr J Aquat Sci 23:95–102

van der Waal B (1998) Survival strategies of sharptooth catfish *Clarias gariepinus* in desiccating pans in the northern Kruger National Park. Koedoe 41:131–138

Vicuna S, Gironas J, Meza FJ, Cruzat ML, Jelinek M, Bustos E, Poblete D, Bambach N (2013) Exploring possible connections between hydrological extreme events and climate change in central south Chile. Hydrolog Sci J 58:1598–1619

Watters B (2009) The ecology and distribution of *Nothobranchius* fishes. J Am Killifish Assoc 42:37–76

Wedderburn SD, Barnes TC, Hillyard KA (2014) Shifts in fish assemblages indicate failed recovery of threatened species following prolonged drought in terminating lakes of the Murray-Darling Basin, Australia. Hydrobiologia 730:179–190

White S, Ondračková M, Reichard M (2012) Hydrologic connectivity affects fish assemblage structure, diversity and ecological traits in the unregulated Gambia River, West Africa. Biotropica 44:521–530

Wildekamp RH (1983a) *Pronotbranchius kyiawensis* (Ahl, 1928). Aquarien- und Terrarien-Zeitschrift 36:339–341

Wildekamp RH (1983b) Preliminary study of the Somalian *Nothobranchius* species of larvivorous fish. WHO report for Anti-Malaria Service of the Somali Democratic Republic

Wildekamp RH (2004) A world of killies: atlas of the oviparous cyprinodontiform fishes of the world, vol 4. American Killifish Association, Elyria, OH

Williams WD (1985) Biotic adaptations in temporary lentic waters, with special reference to those in semi-arid and arid regions. Hydrobiologia 125:85–110

Winemiller KO, Rose KA (1992) Patterns of life history diversification in North American fishes: implications for population regulation. Can J Fish Aquat Sci 49:2196–2218

Wourms JP (1972a) Developmental biology of annual fishes I. Stages in the normal development of *Austrofundulus myersi* Dahl. J Exp Zool 182:143–168

Wourms JP (1972b) The developmental biology of annual fish II. Naturally occurring dispersion and reaggregation of blastomeres during the development of annual fish eggs. J Exp Zool 182:169–200

Wourms JP (1972c) The developmental biology of annual fishes III. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. J Exp Zool 182:389–414

# Evolutionary Toxicology: Population Adaptation in Response to Anthropogenic Pollution

**Elias M. Oziolor and Cole W. Matson**

**Abstract** Anthropogenic activity has affected nearly every environment on the planet. The changes that have occurred as a consequence of human activities have altered aquatic habitats by exacerbating already existing extreme environments and by introducing novel stressors. In some cases, particularly adjacent to heavily industrialized areas, these changes have introduced sufficient novel selective pressures to drive resident populations to genetically adapt in order to survive in the altered habitats, while species that were unable to adapt have been extirpated from these extreme environments. In this chapter, we aim to explore the effects of natural and novel stressors, resulting from anthropogenic activity, on fish populations. We will provide an overview of the possible multi-generational outcomes of anthropogenic contamination, as well as explore documented examples of population-wide changes that have occurred. We present case studies, including population responses to UV light, radionuclides, and metals contamination, as well as adaptational responses to persistent organic pollutants. Through this examination, we aim to not only give an overview of the existing evolutionary changes in fish populations in response to anthropogenic contamination, but also identify future areas of research on the impacts, long-term persistence, and ecological significance of these effects.

## 1 Anthropogenic Changes to Aquatic Environments

There is great natural variability in aquatic environments, which has provided the grounds for the phenomenal diversification and adaptation of fish species to fill available ecological niches. In addition to adaptation along natural environmental gradients, some fish possess unique phenotypes, which allow them to survive under the most extreme environmental conditions. Modern extreme environments include many aquatic habitats that have been so thoroughly contaminated by human activities that many aquatic species are unable to survive or reproduce in them.

---

E.M. Oziolor • C.W. Matson (✉)
Department of Environmental Science, Institute for Biomedical Studies, and Center for Reservoir and Aquatic Systems Research (CRASR), Baylor University, Waco, TX, USA
e-mail: cole_matson@baylor.edu

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_11

These are generally located in heavily industrialized areas or in waterways downstream of industrial activity, and are characterized by dramatic reductions in species diversity. Anthropogenic changes to the climate and environment are increasingly altering freshwater and marine habitats, and will require adaptive responses from resident fish populations to allow for their persistence (Moe et al. 2013; Rosenzweig and Neofotis 2013; Anderegg et al. 2010). As adaptation involves allele frequency changes over many generations, species with shorter generation times are likely to adapt more rapidly to ongoing anthropogenic environmental alterations. Thus, human-induced evolutionary change has been observed more often in simple unicellular or invertebrate species (Palumbi 2001; Medina et al. 2007).

Human-induced environmental changes can act to exacerbate preexisting extreme environments by increasing natural stressors, which include, but are not limited to, salinity/conductivity, temperature, metals, UV radiation, and pH balance (Kaushal et al. 2005; Poloczanska et al. 2013; Core Writing Team et al. 2007; Dijkstra et al. 2013; Veron 2008). In addition to augmenting existing extreme environments, anthropogenic activities have caused compounds with low natural occurrences to reach concentrations high enough to cause toxicity, thus creating new selective pressures shaping evolutionary change in natural populations. These new selective pressures will be referred to hereafter as novel stressors. Some pollutants, such as synthetic estrogenic compounds, pharmaceuticals, polychlorinated dibenzo dioxins/furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), have known modes of action affecting the behavior, reproduction, and survival of fish (Knecht et al. 2013; Incardona et al. 2004, 2011; Clark et al. 2010; Matson et al. 2009; Wassenberg and Di Giulio 2004a; Nebert et al. 2004; Mennigen et al. 2011). Sources for these contaminants include heavy industrial activity, which leads to the production of many persistent organic pollutants (POPs) (Wenning et al. 1993; Walker et al. 2005), and urban water use, including the release of pharmaceuticals into freshwater and marine environments (Camacho-Munoz et al. 2014). Many of these compounds have been shown to adversely affect developmental processes (Wassenberg et al. 2002), survival (Nacci et al. 2010), and reproduction (Valenti et al. 2012) at environmentally relevant concentrations. As a result, environments with high concentrations of contamination pose a threat to resident aquatic biota (Wassenberg et al. 2002). Environmentally relevant concentrations of estrogenic compounds have been tested under realistic exposure conditions and have been shown to be detrimental to populations of fish (Kidd et al. 2007), whereas some persistent pollutants have already led to evolutionary adaptation in fish populations in the wild (Wirgin et al. 2011; Nacci et al. 2010). By focusing on the population-level responses of aquatic organisms to anthropogenic pollution, we account for the cumulative impacts of these novel extreme environments on organismal survival and reproduction. As discovery of human-induced phenotypic and genetic change has increased, attention to the developing field of evolutionary toxicology has also expanded and is likely to continue to grow, as new anthropogenic stressors are unleashed upon the environment (Bickham et al. 2000).

The effects of environmental stressors on populations of fish depend largely on the magnitude, duration, and frequency of the exposure. Even short-term exposures, such as transient oil spills or temporary encounters of a contaminated environment by a migratory species, can lead to trans- or multi-generational effects in organisms (Dubansky et al. 2013; Everett et al. 2012; Gao et al. 2011). Chronic exposure of sufficient magnitude to affect reproductive success or the integrity of genetic material in individuals can lead to evolutionary adaptation, making individuals more fit for those environments (Bickham 2011). Evolutionary theory would predict rapid spread and fixation of preexisting alleles (even when initially rare), if they conferred a selective advantage in responding to a new anthropogenic stressor (Bickham 2011; Bickham et al. 2000), while alternatively, the likelihood of a beneficial de novo mutation becoming common within a fish population in a century or less is less likely. Either way, we can only evaluate the adaptive changes after many generations, which makes evolutionary studies difficult when we are considering anthropogenic stressors introduced into the environment generally less than a century ago.

## 2 Organismal Responses to Stressors

When attempting to observe the effects of a stressor on populations of fish, we need to consider not only the characteristics of the event itself but also the various ways by which organisms are able to respond. Acclimation refers to a response in which exposed individuals are capable of dealing with the stressor by altering gene expression, protein levels or activity, or other physiological processes. In this case, the organisms can tolerate the stressful event depending on their genetic makeup and the degree of plasticity of relevant traits. Such changes in expression or activity can have trans-generational effects, but they are not genetically heritable. Thus, in the absence of the stressor, the changes in gene expression or physiology are likely to disappear within two generations. Mounting a physiological response can have various consequences, including higher energy expenditure, and long-term costs often depend on the severity and duration of the response.

There exists a middle ground between acclimation and adaptation that may result in long-term or multi-generational effects of stressors on populations of fish. The group of responses that encompass non-genetic, yet heritable, changes that affect the expression or behavior of genes are called epigenetic alterations (Laird 2003). Epigenetic events are often environmentally driven and include, but are not limited to, DNA methylation on cytosine nucleotides, acetylation on lysine residues of histones, and differential microRNA expression (Wolffe and Matzke 1999). Events such as the methylation of cytosine have been observed to increase mutation rates in those specific genomic regions and provide one of the connections between short-term exposures and resulting DNA alterations (Fryxell and Moon 2005; Gonzalgo and Jones 1997). Thus, with epigenetics in mind, even short-term environmental events can result in heritable multi-generational outcomes, including altered phenotypes resulting from differential gene expression (Guerrero-Bosagna and Skinner 2012; Pfennig and Servedio 2013). The field of epigenetics is very young, but researchers are making major strides in the understanding of this bridge between acclimation and adaptation. Even though we have a limited understanding of the impacts of these heritable non-genetic changes, they provide a potentially very important mechanism by which populations can respond to new stressors more rapidly than through more traditional evolutionary processes. Including epigenetic processes within the field of evolutionary toxicology or human-induced evolution radically expands the number of populations that could potentially be studied.

To study adaptive responses to anthropogenic contaminants, a model organism is needed that has sufficient genetic variability to provide the capacity for rapid adaptation to varying selective pressures. Teleost fishes are a unique and rich system to study novel gene function, partially because of the multiple gene duplications that have occurred in some lineages, resulting in as many as eight gene copies of some genes (Pittman et al. 2013). The availability of multiple gene copies reduces the selective disadvantage normally associated with new mutations. It also increases the opportunity for differential gene regulation, alternative splicing, or other post-translational modifications (Pittman et al. 2013). In addition to gene duplications, the potential for rapid environmental changes in aquatic systems has also selected for species with the capacity to acclimate and adapt to fluctuating environmental conditions. One example of this would be the highly variable nature of estuarine environments favoring euryhaline fish species, which are able to survive dramatic swings in salinity. Such conditions seem to produce robust species that have the genetic capability to respond relatively quickly to changes in environmental stressors (Pittman et al. 2013). Some euryhaline teleosts have previously been studied for their capability to tolerate high variability in temperature, salinity, and pollutants (Crawford and Powers 1992; Whitehead et al. 2011; Nacci et al. 1999). In the case of thermal adaptation, it has been shown that both acclimation and genetic alterations contribute to the physiological adaptation in Atlantic killifish (*Fundulus heteroclitus*) from different sites (Crawford and Powers 1989). Multiple similar studies suggest that estuarine teleosts may be valuable model organisms for identifying genetic and physiological responses to novel stressors. Their adaptability, ubiquitous presence, and short generation times allow for the investigation of the evolutionary effects of contaminants over relatively short time frames.

A previous summary focused on the mechanistic details of specific examples of adaptation to contaminants in North American fish populations (Wirgin and Waldman 2004). In this chapter, we aim to give an overview of anthropogenic alterations to the environment that are likely to or have already driven evolutionary adaptations in fish. We will discuss two major categories of anthropogenic environmental alterations: alteration of existing natural stressors and the introduction of novel stressors. In order to more comprehensively cover the possible genetic influences, we will discuss both short-term responses with multi-generational effects as well as mutations and shifts in allele frequencies that have led to evolutionary adaptation in fish species.

# 3 Altered Natural Stressors

## *3.1 UV Light*

One of the many natural stressors that has been altered by anthropogenic activity is UV light penetration into aquatic environments. Factors such as the thickness of the ozone layer, amount of colored dissolved organic matter, and cloud density dictate the intensity and the amount of UV light that reaches aquatic organisms (Hader et al. 2011). Among the well-studied anthropogenic contributions to the increase of both UV-A (315–400 nm) and UV-B (280–315 nm) radiation is ozone depletion (Hader et al. 2011; Core Writing Team et al. 2007). The mechanisms of UV toxicity have been well documented to include dimerizations of thymine DNA bases, increase of reactive oxygen species (ROS) production, and an increased production of the adduct 8-hydroxyguanosine—a biomarker of oxidative stress (Durbeej and Eriksson 2002; Zhang et al. 2004). In addition, UV light has been known to exasperate the toxicity of some PAHs in various organisms because of common modes of toxicity or photoactivation of these compounds (Marquis et al. 2009; Nikkila et al. 1999; Shemer and Linden 2007; Wei et al. 2007). The synergistic increase in toxicity, however, is also accompanied by higher rates of degradation of PAHs due to UV light (Xu and Li 2014; Buth et al. 2010; Peachey 2005). Given these possible adverse effects of UV light, aquatic organisms that are dependent on light, such as algae, have developed methods to minimize UV exposure via mycosporine-like amino acids or absorptive surface pigments (Hader et al. 2011). On the other hand, some fish spend sensitive portions of their development exposed to sunlight in shallow waters, which leaves them vulnerable to the harmful effects of UV radiation (Hakkinen et al. 2004).

Since UV exposure can alter diverse molecular pathways, the effects of this stressor have been studied in various organisms. Increased mortality in aquatic invertebrates and fish has been observed as an effect of UV exposure, which suggests that it may be the driver behind evolutionary change (Nikkila et al. 1999; Jokinen et al. 2011). An example is the intertidal fish *Girella laevifrons*, which has been observed to spend large portions of its development in waters exposed to high levels of UV radiation (Carrasco-Malio et al. 2014). Since Chilean waters accommodate a multitude of migratory and resident species, biomonitoring efforts are in place to follow the effects of increasing UV. However, recent investigations suggest that, despite being one of the few resident species, *G. laevifrons* may be an unsuitable species for biomonitoring, due to an apparent resistance to UV-induced mortality (Carrasco-Malio et al. 2014). Even though increased enzymatic activity against ROS was uncovered (including superoxide dismutase, catalase, and lipid peroxidation) along with an increase in DNA damage in the liver, the researchers did not observe the previously reported mortality in *G. laevifrons* in response to larval UV exposure in the laboratory (Carrasco-Malio et al. 2014). Unfortunately, the results of this experiment are not enough to allow discrimination between acclimation, epigenetic mechanisms, or genetic causes of this apparent protection. A mechanistic explanation or a comparison with a similar species could allow an in-depth interpretation of the results, but the current evidence is promising in terms of documenting adaptation to UV stress. The hypothesized explanation for the lack of mortality in *G. laevifrons* is that increasing UV radiation in this southern hemisphere intertidal zone acted as a selective pressure to drive this species to adapt to the potentially lethal effects of high UV exposures (Carrasco-Malio et al. 2014).

## *3.2 Radionuclides*

Another source of radiation pollution, which has increased dramatically through human activity, is radionuclide contamination. Natural sources of radioactivity exist and have been shown to have genotoxic effects on human populations resident in naturally radioactive areas (Forster et al. 2002). On the other hand, anthropogenic radionuclide contamination has been widely studied and often stems from nuclear power plant waste or in a few cases from significant disasters, like the Chernobyl and Fukushima nuclear disasters from 1986 and 2011, respectively (Steinhauser et al. 2014). As with radiation-exposed human populations, radionuclides have genotoxic effects on contaminated aquatic biota, with the study focus mainly being DNA damage (Theodorakis et al. 1997).

An investigation of channel catfish (*Ictalurus punctatus*) populations from radiocesium-contaminated nuclear reactor cooling ponds at Chernobyl showed that fish appear to exhibit higher levels of double-stranded DNA damage, but not an increase in micronuclei (Sugg et al. 1996). The study was unable to determine whether adaptation or acclimation was the cause of the lower apparent micronucleus formation, but it confirmed that radiation contamination could have effects on fish populations at the genetic level (Sugg et al. 1996). A second study focused on the decades-old radionuclide contamination of ponds at the U.S. Department of Energy's Savannah River Site. Theodorakis and Shugart (1998) investigated populations of mosquitofishes, *Gambusia affinis* and *G. holbrooki*, to determine whether long-term exposure in contaminated ponds had affected the genetic integrity of resident populations. These species have short generation times, and their populations were restricted to the study ponds. Researchers found that after multiple generations of exposure, populations of exposed mosquitofishes exhibit higher levels of DNA strand breakage as well as reduced fertility (Theodorakis and Shugart 1998). On the other hand, some individuals in the contaminated sites exhibited lower DNA strand breakage and their genotypes, as determined by random amplified polymorphic DNA (RAPD) assay, were different from the rest of the sampled fish, which suggested a genetic basis for the phenotypic differences (Theodorakis et al. 1999). These studies imply that there may be a possibility of different genotypes providing different protection from radionuclide contamination in mosquitofishes and that human-induced evolution has occurred (Theodorakis et al. 1999).

There are a multitude of existing selective pressures on the planet and anthropogenic events are intentionally or unintentionally contributing to their magnitude. The example studies included in this chapter suggest that the increases of some stressors have led to emerging selective pressures or to the amplification of existing pressures through human activity. Aquatic organisms faced with such challenges may or may not have the capacity to adapt, depending on the natural history of the organism, the availability of genetic resources, and/or the complexity of altered selective pressure. Anthropogenic activities have led to significant alterations in the selection landscape including the increased release of estrogenic compounds, changes in temperature, UV permeability, radionuclide contamination, salinity, dissolved oxygen, and pH. Unfortunately, it is currently not possible to accurately predict the evolutionary impacts of anthropogenic alterations to the existing stressors. However, we are rapidly gaining insight into the mechanisms through which selection may act. This process is even more difficult when considering the potential evolutionary impacts of emerging contaminants.

## 4 Novel Stressors

This section considers stressors that prior to anthropogenic influences may have been present in the environment, but not at levels likely to have had significant toxicological effects on fish. These are often chemicals that are persistent in the environment and are highly toxic to fish, allowing those pollutants to act as selective pressures for extended periods of time. We will discuss most of these compounds in terms of putative evolutionary adaptations in fish. While there is certainly interest in the short-term impacts of many of these classes of contaminants, we will only focus on observed and potential adaptation in fish populations in response to chronic exposures.

### *4.1 Metals*

The concentrations and bioavailability of many toxic metals have increased dramatically in aquatic environments as a result of human activities. Various metals have toxic properties due to interference with biological pathways in fish (Strydom et al. 2006). There are multiple sources of metals, both natural and more importantly anthropogenic, and each of them produces unique compositions of metals pollution that aquatic organisms experience. Mining for coal and minerals is an environmental concern in many parts of the world. For example, acid mine drainage often leaves environments heavily contaminated with metals (Griffith et al. 2012; Pumure et al. 2010). Concern for the integrity of the impacted environments is substantial, which often leads to wetlands being constructed to attempt to contain the considerable contaminant loads being released, and reduce impacts on downstream habitats (Turker et al. 2014; Guittonny-Philippe et al. 2014). The biological implications of high metals concentrations have been investigated for many years, and some studies have suggested that adaptational processes may have led to lower retention rates of metals in fish populations chronically exposed to high metals concentrations for over 50 years (Jeffree et al. 2014). These studies are not conclusive regarding the causality or mechanisms of this adaptation, but the increasing contamination and frequency of acid mine drainage and mountaintop removal beget the study of the biological impacts of observed adaptation.

A large portion of evolutionary toxicology studies focus on finding adaptive responses in the field by comparing populations of contaminant-exposed fish to reference populations, assuming that reference sites represent ancestral populations prior to the impact of contamination (Klerks et al. 2011). Such assays carry high environmental relevance, but sometimes lack the ability to establish causality. Another set of investigations focus on establishing and quantifying the causal links between contamination and adaptation through artificial selection experiments in the laboratory (Klerks et al. 2011). One significant advantage of these studies is that a true control can be included (i.e., a subset of individuals is not exposed while another subset is exposed to the contaminant for multiple generations). This allows for the derivation of true quantitative knowledge about the causality and heritability of adaptive traits (Klerks et al. 2011). On the other hand, long generation turnover times and a simplified exposure scenario are challenges inherent within such experiments, particularly when using vertebrates (Klerks et al. 2011). An artificial selection experiment exposed a population of least killifish, *Heterandria formosa*, to $LC_{50}$ values of cadmium for six consecutive generations, continuing exposures of each generation until at least 50 % of the generation died from Cd exposures (Xie and Klerks 2003). The surviving individuals (generally 15–25 %) were used to produce consecutive generations. The final generation (sixth) produced in this long-term selection study exhibited a threefold increase in Cd resistance, relative to control fish (Xie and Klerks 2003). The realized heritability of the resistance was relatively high ($h^2 = 0.5$), allowing it to be carried to further generations quite rapidly (Xie and Klerks 2003).

When observing evolutionary events, one also needs to consider the costs that may be associated with the newly acquired phenotype. In the case of least killifish, the cadmium-resistant populations had lower heat tolerance, smaller size, as well as lower lifetime fecundity, brood size, and female life span (Xie and Klerks 2003, 2004). These results demonstrate that the effects of adaptation can be complex and often unpredictable. Populations of adapted fish may be compromised unexpectedly, without visible mortality from the selective stressor, leading to a multitude of possible susceptibilities. In this case, an artificial selection experiment was able to quantify and characterize the ability of organisms to adapt to a single contaminant, while other environmental studies have to deal with complex mixtures and their possible adaptational effects.

A separate study identified populations of Atlantic killifish (*F. heteroclitus*), which were present at a site with high concentrations of dioxin and metals, to have developed resistance to methylmercury toxicity (Weis et al. 1981a, b). In addition to the protection from the early teratogenic effects of methylmercury, $F_1$-larvae from contaminated site fish also retained higher prey capture efficiency when dosed with meHg, compared to larvae from reference sites (Zhou et al. 1996). These results suggest that adaptive responses to metals can be observed in field-exposed populations, supporting the idea that metals contamination can have evolutionary impacts on aquatic organisms.

