

# Hearing in Cavfishes

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**Abstract** Caves and associated subterranean habitats represent some of the harshest environments on Earth, yet many organisms, including fishes, have colonized and thrive in these habitats despite the complete absence of light, and other abiotic and biotic constraints. Over 170 species of fishes are considered obligate subterranean inhabitants (stygobionts) that exhibit some degree of troglomorphy, including degeneration of eyes and reduction in pigmentation. To compensate for lack of vision, many species have evolved constructive changes to non-visual sensory modalities. In this chapter we review hearing in cavfishes, with particular emphasis on our own studies on amblyopsid cavfishes. Hearing in cavfishes has not been well studied to date, as hearing ability has only been examined in four species. Two species show no differences in hearing ability relative to their surface relatives, while the other two species (family Amblyopsidae) exhibit regression in the form of reduced hearing range and reduction in hair cell densities on sensory epithelia. In addition to reviewing our current knowledge on cavfish hearing, we offer suggestions for future avenues of research on cavfish hearing and discuss the influence of Popper and Fay on the field of cavfish bioacoustics.

**Keywords** Acoustic • Auditory • Evolution • Fish • Subterranean

## 1 Introduction

Caves have been shown to be rewarding environments for the study of ecology, evolution, and speciation (Poulson and White 1969; Culver 1976, 1982; Christman and Culver 2001; Christman et al. 2005; Niemiller et al. 2008). Yet, subterranean

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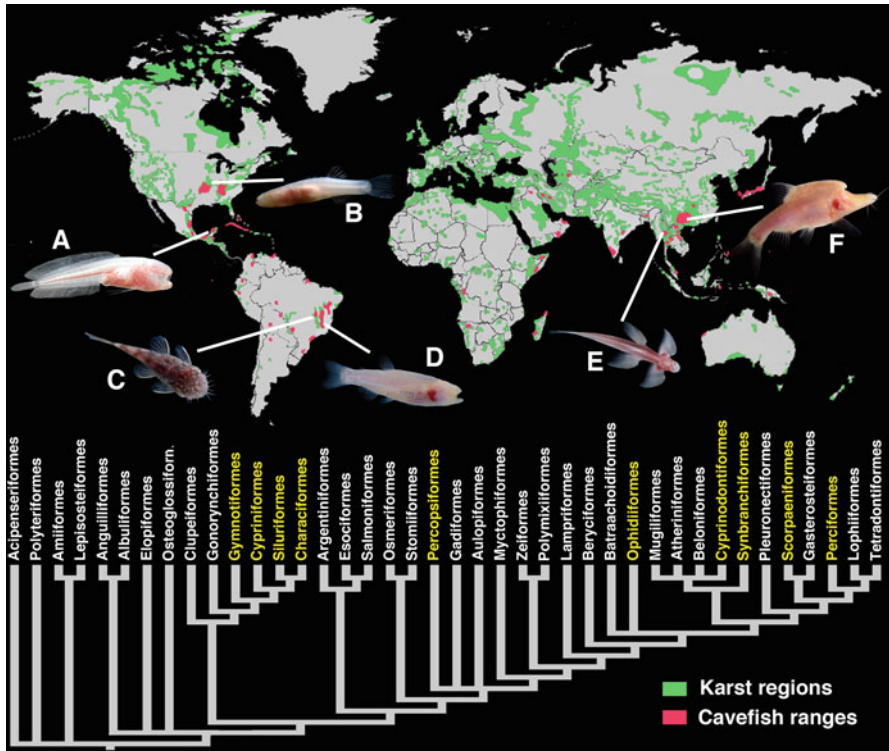
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organisms remain the most poorly understood fauna on the planet and little is known about their sense of hearing. Animals that thrive in a cave not only have evolved cave-specific morphological specializations but also have selective behaviors that allow for their survival in complete darkness. Cavefishes (obligate cave-dwelling fishes) are replicate ecological and evolutionary experiments in adaptation to this extreme environment. Because the direction of evolution is known (i.e., surface to subterranean), the colonization of a subterranean environment affords us the opportunity to examine species that have survived similar evolutionary pressures in parallel and converging ways.