## *4.2 Polycyclic Aromatic Hydrocarbons*

Polycyclic aromatic hydrocarbons (PAHs) are a class of widely distributed contaminants, formed by human activities as a by-product of incomplete combustion. They can also be found naturally in oil, natural gas, and coal, and can be produced by forest fires and volcanoes (Walker et al. 2005). These compounds are always found as complex mixtures, and several PAHs are known to be carcinogenic to a wide variety of organisms, including fish and mammals (Schneider et al. 2002; Hermann 1981; Karahalil et al. 1998). Mixtures of PAHs have also been found to have immunotoxic effects (Reynaud and Deschaux 2006; Carlson et al. 2004) and to cause DNA adducts (Jung et al. 2009) and cardiac deformities in aquatic organisms (Clark et al. 2010). Wastewater runoff, in addition to other anthropogenic activities, has been shown to move complex mixtures of PAHs, allowing them to deposit in coastal regions and estuaries (Walker et al. 2005; Menzie et al. 2002; Daskalakis and Oconnor 1995) and causing them to concentrate in surface sediment (Viguri et al. 2002). Thus, the highly teratogenic properties of PAH mixtures to fish embryos can introduce new selective pressures for estuarine fish (Wassenberg and Di Giulio 2004b).

The Atlantic Wood Industries Superfund site (AWI) on the Elizabeth River in Virginia has been on the National Priorities List since 1990 (Fig. 1) (Landers 2006). The contamination at the AWI mainly consists of PAHs (Mitra et al. 1999). PAHs are generally readily biotransformed by fish, but this process involves the activation of the aryl hydrocarbon receptor (AHR) pathway and has been linked to cardiovascular deformities in developing fish embryos, a shared mode of toxicity with some dioxins and co-planar PCBs (Heinrich et al. 1986; Ashurst et al. 1983; Wassenberg and Di Giulio 2004b). The source of the PAH contamination at the AWI was a wood treatment facility releasing creosote, primarily made up of heavy pyrogenic PAHs, into the river and contaminating aquatic life from 1926 to 1992 (Mitra et al. 1999). The incomplete remediation efforts to reduce PAH concentrations at the AWI included a capping of the old wood treatment facility location in 2002 and a dredging campaign in 2007 (Landers 2006). Additional dredging efforts have been planned for the contaminated sediment including the construction of a steel wall to contain the sediment, but these efforts have not yet been completed.

The first indication that fish were being impacted by contamination in the Elizabeth River was the discovery that mummichog (*F. heteroclitus*) from the AWI had a higher occurrence of hepatic neoplasms (Van Veld et al. 1991;

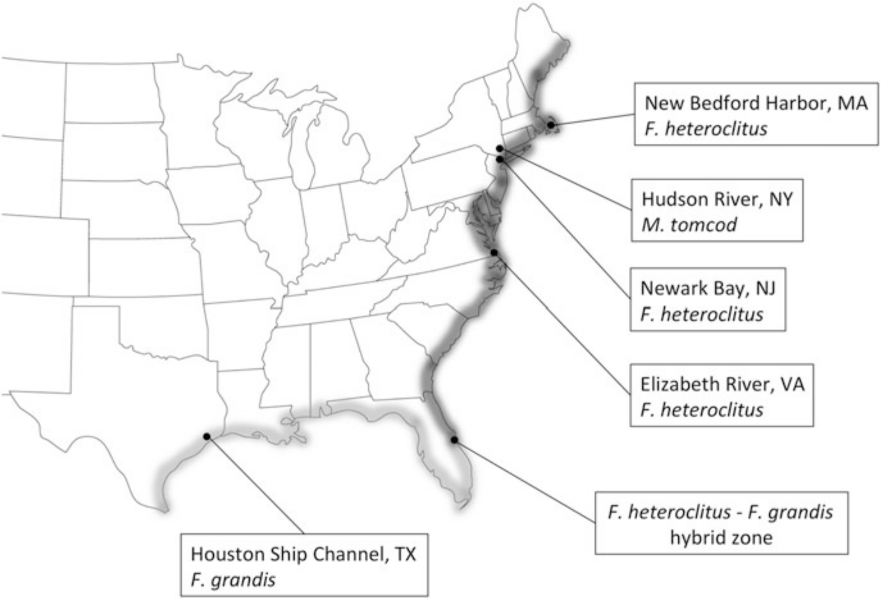


**Fig. 1** Map of the locations where adaptations in response to anthropogenic events have been documented in natural populations of fish. *Darker shading* represents zones occupied mainly by *F. heteroclitus*, while *lighter shading* represents zones primarily occupied by *F. grandis*

Vogelbein et al. 1990). The cause of those lesions was not found, but was attributed to the PAH mixtures found throughout the Elizabeth River, particularly the high concentrations found at the AWI. Following this discovery, the same investigators tested the activity of the main enzyme involved in the biotransformation of large PAHs, cytochrome P4501A (CYP1A). The expected response following PAH exposure is a significant induction of CYP1A, which is why CYP1A is used as a biomarker of PAH exposure (Nacci et al. 1998; Matson et al. 2009). An example dataset with *Fundulus grandis* shows the induction of CYP1A activity, measured through a standard ethoxyresorufin-*O*-deethylase (EROD) assay, with a chosen PCB agonist (Fig. 2). This activity is commonly seen to diminish with the onset of cardiac deformities in embryos, while the causes of this relationship are not fully understood (Fig. 2). Despite the expectation that CYP1A should be elevated in AWI, *F. heteroclitus* from this highly contaminated site had depressed levels of CYP1A (Van Veld and Westbrook 1995). These results were confirmed in $F_1$-progeny (Wills et al. 2010). In addition, protection from cardiac, tail, and bladder abnormalities in embryos was discovered and linked to the reduced activity of CYP1A, and more specifically a recalcitrant AHR pathway (Meyer et al. 2002; Ownby et al. 2002; Clark et al. 2010). Further studies went on to confirm that altered AHR2 activity was the specific AHR gene alteration likely responsible for the observed resistance, and not simply reduced CYP1A activity (Clark et al. 2010; Matson et al. 2008). These findings were consistent with mechanistic studies

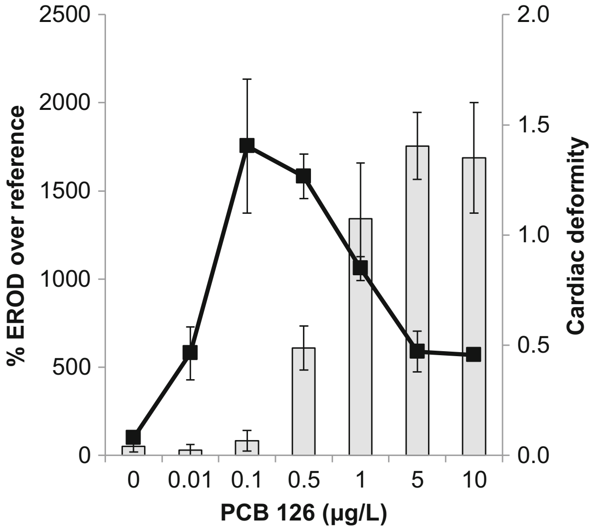


**Fig. 2** Relationship between CYP1A activity levels and developmental cardiac deformities in response to a common AHR agonist, PCB 126. When F1 embryos from a reference *F. grandis* population are dosed with PCB 126, the CYP1A activity (*black line*) is induced as part of the AHR pathway response to the toxicant. At higher concentrations, CYP1A has lower activity levels, and embryos develop cardiac deformities (*gray bars*). The inverse relationship between the two is a well-documented occurrence, while the mechanistic connection is yet to be elucidated. A subset of these data was presented in Oziolor et al. (2014)

previously done in zebrafish (*Danio rerio*; Carney et al. 2004). The control and the mechanisms by which reduced responsiveness of the AHR pathway leads to protection from PAH toxicity in development remain unknown. Since the generation turnover time for *F. heteroclitus* is close to a year, there have likely been more than 50 generations, and perhaps as many as 85, since the onset of pollution at the AWI. The identification of an adaptation in a vertebrate species to novel toxicants is a rare and interesting event.

Results documenting PAH resistance in $F_1$-mummichog embryos from the AWI were quite interesting, but failed to clarify whether they represent evolutionary adaptation or a trans-generational epigenetic response to heavy contamination. In order to distinguish between the two options, later generations reared under common garden conditions (i.e., no PAH exposure) needed to be tested. However, studies conducted to date differ somewhat in their findings regarding the heritability of protection to $F_2$-progeny (Ownby et al. 2002; Meyer and Di Giulio 2002; Clark et al. 2013a). Ownby et al. (2002) found that the resistance was maintained completely in $F_2$-progeny, whereas Meyer and Di Giulio (2002) concluded that, while there was still resistance, $F_2$-progeny were less resistant than the $F_1$-generation. Nonetheless, high levels of mortality in AWI fish in the study seem to suggest that the more resistant fish might have been eliminated from contributing to the tested $F_2$-progeny because of fitness costs associated with the resistance, and thus, the protective phenotype may have only appeared to not be heritable (Meyer and Di Giulio 2002). In addition, it is possible that epigenetic effects could be interacting with the adaptive change. The most thorough investigation of the heritability of PAH resistance in AWI mummichog was performed by Clark et al. (2013a). They found an interesting disconnect between the recalcitrance of the molecular pathway believed to be responsible for observed resistance, the AHR pathway, and the actual embryo resistance to cardiovascular defects. The $F_2$-progeny in their study regained partial AHR pathway responsiveness, yet fully retained their protection from embryotoxicity. They concluded that multiple pathways may be involved in PAH resistance, but that none of them seem to be fully genetically heritable (Clark et al. 2013a).

In addition to the physiological differences, which were shown to be at least partially heritable, *F. heteroclitus* from the AWI were genetically distant from geographically proximate populations (Mulvey et al. 2002). Combined, these results support the hypothesis that there has been a selective sweep caused by the high contamination at the location, causing these non-migratory fish to become resistant to PAH toxicity. As with most adaptations, fitness costs are to be expected, and a few have been found within the AWI population. Fluoranthene, a CYP1A-inhibiting PAH, which is also phototoxic, was found to affect AWI populations more severely in combination with UV light than observed for a reference population (Meyer and Di Giulio 2003). In addition, AWI larvae have been documented to have a reduced resistance to short-term hypoxia exposures (see chapter “Low-Oxygen Lifestyles”), as well as exhibiting decreased survival under common-garden laboratory conditions (see above), suggesting a general reduction in fitness (Meyer and Di Giulio 2003). While some fitness costs have been identified, several other attempts to identify further fitness costs have been unsuccessful. Glutathione expression is on average lower in polluted site fish, but sex and present exposure confound the significance of those results (Bacanskas et al. 2004). Some specific fitness costs were hypothesized as a result of the mechanistic basis for the PAH resistance; because of the lower CYP1A activity, *F. heteroclitus* from polluted sites were hypothesized to be more susceptible to pesticides deactivated by that enzyme (Clark and Di Giulio 2012). Contrary to this expectation, polluted site mummichogs were shown to be highly cross-resistant to the acute toxicity of two CYP1A-detoxified pesticides, a carbamate and a pyrethroid (Clark and Di Giulio 2012). In addition, even though the main contaminant load at the AWI stems from PAHs, *F. heteroclitus* from that site are also protected from the effects of PCBs (Clark et al. 2013b). Other research suggests that field-collected fish may have an impaired reproductive system and increased chromosomal damage compared to reference populations (Frederick et al. 2007; Jung et al. 2011). The issue with these studies is that they often have a difficulty differentiating between the effects of PAHs on fish in the field, versus the fitness costs driven by the adaptation of the fish. Thus, the potential adverse effects of PAHs in AWI mummichog may involve multiple physiological changes, some of which are caused by genetic alterations in the fish, while others could be caused simply by the exposure to a toxic mixture with very diverse toxicity pathways.

## *4.3 Polychlorinated Biphenyls*

There are 209 known congeners of PCBs that can be produced to create mixtures of various properties, dependent on their chlorine content (Safe 1984). They were produced in the USA until the early 1970s with known uses in plastics, insulation, various dyes, carbonless paper, and transformers (ATSDR 2000). Because of their stability and resilience to chemical, thermal, and photo-degradation, PCBs are persistent in the environment (Buckman et al. 2004; ATSDR 2000). Nevertheless, they are still found in the production of certain dyes and paint (Hu and Hornbuckle 2010). Resembling 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), some co-planar PCB congeners can upregulate the aryl hydrocarbon receptor (AHR) pathway constitutively and cause cancer, apoptosis, and cardiac deformity in aquatic organisms and mammals (Zhang et al. 2012; Thackaberry et al. 2005; Gao et al. 2011; Jonsson et al. 2007; Antkiewicz et al. 2006; Carney et al. 2004). These properties of co-planar PCBs make them likely selective agents in heavily PCB-contaminated environments (U.S. Environmental Protection Agency 2004).

### 4.3.1 Atlantic Tomcod (*Microgadus tomcod*)

Heavy PCB contamination often occurs in urban estuarine regions. A well-known site with historic PCB contamination is the Hudson River (Fig. 1; Feng et al. 1998). Following 70 years of contamination, including the release of an estimated 1.3 million pounds of PCBs, a 200-mile stretch of the Hudson River was put on the National Priorities List as a Superfund site in 1984 (U.S. General Accounting Office 2000). Since then, the fate and distribution of these compounds have been modeled and studied extensively (Connolly et al. 2000). Accumulation and toxicity have been seen both in fish and migratory piscivorous birds in the area (Custer et al. 2012; Deshpande et al. 2013; Koenig et al. 2013; Fernandez et al. 2004). The historic contamination with highly toxic PCBs in the Hudson River resulted in multi-generational exposures and the subsequent evolutionary adaptation of Atlantic tomcod, providing resistance to many of the toxic effects associated with PCBs (Roy and Wirgin 1997). Despite the infamy of this site as a PCB-contaminated site, there have also been levels of TCDD found in juvenile fish collected from the Hudson River that may be among some of the highest levels of this contaminant found in natural populations (Fernandez et al. 2004). Thus, the contamination at this site is complicated by multiple highly toxic contaminants at very high concentrations, many of which have similar ecotoxicological properties.

Resistance to PCBs by Atlantic tomcod is one of the clearest and mechanistically best-elucidated investigations of human-induced evolution through contaminants (Wirgin et al. 2011). The extraordinary concentrations of PCBs in the Hudson River were predicted to be highly detrimental to aquatic life, including the resident Atlantic tomcod (Fernandez et al. 2004). Not unexpectedly, initial investigations identified increased levels of hepatic tumors and decreased life spans of populations living in the Hudson River (Dey et al. 1993). Since a primary molecular mechanism linked to PCB toxicity is the activation of the AHR pathway, research quickly focused on potential alterations to this important pathway. Cytochrome P450 1A (CYP1A) was already discussed as a downstream regulated enzyme responsible for PAH degradation, but its activity is also highly induced in aquatic organisms exposed to co-planar PCBs and dioxins (Nacci et al. 1998; Courtenay et al. 1999). Populations of tomcod collected from the Hudson River did not increase the expression of CYP1A after exposure to PCBs, in contrast to fish from a proximate reference site (Roy and Wirgin 1997). Both populations had similarly increased levels of AHR expression, a normal response to PAHs, but their CYP1A expression was recalcitrant (Roy and Wirgin 1997). The downregulation of the pathway could not be attributed to the AHR repressor (AHRR), which was also found to be downregulated in all tissues, similar to CYP1A (Roy et al. 2006). Following these results, researchers sequenced AHR2, the more active form in fish, for tomcod from four reference populations and two populations in the polluted Hudson River. The sequence data elucidated the mechanistic basis of the phenotypic adaptation of these fish (Wirgin et al. 2011). A six base pair deletion in exon 10 was discovered in Hudson River tomcod, and kinetic tests confirmed that the mutated AHR2 protein had more than sevenfold reduced binding to a model dioxin (Wirgin et al. 2011). This mutation was close to fixation in polluted site populations, whereas it was extremely rare and was always heterozygous in reference site tomcod (Wirgin et al. 2011). Mitochondrial genome diversity revealed that the adaptation in polluted sites did not lead to a decrease in haplotype diversity, confirmed gene flow between resistant populations, and indicated the lack of significant gene flow to reference sites (Wirgin et al. 2011). However, Wirgin et al. (2011) concluded that the variant AHR2 allele, which is also present in some reference populations at low frequencies, likely predates anthropogenic pollution.

The identification of a molecular mechanism and the attribution of this change to anthropogenic pollution is the ultimate goal of evolutionary toxicology (Bickham 2011). Research on Atlantic tomcod populations in the Hudson River has quickly reached that target. In this case, the PCB pollution, likely with help from TCDD, in the Hudson River was intense enough to cause mortality in the resident Atlantic tomcod populations. In addition, PCBs are persistent enough to remain in the environment for enough time such that many generations of tomcod were exposed to the stress of pollution. Therefore, it seems that when a beneficial mutant allele is available in the populations originally exposed to the contaminants, as in the Atlantic tomcod, novel selective pressures can allow for the rapid fixation of this trait and for the success of populations that would normally collapse under these extreme anthropogenic selective pressures (Wirgin et al. 2011).

Estuaries polluted with elevated levels of PCBs are present throughout the world, and they have provided the opportunity to examine the possibility for additional phenotypic adaptations to anthropogenic contaminants (Nelson and Bergen 2012; Lakshmanan et al. 2010). Two species of killifish in the USA, both in the genus *Fundulus*, have also been found to have repeatedly evolved an increased ability to survive in estuaries with high PCB contaminations (Oziolor et al. 2014; Nacci et al. 1999). Despite being genetically isolated, both species include multiple, disparate populations that have apparently evolved phenotypes resistant to PCB toxicity independently. Unfortunately, the exact molecular mechanism responsible for tolerance remains unknown in either species, but the rapid evolution that has occurred in both suggests that it results from selection on standing genetic variation present in ancestral populations, which—if it turns out to be similar in both species—could predate the *Fundulus heteroclitus*–*F. grandis* split (Whitehead et al. 2010; Oziolor et al. 2014). Research on *F. heteroclitus* and *F. grandis* reveals a diverse side of human-induced evolution, which has the potential to contribute greatly to our understanding of adaptational processes in response to anthropogenic pollution.

### 4.3.2 New Bedford Harbor Atlantic Killifish (*Fundulus heteroclitus*)

Another site on the Atlantic Coast of the United States, New Bedford Harbor (NBH), Massachusetts, was identified as a highly PCB-polluted estuary and placed on the National Priorities List in 1983 (Fig. 1; Nelson and Bergen 2012). Because of the high projected costs of remediating this site, it was placed on the EPA's Long Term Monitoring program, which allowed for frequent tracking of the contamination at the site (Nelson and Bergen 2012). The first dredging event removed 14,000 cubic yards of sediment with concentrations of over 4,000 μg/g of PCBs in 1995 (Bergen et al. 2005). Through 2009, approximately 200,000 cubic yards of sediment had been dredged from NBH, leading to a significant decrease in PCB concentrations (Nelson and Bergen 2012).

Following 2009, the projected 20-year remediation process led to multiple investigations of toxicity to aquatic organisms at NBH (Nelson and Bergen 2012). To investigate potential population-level impacts, *F. heteroclitus* was used as a model for effects on fish, since it is a resident non-migratory species in NBH (Munns et al. 1997). Laboratory studies suggested that *F. heteroclitus* reproduction might be impaired by exposure to high PCB concentrations, signifying that the toxicants could act as a selective pressure in the polluted estuary (Black et al. 1998). Interest in the effects of chronic PCB contamination on *F. heteroclitus* finally led to the discovery that NBH populations had adapted to resist the developmental toxicity of PCBs (Nacci et al. 1999).

Populations of *F. heteroclitus* collected from NBH had lower embryonic and larval mortality in comparison with reference site populations (Nacci et al. 1999). This resistance extended to at least the $F_2$-generation raised under common-garden conditions, confirming that the trait is genetic, rather than an example of physiological acclimation (Nacci et al. 2010). In addition, the resistant phenotype was also correlated with a reduced responsiveness of CYP1A activity, much like in the case of Atlantic tomcod from the Hudson River (Nacci et al. 1999). CYP1A has also been shown to be a sensitive biomarker of dioxin exposure and toxicity in *F. heteroclitus* (Toomey et al. 2001). In the process of studying the control of

CYP1A by the AHR pathway, initial tests identified AHR1 to be more strongly and ubiquitously expressed in tissues of NBH fish, while not being responsive to dioxins or PCBs (Karchner et al. 1999). Adult fish and hepatocyte cell cultures also revealed that NBH populations were much less sensitive in inducing CYP1A mRNA expression or enzyme activity when exposed to dioxins and a model PAH (Bello et al. 2001). The AHR pathway was capable of being activated to the same magnitude, but it only occurred at much higher doses of pollutants (Bello et al. 2001). Results in zebrafish suggest that dioxin-mediated cardiovascular defects are AHR2 dependent, and that the heart deformities are prevented when AHR2, and thus its downstream genes, was knocked down (Carney et al. 2004). On the other hand, knocking down only CYP1A had no effect (Carney et al. 2004). These results support the hypothesis that the resistance of NBH populations of *F. heteroclitus* to PCB-mediated deformities is based on a recalcitrant AHR pathway and, by extension, its main inducible downstream enzymes (Toomey et al. 2001; Bello et al. 2001).

One factor that makes this case particularly interesting is that there are very few identified fitness costs associated with adaptation to PCBs in *F. heteroclitus*. Studies have identified the lower capacity of NBH killifish to deal with oxidative stress (Harbeitner et al. 2013). Resistant populations were also found to have lower hepatic, but higher intestinal, expression of P-glycoprotein, an enzyme responsible for the excretion of moderately hydrophobic compounds (Bard et al. 2002). This difference between populations diminished after they were placed in common-garden laboratory conditions for more than 8 days (Bard et al. 2002). Other studies dealing with retinoid depletion by PCB exposures have been unable to identify a difference between reference and resistant populations of *F. heteroclitus* (Nacci et al. 2001). In addition, it was found that the PCB sensitivity of populations of *F. heteroclitus* correlated with the concentrations of PCBs at the sites of their collection (Nacci et al. 2002). Further studies into that trend determined that there were several exceptions to the correlation between contamination levels and resistance, which may result from the vast variability of contaminants present at different locations (Nacci et al. 2010).

The lack of mechanistic evidence to explain the differential sensitivity to dioxins in NBH populations led investigators to explore genetic and epigenetic markers to better understand the possible shifts in genes or their control. The efforts began with the sequencing of the CYP1A promoter region, but after CYP1A knockdown was identified to not be protective of PCB-induced teratogenesis, efforts were shifted to the AHR (Powell et al. 2004). The methylation patterns of AHR1 and AHR2 were not observed to have an effect on the PCB sensitivity of *F. heteroclitus*, but rather to affect the gene expression patterns in the fish (Aluru et al. 2011). With the development of new technology, population resequencing studies have become more affordable, and investigations have begun into the genotypic differences between the AHR pathway genes of these fish (Reitzel et al. 2014). These studies identified a significant population genetic differentiation between resistant and reference populations (Reitzel et al. 2014). The results suggest that selection is possible for the tested genes (AHR1, AHR2, and AHRR), but with the current results, the study was unable to show strong significant differences that could identify the genetic basis of protection (Reitzel et al. 2014). Another exploration of the genetic variation between reference and resistant site fish through single-nucleotide polymorphism (SNP) analysis identified signs of positive selection in SNPs of AHR2 and CYP1A between these populations (Proestou et al. 2014). Such variation may suggest that the AHR pathway still represents a potential mechanism to explain part of the phenotypic differences in the responses of resistant killifish to contaminant-induced damage. Population genomics is a promising tool for the exploration of genetic effects on populations, and the *F. heteroclitus* populations at New Bedford Harbor are a promising start for this endeavor, but adaptation in killifish contains more complexity that still stands to be unraveled.

### 4.3.3 Newark Bay Atlantic Killifish (*F. heteroclitus*)

Shortly after the identification of the first adapted populations at NBH, another group was able to find a similar recalcitrant AHR phenotype in *F. heteroclitus* collected from Newark Bay (NB) (Fig. 1; Arzuaga and Elskus 2002). The NB estuary, part of the Passaic River in New Jersey, is a heavily contaminated area, which has been on the National Priorities List since 1984 as the "Diamond Alkali Superfund Site" (Crawford et al. 1995). In addition, the Passaic River runs parallel to the Hudson River discussed previously in terms of Atlantic tomcod adaptation. The PCB levels at the NB site are not as high as the ones at NBH, but the type of contamination is much more complex, involving high levels of polychlorinated dibenzo-dioxins and furans (PCDD/Fs), PCBs, and heavy metals (Crawford et al. 1995; Wenning et al. 1993; Armstrong et al. 2005). This has led to multiple fish consumption advisories for this area and to the identification of another population of contaminant-adapted *F. heteroclitus* (Pflugh et al. 2011; Arzuaga and Elskus 2002).

The initial studies identified a similar phenotype of resistance associated with protection from developmental toxicity and reduced CYP1A activity in *F. heteroclitus* populations collected from NB (Arzuaga et al. 2004; Arzuaga and Elskus 2002). In addition, de-methylating agents failed to revert either of those phenotypes, suggesting that epigenetics may not play a strong role in the observed resistance (Arzuaga et al. 2004). Similar to other adapted populations, NB fish were resistant not only to cardiovascular teratogenesis, but also to the induction of ROS by a PCB-simulating compound (Arzuaga and Elskus 2010; Arzuaga et al. 2006). These findings point to a similar phenotype of protection and shared fitness costs with *F. heteroclitus* populations from NBH. Additional studies on NB fish confirmed that complex contaminant exposures impose selection on populations by reducing the reproductive fitness of chronically exposed individuals (Bugel et al. 2010, 2011).

The discovery of three, geographically distant, populations of *F. heteroclitus* that have adapted to chronic contamination in a physiologically similar manner distinguishes this system from Atlantic tomcod. The tomcod population fixed a rare mutation that allowed it to survive in conditions of high contamination. Conversely, *F. heteroclitus* seems to have been able to adapt at multiple locations, and gene flow among adapted populations is extremely unlikely. In addition, the genetic basis of this adaptation or the full extent of the adaptation has still not been fully determined.

One of the most fascinating findings about *F. heteroclitus* is that they seem to have adapted to heavy PCB pollution in a very similar manner to the way they have adapted to PAHs, including recalcitrance in AHR pathway enzymes and protection from cardiac deformities (Arzuaga and Elskus 2002; Van Veld and Westbrook 1995; Roy and Wirgin 1997). The vast geographic separation has been shown to prevent gene flow among these distant adapted populations, as would be expected for this species (Duvernell et al. 2008). In addition, it has been shown that selection at NBH and AWI has had an influence on the population genetic differentiation between adapted and non-adapted populations (Duvernell et al. 2008). Other populations of *F. heteroclitus* have also been shown to exhibit resistance, with levels of pollution similar to those previously described at NBH and NB (Nacci et al. 2010). The vast genetic and geographic distance between these locations and the similar mode of adaptation between sites with varying pollution led to the hypothesis that standing genetic variation in ancestral populations is responsible for the rapid evolution of protection in *F. heteroclitus* from contaminated habitats (Whitehead et al. 2012). Populations from various locations were compared in terms of their resistance and their transcriptomic profiles, revealing that distant resistant populations had profiles more similar to each other than to their most geographically proximate reference sites (Whitehead et al. 2012). This could allow for the rapid fixation of the trait and the possibility for multiple populations to acquire this resistance, despite low migration rates in *F. heteroclitus*.

### 4.3.4 Houston Ship Channel Gulf Killifish (*Fundulus grandis*)

While the *F. heteroclitus* case is fascinating at its current intricacy, there is another level of complexity—*F. grandis*. Gulf killifish are the sister species of *F. heteroclitus* and are found primarily along the US Gulf Coast (Fig. 1; Gonzalez et al. 2009). These two species have been shown to overlap and hybridize along a short portion of the northeastern Florida Atlantic coast (Gonzalez et al. 2009). *Fundulus grandis* is found ubiquitously along the Gulf of Mexico coast and has been studied as a relevant environmental model for hypoxia exposures, osmoregulation in euryhaline fishes, as well as physiological and toxicological responses to the Deepwater Horizon Oil Spill (Dubansky et al. 2013; Landry et al. 2003; Love and Rees 2002; Virani and Rees 2000). As an ecologically important coastal fish in the Gulf of Mexico, it was of particular interest that populations of *F. grandis* were identified to be resident, and in fact quite common, in the Houston Ship Channel (HSC) (Fig. 1; Oziolor et al. 2014).

The HSC is a heavily industrialized commercial waterway, heavily polluted with a mixture of PCBs, dioxins, and PAHs (Lakshmanan et al. 2010). These compounds

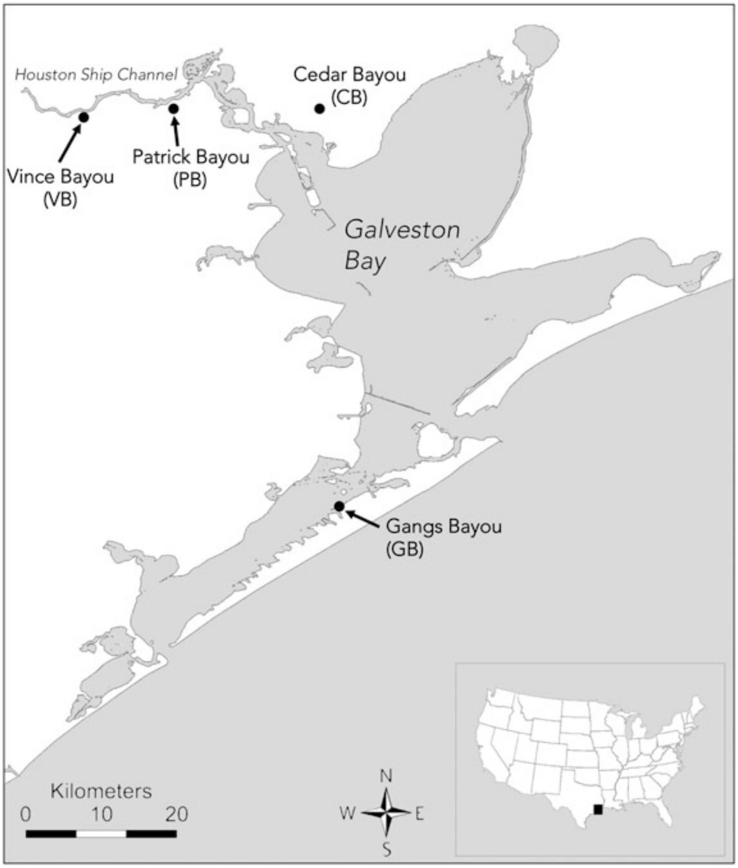


**Fig. 3** Sampling sites from two Superfund sites within the industrialized portion of the Houston Ship Channel, Vince Bayou (VB), and Patrick Bayou (PB). A site with predicted intermediate contamination levels, Cedar Bayou (CB), was collected from a portion proximate to the HSC, while a reference population, Gangs Bayou (GB), was collected far from the high contamination regions

have been observed to accumulate in catfish (*Ictalurus punctatus*), reaching total PCB concentrations of 37 pg/g (Subedi and Usenko 2012). The spatial distributions of PCBs and PCDD/Fs have been studied intensely and were found to diminish as the channel continues through Galveston Bay (Howell et al. 2008, 2011). In addition, these levels of contamination have been observed historically over the last four decades allowing for chronic contamination of aquatic organisms over many generations (Yeager et al. 2007).

Recently, populations of *F. grandis*, collected from heavily contaminated areas in the HSC, were shown to exhibit a similar phenotypic resistance to PCB- and PAH-induced cardiovascular teratogenesis (Fig. 3; Oziolor et al. 2014). Cardiovascular teratogenesis occurs in embryos, if they come in contact with a toxicant at

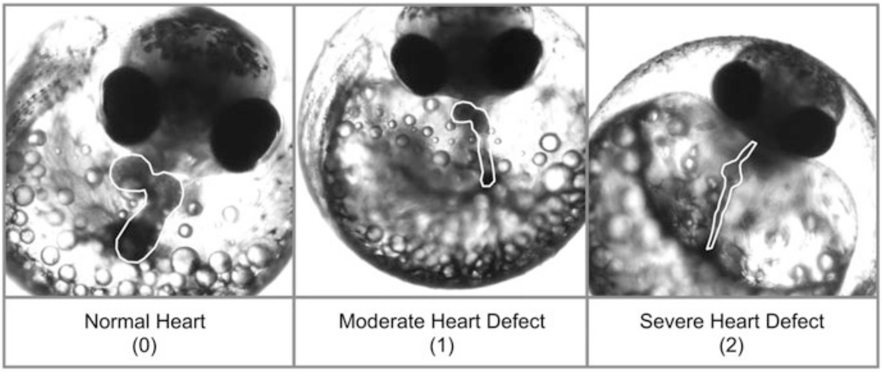


**Fig. 4** Scoring scale for cardiovascular teratogenesis. Embryos were dosed at 24 h post-fertilization (hpf) with a toxicant and screened at 144 hpf. The scores were recorded blind to avoid bias for population or contamination levels from researcher performing the screening

sensitive developmental stages. Often, these deformities are scored qualitatively from a normal, two-chambered heart (0) to severely deformed string-heart (2) (Fig. 4). The protection from PCBs in HSC *F. grandis* was of much higher magnitude than that observed for PAHs, which correlated well with the toxicity equivalency of these classes of compounds in the HSC (Oziolor et al. 2014). The protection from cardiovascular teratogenesis in *F. grandis* population also correlated highly with a recalcitrant AHR pathway, as measured via CYP1A activity, suggesting a similar mode of action as seen in adapted *F. heteroclitus* populations (Oziolor et al. 2014). The different levels of resistance to PCBs and PAHs, even when evaluating a known AHR-mediated toxicity endpoint, suggest that there may be additional complexity to the observed adaptation, beyond a simple AHR recalcitrance. Biparental crosses of reference and resistant populations suggest that each parent contributes equally to the resistant phenotype, suggesting a genetic basis of this adaptation (Oziolor et al. 2014). More recently, a gradient of this adaptation has been found, where populations from the HSC with lower predicted contaminant exposure exhibit lower levels of protection and lower levels of AHR recalcitrance (Fig. 5).

The identification of a sister species that has undergone a phenotypically similar genetic adaptation to human-induced pollution suggests that the hypothesis of adaptation through preexisting genetic variation would now extend to their shared common ancestor. Thus, the alleles responsible for this adaptation may have been carried through populations and generations of *Fundulus* even before the split of the two species. Such an extended timeframe begets multiple questions, including the question of why these alleles persisted through time, the nature of the fitness costs associated with them, and the natural stressors that may have led to their evolution.

Expanding adaptation to human-induced novel selective pressures in *Fundulus* to multiple species has the potential to answer questions extending from toxicology to the basics of evolutionary biology. Through these adaptations, we can study not

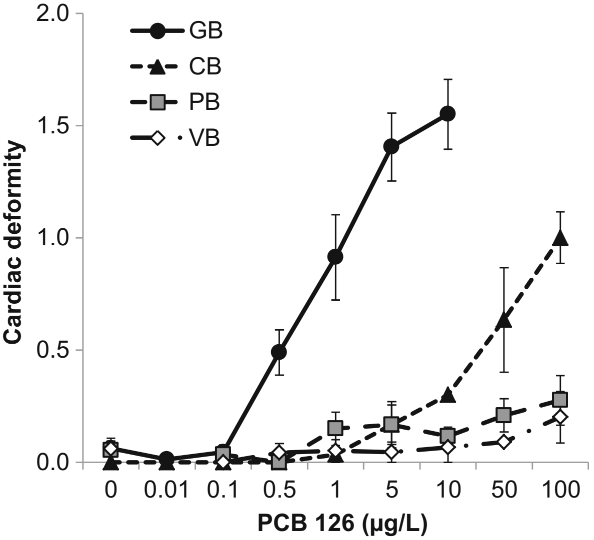


**Fig. 5** Populations of *F. grandis* from chronically contaminated sites exhibit protection from cardiovascular deformities in a gradient-dependent manner. F1 embryos from the reference site, GB, show a normal dose–response curve for cardiovascular deformities in response to PCB 126, a known AHR agonist. The population from a site with predicted intermediate contamination, CB, develops deformities at higher concentrations of contaminant, showing significant levels of protection. On the other hand, sites within the industrialized portion of the HSC show no significant cardiac defects in response to PCB 126, revealing a >1,000× protection from contaminant induced deformities. A subset of these data was presented in Oziolor et al. (2014)

only the effect of current toxicants in the environment but also about the nature of evolutionary processes that have led to the continuous selection of alleles that would provide some protection from xenobiotics. This should lead to a better understanding of how natural biological systems may respond to current anthropogenic pollutants and how they cross-react with historical biological selective pressures. The study of these two species is at the forefront of evolutionary toxicology and the discovery of the molecular mechanisms and history of these adaptations will significantly improve our understanding of the evolutionary effects of anthropogenic contamination.

## 5 Conclusions and Outlook

While research has identified some long-term effects of legacy contaminants following chronic exposures, there are many chemicals that have not been investigated, and novel stressors being released every year. For most systems, we are far from having a comprehensive understanding of how organisms will respond to the changing selective landscape in their natural environments. A crucial misconception with finding resistance to high concentrations of contaminants is that the public may interpret this to mean that contamination is acceptable or less of a problem, because organisms can adapt to it. Unfortunately, this is a misinterpretation of such results for two primary reasons. First, the fact that some species are able to adapt to pollution does not imply that it will happen in every organism, population, or pollution scenario. Second, the resistant population often has been changed in terms of its phenotypic response to more than just the compound it adapted to; in other words, adaptation to anthropogenic stressors almost certainly has fitness costs. Whether and how fish will respond to rising salinities in freshwater bodies and whether they will cope with higher radiation or temperature increases are questions stemming from already existing sources of human-induced alterations of natural selective regimes. Along with current levels of toxicity from pesticides or persistent pollutants that have already been introduced in the environment, a new direction of research will be to study the evolutionary consequences of constantly increasing levels of pharmaceuticals in aquatic environments. The toxicity of pharmaceuticals in the environment is being widely studied; but in terms of population adaptation, the effects of these compounds are still entirely unknown. There are multiple classes and types of anthropogenic effects on the environment, but our knowledge of how they shape fish populations is often limited to short exposure durations. In addition, as most of these compounds are found in very complex mixtures, it is of interest to understand how they interact with existing environmental stressors to change the selective landscape of resident organisms at varying locations. Future extremophile fish may evolve in response to already existing levels of alteration in the selective landscape within aquatic environments, and if the populations have sufficient standing genetic variation to adapt to these changes.

## References

Aluru N, Karchner SI, Hahn ME (2011) Role of DNA methylation of AHR1 and AHR2 promoters in differential sensitivity to PCBs in Atlantic Killifish, *Fundulus heteroclitus*. Aquat Toxicol 101:288–294

Anderegg WRL, Prall JW, Harold J, Schneider SH (2010) Expert credibility in climate change. Proc Natl Acad Sci 107:12107–12109

Antkiewicz DS, Peterson RE, Heideman W (2006) Blocking expression of AHR2 and ARNT1 in zebrafish larvae protects against cardiac toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Sci 94:175–182

Armstrong TN, Iannuzzi TJ, Thelen JB, Ludwig DF, Firstenberg CE (2005) Characterization of chemical contamination in shallow-water estuarine habitats of an industrialized river. Part II. Metals. Soil Sediment Contam 14:35–52

Arzuaga X, Elskus A (2002) Evidence for resistance to benzo a pyrene and 3,4,3′,4-′-tetrachlorobiphenyl in a chronically polluted *Fundulus heteroclitus* population. Mar Environ Res 54:247–251

Arzuaga X, Elskus A (2010) Polluted-site killifish (*Fundulus heteroclitus*) embryos are resistant to organic pollutant-mediated induction of CYP1A activity, reactive oxygen species, and heart deformities. Environ Toxicol Chem/SETAC 29:676–682

Arzuaga X, Calcano W, Elskus A (2004) The DNA de-methylating agent 5-azacytidine does not restore CYP1A induction in PCB resistant Newark Bay killifish (*Fundulus heteroclitus*). Mar Environ Res 58:517–520

Arzuaga X, Wassenberg D, Di Giulio R, Elskus A (2006) The chlorinated AHR ligand 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) promotes reactive oxygen species (ROS) production during embryonic development in the killifish (*Fundulus heteroclitus*). Aquat Toxicol 76:13–23

Ashurst SW, Cohen GM, Nesnow S, Digiovanni J, Slaga TJ (1983) Formation of benzo[a]pyrene DNA adducts and their relationship to tumor initiation in mouse epidermis. Cancer Res 43:1024–1029

ATSDR (2000) Toxicological profile for polychlorinated biphenyls (PCBs). Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry (ATSDR)

Bacanskas LR, Whitaker J, Di Giulio RT (2004) Oxidative stress in two populations of killifish (*Fundulus heteroclitus*) with differing contaminant exposure histories. Mar Environ Res 58:597–601

Bard SM, Bello SM, Hahn ME, Stegeman JJ (2002) Expression of P-glycoprotein in killifish (*Fundulus heteroclitus*) exposed to environmental xenobiotics. Aquat Toxicol 59:237–251

Bello SM, Franks DG, Stegeman JJ, Hahn ME (2001) Acquired resistance to Ah receptor agonists in a population of Atlantic killifish (*Fundulus heteroclitus*) inhabiting a marine superfund site: In vivo and in vitro studies on the inducibility of xenobiotic metabolizing enzymes. Toxicol Sci 60:77–91

Bergen BJ, Nelson WG, Mackay J, Dickerson D, Jayaraman S (2005) Environmental monitoring of remedial dredging at the New Bedford Harbor, MA, Superfund site. Environ Monit Assess 111:257–275

Bickham JW (2011) The four cornerstones of evolutionary toxicology. Ecotoxicology 20:497–502

Bickham JW, Sandhu S, Hebert PDN, Chikhi L, Athwal R (2000) Effects of chemical contaminants an genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. Mutat Res Rev Mutat Res 463:33–51

Black DE, Gutjahr-Gobell R, Pruell RJ, Bergen B, McElroy AE (1998) Effects of a mixture of non-ortho-and mono-ortho-polychlorinated biphenyls on reproduction in *Fundulus heteroclitus* (Linnaeus). Environ Toxicol Chem 17:1396–1404

Buckman AH, Norstrom RJ, Hobson KA, Karnovsky NJ, Duffe J, Fisk AT (2004) Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. Environ Pollut 128:327–338

Bugel SM, White LA, Cooper KR (2010) Impaired reproductive health of killifish (*Fundulus heteroclitus*) inhabiting Newark Bay, NJ, a chronically contaminated estuary. Aquat Toxicol 96:182–193

Bugel SM, White LA, Cooper KR (2011) Decreased vitellogenin inducibility and 17 beta-estradiol levels correlated with reduced egg production in killifish (*Fundulus heteroclitus*) from Newark Bay, NJ. Aquat Toxicol 105:1–12

Buth JM, Steen PO, Sueper C, Blumentritt D, Vikesland PJ, Arnold WA, McNeill K (2010) Dioxin photoproducts of triclosan and its chlorinated derivatives in sediment cores. Environ Sci Technol 44:4545–4551

Camacho-Munoz D, Martin J, Santos JL, Aparicio I, Alonso E (2014) Concentration evolution of pharmaceutically active compounds in raw urban and industrial wastewater. Chemosphere 111:70–79

Carlson EA, Li Y, Zelikoff JT (2004) Benzo a pyrene-induced immunotoxicity in Japanese medaka (*Oryzias latipes*): relationship between lymphoid CYP1A activity and humoral immune suppression. Toxicol Appl Pharmacol 201:40–52

Carney SA, Peterson RE, Heideman W (2004) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin activation of the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator pathway causes developmental toxicity through a CYP1A-independent mechanism in zebrafish. Mol Pharmacol 66:512–521

Carrasco-Malio A, Diaz M, Mella M, Montoya MJ, Miranda A, Landaeta MF, Sanchez G, Hidalgo ME (2014) Are the intertidal fish highly resistant to UV-B radiation? A study based on oxidative stress in *Girella laevifrons* (Kyphosidae). Ecotoxicol Environ Saf 100:93–98

Clark BW, Di Giulio RT (2012) *Fundulus heteroclitus* adapted to PAHs are cross-resistant to multiple insecticides. Ecotoxicology 21:465–474

Clark BW, Matson CW, Jung D, Di Giulio RT (2010) AHR2 mediates cardiac teratogenesis of polycyclic aromatic hydrocarbons and PCB-126 in Atlantic killifish (*Fundulus heteroclitus*). Aquat Toxicol 99:232–240

Clark BW, Bone AJ, Giulio RT (2013a) Resistance to teratogenesis by F1 and F2 embryos of PAH-adapted *Fundulus heteroclitus* is strongly inherited despite reduced recalcitrance of the AHR pathway. Environ Sci Pollut Res Int 21:13898–13908

Clark BW, Cooper EM, Stapleton HM, Di Giulio RT (2013b) Compound- and mixture-specific differences in resistance to polycyclic aromatic hydrocarbons and PCB-126 among *Fundulus heteroclitus* subpopulations throughout the Elizabeth River Estuary (Virginia, USA). Environ Sci Tech 47:10556–10566

Connolly JP, Zahakos HA, Benaman J, Ziegler CK, Rhea JR, Russell K (2000) A model of PCB fate in the Upper Hudson River. Environ Sci Technol 34:4076–4087

Core Writing Team, Pachauri RK, Reisinger A (eds) (2007) IPCC fourth assessment report: climate change 2007. IPCC, Geneva, Switzerland

Courtenay SC, Grunwald CM, Kreamer GL, Fairchild WL, Arsenault JT, Ikonomou M, Wirgin II (1999) A comparison of the dose and time response of CYP1A1 mRNA induction in chemically treated Atlantic tomcod from two populations. Aquat Toxicol 47:43–69

Crawford DL, Powers DA (1989) Molecular basis of evolutionary adaptation at the lactate dehydrogenase-B locus in the fish *Fundulus heteroclitus*. Proc Natl Acad Sci U S A 86:9365–9369

Crawford DL, Powers DA (1992) Evolutionary adaptation to different thermal environments via transcriptional regulation. Mol Biol Evol 9:806–813

Crawford DW, Bonnevie NL, Wenning RJ (1995) Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol Environ Saf 30:85–100

Custer CM, Custer TW, Hines JE (2012) Adult tree swallow survival on the polychlorinated biphenyl-contaminated Hudson River, New York, USA, between 2006 and 2010. Environ Toxicol Chem 31:1788–1792

Daskalakis KD, Oconnor TP (1995) Distribution of chemical concentrations in US coastal and estuarine environments. Mar Environ Res 40:381–398

Deshpande AD, Dockum BW, Cleary T, Farrington C, Wieczorek D (2013) Bioaccumulation of polychlorinated biphenyls and organochlorine pesticides in young-of-the-year bluefish (*Pomatomus saltatrix*) in the vicinity of a Superfund Site in New Bedford Harbor, Massachusetts, and in the adjacent waters. Mar Pollut Bull 72:146–164

Dey WP, Peck TH, Smith CE, Kreamer GL (1993) Epizoology of hepatic neoplasia in Atlantic tomcod (*Microgadus tomcod*) from the Hudson River estuary. Can J Fish Aquat Sci 50:1897–1907

Dijkstra JA, Buckman KL, Ward D, Evans DW, Dionne M, Chen CY (2013) Experimental and natural warming elevates mercury concentrations in estuarine fish. PLoS One 8:e58401

Dubansky B, Whitehead A, Miller JT, Rice CD, Galvez F (2013) Multitissue molecular, genomic, and developmental effects of the deepwater horizon oil spill on resident gulf killifish (*Fundulus grandis*). Environ Sci Technol 47:5074–5082

Durbeej B, Eriksson LA (2002) Reaction mechanism of thymine dimer formation in DNA induced by UV light. J Photochem Photobiol A Chem 152:95–101

Duvernell DD, Lindmeier JB, Faust KE, Whitehead A (2008) Relative influences of historical and contemporary forces shaping the distribution of genetic variation in the Atlantic killifish, *Fundulus heteroclitus*. Mol Ecol 17:1344–1360

Everett MV, Antal CE, Crawford DL (2012) The effect of short-term hypoxic exposure on metabolic gene expression. J Exp Zool A Ecol Genet Physiol 317A:9–23

Feng H, Cochran JK, Lwiza H, Brownawell BJ, Hirschberg DJ (1998) Distribution of heavy metal and PCB contaminants in the sediments of an urban estuary: The Hudson River. Mar Environ Res 45:69–88

Fernandez MP, Ikonomou MG, Courtenay SC, Wirgin II (2004) Spatial variation in hepatic levels and patterns of PCBs and PCDD/Fs among young-of-the-year and adult Atlantic tomcod (*Microgadus tomcod*) in the Hudson River estuary. Environ Sci Technol 38:976–983

Forster L, Forster P, Lutz-Bonengel S, Willkomm H, Brinkmann B (2002) Natural radioactivity and human mitochondrial DNA mutations. Proc Natl Acad Sci U S A 99:13950–13954

Frederick LA, Van Veld PA, Rice CD (2007) Bioindicators of immune function in creosote-adapted estuarine killifish, *Fundulus heteroclitus*. J Toxicol Environ Health A 70:1433–1442

Fryxell KJ, Moon WJ (2005) CpG mutation rates in the human genome are highly dependent on local GC content. Mol Biol Evol 22:650–658

Gao K, Brandt I, Goldstone JV, Jonsson ME (2011) Cytochrome P450 1A, 1B, and 1C mRNA induction patterns in three-spined stickleback exposed to a transient and a persistent inducer. Comp Biochem Physiol C Toxicol Pharmacol 154:42–55

Gonzalez I, Levin M, Jermanus S, Watson B, Gilg MR (2009) Genetic assessment of species ranges in *Fundulus heteroclitus* and *F. grandis* in northeastern Florida salt marshes. Southeast Nat 8:227–244

Gonzalgo ML, Jones PA (1997) Mutagenic and epigenetic effects of DNA methylation. Mutat Res Rev Mutat Res 386:107–118

Griffith MB, Norton SB, Alexander LC, Pollard AI, LeDuc SD (2012) The effects of mountaintop mines and valley fills on the physicochemical quality of stream ecosystems in the central Appalachians: a review. Sci Total Environ 417:1–12

Guerrero-Bosagna C, Skinner MK (2012) Environmentally induced epigenetic transgenerational inheritance of phenotype and disease. Mol Cell Endocrinol 354:3–8

Guittonny-Philippe A, Masotti V, Hohener P, Boudenne JL, Viglione J, Laffont-Schwob I (2014) Constructed wetlands to reduce metal pollution from industrial catchments in aquatic Mediterranean ecosystems: A review to overcome obstacles and suggest potential solutions. Environ Int 64:1–16

Hader DP, Helbling EW, Williamson CE, Worrest RC (2011) Effects of UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci 10:242–260

Hakkinen J, Vehniainen E, Oikari A (2004) High sensitivity of northern pike larvae to UV-B but no UV-photoinduced toxicity of retene. Aquat Toxicol 66:393–404

Harbeitner RC, Hahn ME, Timme-Laragy AR (2013) Differential sensitivity to pro-oxidant exposure in two populations of killifish (*Fundulus heteroclitus*). Ecotoxicology 22:387–401

Heinrich U, Pott F, Mohr U, Fuhst R, Konig J (1986) Lung tomors in rats and mice after inhalation of PAH rich emissions. Exp Pathol 29:29–34

Hermann M (1981) Synergistic effects of individual polycyclic aromatic hydrocarbons on the mutagenicity of their mixtures. Mutat Res 90:399–409

Howell NL, Suarez MP, Rifai HS, Koenig L (2008) Concentrations of polychlorinated biphenyls (PCBs) in water, sediment, and aquatic biota in the Houston Ship Channel, Texas. Chemosphere 70:593–606

Howell NL, Rifai HS, Koenig L (2011) Comparative distribution, sourcing, and chemical behavior of PCDD/Fs and PCBs in an estuary environment. Chemosphere 83:873–881

Hu DF, Hornbuckle KC (2010) Inadvertent polychlorinated biphenyls in commercial paint pigments. Environ Sci Technol 44:2822–2827

Incardona JP, Collier TK, Scholz NL (2004) Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol Appl Pharmacol 196:191–205

Incardona JP, Linbo TL, Scholz NL (2011) Cardiac toxicity of 5-ring polycyclic aromatic hydrocarbons is differentially dependent on the aryl hydrocarbon receptor 2 isoform during zebrafish development. Toxicol Appl Pharmacol 257:242–249

Jeffree RA, Markich SJ, Twining JR (2014) Diminished metal accumulation in riverine fishes exposed to acid mine drainage over five decades. PLoS One 9:e91371

Jokinen IE, Salo HM, Markkula E, Rikalainen K, Arts MT, Browman HI (2011) Additive effects of enhanced ambient ultraviolet B radiation and increased temperature on immune function, growth and physiological condition of juvenile (parr) Atlantic Salmon, Salmo salar. Fish Shellfish Immunol 30:102–108

Jonsson ME, Jenny MJ, Woodin BR, Hahn ME, Stegeman JJ (2007) Role of AHR2 in the expression of novel cytochrome p450 1 family genes, cell cycle genes, and morphological defects in developing zebrafish exposed to 3,3′,4,4′,5-pentachlorobiphenyl or 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Sci 100:180–193

Jung D, Cho Y, Collins LB, Swenberg JA, Di Giulio RT (2009) Effects of benzo a pyrene on mitochondrial and nuclear DNA damage in Atlantic killifish (*Fundulus heteroclitus*) from a creosote-contaminated and reference site. Aquat Toxicol 95:44–51

Jung DW, Matson CW, Collins LB, Laban G, Stapleton HM, Bickham JW, Swenberg JA, Di Giulio RT (2011) Genotoxicity in Atlantic killifish (*Fundulus heteroclitus*) from a PAH-contaminated Superfund site on the Elizabeth River, Virginia. Ecotoxicology 20:1890–1899

Karahalil B, Burgaz S, Fisek G, Karakaya AE (1998) Biological monitoring of young workers exposed to polycyclic aromatic hydrocarbons in engine repair workshops. Mutat Res Genet Toxicol Environ Mutagen 412:261–269

Karchner SI, Powell WH, Hahn ME (1999) Identification and functional characterization of two highly divergent aryl hydrocarbon receptors (AHR1 and AHR2) in the teleost *Fundulus heteroclitus* – evidence for a novel subfamily of ligand-binding basic helix loop helix-Per-ARNT-Sim (bHLH-PAS) factors. J Biol Chem 274:33814–33824

Kaushal SS, Groffman PM, Likens GE, Belt KT, Stack WP, Kelly VR, Band LE, Fisher GT (2005) Increased salinization of fresh water in the northeastern United States. Proc Natl Acad Sci U S A 102:13517–13520

Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW (2007) Collapse of a fish population after exposure to a synthetic estrogen. Proc Natl Acad Sci U S A 104:8897–8901

Klerks PL, Xie L, Levinton JS (2011) Quantitative genetics approaches to study evolutionary processes in ecotoxicology; a perspective from research on the evolution of resistance. Ecotoxicology 20:513–523

Knecht AL, Goodale BC, Truong L, Simonich MT, Swanson AJ, Matzke MM, Anderson KA, Waters KM, Tanguay RL (2013) Comparative developmental toxicity of environmentally relevant oxygenated PAHs. Toxicol Appl Pharmacol 271:266–275

Koenig S, Fernandez P, Company JB, Huertas D, Sole M (2013) Are deep-sea organisms dwelling within a submarine canyon more at risk from anthropogenic contamination than those from the adjacent open slope? A case study of Blanes canyon (NW Mediterranean). Prog Oceanogr 118:249–259

Laird PW (2003) The power and the promise of DNA methylation markers. Nat Rev Cancer 3:253–266

Lakshmanan D, Howell NL, Rifai HS, Koenig L (2010) Spatial and temporal variation of polychlorinated biphenyls in the Houston Ship Channel. Chemosphere 80:100–112

Landers J (2006) Elizabeth River to be focus of major cleanup effort. Civil Eng 76:22–23

Landry CA, Manning S, Cheek AO (2003) Hypoxia affects reproduction in gulf killifish, *Fundulus grandis*. Integr Comp Biol 43:812

Love JW, Rees BB (2002) Seasonal differences in hypoxia tolerance in gulf killifish, *Fundulus grandis* (*Fundulidae*). Environ Biol Fishes 63:103–115

Marquis O, Miaud C, Ficetola GF, Bocher A, Mouchet F, Guittonneau S, Devaux A (2009) Variation in genotoxic stress tolerance among frog populations exposed to UV and pollutant gradients. Aquat Toxicol 95:152–161

Matson CW, Clark BW, Jenny MJ, Fleming CR, Hahn ME, Di Giulio RT (2008) Development of the morpholino gene knockdown technique in *Fundulus heteroclitus*: a tool for studying molecular mechanisms in an established environmental model. Aquat Toxicol 87:289–295

Matson CW, Gillespie AM, McCarthy C, McDonald TJ, Bickham JW, Sullivan R, Donnelly KC (2009) Wildlife toxicology: biomarkers of genotoxic exposures at a hazardous waste site. Ecotoxicology 18:886–898

Medina MH, Correa JA, Barata C (2007) Micro-evolution due to pollution: Possible consequences for ecosystem responses to toxic stress. Chemosphere 67:2105–2114

Mennigen JA, Stroud P, Zamora JM, Moon TW, Trudeau VL (2011) Pharmaceuticals as neuroendocrine disruptors: lessons learned from fish on prozac. J Toxicol Environ Health B Crit Rev 14:387–412

Menzie CA, Hoeppner SS, Cura JJ, Freshman JS, LaFrey EN (2002) Urban and suburban storm water runoff as a source of polycyclic aromatic hydrocarbons (PAHs) to Massachusetts estuarine and coastal environments. Estuaries 25:165–176

Meyer J, Di Giulio R (2002) Patterns of heritability of decreased EROD activity and resistance to PCB 126-induced teratogenesis in laboratory-reared offspring of killifish (*Fundulus heteroclitus*) from a creosote-contaminated site in the Elizabeth River, VA, USA. Mar Environ Res 54:621–626

Meyer JN, Di Giulio RT (2003) Heritable adaptation and fitness costs in killifish (*Fundulus beteroclitus*) inhabiting a polluted estuary. Ecol Appl 13:490–503

Meyer JN, Nacci DE, Di Giulio RT (2002) Cytochrome P4501A (CYP1A) in killifish (*Fundulus heteroclitus*): heritability of altered expression and relationship to survival in contaminated sediments. Toxicol Sci 68:69–81

Mitra S, Dickhut RM, Kuehl SA, Kimbrough KL (1999) Polycyclic aromatic hydrocarbon (PAH) source, sediment deposition patterns, and particle geochemistry as factors influencing PAH distribution coefficients in sediments of the Elizabeth River, VA, USA. Mar Chem 66:113–127

Moe SJ, De Schamphelaere K, Clements WH, Sorensen MT, Van den Brink PJ, Liess M (2013) Combined and interactive effects of global climate change and toxicants on populations and communities. Environ Toxicol Chem 32:49–61

Mulvey M, Newman MC, Vogelbein W, Unger MA (2002) Genetic structure of *Fundulus heteroclitus* from PAH-contaminated and neighboring sites in the Elizabeth and York Rivers. Aquat Toxicol 61:195–209

Munns WR, Black DE, Gleason TR, Salomon K, Bengtson D, GutjahrGobell R (1997) Evaluation of the effects of dioxin and PCBs on *Fundulus heteroclitus* populations using a modeling approach. Environ Toxicol Chem 16:1074–1081

Nacci D, Coiro L, Kuhn A, Champlin D, Munns W, Specker J, Cooper K (1998) Nondestructive indicator of ethoxyresorufin-*O*-deethylase activity in embryonic fish. Environ Toxicol Chem 17:2481–2486

Nacci D, Coiro L, Champlin D, Jayaraman S, McKinney R, Gleason TR, Munns WR, Specker JL, Cooper KR (1999) Adaptations of wild populations of the estuarine fish *Fundulus heteroclitus* to persistent environmental contaminants. Mar Biol 134:9–17

Nacci D, Jayaraman S, Specker J (2001) Stored retinoids in populations of the estuarine fish *Fundulus heteroclitus* indigenous to PCB-contaminated and reference sites. Arch Environ Contam Toxicol 40:511–518

Nacci DE, Champlin D, Coiro L, McKinney R, Jayaraman S (2002) Predicting the occurrence of genetic adaptation to dioxinlike compounds in populations of the estuarine fish *Fundulus heteroclitus*. Environ Toxicol Chem 21:1525–1532

Nacci DE, Champlin D, Jayaraman S (2010) Adaptation of the Estuarine Fish Fundulus heteroclitus (Atlantic Killifish) to Polychlorinated Biphenyls (PCBs). Estuaries Coasts 33:853–864

Nebert DW, Dalton TP, Okey AB, Gonzalez FJ (2004) Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. J Biol Chem 279:23847–23850

Nelson WG, Bergen BJ (2012) The New Bedford Harbor Superfund site long-term monitoring program (1993–2009). Environ Monit Assess 184:7531–7550

Nikkila A, Penttinen S, Kukkonen JVK (1999) UV-B-induced acute toxicity of pyrene to the waterflea Daphnia magna in natural freshwaters. Ecotoxicol Environ Saf 44:271–279

Ownby DR, Newman MC, Mulvey M, Vogelbein WK, Unger MA, Arzayus LF (2002) Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote-contaminated sediments. Environ Toxicol Chem 21:1897–1902

Oziolor EM, Bigorgne E, Aguilar L, Usenko S, Matson CW (2014) Evolved resistance to PCB- and PAH-induced cardiac teratogenesis, and reduced CYP1A activity in Gulf killifish (*Fundulus grandis*) populations from the Houston Ship Channel, Texas. Aquat Toxicol 150:210–219

Palumbi SR (2001) Evolution – humans as the world's greatest evolutionary force. Science 293:1786–1790

Peachey RBJ (2005) The synergism between hydrocarbon pollutants and UV radiation: a potential link between coastal pollution and larval mortality. J Exp Mar Biol Ecol 315:103–114

Pfennig DW, Servedio MR (2013) The role of transgenerational epigenetic inheritance in diversification and speciation. Nongenet Inheritance 1:17–26

Pflugh KK, Stern AH, Nesposudny L, Lurig L, Ruppel B, Buchanan GA (2011) Consumption patterns and risk assessment of crab consumers from the Newark Bay Complex, New Jersey, USA. Sci Total Environ 409:4536–4544

Pittman K, Yufera M, Pavlidis M, Geffen AJ, Koven W, Ribeiro L, Zambonino-Infante JL, Tandler A (2013) Fantastically plastic: fish larvae equipped for a new world. Rev Aquaculture 5:S224–S267

Poloczanska ES, Brown CJ, Sydeman WJ, Kiessling W, Schoeman DS, Moore PJ, Brander K, Bruno JF, Buckley LB, Burrows MT, Duarte CM, Halpern BS, Holding J, Kappel CV, O'Connor MI, Pandolfi JM, Parmesan C, Schwing F, Thompson SA, Richardson AJ (2013) Global imprint of climate change on marine life. Nat Clim Change 3:919–925

Powell WH, Morrison HG, Weil EJ, Karchner SI, Sogin ML, Stegeman JJ, Hahn ME (2004) Cloning and analysis of the CYP1A promoter from the atlantic killifish (*Fundulus heteroclitus*). Mar Environ Res 58:119–124

Proestou DA, Flight P, Champlin D, Nacci D (2014) Targeted approach to identify genetic loci associated with evolved dioxin tolerance in Atlantic Killifish (*Fundulus heteroclitus*). BMC Evol Biol 14:17

Pumure I, Renton JJ, Smart RB (2010) Ultrasonic extraction of arsenic and selenium from rocks associated with mountaintop removal/valley fills coal mining: Estimation of bioaccessible concentrations. Chemosphere 78:1295–1300

Reitzel AM, Karchner SI, Franks DG, Evans BR, Nacci D, Champlin D, Vieira VM, Hahn ME (2014) Genetic variation at aryl hydrocarbon receptor (AHR) loci in populations of Atlantic killifish (*Fundulus heteroclitus*) inhabiting polluted and reference habitats. BMC Evol Biol 14:6

Reynaud S, Deschaux P (2006) The effects of polycyclic aromatic hydrocarbons on the immune system of fish: a review. Aquat Toxicol 77:229–238

Rosenzweig C, Neofotis P (2013) Detection and attribution of anthropogenic climate change impacts. Wiley Interdiscip Rev Clim Change 4:121–150

Roy NK, Wirgin I (1997) Characterization of the aromatic hydrocarbon receptor gene and its expression in Atlantic tomcod. Arch Biochem Biophys 344:373–386

Roy NK, Courtenay SC, Chambers RC, Wirgin II (2006) Characterization of the aryl hydrocarbon receptor repressor and a comparison of its expression in atlantic tomcod from resistant and sensitive populations. Environ Toxicol Chem 25:560–571

Safe S (1984) Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) – biochemistry, toxicology and mechanism of action. CRC Crit Rev Toxicol 13:319–395

Schneider K, Roller M, Kalberlah F, Schuhmacher-Wolz U (2002) Cancer risk assessment for oral exposure to PAH mixtures. J Appl Toxicol 22:73–83

Shemer H, Linden KG (2007) Photolysis, oxidation and subsequent toxicity of a mixture of polycyclic aromatic hydrocarbons in natural waters. J Photochem Photobiol A Chem 187:186–195

Steinhauser G, Brandl A, Johnson TE (2014) Comparison of the Chernobyl and Fukushima nuclear accidents: a review of the environmental impacts. Sci Total Environ 470:800–817

Strydom C, Robinson C, Pretorius E, Whitcutt JM, Marx J, Bornman MS (2006) The effect of selected metals on the central metabolic pathways in biology: a review. Water SA 32:543–554

Subedi B, Usenko S (2012) Enhanced pressurized liquid extraction technique capable of analyzing polychlorodibenzo-p-dioxins, polychlorodibenzofurans, and polychlorobiphenyls in fish tissue. J Chromatogr 1238:30–37

Sugg DW, Bickham JW, Brooks JA, Lomakin MD, Jagoe CH, Dallas CE, Smith MH, Baker RJ, Chesser RK (1996) DNA damage and radiocesium in channel catfish from Chernobyl. Environ Toxicol Chem 15:1057–1063

Thackaberry EA, Nunez BA, Ivnitski-Steele ID, Friggins M, Walker MK (2005) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on murine heart development: alteration in fetal and postnatal cardiac growth, and postnatal cardiac chronotropy. Toxicol Sci 88:242–249

Theodorakis CW, Shugart LR (1998) Genetic ecotoxicology III: the relationship between DNA strand breaks and genotype in mosquito fish exposed to radiation. Ecotoxicology 7:227–236

Theodorakis CW, Blaylock BG, Shugart LR (1997) Genetic ecotoxicology I: DNA integrity and reproduction in mosquitofish exposed in situ to radionuclides. Ecotoxicology 6:205–218

Theodorakis CW, Elbl T, Shugart LR (1999) Genetic ecotoxicology IV: survival and DNA strand breakage is dependent on genotype in radionuclide-exposed mosquitofish. Aquat Toxicol 45:279–291

Toomey BH, Bello S, Hahn ME, Cantrell S, Wright P, Tillitt DE, Di Giulio RT (2001) 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces apoptotic cell death and cytochrome P4501A expression in developing *Fundulus heteroclitus* embryos. Aquat Toxicol 53:127–138

Turker OC, Vymazal J, Ture C (2014) Constructed wetlands for boron removal: a review. Ecol Eng 64:350–359

U.S. Environmental Protection Agency (2004) Exposure and human health reassessment of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. National Academy Sciences (External Review Draft), EPA/600/P-00/001Cb. U.S. Environmental Protection Agency, Washington, DC

U.S. General Accounting Office (2000) Superfund: information regarding EPA's cleanup decision process on the Hudson River site, GAO/RCED-00-193. U.S. General Accounting Office, Washington, DC

Valenti TW, Gould GG, Berninger JP, Connors KA, Keele NB, Prosser KN, Brooks BW (2012) Human therapeutic plasma levels of the selective serotonin reuptake inhibitor (SSRI) sertraline decrease serotonin reuptake transporter binding and shelter-seeking behavior in adult male fathead minnows. Environ Sci Technol 46:2427–2435

Van Veld PA, Westbrook DJ (1995) Evidence for depression of cytochrome P4501A in population of chemically resistant mummichog (*Fundulus heteroclitus*). Environ Sci 3:221–234

Van Veld PA, Ko UC, Vogelbein WK, Westbrook DJ (1991) Glutathione-S-transferase in intestine, liver and hepatic lesions of mummichog (*Fundulus heteroclitus*) from a creosote-contaminated environment. Fish Physiol Biochem 9:369–376

Veron JEN (2008) Mass extinctions and ocean acidification: biological constraints on geological dilemmas. Coral Reefs 27:459–472

Viguri J, Verde J, Irabien A (2002) Environmental assessment of polycyclic aromatic hydrocarbons (PAHs) in surface sediments of the Santander Bay, Northern Spain. Chemosphere 48:157–165

Virani NA, Rees BB (2000) Oxygen consumption, blood lactate and inter-individual variation in the gulf killifish, *Fundulus grandis*, during hypoxia and recovery. Comp Biochem Physiol A Mol Integr Physiol 126:397–405

Vogelbein WK, Fournie JW, Van Veld PA, Huggett RJ (1990) Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. Cancer Res 50:5978–5986

Walker SE, Dickhut RM, Chisholm-Brause C, Sylva S, Reddy CM (2005) Molecular and isotopic identification of PAH sources in a highly industrialized urban estuary. Org Geochem 36:619–632

Wassenberg DM, Di Giulio RT (2004a) Synergistic embryotoxicity of polycyclic aromatic hydrocarbon aryl hydrocarbon receptor agonists with cytochrome P4501A inhibitors in *Fundulus heteroclitus*. Environ Health Perspect 112:1658–1664

Wassenberg DM, Di Giulio RT (2004b) Teratogenesis in *Fundulus heteroclitus* embryos exposed to a creosote-contaminated sediment extract and CYP1A inhibitors. Mar Environ Res 58:163–168

Wassenberg DM, Swails EE, Di Giulio RT (2002) Effects of single and combined exposures to benzo(a)pyrene and 3,3′,4,4′,5-pentachlorobiphenyl on EROD activity and development in *Fundulus heteroclitus*. Mar Environ Res 54:279–283

Wei M, Wang LP, Zheng BH (2007) Photoinduced toxicity single and binary mixtures of four polycyclic aromatic hydrocarbons to the marine diatom *Skeletonema costatum*. Acta Oceanol Sin 26:41–50

Weis JS, Weis P, Heber M, Vaidya S (1981a) Methylmercury tolerance of killifish (*Fundulus heteroclitus*) embryos from a polluted *vs* non-polluted environment. Mar Biol 65:283–287

Weis P, Weis JS, Heber MA (1981b) Variation in teratogenic response to methylmercury by killifish (*Fundulus heteroclitus*) embryos – differences within and between populations. Anat Rec 199:A270–A271

Wenning R, Paustenbach D, Johnson G, Ehrlich R, Harris M, Bedbury H (1993) Chemometric analysis of potential sources of polychlorinated dibenzo-p-dioxins and dibenzofurans in surficial sediments from Newark Bay, New-Jersey. Chemosphere 27:55–64

Whitehead A, Triant DA, Champlin D, Nacci D (2010) Comparative transcriptomics implicates mechanisms of evolved pollution tolerance in a killifish population. Mol Ecol 19:5186–5203

Whitehead A, Roach JL, Zhang SJ, Galvez F (2011) Genomic mechanisms of evolved physiological plasticity in killifish distributed along an environmental salinity gradient. Proc Natl Acad Sci U S A 108:6193–6198

Whitehead A, Pilcher W, Champlin D, Nacci D (2012) Common mechanism underlies repeated evolution of extreme pollution tolerance. Proc Roy Soc B Biol Sci 279:427–433

Wills LP, Matson CW, Landon CD, Di Giulio RT (2010) Characterization of the recalcitrant CYP1 phenotype found in Atlantic killifish (*Fundulus heteroclitus*) inhabiting a Superfund site on the Elizabeth River, VA. Aquat Toxicol 99:33–41

Wirgin I, Waldman JR (2004) Resistance to contaminants in North American fish populations. Mutat Res Fundam Mol Mech Mutag 552:73–100

Wirgin I, Roy NK, Loftus M, Chambers RC, Franks DG, Hahn ME (2011) Mechanistic basis of resistance to PCBs in Atlantic Tomcod from the Hudson River. Science 331:1322–1325

Wolffe AP, Matzke MA (1999) Epigenetics: regulation through repression. Science 286:481–486

Xie L, Klerks PL (2003) Responses to selection for cadmium resistance in the least killifish, *Heterandria formosa*. Environ Toxicol Chem/SETAC 22:313–320

Xie LT, Klerks PL (2004) Fitness cost of resistance to cadmium in the least killifish (*Heterandria formosa*). Environ Toxicol Chem 23:1499–1503

Xu X, Li XG (2014) QSAR for photodegradation activity of polycyclic aromatic hydrocarbons in aqueous systems. J Ocean Univ 13:66–72

Yeager KM, Santschi PH, Rifai HS, Suarez MP, Brinkmeyer R, Hung CC, Schindler KJ, Andres MJ, Weaver EA (2007) Dioxin chronology and fluxes in sediments of the Houston Ship Channel, Texas: influences of non-steady-state sediment transport and total organic carbon. Environ Sci Tech 41:5291–5298

Zhang XW, Wu RSS, Fu WY, Xu LH, Lam PKS (2004) Production of reactive oxygen species and 8-hydroxy-2′ deoxyguanosine in KB cells co-exposed to benzo a pyrene and UV-A radiation. Chemosphere 55:1303–1308

Zhang WS, Sargis RM, Volden PA, Carmean CM, Sun XJ, Brady MJ (2012) PCB 126 and other dioxin-like PCBs specifically suppress hepatic PEPCK expression via the aryl hydrocarbon receptor. PLoS One 7:e37103

Zhou T, Scali R, Weis P, Weis JS (1996) Behavioral effects in mummichog larvae (*Fundulus heteroclitus*) following embryonic exposure to methylmercury. Mar Environ Res 42:45–49

# Extremophile Fishes: An Integrative Synthesis

**Michael Tobler, Rüdiger Riesch, and Martin Plath**

> *The history of evolution is that life escapes all barriers. Life breaks free. Life expands to new territories. Painfully, perhaps even dangerously. But life finds a way.*
> —Dr. Ian Malcolm (Jeff Goldblum) in *Jurassic Park*

**Abstract** Extremophile fishes have emerged as veritable models for investigations in integrative biology. They not only provide insights into biochemical, physiological, and developmental processes that govern life, but also allow for the elucidation of life's capacities and limitations to adapt to extreme environmental conditions. Over the past decades, researchers have made substantial progress towards understanding mechanisms underlying adaptation to extreme conditions mediated through a wide variety of physicochemical stressors. This chapter reviews some of the common themes and approaches used in the investigation of extremophiles and highlights several of the major open questions in this field: (1) Why do fish colonize extreme environments? (2) How can we gain an understanding of the mechanistic links between genomes and fitness of extremophiles in their natural environment? (3) How common is convergent evolution in extreme environments? (4) How do physicochemical stressors shape macroevolutionary processes? (5) How does acknowledging environmental and organismal complexity change our knowledge of evolution in extreme environments? Finally, (6) how can we make basic research on extremophiles applicable to solving major scientific challenges of our time and the coming decades?

---

M. Tobler (✉)
Division of Biology, Kansas State University, 116 Ackert Hall, Manhattan, KS 66506, USA
e-mail: tobler@ksu.edu

R. Riesch
School of Biological Sciences, Centre for Ecology, Evolution and Behaviour, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK

M. Plath
Sino-German Animal Research Center, College of Animal Science and Technology, Northwest A&F University, Yangling 712100, PR China

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_12

# 1 Introduction

Life in extreme environments has long captured the imagination of people. This is perhaps best exemplified by some of Jules Verne's "Voyages Extraordinaires" (Extraordinary Journeys), a series of novels that—amongst others—describes explorations of earth's poles and the deep sea that have profoundly influenced scientific inquiry (Verne 1870, 1889, 1897). In the scientific realm, life in extreme environments has sparked research on the origin of life (Mulkidjanian et al. 2012; Novikov and Copley 2013) and the exploration of potential extraterrestrial life (Cavicchioli 2002; Javaux 2006). Organisms thriving in environments that few life forms can tolerate are predominantly microbes (Rothschild and Mancinelli 2001; Pikuta and Hoover 2007), but metazoans—like the fishes emphasized in this volume—are increasingly recognized for their ability to adapt to abiotic stressors lethal to most other organisms (Waterman 1999, 2001; Bell 2012). These extremophiles allow for the investigation of life's capacities and limitations to deal with far-from-average conditions and have emerged as veritable models for investigations in integrative biology.

Physicochemical stressors typically have clear-cut and predictable physical, chemical, or physiological effects purportedly influencing biological processes at all levels of organization. They provide strong sources of selection shaping evolutionary trajectories of populations and can modulate ecological interactions and processes (Nevo 2011; Steinberg 2012). Consequently, they provide unique opportunities to address major questions in organismal biology: What are the mechanisms that link genomic variation to fitness (Barrett and Hoekstra 2011)? How do ecological and evolutionary processes interact to generate diversity (Nosil 2012)? What factors influence the repeatability and predictability of evolutionary change (Stern and Orgogozo 2009; Riesch et al. 2014)? Does evolutionary change affect ecological dynamics (Schoener 2011)? And finally, what factors constrain the fundamental niches and shape distribution patterns of organisms (Sexton et al. 2009)?

Extremophile fishes provide tangible study systems to address such questions particularly because of their high diversity. These systems frequently offer replicated, closely related lineages inhabiting both extreme and adjacent benign habitats, allowing for powerful comparative approaches. Furthermore, the ability to cultivate fishes in captivity facilitates combining field-based studies with laboratory experimentation, and the increasing availability of research tools, including genomic resources and other high throughput methods for data acquisition, consistently increases the power and reach of extremophiles to address fundamental questions in biology (e.g., Cossins and Crawford 2005; Crollius and Weissenbach 2005; Carvan 2007; Pardo-Martin et al. 2013; Jeyasingh et al. 2014; Gerlai 2015). Extremophile fishes also provide opportunities for translational science, because understanding the ways organisms function in the context of natural stressors provides basic insights into the biochemical, physiological, and developmental processes that govern life. For example, blind Mexican cavefish already serve as a model for eye development and degeneration (Jeffery 2001; O'Quin et al. 2013), and annual killifishes of the genus *Nothobranchius* are emerging as a study system for aging-related research (Genade et al. 2005; Terzibasi et al. 2007). Furthermore, fishes adapted to sulfide and anthropogenically toxic environments may provide insights into biomedical applications associated with the disruption of $H_2S$ homeostasis (Szabo 2007; Li et al. 2011) and the organismal processing of xenobiotics (van der Oost et al. 2003; Ferreira et al. 2014).

## 2 Fish Adaptation to Extreme Environments: Research Approaches

The chapters included in this volume are testament to the great advancement in knowledge about mechanisms underlying fish adaptation to extreme environments. Nonetheless, how and what we know about strategies that allow fishes to cope with these conditions varies widely among physicochemical stressors and taxonomic groups. This is in part for historical reasons, with different scientific groups applying particular skill sets to investigate adaptation to extreme environments, and in part a consequence of idiosyncrasies among different physicochemical stressors, which affect particular aspects of phenotypes and accordingly lend themselves to the application of certain modes of scientific inquiry. Here, we first review the general approaches employed to date and then highlight some gaps in our current knowledge that will necessitate the integration of analyses across levels of biological organization and taxonomic groups.

Consequences of some physicochemical stressors have been particularly investigated at or below the individual level. Such studies have often relied on the dose-dependent exposure of individuals to stressors to quantify subsequent biochemical and physiological responses (see Fig. 1a). For example, this allowed for the understanding of the modulation of metabolic physiology in dependence of dissolved oxygen concentrations (see chapter “Low-Oxygen Lifestyles”), the significance of antifreeze glycoproteins in the extreme cold of Antarctic waters (see chapter “The Adaptive Radiation of Notothenioid Fishes in the Waters of Antarctica”), ion transport mechanisms underlying tolerance to hypersaline (see chapter “Hypersaline Environments”) and acidic (see chapter “Pickled Fish Anyone?”) environments, and organismal processing of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in habitats affected by anthropogenic pollution (see chapter “Evolutionary Toxicology: Population Adaptation in Response to Anthropogenic Pollution”). Other systems have been investigated through the comparison of phenotypes in closely related populations occupying adjacent habitats differing in the presence or absence of a physicochemical stressor to make inferences about adaptation through the documentation of trait–environment correlations and subsequent studies on trait function (Fig. 1a, b). This approach has uncovered complex patterns of plastic and heritable modifications associated with the colonization of and adaptation to hypoxic (see chapter “Low-Oxygen Lifestyles”), desert (see chapter “Desert Environments”), sulfidic (see chapter “Hydrogen Sulfide-Toxic Habitats”), and cave (see chapter “Cave Environments”) environments. Finally, the ecological and evolutionary effects of some physicochemical stressors have predominantly been investigated through the application of

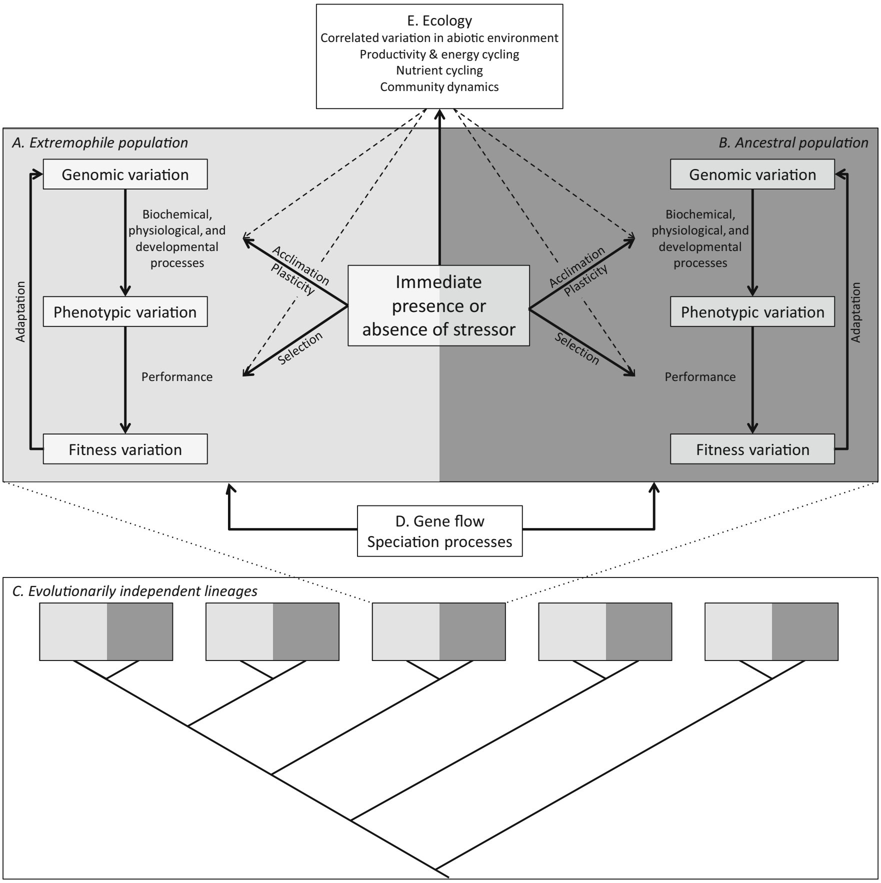


**Fig. 1** Our grand vision for understanding life in extreme environments emphasizes the integrative analysis of the effects of physicochemical stressors on different levels of biological organization and among disparate taxa. See text for details

broad scale comparative analyses among species and wider taxonomic groups (Fig. 1c). Such comparisons have revealed morphological modifications in rheophilic fishes (see chapter “Life in the Fast Lane: A Review of Rheophily in Freshwater Fishes”) and unique life-history strategies in fishes from temporary habitats (see chapter “Temporary Environments”).

Although the general approaches outlined above have been unevenly applied to understand the biology of fishes in extreme environments, it is important to note that the investigation of a growing number of systems has heavily relied on the integration of diverse methods to elucidate consequences of physicochemical stressors at multiple levels of biological organization. Such combinations of approaches have led to a rather sophisticated understanding about the biological consequences of life in some extreme environments. For example, we not only understand the physiological consequences of hypoxia exposure in adapted and non-adapted fishes, but studies have illuminated how genetic variation and hypoxia-induced phenotypic plasticity interact to shape phenotypic variation in natural populations of the same species, how different taxonomic groups vary in their strategies to cope with oxygen depletion, and how hypoxic conditions affect the composition of fish communities in different ecosystems (see chapter “Low-Oxygen Lifestyles”). Similarly, investigation of cavefishes of disparate evolutionary lineages has shed light into the genomic and developmental basis of phenotypic evolution, uncovered complex phenotypic modifications resulting from direct and indirect effects of the absence of light, and revealed striking patterns of convergent evolution among different taxonomic groups (see chapter “Cave Environments”).

## 3 A Grand Vision for Understanding Metazoan Life in Extreme Environments

A major theme emerging from the research on extremophile fishes is the complexity—and in some instances the diversity—of organismal responses to immediate and evolutionary exposures to stressors, despite their apparently clear-cut physical and chemical effects. This complexity and diversity is likely a function of three interacting factors: (1) Variation in genomic architecture and resulting genetic constraints can limit the expression of possible phenotypes and bias responses to selection (Schluter 1996; Simoes et al. 2008; Eroukhmanoff and Svensson 2011). (2) Phenotypic integration can cause functionally related traits to co-vary despite being genetically and developmentally independent and can generate alternative phenotypes with comparable performance (Arnold 1983; Sinervo and Svensson 2002; Wainwright et al. 2005). (3) Environmental complexity associated with the presence of physicochemical stressors can cause multifarious selective regimes through direct and indirect effects on abiotic and biotic components of an ecosystem (Grether et al. 2001; Tobler and Plath 2011; Kaeuffer et al. 2012). While there is a wealth of empirical data documenting the complexity and diversity of adaptive solutions to life in extreme environments, we still know little about the relative importance of underlying mechanisms driving these patterns. To discover commonalities in ecological and evolutionary consequences of physicochemical stressors, future work needs to strive for vertical integration of analyses across levels of organization to gain a mechanistic understanding of how genomic variation translates to phenotypes and fitness of organisms in their natural environment, and for horizontal integration among study systems to uncover general themes of ecological and evolutionary patterns in extreme environments. Below, we review what we consider some of the major open questions in our understanding of extremophile fishes, well knowing that some research groups have made tangible progress toward addressing these problems.

### *3.1 Why Live in Extreme Environments?*

Most faunal elements found in extreme environments are derived from ancestors that inhabit ecosystems with environmental parameters that we consider “normal” or “benign”. Considering the costs associated with exposure to physicochemical stressors, which can range from energetic costs required for the maintenance of homeostasis to drastically increased likelihoods of mortality (Calow 1989; Sibly and Calow 1989) and result in avoidance behaviors documented in many organisms (Schreck et al. 1997), a major question is how and why extreme environments were initially colonized. The colonization of extreme environments could occur as a passive process, where organisms haphazardly disperse into and get trapped in habitats characterized by the presence of a physicochemical stressor and subsequently adapt to the novel environmental conditions (e.g., Wilkens 1979; Holsinger 2000). Alternatively, extreme environments could offer open niches and be actively colonized by organisms, because they could gain direct fitness benefits that mitigate initial costs through the exploitation of novel trophic resources, minimizing competition, or avoiding natural enemies such as predators and parasites (e.g., Tobler et al. 2007; Springer 2009). While elucidating modes of colonization is inherently difficult, analyses of costs and benefits associated with life in extreme environments may provide a tangible avenue for differentiating between alternative hypotheses (see chapter “Low-Oxygen Lifestyles”).

Irrespective of whether the initial colonization was passive or active, population persistence in extreme environments obviously requires some degree of tolerance in invaders. In this context, we still lack rigorous analyses of what characteristics predispose certain organismal groups for being successful in the colonization of these marginal habitats. The fish faunas of some extreme habitats are clearly skewed taxonomically (e.g., sulfide-rich freshwaters globally are dominated by cyprinodontiform fishes; see chapter “Hydrogen Sulfide-Toxic Habitats” and Greenway et al. 2014) or functionally (e.g., many cavefishes have been hypothesized to be derived from nocturnal, benthic, or epibenthic ancestors; see chapter “Cave Environments” and Holsinger 2000). Comparative studies between groups that have successfully colonized extreme environments and other faunal elements that live in adjacent habitats could shed light into what traits could putatively represent exaptations facilitating persistence under stressful conditions and into fundamental constraints limiting evolution in and adaptation to extreme environments. Furthermore, phenotypic plasticity could play a critical role in the colonization of extreme environments, but remains largely unexplored. Plasticity in physiological, morphological, life-history, and behavioral traits may be induced by the presence of physicochemical stressors, facilitating the initial persistence of colonizing organisms and generating novel phenotypic variants for selection to act upon (Ghalambor et al. 2007; Pfennig et al. 2010). Through evolutionary time, initially plastic traits subsequently could be genetically assimilated, allowing for the expression of beneficial traits without the previously required environmental stimulus, and further refined by natural selection, ultimately creating locally adapted populations that now thrive in extreme environments (West-Eberhard 1989; Schlichting 2004).

### 3.2 Understanding the Hierarchical Effects of Physicochemical Stressors and Establishing Mechanistic Links Between Genomes and Fitness

Elucidating the mechanisms that link genomic variation to the expression of phenotypes and ultimately to the fitness of individuals in their natural environments is a major goal in current evolutionary biology (Barrett and Hoekstra 2011). This is a phenomenally complex task, because adaptation to physicochemical stressors can occur at multiple hierarchical levels, including short-term acclimatization, developmental plasticity, and genetic variation, as well as their interactions (Whitehead 2012). While the research reviewed in this volume provides clear evidence for the importance of all of these mechanisms in terms of their potential for shaping phenotypic variation relevant to coping with stressful environmental conditions, the current knowledge remains fragmentary because of a trend of compartmentalization in analytical approaches.

Responses to some physicochemical stressors have been primarily studied in the laboratory, focusing on the elucidation of links between genomes and phenotypes (Fig. 1). This has provided insight into the roles of acclimatization responses, developmental plasticity, and genetic variation in mounting responses to particular stressors (Hofmann and Todgham 2010; Schulte 2014), and has allowed for the identification of adaptive modifications down to particular physiological pathways, enzymes, and even genes (Bucheli 1995; Yeh and Feeney 1996; Pfenninger et al. 2014). Despite the obvious strengths of this approach, there are limitations that raise several intriguing problems. (1) Laboratory studies have not always relied on comparisons between populations adapted to extreme conditions and their putative ancestors, which limits inferences about how evolutionary processes have shaped adaptation; i.e., we may have an understanding of mechanisms conferring tolerance to certain stressors, but not necessarily how such modifications compare to ancestral, susceptible populations. In addition, laboratory-based studies that attempt to elucidate mechanisms underlying the expression of relevant phenotypic traits often fail to identify potential interactions between mechanisms (e.g., genetic variation in acclimatization responses). (2) Research disentangling the mechanisms of tolerance to physicochemical stress has frequently—and necessarily—focused on a small set of species (or even populations) inhabiting extreme environments. This narrow taxonomic scope has often prevented generalizations beyond rigorous case studies, which is a relevant limitation because even closely related evolutionary lineages exposed to the same physicochemical stressor can differ in mechanisms that give rise to similar phenotypic variants observed in natural systems (Langerhans and Riesch 2013; Pfenninger et al. 2014). (3) It is not always clear whether and how organismal responses quantified during exposure experiments in the laboratory translate to performance and fitness differences in natural environments, where multiple sources of selection and their interactions can shape fitness in unpredictable ways (Christensen et al. 2006; Holmstrup et al. 2010).

Much of what we know about adaptation to other extreme environments has been inferred from field-based studies. These have provided tremendous insights about phenotypic differences between extremophile populations and their ancestors, whether and how phenotypic responses vary among evolutionarily independent lineages, and how phenotypic modifications affect individual performance under stressful environmental conditions (Tobler et al. 2011; Soares and Niemiller 2013; Riesch et al. 2014). Field-based studies, however, have inherent limitations in terms of untangling mechanisms linking genomic and phenotypic variation. Most importantly, it often remains unclear whether trait divergence documented in nature is primarily caused by genetic variation among populations, or whether short-term acclimatization, developmental plasticity, and epigenetic effects contribute significantly to phenotypic variation in the wild. Even if the functional significance of trait modifications has been established, a lack of knowledge about mechanisms shaping trait variation tremendously constrains inferences about evolutionary mechanisms driving phenotypic evolution. Furthermore, it is not always straightforward to infer cause and effect relationships between sources of selection and specific traits. This is especially true in cases with complex phenotypic differences between extremophile and ancestral populations that are exposed to multifarious selective regimes (see Sect. 3.5).

While not all extremophile fishes lend themselves to investigating questions about mechanistic links between genomic variation and fitness, the availability of closely related lineages inhabiting extreme and benign environments associated with hypoxia (see chapter “Low-Oxygen Lifestyles”), hypersalinity (see chapter “Hypersaline Environments”), the absence of light in caves (see chapter “Cave Environments”), acidity (see chapter “Pickled Fish Anyone?”), as well as a variety of toxicants (see chapters “Hydrogen Sulfide-Toxic Habitats” and “Evolutionary Toxicology: Population Adaptation to Anthropogenic Pollution”) presents unique models to understand the effects of physicochemical stressors across levels of organization. Contrasting processes in adapted and ancestral (i.e., non-adapted) populations both in the presence and absence of a stressor will provide unprecedented insights into mechanisms underlying trait expression, trait functionality, and potential evolutionary consequences of adaptation. This will require factorial common garden experiments in the laboratory that simultaneously control environmental exposures for both adapted and ancestral populations and quantifying responses at relevant levels of biological organization. Complementing such experiments with measurements of organismal performance in the laboratory and field will also shed light into the relationship between phenotypic variation and fitness under different environmental conditions. Such approaches are facilitated by recent methodological breakthroughs that are increasingly accessible even for non-model organisms. Large-scale genomic analyses are becoming routine, and methods for assessing phenotypic variation at the level of gene transcription, proteins, metabolic processing, and more recently even developmental and structural traits are rapidly advancing.

### *3.3 Understanding the Nature of Evolutionary Convergence*

Convergent evolution, the evolution of similar traits in disparate lineages exposed to similar environmental conditions, has been documented in a wide variety of traits and taxa associated with nearly every physicochemical stressor highlighted in this volume, undoubtedly rendering it a major theme of life in extreme environments. Nonetheless, there is considerable variation in the degree of convergence documented to date. On one end of the spectrum, we have evidence for convergence at a functional level in many extremophiles. For example, fish have evolved a diversity of strategies for extracting atmospheric oxygen when living in hypoxic waters (see chapter "Low-Oxygen Lifestyles") or for coping with high water flows in rapids around the world (see chapter "Life in the Fast Lane: A Review of Rheophily in Freshwater Fishes"). Such functional convergence—particularly when achieved through different phenotypic modifications—is usually interpreted as an indicator for adaptation and for the deterministic nature of natural selection; i.e., organisms evolve shared solutions to shared problems, but sometimes in very different ways (Endler 1986). At the other end of the spectrum, convergence has been documented at a molecular level, where different lineages exposed to the same physicochemical stressors have independently evolved the same amino acid substitutions with concurrent changes in enzyme function (e.g., see chapter "Hydrogen Sulfide-Toxic Habitats"). Such convergence at a molecular level may at least in part reflect genetic constraints that limit organisms to few alternative phenotypes rather than solely being a signal of adaptive evolution (Wake 1991; Arthur 2001).

Clearly, the presence of evolutionary convergence is critically dependent on the level of biological organization investigated, because convergence at a functional level does not necessitate convergence on a trait level, and convergence on a trait level does not necessitate convergence in genes and developmental mechanisms underlying these traits (Arendt and Reznick 2008; Elmer and Meyer 2011). Furthermore, there is an increasing awareness that convergent evolution is not omnipresent, but organisms in some instances may have evolved very different solutions to a shared problem (Langerhans and Riesch 2013), although such non-convergent evolutionary change has not been investigated systematically in a large number of study systems (Rosenblum and Harmon 2011; Kaeuffer et al. 2012). Research on evolutionary convergence has accordingly shifted to the simultaneous quantification of shared (convergent) and unique (non-convergent) aspects of evolutionary diversification to estimate the relative importance of the two processes as well as simultaneous analyses across multiple levels of organization (Langerhans and DeWitt 2004). Extremophile fishes represent formidable models to build upon further investigations of convergent evolution, because physicochemical stressors provide high reproducibility as an environmental factor, thus facilitating comparative analyses across broad spatial and taxonomic scales. Extending approaches outlined in Sect. 3.2 across independent lineages inhabiting similar environments would allow testing how genetic and ecological factors, as well as functional constraints determine the balance between convergent and non-convergent evolutionary patterns, and provide insights into the question of the predictability and repeatability of evolution at varying taxonomic scales.

### 3.4 Key Innovations and the Role of Physicochemical Stressors in Driving Diversification

Extreme environments have not only been associated with microevolutionary processes, but the presence of stressors has also shaped macroevolutionary diversification in a variety of fish groups. This is evidenced by the wealth of endemic species, and in some instances even genera and families, that exclusively inhabit extreme environments. Such endemism is found both in extreme environments that cover vast spatial scales, including Antarctic waters (see chapter "The Adaptive Radiation of Notothenioid Fishes in the Waters of Antarctica"), as well as small, locally restricted habitats nested within benign environments, such as sulfide springs (see chapter "Hydrogen Sulfide-Toxic Habitats") and caves (see chapter "Cave Environments").

Endemism in extreme environments at a local scale is mostly congruent with the process of ecological speciation, where local adaptation driven by divergent selection along environmental gradients connecting benign and extreme habitat patches causes the evolution of reproductive isolation as a by-product (Schluter 2000; Rundle and Nosil 2005; Nosil 2012). In its simplest form, reproductive isolation can emerge simply as a consequence of adaptation, where costs associated with performance trade-offs of locally adapted individuals under different environmental conditions constrain gene flow even in the absence of physical barriers preventing migration (Nosil et al. 2005). Ecological speciation processes have been particularly well studied in sulfide spring and—to some degree—cavefishes (Borowsky and Cohen 2013; Plath et al. 2013), but it is tempting to speculate that ecological speciation may also occur in other locally restricted extreme habitats that have not been investigated in depth yet. For example, significant genetic differentiation has been documented between populations in the highly acidic waters of the Rio Negro and adjacent populations in the Solimoes/Amazon River of South America for several species of fish (Cooke et al. 2012a, b, 2014). In addition, gene flow has been shown to be low among populations of a cyprinid occupying habitats with different oxygen regimes (Harniman et al. 2013). However, how adaptation to acidic and hypoxic environments relates to potential speciation processes in these species remains to be investigated in detail. It is also important to note that genetic differentiation in response to divergent selection between extreme and benign habitats is not omnipresent (Crispo and Chapman 2008), and it has been hypothesized that phenotypic plasticity induced by physicochemical stressors may facilitate gene flow among populations in contrasting habitat types (Crispo 2008). Consequently, extremophile fishes provide exciting models to study speciation "in action", and future studies will need to illuminate the dynamic links between plasticity, gene flow, genetically determined local adaptation, and extrinsic as well as intrinsic reproductive isolation mechanisms in extreme environments. This will help understanding how and under what conditions speciation in extreme environments actually occurs and will also further our knowledge about the process of speciation in general.

The potential role of physicochemical stressors as a driving force in macroevolutionary diversification is also evident on larger spatial scales, where some fish groups that have evolved the ability to cope with extreme environmental conditions radiated from a single ancestor into multiple species. Such an adaptive radiation can, for example, be found in Antarctic waters (see chapter "The Adaptive Radiation of Notothenioid Fishes in the Waters of Antarctica"): The evolution of antifreeze proteins represents a key innovation that has allowed notothenioid fishes to persist at subzero temperatures and subsequently diversify into multiple species that occupy distinct ecological niches (Matschiner et al. 2011). The emergence of key innovations and the subsequent diversification of colonizing lineages remain to be studied rigorously in a variety of others systems, but there is compelling circumstantial evidence that might indicate that (adaptive) radiations in extreme environments may be more common than currently assumed. For example, pupfish of the genus *Cyprinodon* that have colonized hypersaline habitats in San Salvador and the calcium sulfate-rich Laguna Chichancanab in Mexico have sympatrically diverged into multiple species occupying different microhabitats and/or exploiting distinct trophic resources (Humphries and Miller 1981; Strecker 2006; Martin and Wainwright 2013; Martin and Feinstein 2014). Similarly, pupfish have diversified into multiple species across desert springs in North America (Soltz and Naiman 1978; Echelle and Dowling 1992), and temporary habitats in African and South American savannas harbor a diversity of annual killifishes (Costa 2009; Dorn et al. 2011; Garcia et al. 2014; Polacik et al. 2014), although speciation in these cases appears to have occurred largely in allopatry. All of these systems provide an intriguing opportunity to test whether and how the evolution of potential key innovations that allow for survival in the presence of physicochemical stressors may have facilitated the diversification of fishes in extreme environments.

## *3.5 Acknowledging and Identifying Environmental and Organismal Complexity*

Evolutionary responses to divergent selection are often studied in proximate habitats that differ in one or a few key environmental parameters, whereby habitats are typically classified into discrete categories. This approach has not only been used for the study of extremophiles but is also common in evolutionary ecology through the comparison of populations in low *vs.* high predation environments (e.g., Langerhans et al. 2007) or in marine *vs.* freshwater (e.g., Jones et al. 2012), lotic *vs.* lentic (e.g., Krabbenhoft et al. 2009), or benthic *vs.* limnetic habitats (e.g., Bernatchez et al. 1996). Simplifying environmental variation by classifying habitats into discrete categories has been extremely fruitful, because it has precipitated in the documentation of environmental factors shaping phenotypic variations in a wide variety of study systems, and gaining an understanding of both the functional significance of divergent traits (Ghalambor et al. 2004; Langerhans 2009) and their genomic underpinnings (Renaut et al. 2011; Jones et al. 2012). Nonetheless, extreme and benign habitats usually exhibit complex environmental differences, and suites of ecologically relevant factors co-vary between them (Tobler and Plath

2011). The presence of a physicochemical stressor can directly and indirectly affect other abiotic parameters as well as biotic components of the extreme environment (Fig. 1e). For example, the presence of hydrogen sulfide also affects oxygen availability in sulfidic habitats because of spontaneous oxidation in aqueous solution (see chapter "Hydrogen Sulfide-Toxic Habitats"). Moreover, because physicochemical stressors impose constraints for the persistence of populations, they act as a filter affecting the composition of biological communities (Belyea and Lancaster 1999). Accordingly, extreme habitats are usually less productive and characterized by truncated species diversity. Life in extreme environments may therefore be associated with changes in resource availability and quality, shifts from inter- to intraspecific competition, and changes in the exposure to predators and parasites. Any of these factors may in turn provide environmental stimuli that affect the expression of phenotypic traits and represent sources of selection that influence evolutionary differentiation among populations exposed to contrasting environmental conditions (Fig. 1e). Hence, while physicochemical stressors undoubtedly are primary candidates for shaping the ecology and evolution of extremophiles, selective regimes in extreme environments are invariably multifarious, which could ultimately facilitate or impede adaptation to stressors per se. Acknowledging and understanding the complexity of environmental variation and concomitant phenotypic adaptations has, for example, been a key theme in the investigation of cavefishes (see chapter "Cave Environments"). However, it remains largely unknown how the presence of physicochemical stressors affects additional environmental variables in other extreme habitats, and whether such co-varying factors are important in driving adaptive evolution.

Phenotypes, just like environments, are also notoriously complex. Phenotypic complexity spans traits ranging from gene expression patterns, physiology, morphology, and life history to behavior. In comparison to our ability to characterize genomes, the integrative analysis of traits across organismal levels of organization (i.e., the quantification of phenomes) has not kept pace over the past decades (Houle et al. 2010). Acknowledging phenotypic complexity and addressing it explicitly during empirical inquiry of adaptation to extreme environments will be critical to understand the multiple, layered strategies organisms use to cope with stressful environmental conditions. Although a wide variety of biochemical, physiological, structural, and behavioral coping strategies have been discovered for specific physicochemical stressors, disentangling the relative contributions of these traits to overall organismal performance remains a major task for the field and should reveal potential synergies and redundancies among strategies. Elucidating patterns of trait co-variation, as well as environmental and genetic mechanisms underlying it, is also important from an evolutionary perspective in order to make inferences about cause and effect relationships between sources of selection and trait divergence. This is exemplified by a recent study investigating evolutionary divergence in jaw morphology, taste bud expression, and eye development in *Astyanax* cavefish (Yamamoto et al. 2009). While divergence in these traits has previously been attributed to the action of selection from correlated environmental factors (and potentially genetic drift) on different parts of the genome, the discovery that these traits are modulated through the same developmental signaling pathway (*sonic* *hedgehog*) indicated that eye regression may be a mere pleiotropic consequence of selection for an increased number of taste buds and/or different jaw morphology (Yamamoto et al. 2009). Hence, integrating analyses of environmental and phenotypic complexity promises to gain a new level of sophistication in our understanding of life in extreme environments. Disentangling such effects would necessarily require the availability and investigation of multiple, independent lineages inhabiting extreme environments (see Sect. 3.3).

### *3.6 Making Basic Research Applicable*

Undoubtedly, research on extremophile fishes has contributed substantial knowledge to a wide variety of biological disciplines. We would like to stress, however, that many extremophiles also have a largely unrealized potential to contribute to applied research. This does not only include potential contributions to biomedical sciences discussed above in Sect. 1, but also pertains to probably the biggest popular scientific challenge of our time, the unprecedented effects of human activity on natural ecosystems. Human-induced environmental change has effectively created more and more extreme habitats, altering environmental conditions beyond what many organisms can tolerate and potentially causing the next mass extinction (Pimm et al. 2014). This is true not only for some physicochemical stressors discussed here, including the expansion of hypoxic zones coinciding with eutrophication (see chapter “Low-Oxygen Lifestyles”), the acidification of lakes through sulfur emissions into the atmosphere (see chapter “Pickled Fish Anyone?”), and of course the introduction of myriad xenobiotics from pharmaceutical and industrial applications (see chapter “Evolutionary Toxicology: Population Adaptation in Response to Anthropogenic Pollution”), but also for many other environmental parameters that were beyond the scope of this book. Studying how fishes and other organisms cope with naturally occurring extreme habitats will provide important information that can be applied to ecological risk assessment and to model and predict potential future patterns of biodiversity and ecosystem functions in the age of anthropogenic environmental change. Thus, studying the ecology, evolution, and physiology of organisms living in extreme environments also is of great relevance to the conservation of non-extremophile species.

## 4 Conclusions

Moyle and Cech (2000) famously stated “Humans are not the pinnacle of evolutionary progress but only an aberrant side branch of fish evolution.” Extremophile fish, on the other hand, may be veritable candidates for representing a pinnacle of evolutionary progress; the adverse environments they inhabit, and the sometimes-bizarre adaptations they have evolved, would indeed provide inspiration for science fiction movies. We hope that this volume will provide a useful resource for students and scientists interested in evolutionary and behavioral ecology, in extremophile life and organism–environment interactions, and in environmental science. Moreover, we hope this compilation of articles on fishes in extreme environments provides additional incentive for the continued use of extremophile fishes in basic and applied research.

**Acknowledgments** Z. Culumber and G. Hopper kindly provided comments on an earlier manuscript draft. We would like to thank all the contributing authors without whom this book would not have been possible. Our thanks are also extended to the numerous scientists who agreed to serve as external reviewers for the contributed chapters. Funding for this project was provided by the National Science Foundation (IOS-1121832) and Kansas State University (to MT) and the "1000 talents program" of the People's Republic of China (to MP).

## References

Arendt J, Reznick D (2008) Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? Trends Ecol Evol 23:26–32

Arnold SJ (1983) Morphology, performance and fitness. Am Zool 23:347–361

Arthur W (2001) Developmental drive: an important determinant of the direction of phenotypic evolution. Evol Dev 3:271–278

Barrett RDH, Hoekstra HE (2011) Molecular spandrels: tests of adaptation at the genetic level. Nat Rev Genet 12:767–780

Bell EM (ed) (2012) Life at extremes: environments, organisms and strategies for survival. CABI, Oxfordshire

Belyea LR, Lancaster J (1999) Assembly rules with a contingent ecology. Oikos 86:402–416

Bernatchez L, Vuorinen JA, Bodaly RA, Dodson JJ (1996) Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (Coregonus). Evolution 50:624–635

Borowsky R, Cohen D (2013) Genomic consequences of ecological speciation in *Astyanax* cavefish. PLoS One 8:e79903

Bucheli TD (1995) Induction of cytochrome-P450 as a biomarker for environmental contamination in aquatic ecosystems. Crit Rev Environ Sci Technol 25:201–268

Calow P (1989) Proximate and ultimate responses to stress in biological systems. Biol J Linn Soc 37:173–181

Carvan MJ III (2007) Fish models in toxicology. Zebrafish 4:9–20

Cavicchioli R (2002) Extremophiles and the search for extraterrestrial life. Astrobiology 2:281–292

Christensen MR, Graham MD, Vinebrooke RD, Findlay DL, Paterson MJ, Turner MA (2006) Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Glob Chang Biol 12:2316–2322

Cooke GM, Chao NL, Beheregaray LB (2012a) Marine incursions, cryptic species and ecological diversification in Amazonia: the biogeographic history of the croaker genus *Plagioscion* (Sciaenidae). J Biogeogr 39:724–738

Cooke GM, Chao NL, Beheregaray LB (2012b) Divergent natural selection with gene flow along major environmental gradients in Amazonia: insights from genome scans, population genetics and phylogeography of the characin fish *Triportheus albus*. Mol Ecol 21:2410–2427

Cooke GM, Landguth EL, Beheregaray LB (2014) Riverscape genetics identified replicated ecological divergence across an Amazonian ecotone. Evolution 68:1947–1960

Cossins AR, Crawford DL (2005) Fish as models for environmental genomics. Nat Rev Genet 6:324–340

Costa WJEM (2009) Trophic radiation in the South American annual killifish genus Austrolebias (Cyprinodontiformes: Rivulidae). Ichthyol Explor Freshw 20:179–191

Crispo E (2008) Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. J Evol Biol 21:1460–1469

Crispo E, Chapman LJ (2008) Population genetic structure across dissolved oxygen regimes in an African cichlid fish. Mol Ecol 17:2134–2148

Crollius HR, Weissenbach J (2005) Fish genomics and biology. Genome Res 15:1675–1682

Dorn A, Ng'oma E, Janko K, Reichwald K, Polacik M, Platzer M, Cellerino A, Reichard M (2011) Phylogeny, genetic variability and color polymorphism of an emerging animal model: the short-lived annual *Nothobranchius* fishes from southern Mozambique. Mol Phylogenet Evol 61:739–749

Echelle AA, Dowling TE (1992) Mitochondrial DNA variation and evolution of the Death Valley pupfishes (*Cyprinodon*, Cyprinodontidae). Evolution 46:193–206

Elmer KR, Meyer A (2011) Adaptation in the age of genomics: insights from parallelism and convergence. Trends Ecol Evol 26:298–306

Endler JA (1986) Natural selection in the wild. Princeton University Press, Princeton, NJ

Eroukhmanoff F, Svensson EI (2011) Evolution and stability of the G-matrix during the colonization of a novel environment. J Evol Biol 24:1363–1373

Ferreira M, Costa J, Reis-Henriques MA (2014) ABC transporters in fish species: a review. Front Physiol 5:266

Garcia G, Gutierrez V, Rios N, Turner B, Santunaque F, Lopez-Carro B, Folle G (2014) Burst speciation processes and genomic expansion in the neotropical annual killifish genus Austrolebias (Cyprinodontiformes, Rivulidae). Genetica 142:87–98

Genade T, Benedetti M, Terzibasi E, Roncaglia P, Valenzano DR, Cattaneo A, Cellerino A (2005) Annual fishes of the genus *Nothobranchius* as a model system for aging research. Aging Cell 4:223–233

Gerlai R (2015) Zebrafish phenomics: behavioral screens and phenotyping of mutagenized fish. Curr Opin Behav Sci 2:21–27

Ghalambor CK, Reznick DN, Walker JA (2004) Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (Poecilia reticulata). Am Nat 164:38–50

Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol 21:394–407

Greenway R, Arias-Rodriguez L, Diaz P, Tobler M (2014) Patterns of macroinvertebrate and fish diversity in freshwater sulphide springs. Diversity 6:597–632

Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W (2001) Rain forest canopy cover, resource availability, and life history evolution in guppies. Ecology 82:1546–1559

Harniman R, Merritt TJS, Chapman LJ, Lesbarreres D, Martinez ML (2013) Population differentiation of the African cyprinid *Barbus neumayeri* across dissolved oxygen regimes. Ecol Evol 3:1495–1506

Hofmann GE, Todgham AE (2010) Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annu Rev Physiol 72:127–145

Holmstrup M, Bindesbol A-M, Janneke Oostingh G, Duschl A, Scheil V, Kohler HR, Loureiro S, Soares AMVM, Ferreira ALG, Kienle C, Gerhardt A, Laskowski R, Kramarz PE, Bayley M, Svendsen C, Spurgeon DJ (2010) Interactions between effects of environmental chemicals and natural stressors: a review. Sci Total Environ 408:3746–3762

Holsinger JR (2000) Ecological derivation, colonization, and speciation. In: Wilkens H, Culver DC, Humphreys WF (eds) Ecosystems of the world 30. Subterranean ecosystems. Elsevier, Amsterdam

Houle D, Govindaraju DR, Omholt S (2010) Phenomics: the next challenge. Nat Rev Genet 11:855–866

Humphries J, Miller RR (1981) A remarkable species flock of pupfishes, genus *Cyprinodon*, from Yucatán, México. Copeia 1981:52–64

Javaux EJ (2006) Extreme life on Earth: past, present, and possibly beyond. Res Microbiol 157:37–48

Jeffery WR (2001) Cavefish as a model system in evolutionary developmental biology. Dev Biol 231:1–12

Jeyasingh PD, Cothran RD, Tobler M (2014) Testing the ecological consequences of evolutionary change using elements. Ecol Evol 4:528–538

Jones FC, Grabherr MG, Chan YF, Russell P, Mauceli E, Johnson J, Swofford R, Pirun M, Zody MC, White S, Birney E, Searle S, Schmutz J, Grimwood J, Dickson MC, Myers RM, Miller CT, Summers BR, Knecht AK, Brady SD, Zhang H, Pollen AA, Howes T, Amemiya C, Lander ES, di Palma F, Lindblad-Toh K, Kingsley DM (2012) The genomic basis of adaptive evolution in threespine stickleback. Nature 484:55–61

Kaeuffer R, Peichel CL, Bolnick DI, Hendry AP (2012) Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. Evolution 66:402–418

Krabbenhoft TJ, Collyer ML, Quattro JM (2009) Differing evolutionary patterns underlie convergence on elongate morphology in endemic fishes of Lake Waccamaw, North Carolina. Biol J Linn Soc 98:636–645

Langerhans RB (2009) Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. J Evol Biol 22:1057–1075

Langerhans RB, DeWitt TJ (2004) Shared and unique features of evolutionary diversification. Am Nat 164:335–349

Langerhans RB, Riesch R (2013) Speciation by selection: a framework for understanding ecology's role in speciation. Curr Zool 59:31–52

Langerhans RB, Gifford M, Joseph E (2007) Ecological speciation in *Gambusia* fishes. Evolution 61:2056–2074

Li L, Rose P, Moore PK (2011) Hydrogen sulfide and cell signaling. Annu Rev Pharmacol Toxicol 51:169–187

Martin CH, Feinstein LC (2014) Novel trophic niches drive variable progress towards ecological speciation within an adaptive radiation of pupfishes. Mol Ecol 23:1846–1862

Martin CH, Wainwright PC (2013) A remarkable species flock of *Cyprinodon* pupfishes endemic to San Salvador island, Bahamas. Bulletin of the Peabody Museum of Natural History 54:231–241

Matschiner M, Hanel R, Salzburger W (2011) On the origin and trigger of the notothenioid adaptive radiation. PLoS One 6:e18911

Moyle PB, Cech JJ Jr (2000) Fishes: an introduction to ichthyology. Trentice Hall, Upper Saddle River

Mulkidjanian AY, Bychkov AY, Dibrova DV, Galperin MY, Koonin EV (2012) Origin of first cells at terrestrial, anoxic geothermal fields. Proc Natl Acad Sci U S A 109:E821–E830

Nevo E (2011) Evolution under environmental stress at macro and microscales. Genome Biol Evol 3:1039–1052

Nosil P (2012) Ecological speciation. Oxford University Press, Oxford

Nosil P, Vines TH, Funk DJ (2005) Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution 59:705–719

Novikov Y, Copley SD (2013) Reactivity landscape of pyruvate under simulated hydrothermal vent conditions. Proc Natl Acad Sci U S A 110:13283–13288

O'Quin KE, Yoshizawa M, Doshi P, Jeffery WR (2013) Quantitative genetic analysis of retinal degeneration in the blind cave fish *Astyanax mexicanus*. PLoS One 8:e57281

Pardo-Martin C, Allalou A, Medina J, Eimon PM, Wahlby C, Yanik MF (2013) High-throughput hyperdimensional vertebrate phenotyping. Nat Commun 4:1467

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol Evol 25:459–467

Pfenninger M, Lerp H, Tobler M, Passow C, Kelley JL, Funke E, Greshake B, Erkoc UK, Berberich T, Plath M (2014) Parallel evolution of *cox* genes in $H_2S$-tolerant fish as key adaptation to a toxic envrionment. Nat Commun 5:3873

Pikuta EV, Hoover RB (2007) Microbial extremophiles at the limits of life. Crit Rev Microbiol 33:183–209

Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, Raven PH, Roberts CM, Sexton JO (2014) The biodiversity of species and their rates of extinction, distribution, and protection. Science 344:1246752

Plath M, Pfenninger M, Lerp H, Riesch R, Eschenbrenner C, Slattery P, Bierbach D, Herrmann N, Schulte M, Arias-Rodriguez L, Indy JR, Passow C, Tobler M (2013) Genetic differentiation and selection against migrants in evolutionarily replicated extreme environments. Evolution 67:2647–2661

Polacik M, Harrod C, Blazek R, Reichard M (2014) Trophic niche partitioning in communities of African annual fish: evidence from stable isotopes. Hydrobiologia 721:99–106

Renaut S, Nolte AW, Rogers SM, Derome N, Bernatchez L (2011) SNP signatures of selection on standing genetic variation and their association with adaptive phenotypes along gradients of ecological speciation in lake whitefish species pairs (*Coregonus* spp.). Mol Ecol 20:545–559

Riesch R, Plath M, Schlupp I, Tobler M, Langerhans RB (2014) Colonization of toxic springs drives predictable life-history shift in livebearing fishes (Poeciliidae). Ecol Lett 17:65–71

Rosenblum EB, Harmon LJ (2011) "Same but different": replicated ecological speciation at White Sands. Evolution 65:946–960

Rothschild L, Mancinelli R (2001) Life in extreme environments. Nature 409:1092–1101

Rundle HD, Nosil P (2005) Ecological speciation. Ecol Lett 8:336–352

Schlichting CD (2004) The role of phenotypic plasticity in diversification. In: DeWitt TJ, Scheiner SM (eds) Phenotypic plasticity: functional and conceptual approaches. Oxford University Press, Oxford, pp 191–200

Schluter D (1996) Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, Oxford

Schoener T (2011) The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. Science 331:426–429

Schreck CB, Olla BL, Davis MW (1997) Behavioral responses to stress. In: Iwama GK, Pickering AD, Sumpter JP, Schreck CB (eds) Fish stress and health in aquaculture. Cambridge University Press, New York, NY, pp 145–170

Schulte PM (2014) What is environmental stress? Insights from fish living in variable environments. J Exp Biol 217:23–34

Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and ecology of species range limits. Annu Rev Ecol Evol Syst 40:415–436

Sibly RM, Calow P (1989) A life-cycle theory of responses to stress. Biol J Linn Soc 37:101–116

Simoes P, Santos J, Fragata I, Mueller LD, Rose MR, Matos M (2008) How repeatable is adaptive evolution? The role of geographical origin and founder effects in laboratory adaptation. Evolution 62:1817–1829

Sinervo B, Svensson E (2002) Correlational selection and the evolution of genomic architecture. Heredity 89:329–338

Soares D, Niemiller ML (2013) Sensory adaptations of fishes to subterranean environments. Bioscience 63:274–283

Soltz DL, Naiman RJ (1978) The natural history of native fishes in the Death Valley system. Natural History Museum of Los Angeles County, Science Series 30:1–76

Springer YP (2009) Do extreme environments provide a refuge from pathogens? A phylogenetic test using serpentine flax. Am J Bot 96:2010–2021

Steinberg CEW (2012) Stress ecology: environmental stress as ecological driving force and key player in evolution. Springer, Heidelberg

Stern DL, Orgogozo V (2009) Is genetic evolution predictable? Science 323:746–751

Strecker U (2006) Genetic differentiation and reproductive isolation in a *Cyprinodon* fish species flock from Laguna Chichancanab, Mexico. Mol Phylogenet Evol 39:865–872

Szabo C (2007) Hydrogen sulphide and its therapeutic potential. Nat Rev Drug Discov 6:917–935

Terzibasi E, Valenzano DR, Cellerino A (2007) The short-lived fish *Nothobranchius furzeri* as a new model system for aging studies. Exp Gerontol 42:81–89

Tobler M, Plath M (2011) Living in extreme habitats. In: Evans J, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago, IL, pp 120–127

Tobler M, Schlupp I, Garcia FJ, de Leon M, Glaubrecht MP (2007) Extreme habitats as refuge from parasite infections? Evidence from an extremophile fish. Acta Oecologica 31:270–275

Tobler M, Palacios M, Chapman LJ, Mitrofanov I, Bierbach D, Plath M, Arias-Rodriguez L, Garcia de Leon FJ, Mateos M (2011) Evolution in extreme environments: replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. Evolution 65:2213–2228

van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Verne J (1870) Vingt mille lieues sous les mers. Hetzel, Paris

Verne J (1889) Sans dessus dessous. Hetzel, Paris

Verne J (1897) Le sphinx des glaces. Hetzel, Paris

Wainwright PC, Alfaro ME, Bolnick DI, Hulsey CD (2005) Many-to-one mapping of form to function: a general principle of organismal design. Integr Comp Biol 45:256–262

Wake DB (1991) Homoplasy: the result of natural selection or evidence for design limitations? Am Nat 138:543–567

Waterman TH (1999) The evolutionary challenges of extreme environments (part 1). J Exp Zool 285:326–359

Waterman TH (2001) Evolutionary challenges of extreme environments (part 2). J Exp Zool 291:130–168

West-Eberhard M (1989) Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Systemat 20:249–278

Whitehead A (2012) Comparative genomics in ecological physiology: toward a more nuanced understanding of acclimation and adaptation. J Exp Biol 215:884–891

Wilkens H (1979) Reduktionsgrad und phylogenetisches Alter: Ein Beitrag zur Besiedlungsgeschichte der Limnofauna Yukatans. Zeitschrift fur zoologische Systematik und Evolutionsforschung 17:262–272

Yamamoto Y, Byerly MS, Jackman WR, Jeffery WR (2009) Pleiotropic functions of embryonic sonic hedgehog expression link jaw and taste bud amplification with eye loss during cave fish evolution. Dev Biol 330:200–211

Yeh Y, Feeney RE (1996) Antifreeze proteins: structures and mechanisms of function. Chem Rev 96:601–617

# Appendix

**Table 1** Summary of genera containing rheophilic taxa and morphological specializations associated with each

| Ord. Fam. Subf. Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| Petromyzontiformes | | | | | | | | | | | | | | | | | | |
| Petromyzontidae | | | | | | | | | | | | | | | | | | |
| *Ichthyomyzon* | North America | X | X | - | - | - | X | - | - | - | ? | - | - | - | X | - | - | - |
| *Entosphenus**† | Pacific coasts | X | X | - | - | - | X | - | - | - | ? | - | - | - | X | - | - | - |
| Anguilliformes | | | | | | | | | | | | | | | | | | |
| Anguillidae | | | | | | | | | | | | | | | | | | |
| *Anguilla*† | Circumglobal | X | - | - | - | - | X | - | - | - | - | X | - | - | - | - | - | - |
| Osteoglossiformes | | | | | | | | | | | | | | | | | | |
| Mormyridae | | | | | | | | | | | | | | | | | | |
| *Campylomormyrus* | West/Central Africa | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - | - | - |
| *Mormyrops* | West/Central Africa | - | - | - | X | - | X | - | - | X | - | X | X | - | - | - | - | - |
| *Mormyrus** | West/Central Africa | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - | - | - |
| *Paramormyrops** | West/Central Africa | - | - | - | X | - | X | - | - | X | - | X | - | - | - | - | - | - |
| *Petrocephalus** | West/Central Africa | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - | - | - |
| *Pollimyrus** | West/Central Africa | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - | - | - |
| *Stomatorhinus* | West/Central Africa | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - | - | - |
| Clupeiformes | | | | | | | | | | | | | | | | | | |
| Clupeidae | | | | | | | | | | | | | | | | | | |
| *Clupeoides** | South East Asia | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| Gonorynchiformes | | | | | | | | | | | | | | | | | | |
| Kneriidae | | | | | | | | | | | | | | | | | | |
| Kneriinae | | | | | | | | | | | | | | | | | | |
| *Kneria* | Africa | - | X | - | - | - | X | - | - | X | - | X | - | - | - | Xa | - | - |
| *Parakneria* | Africa | - | X | - | - | - | X | - | - | X | X | - | - | - | - | Xa | - | - |

(continued)

R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1

**Table 1** (continued)

| Ord. | Fam. | Subf. | Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| Cypriniformes | | | | | | | | | | | | | | | | | | | | | |
| | Cyprinidae | | | | | | | | | | | | | | | | | | | | |
| | | Cyprininae | | | | | | | | | | | | | | | | | | | |
| | | | *Akrokolioplax* | South East Asia | - | - | X | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Bangana* | South Asia | - | - | X | - | - | - | - | - | X | X | - | - | - | - | Xa | - | - |
| | | | *Barbichthys* | South East Asia | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Barbus* | Eurasia | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | "*Barbus*"* | Africa | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Chuanchia* | China | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Cirrhinus* | South Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Cophecheilus* | China | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Crossocheilus* | South Asia | - | X | X | - | - | - | - | X | - | - | X | - | - | - | Xa | - | - |
| | | | *Cyprinion* | South Asia | - | - | X | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Discherodontus* | South East Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Discocheilus* | China | - | X | - | - | - | - | - | X | - | X | - | - | - | X | Xa | - | - |
| | | | *Discogobio* | South East Asia | - | X | - | - | - | - | - | X | - | X | - | - | - | X | Xa | - | - |
| | | | *Diplocheilichthys* | South East Asia | - | - | X | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Diptychus* | South Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Folifer* | South East Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Garra* | Africa and Asia | - | X | - | - | - | - | X | X | - | X | - | - | - | X | Xa | - | - |
| | | | *Gymnocypris* | China | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Gymnodiptychus* | South Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Gymnostomus* | South East Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Hongshuia* | China | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Incisilabeo* | South East Asia | - | - | X | - | - | - | - | - | X | - | X | - | - | - | Xa | - | - |
| | | | *Labeo** | Africa and Asia | - | X | X | - | - | - | - | X | X | X | X | - | - | - | Xa | - | - |
| | | | *Labeobarbus* | Africa | - | - | X | X | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Laocypris* | Laos | - | - | - | X | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Lobocheilos* | South East Asia | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Mekongina* | South East Asia | - | X | X | - | - | - | - | - | X | X | - | - | - | - | Xa | - | - |
| | | | *Neolissochilus* | South Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Onychostoma* | South East Asia | - | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - |
| | | | *Paracrossocheilus* | South East Asia | - | - | X | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Parapsilorhynchus* | India | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Parasinilabeo* | China | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Placocheilus* | East Asia | - | X | - | - | - | - | X | X | - | X | - | - | - | X | Xa | - | - |
| | | | *Platypharodon* | China | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Probarbus*† | South East Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Pseudocrossocheilus* | China | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Pseudogyrinocheilus* | China | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Ptychidio* | East Asia | - | - | X | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Ptychobarbus* | South Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Scaphiodonichthys* | South East Asia | - | - | X | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Schismatorhynchos* | South Asia | - | X | X | - | - | - | - | X | X | X | X | - | - | - | - | - | - |
| | | | *Schizopygopsis* | South Central Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Schizothorax* | South Central Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Semilabeo* | South East Asia | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Semiplotus* | South Asia | - | - | X | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Sinocrossocheilus* | China | - | X | X | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Varicorhinus** | Africa | - | - | X | - | - | - | - | X | - | X | X | - | - | - | - | - | - |
| | | | *Tor*† | South Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | Danioninae | | | | | | | | | | | | | | | | | | | |
| | | | *Barilius* | South Asia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Devario* | South Asia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Opsaridium* | Africa | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Opsarius* | South Asia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Raiamas* | Africa/South Asia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Rasbora** | South Asia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |

(continued)

**Table 1** (continued)

| Ord. | Fam. | Subf. | Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| | | | *Salmostoma* | South Asia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Securicula* | South Asia | - | - | - | - | X | - | - | X | - | - | - | - | X | - | - | - | - |
| | | Opsarichthyinae | | | | | | | | | | | | | | | | | | | |
| | | | *Opsariichthys* | East Asia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Zacco* | East Asia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Candidia* | East Asia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Nipponocypris* | East Asia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Macrochirichthys* | South East Asia | - | - | - | - | X | - | - | X | - | - | - | - | X | - | - | - | - |
| | | Leuciscinae | | | | | | | | | | | | | | | | | | | |
| | | | *Alburnoides* | Eurasia | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Alburnus**† | Eurasia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Anaecypris* | Europe | - | - | - | X | - | - | - | X | - | - | - | - | X | - | - | - | - |
| | | | *Aspius*† | Eurasia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Ballerus*† | Eurasia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Campostoma* | North America | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Chondrostoma** | Europe | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Cyprinella* | North America | - | - | X | X | - | - | - | X | - | X | X | - | - | - | - | - | - |
| | | | *Erimystax* | North America | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Exoglossum* | North America | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Gila* | North America | - | - | X | X | - | - | - | X | - | - | X | X | - | - | - | - | - |
| | | | *Hybognathus* | North America | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Hybopsis* | North America | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Leuciscus**† | Eurasia | - | - | X | X | - | - | - | X | - | - | X | X | - | - | - | - | - |
| | | | *Luxilus* | North America | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Macrhybopsis* | North America | - | X | X | - | - | - | - | X | - | X | X | - | - | - | - | - | - |
| | | | *Margariscus* | North America | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Nocomis* | North America | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Notropis** | North America | - | - | X | X | - | - | - | X | - | - | X | X | - | - | - | - | - |
| | | | *Parachondrostoma* | Europe | - | - | X | X | - | - | - | X | - | - | X | X | - | - | - | - | - |
| | | | *Phenacobius* | North America | - | X | X | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Phoxinus* | Eurasia | - | - | X | X | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Pimephales** | North America | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Platygobio* | North America | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Protochondrostoma* | Europe | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Pseudochondrostoma* | Europe | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Ptychocheilus* | North America | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Rhinichthys* | North America | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Richardsonius* | North America | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Rutilus**† | Eurasia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Squalius**† | Eurasia | - | - | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Telestes* | Europe | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Tiaroga* | North America | - | X | - | - | - | - | - | X | - | - | X | - | - | - | Xa | - | - |
| | | | *Tropidophoxinellus** | Europe | - | - | - | X | - | - | - | X | - | - | - | X | X | - | - | - | - |
| | | | *Vimba*† | Eurasia | - | - | - | X | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | Gobioninae | | | | | | | | | | | | | | | | | | | |
| | | | *Abbottina* | East Asia | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Belligobio* | China | - | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Biwia* | Japan | - | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Coreius*† | China | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Coreoleuciscus* | Korean Peninsular | - | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Gobio* | Eurasia | - | X | - | - | - | - | - | X | - | X | X | - | - | - | - | - | - |
| | | | *Gobiobotia* | Eurasia | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Hemibarbus* | East Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Huigobio* | China | - | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Ladislavia* | East Asia | - | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Mesogobio* | East Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Microphysogobio* | East Asia | - | X | - | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Platysmacheilus* | China | - | X | - | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Pseudogobio* | East Asia | - | X | - | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Pseudopungtungia* | East Asia | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |

(continued)

**Table 1** (continued)

| Ord. | Fam. | Subf. | Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| | | | *Rhinogobio* | China | - | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Romanogobio* | Eurasia | - | X | - | - | - | - | - | X | - | X | X | - | - | - | - | - | - |
| | | | *Sarcocheilichthys* | East Asia | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | | | *Saurogobio* | East Asia | - | X | X | - | - | - | - | X | - | X | X | - | - | - | - | - | - |
| | | | *Xenophysogobio* | China | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| | Psilorhynchidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Psilorhynchus* | South Asia | - | X | - | - | - | - | X | X | - | X | - | - | - | - | Xa | - | - |
| | Gyrinocheilidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Gyrinocheilus* | South East Asia | - | X | - | - | - | - | - | X | - | X | - | - | - | X | Xa | - | - |
| | Catostomidae | | | | | | | | | | | | | | | | | | | | |
| | | Catostominae | | | | | | | | | | | | | | | | | | | |
| | | | *Xyrauchen* | North America | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | Cycleptinae | | | | | | | | | | | | | | | | | | | |
| | | | *Cycleptus* | North America | - | X | X | - | - | - | - | X | - | X | - | - | - | - | Xc | - | - |
| | | Moxostominae | | | | | | | | | | | | | | | | | | | |
| | | | *Moxostoma** | North America | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Hypentelium* | North America | - | X | - | - | - | - | - | X | - | X | - | - | - | - | Xc | - | - |
| | Cobitidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Acantopsis* | South East Asia | X | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Bibarba* | China | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Canthophrys* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Cobitis** | Eurasia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Iksookimia* | South Korea | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Kichulchoia* | South Korea | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Lepidocephalichthys* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Neoeucirrhichthys* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Niwaella* | East Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Pangio** | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Paralepidocephalus* | China | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Sabanejewia* | Europe | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | Botiidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Botia** | South Asia | - | X | - | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Chromobotia* | South East Asia | - | X | - | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Leptobotia* | China | - | X | - | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Sinibotia* | China | - | X | - | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Syncrossus* | South East Asia | - | X | - | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | | | *Yasuhikotakia* | South East Asia | - | X | - | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| | Nemacheilidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Aborichthys* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Acanthocobitis* | South Asia | X | - | - | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Afronemacheilus* | East Africa | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Barbatula* | Eurasia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Claea* | China | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Dzihunia* | Central Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Homatula* | China | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Ilamnemacheilus* | Iran | - | X | - | - | - | - | - | - | X | X | - | - | - | - | Xa | - | - |
| | | | *Indoreonectes* | India | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Indotriplophysa* | South Asia | - | X | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Iskandaria* | Central Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Labiatophysa* | Central Asia | - | X | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Mesonoemacheilus* | India | X | X | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Metaschistura* | Central Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Nemacheilus** | South East Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Nemachilichthys* | India | X | - | - | - | - | - | - | - | X | X | - | - | - | - | Xa | - | - |
| | | | *Neonoemacheilus* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Oreonectes* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Oxynoemacheilus* | Eurasia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Paracobitis* | West Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Paraschistura* | West Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Physoschistura* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |

(continued)

**Table 1** (continued)

| Ord. | Fam. | Subf. | Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| | | | *Protonemacheilus* | China | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Pteronemacheilus* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Schistura* | South Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Sectoria* | South East Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Seminemacheilus* | Turkey | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Sundoreonectes* | Borneo | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Tarimichthys* | China | - | X | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Traccatichthys* | South East Asia | X | X | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Triplophysa* | Asia | - | X | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Tuberoschistura* | South East Asia | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Turcinoemacheilus* | Middle East/Nepal | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xa | - | - |
| | Balitoridae | | | | | | | | | | | | | | | | | | | | |
| | | | *Balitora* | South Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Balitoropsis* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Bhavania* | India | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Cryptotora* | Thailand | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Hemimyzon* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Homaloptera* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Homalopteroides* | South East Asia | X | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Homalopterula* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Jinshaia* | China | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Lepturichthys* | China | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Metahomaloptera* | China | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | Xa |
| | | | *Neohomaloptera* | South East Asia | X | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Sinogastromyzon* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | Xa |
| | | | *Travancoria* | India | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | Gastromyzontidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Annamia* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Beaufortia* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | Xa |
| | | | *Erromyzon* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Formosania* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Gastromyzon* | Borneo | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | Xa |
| | | | *Glaniopsis* | Borneo | X | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Hypergastromyzon* | Borneo | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | Xa |
| | | | *Katibasia* | Borneo | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Liniparhomaloptera* | China | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Neogastromyzon* | Borneo | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | Xa |
| | | | *Paraprotomyzon* | China | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Parhomaloptera* | Borneo | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Plesiomyzon* | China | - | X | - | - | - | - | - | X | - | X | - | - | - | - | Xa | - | - |
| | | | *Protomyzon* | Borneo | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Pseudogastromyzon* | China | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Sewellia* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Vanmanenia* | South East Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Yaoshania* | China | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | Barbuccidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Barbucca* | South East Asia | X | - | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | Serpenticobitidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Serpenticobitis* | South East Asia | - | X | - | - | - | - | - | - | X | X | - | - | - | - | Xa | - | - |
| Characiformes | | | | | | | | | | | | | | | | | | | | | |
| | Alestidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Bryconaethiops* | West/Central Africa | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| | Bryconidae | | | | | | | | | | | | | | | | | | | | |
| | | Bryconinae | | | | | | | | | | | | | | | | | | | |
| | | | *Brycon* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| | | Salmininae | | | | | | | | | | | | | | | | | | | |
| | | | *Salminus* | Tropical S America | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |

(continued)

**Table 1** (continued)

| Ord. Fam. Subf. Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| Characidae | | | | | | | | | | | | | | | | | | |
| *incertae sedis* | | | | | | | | | | | | | | | | | | |
| *Astyanacinus* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Astyanax* | Central/South America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Attonitus* | Andes Mountains | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| *Bryconamericus* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Bryconexodon* | Lower Amazon | - | - | - | - | X | - | - | - | X | - | - | X | - | - | - | - | - |
| *Bryconops* | Tropical S America | - | - | - | - | X | - | - | X | - | - | - | X | - | - | - | - | - |
| *Deuterodon* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Engraulisoma* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Exodon* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Genycharax* | Upper Cauca River | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Jupiaba* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Knodus* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Piabina* | Southeastern Brazil | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| Agoniatinae | | | | | | | | | | | | | | | | | | |
| *Agoniates* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - |
| Clupeacharacinae | | | | | | | | | | | | | | | | | | |
| *Clupeacharax* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| Aphyocharacinae | | | | | | | | | | | | | | | | | | |
| *Aphyocharax* | Tropical S America | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| Characinae | | | | | | | | | | | | | | | | | | |
| *Galeocharax* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Roeboexodon* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| Stevardiinae | | | | | | | | | | | | | | | | | | |
| *Bryconacidnus* | SW Amazon Basin | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| *Ceratobranchia* | Andes Mountains | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| *Creagrutus* | Tropical S America | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| *Hemibrycon* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| Cynodontidae | | | | | | | | | | | | | | | | | | |
| *Cynodon* | Tropical S America | - | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - |
| *Hydrolycus* | Tropical S America | - | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - |
| *Rhaphiodon* | Tropical S America | - | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - |
| *Roestes* | Tropical S America | - | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - |
| Distichodontidae | | | | | | | | | | | | | | | | | | |
| *Distichodus** | West/Central Africa | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Nannocharax** | West/Central Africa | - | - | X | - | - | - | - | - | X | - | X | - | - | - | Xa | - | - |
| Lebiasinidae | | | | | | | | | | | | | | | | | | |
| *Lebiasina* | Tropical S America | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| *Piabucina* | Tropical S America | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| Parodontidae | | | | | | | | | | | | | | | | | | |
| *Parodon* | Tropical S America | - | - | X | - | - | - | - | X | - | X | - | - | - | - | Xb | - | - |
| *Apareiodon* | Tropical S America | - | - | X | - | - | - | - | X | - | X | - | - | - | - | Xb | - | - |

(continued)

**Table 1** (continued)

| Ord. Fam. Subf. Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| Prochilodontidae | | | | | | | | | | | | | | | | | | |
| *Prochilodus* | Tropical S America | - | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - |
| Crenuchidae | | | | | | | | | | | | | | | | | | |
| *Characidium** | Tropical S America | - | X | - | - | - | - | - | X | - | - | X | - | - | - | Xa | - | - |
| Anostomidae | | | | | | | | | | | | | | | | | | |
| *Abramites* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Anostomoides** | Tropical S America | - | - | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Anostomus* | Tropical S America | - | - | X | - | - | - | - | X | - | - | - | - | X | - | - | - | - |
| *Hypomasticus* | Tropical S America | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| *Leporellus* | Tropical S America | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - |
| *Leporinus** | Tropical S America | - | - | X | - | - | - | - | X | - | - | X | X | - | - | - | - | - |
| *Petulanos* | Tropical S America | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| *Pseudanos* | Tropical S America | - | - | X | - | - | - | - | X | - | - | - | - | X | - | - | - | - |
| *Sartor* | Lower Amazon | - | - | X | - | - | - | - | X | - | - | - | - | X | - | - | - | - |
| *Synaptolaemus* | Guiana/Brazilian Shield | - | - | X | - | - | - | - | X | - | - | - | - | X | - | - | - | - |
| *Gnathodolus* | Orinoco/Casiquiare | - | - | X | - | - | - | - | X | - | - | - | - | X | - | - | - | - |
| Serrasalmidae | | | | | | | | | | | | | | | | | | |
| *Acnodon* | Tropical S America | - | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - |
| *Myleus* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Myloplus* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| *Ossubtus* | Xingu River | - | - | X | - | - | - | - | - | X | X | - | - | - | - | - | - | - |
| *Tometes* | Tropical S America | - | - | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| Siluriformes | | | | | | | | | | | | | | | | | | |
| Diplomystidae | | | | | | | | | | | | | | | | | | |
| *Diplomystes* | Southern Andes | - | - | X | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| Astroblepidae | | | | | | | | | | | | | | | | | | |
| *Astroblepus* | Andes Mountains | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| Trichomycteridae | | | | | | | | | | | | | | | | | | |
| *Trichomycterus** | South America | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xc | - | - |
| *Itugianis* | Tropical S America | X | - | - | - | - | X | - | - | - | X | - | - | - | - | Xc | - | - |
| Loricariidae | | | | | | | | | | | | | | | | | | |
| Ancistrini | | | | | | | | | | | | | | | | | | |
| *Acanthicus* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Ancistrus** | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Baryancistrus* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Chaetostoma* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Cordylancistrus* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Corymbophanes* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Dolichancistrus* | Northern Andes | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Exastilithoxus* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Guyanancistrus* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Hemiancistrus** | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Hopliancistrus* | Brazilian Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| *Lasiancistrus** | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |

(continued)

**Table 1** (continued)

| Ord. | Fam. | Subf. | Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| | | | *Leporacanthicus* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Leptoancistrus* | Northern Andes | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Lithoxancistrus* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Lithoxus* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Megalancistrus* | Paraná/São Francisco | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Neblinichthys* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Oligancistrus* | Brazilian Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Panaqolus* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Panaque* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Parancistrus* | Brazilian Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Paulasquama* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Peckoltia* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Pseudacanthicus* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Pseudancistrus* | Guiana/Brazilian Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Pseudolithoxus* | Orinoco Basin | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Scobinancistrus* | Brazilian Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Spectracanthicus* | Brazilian Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Zonancistrus* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | Neoplecostominae | | | | | | | | | | | | | | | | | | | |
| | | | *Isbrueckerichthys* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Kronichthys* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Neoplecostomus* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Pareiorhaphis* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Pareiorhina* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Pseudotocinclus* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | Lithogeninae | | | | | | | | | | | | | | | | | | | |
| | | | *Lithogenes* | Guiana Shield | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | Delturinae | | | | | | | | | | | | | | | | | | | |
| | | | *Delturus* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Hemipsylichthys* | Southeastern Brazil | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | Loricariinae | | | | | | | | | | | | | | | | | | | |
| | | | *Lamontichthys* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Harttia* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | | | *Spatuloricaria* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xc | - | - |
| | Bagridae | | | | | | | | | | | | | | | | | | | | |
| | | | *Bagrus* | Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | Claroteidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Chrysichthys* | Africa | - | X | X | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Notoglanidium* | Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | Amblycipitidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Amblyceps* | South Asia | X | X | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | | | *Liobagrus* | East Asia | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | Amphiliidae | | | | | | | | | | | | | | | | | | | | |
| | | Amphiliinae | | | | | | | | | | | | | | | | | | | |
| | | | *Amphilius* | Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Paramphilius* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |

(continued)

**Table 1** (continued)

| | | | | | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ord. | Fam. | Subf. | Genus | Distribution | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| | | Doumeinae | | | | | | | | | | | | | | | | | | | |
| | | | *Andersonia* | Northeastern Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Belonoglanis* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Congoglanis* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Doumea* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Phractura* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xa | - | - |
| | | | *Trachyglanis* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | Leptoglanidinae | | | | | | | | | | | | | | | | | | | |
| | | | *Dolichamphilius* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xa | - | - |
| | | | *Leptoglanis* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Psammphiletria* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Tetracamphilius* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Zaireichthys* | Africa | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | Clariidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Clarius** | Paleotropical | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Gymnallabes* | West/Central Africa | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - | - | - |
| | Mochokidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Synodontis** | Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | X | - | - | - |
| | | Chiloglaninae | | | | | | | | | | | | | | | | | | | |
| | | | *Atopochilus* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | X | - | - | - |
| | | | *Atopodontus* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | X | - | - | - |
| | | | *Chiloglanis* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | X | - | - | - |
| | | | *Euchilichthys* | West/Central Africa | - | X | - | - | - | - | X | - | - | X | - | - | - | X | - | - | - |
| | Sisoridae | | | | | | | | | | | | | | | | | | | | |
| | | Sisorinae | | | | | | | | | | | | | | | | | | | |
| | | | *Bagarius* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Caelatoglanis* | SE Asia | - | X | - | - | - | - | X | X | - | X | - | - | - | - | - | X | - |
| | | | *Conta* | India/SE Asia | - | X | - | - | - | - | X | X | - | X | - | - | - | - | - | X | - |
| | | | *Nangra* | South Asia | - | X | - | - | - | - | - | X | - | X | - | - | - | - | - | - | - |
| | | | *Pseudolaguvia* | India/SE Asia | X | X | - | - | - | - | X | X | - | X | - | - | - | - | - | X | - |
| | | | *Glyptothorax* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | X | - |
| | | Glyptosterninae | | | | | | | | | | | | | | | | | | | |
| | | | *Creteuchiloglanis* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xa | - | - |
| | | | *Euchiloglanis* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Exostoma* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xa | - | - |
| | | | *Glaridoglanis* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Glyptosternon* | South Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | X | - |
| | | | *Myersglanis* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Oreoglanis* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xa | - | - |
| | | | *Pareuchiloglanis* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | - | - |
| | | | *Pseudecheneis* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | - | Xa | X | - |
| | | | *Pseudexostoma* | India/SE Asia | - | X | - | - | - | - | X | - | - | X | - | - | - | X | Xa | - | - |
| | Heptapteridae | | | | | | | | | | | | | | | | | | | | |
| | | | *Chasmocranus* | Tropical S America | X | - | - | - | - | X | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Leptorhamdia* | Tropical S America | X | - | - | - | - | X | X | - | - | X | - | - | - | - | - | - | - |
| | Pimelodidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Brachyplatystoma* | Tropical S America | - | X | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | Ictaluridae | | | | | | | | | | | | | | | | | | | | |
| | | | *Noturus** | North America | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |

(continued)

**Table 1** (continued)

| | | | | | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ord.** | **Fam.** | **Subf.** | **Genus** | **Distribution** | **Hyp.** | **Ben.** | **B.P.** | **M.P.** | **T.P.** | **Ang.** | **Dep.** | **Fus.** | **Com.** | **Inf.** | **Sub.** | **Ter.** | **Sup.** | **O.D.** | **P.-F.P.** | **T.P.** | **P.-F.D.** |
| | Aspredinidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Hoplomyzon* | Northern Andes | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| | | | *Ernstichthys* | Andes | X | - | - | - | - | - | X | - | - | X | - | - | - | - | - | - | - |
| Gymnotiformes | | | | | | | | | | | | | | | | | | | | | |
| | Gymnotidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Gymnotus** | Tropical S America | - | - | - | X | - | X | - | - | - | - | - | - | X | - | - | - | - |
| | | | *Sternarchogiton* | Tropical S America | - | - | - | X | - | - | - | - | X | - | X | - | - | - | - | - | - |
| | | | *Sternarchorhynchus* | Tropical S America | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - | - | - |
| Osmeriformes | | | | | | | | | | | | | | | | | | | | | |
| | Plecoglossidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Plecoglossus**† | East Asia | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | Galaxiidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Galaxias**† | Southern Hemisphere | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| Salmoniformes | | | | | | | | | | | | | | | | | | | | | |
| | Salmonidae | | | | | | | | | | | | | | | | | | | | |
| | | Salmoninae | | | | | | | | | | | | | | | | | | | |
| | | | *Brachymystax* | North East Asia | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Hucho** | Eurasia | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Oncorhynchus**† | North America | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Salmo*† | Europe | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | | Thymallinae | | | | | | | | | | | | | | | | | | | |
| | | | *Thymallus* | Northern Hemisphere | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| Mugiliformes | | | | | | | | | | | | | | | | | | | | | |
| | Mugilidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Agonostomus*† | W Central Atlantic/E Central Pacific/Madagascar | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| Atheriniformes | | | | | | | | | | | | | | | | | | | | | |
| | Melanotaenidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Chilatherina** | Australia and New Guinea | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | - | - |
| Synbranchiformes | | | | | | | | | | | | | | | | | | | | | |
| | Mastacembelidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Mastacembelus** | Africa/Asia | X | - | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - |
| | | | *Sinobdella* | Southeast Asia | X | - | - | - | - | X | - | - | - | - | X | X | - | - | - | - | - |
| Scorpaeniformes | | | | | | | | | | | | | | | | | | | | | |
| | Cottidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Cottus** | Northern Hemisphere | X | X | - | - | - | - | X | - | X | - | - | X | - | - | Xd | - | - |
| "Perciformes" | | | | | | | | | | | | | | | | | | | | | |
| | Badidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Badis** | South Asia | - | X | - | - | - | - | - | X | X | - | - | X | - | - | Xd | - | - |
| | Percidae | | | | | | | | | | | | | | | | | | | | |
| | | Etheostomatinae | | | | | | | | | | | | | | | | | | | |
| | | | *Ammocrypta* | North America | - | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | | | *Crystallaria* | North America | X | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | | | *Etheostoma** | North America | X | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | | | *Nothonotus* | North America | X | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | | | *Percina* | North America | - | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | | Luciopercinae | | | | | | | | | | | | | | | | | | | |
| | | | *Romanichthys* | Danube River | X | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | | | *Zingel* | Southern Europe | - | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | Cheimarrichthyidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Cheimarrichthys*† | New Zealand | - | X | - | - | - | - | X | - | X | - | X | - | - | - | Xd | - | - |
| | Cichlidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Gobiocichla* | West Africa | - | - | X | - | - | X | - | - | - | - | X | - | - | - | - | - | - |
| | | | *Hemichromis** | West Africa | - | - | X | - | - | - | - | X | X | - | - | X | - | - | - | - | - |
| | | | *Lamprologus** | Central Africa | X | - | X | - | - | X | - | X | - | - | X | X | - | - | - | - | - |
| | | | *Nanochromis* | Central Africa | - | - | X | - | - | - | - | X | - | - | - | X | - | - | - | - | - |

(continued)

**Table 1** (continued)

| Ord. | Fam. | Subf. | Genus | Distribution | Microhabitat | | | | | Body Shape | | | | Mouth Position | | | | Attachment Organ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hyp. | Ben. | B.P. | M.P. | T.P. | Ang. | Dep. | Fus. | Com. | Inf. | Sub. | Ter. | Sup. | O.D. | P.-F.P. | T.P. | P.-F.D. |
| | | | *Paraneetroplus** | Central America | - | - | X | X | - | - | - | - | X | - | - | X | - | - | - | - | - |
| | | | *Retroculus* | South America | - | - | X | - | - | - | - | - | X | - | X | X | - | - | - | - | - |
| | | | *Steatocranus* | Central Africa | - | X | X | - | - | X | - | X | - | - | - | X | - | - | - | - | - |
| | | | *Teleocichla* | South America | - | X | X | - | - | X | - | X | - | - | - | X | - | - | Xd | - | - |
| | | | *Teleogramma* | Central Africa | - | X | X | - | - | X | - | X | - | - | - | X | X | - | - | - | - |
| | | | *Theraps** | North/Central America | - | - | X | X | - | - | - | X | X | - | - | X | - | - | - | - | - |
| | Blenniidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Salaria**† | Mediterranean | - | X | - | - | - | - | - | X | - | - | - | X | - | - | Xd | - | - |
| | Gobiesocidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Gobiesox**† | Central/Northern S America | - | X | - | - | - | - | X | - | - | - | - | X | - | - | - | - | Xb |
| | Rhyacichthyidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Protogobius*† | New Caladonia | - | X | - | - | - | - | X | X | - | - | X | - | - | - | Xd | - | - |
| | | | *Rhyacichthys*† | Southeast Asia/Oceania | - | X | - | - | - | - | X | - | - | - | X | - | - | - | Xd | - | - |
| | Eleotridae | | | | | | | | | | | | | | | | | | | | |
| | | | *Eleotris**† | Pantropical | - | X | - | - | - | - | - | X | - | - | - | X | X | - | - | - | - |
| | | | *Gobiomorphus**† | Australasia | - | X | - | - | - | - | - | X | - | - | - | X | X | - | - | - | - |
| | Odontobutidae | | | | | | | | | | | | | | | | | | | | |
| | | | *Odontobutis** | East Asia | - | X | - | - | - | - | - | X | - | - | - | X | - | - | - | - | - |
| | Gobiidae | | | | | | | | | | | | | | | | | | | | |
| | | Gobionellinae | | | | | | | | | | | | | | | | | | | |
| | | | *Awaous*† | Pantropical | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Parawaous*† | Borneo | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Rhinogobius* | East Asia | - | X | - | - | - | - | - | X | X | - | X | X | - | - | - | - | Xc |
| | | | *Schismatogobius**† | Asia/Oceania | X | X | - | - | - | X | - | - | - | - | - | X | - | - | - | - | Xc |
| | | Sicydiinae | | | | | | | | | | | | | | | | | | | |
| | | | *Akihito*† | South Pacific | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Cotylopus*† | Indian Ocean | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Lentipes*† | Southeast Asia/Oceania | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Parasicydium*† | West Africa | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Sicyopterus*† | Indo_Pacific | - | X | - | - | - | - | - | X | X | - | X | - | - | X | - | - | Xc |
| | | | *Sicyopus*† | Southeast Asia/Oceania | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Stiphodon*† | Southeast Asia/Oceania | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |
| | | | *Sycidium*† | Tropical Atlantic/E Pacific | - | X | - | - | - | - | - | X | X | - | X | - | - | - | - | - | Xc |

See text for further explanation. Taxonomic classification generally follows that of Nelson (2006). 'Perciformes' follows Wiley and Johnson (2011). Abbreviations (listed from left to right across table): Hyp., hyporeic; Ben., benthic; B.P., bentho-pelagic; M.P., midwater-pelagic; T.P., topwater-pelagic; Ang., anguilliform; Dep., depressed; Fus., fusiform; Com., compressed; Inf., inferior; Sub., subterminal; Ter., terminal; Sup., superior; O.D., oral disc; P.-F.P., paired-fin pads; T.P., thoracic pad; P.-F.D., paired-fin disc. Paired-fin pads are divided between the following types: Xa, pads that are restricted to the ventral surface of rays, equipped with a superficial unculiferous layer; Xb, as in Xa but without an unculiferous layer; Xc, as in Xb but with odontodes; Xd, thickened skin continuous around ray, without forming an obvious pad and without an unculiferous layer (for detailed discussion of paired-fin pads see Conway et al. 2012a). Paired-fin discs are divided into the following types: Xa, balitorid type; Xb, gobiesocid type; Xc, gobiid type. An asterisk (*) after a generic name indicates that only some members of genus are rheophilic and a cross (†) indicates that members of this genus are migratory (either within freshwater or diadromous)

# Index

R. Riesch et al. (eds.), *Extremophile Fishes,* DOI 10.1007/978-3-319-13362-1