Of the approximately 32,000 fish species globally, over 300 species have been reported to live in subterranean habitats with more than 170 described species living obligate underground with some degree of troglomorphy (features related to cave adaptation; Proudlove 2006, 2010). The phylogenetic diversity of cavefishes representing 10 orders and 21 families distributed on every continent but Europe and Antarctica (Fig. 1) and the assortment of aquatic subterranean habitats from fast flowing streams and waterfalls to quiet phreatic waters provide excellent examples for the study of independent responses to subterranean environments. Many species are monotypic and their relationships to surface forms are unknown; in other cases cave forms are grouped together based on convergent morphologies (Niemiller and Poulson 2010; Niemiller et al. 2013). However, the surface ancestors of all cavefish species had to adapt to the strict constraints imposed by caves, particularly perpetual darkness and limited energy resources. As a result, a suite of unique phenotypes associated with subterranean adaptation has emerged, with loss of pigmentation and eyes being the most conspicuous. Cavefishes, however, are outcomes of not just regressive evolution but also constructive adaptation. For example, several cavefishes exhibit enhancement of the mechanosensory lateral line system relative to their surface relatives (Culver and Pipan 2009; Soares and Niemiller 2013), and a cave catfish, *Astroblepus pholeter*, has adapted skin-teeth to sense water flow (Haspel et al. 2012). Despite the obvious power of using cavefish as a natural experiment, to date there have been few comparative studies in cavefish ecology and sensory biology (Trajano 1991, 1997, 2001; Niemiller et al. 2013; Soares and Niemiller 2013).

There is strong selection to develop and enhance non-visual sensory modalities in subterranean habitats, with enhanced hearing ability being one possible modification (see below for a review on possible lateral line enhancement). Lower auditory thresholds and greater frequency ranges in subterranean habitats should be adaptive for several reasons, including integrating with other non-visual senses to detect prey, predators, or conspecifics. However, hearing sensitivity and range, and acoustic communication for that matter, have received little attention in cavefishes—with studies limited to just three groups. Popper (1970) found no differences in hearing sensitivities between cave and surface forms of the characid *Astyanax mexicanus*. Both forms had sensitivities comparable to other otophysan (Actinopterygii: Teleostei: Ostariophysi) fishes with a threshold at 1000 Hz. The best-studied cavefish with respect to hearing are cave and surface ecotypes of the Atlantic Molly (*Poecilia mexicana*). Schulz-Mirbach et al. (2008) documented pronounced



**Fig. 1** Cavefishes are known from 10 teleost fish orders (highlighted in yellow) and occur on every continent but Europe and Antarctica: (a) *Typhliasina pearsei* (Ophidiiformes: Bythitidae) from Mexico. (b) *Troglichthys rosae* (Percopsiformes: Amblyopsidae) from the USA. (c) *Ancistrus cryptophthalmus* (Siluriformes: Loricariidae) from Brazil. (d) *Stygichthys typhlops* (Characiformes incertae sedis) from Brazil. (e) *Cryptotora thamicola* (Cypriniformes: Balitoridae) from Thailand. (f) *Sinocyclocheilus furcodorsalis* (Cypriniformes: Cyprinidae) from China. Sources: The karst regions were modified from those in Williams and Ford (2006), and the phylogenetic relationships are based on those in Li et al. (2008). Photographs: Thomas M Illiffe (a) Dante B. Fenolio (b-f). Modified from Soares and Niemiller (2013)

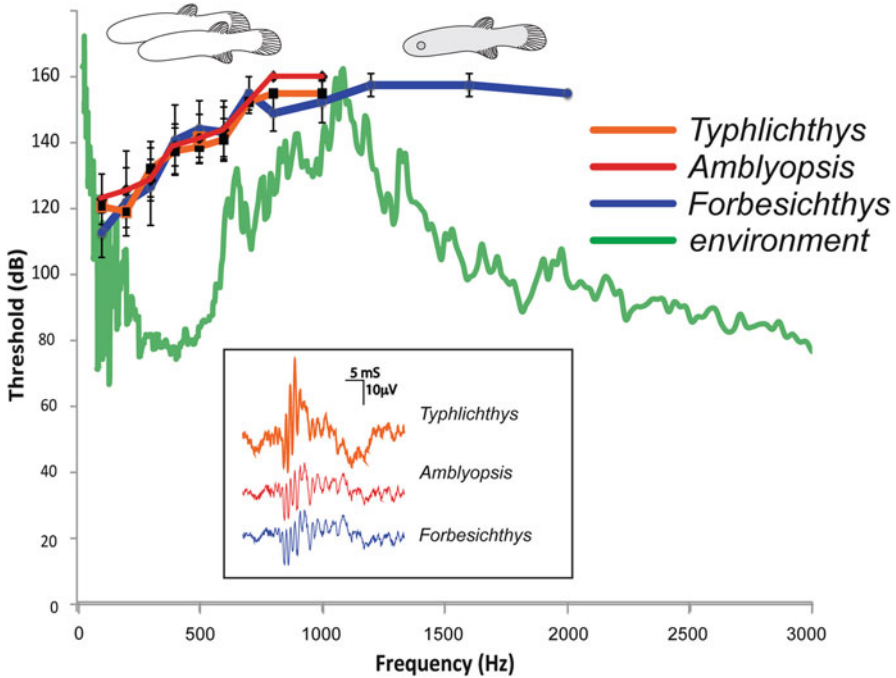
morphological differences between the sagittal otoliths of cave- and surface-dwelling mollies, with cave forms having heavier otoliths and a deeper auditory sulcus than surface forms. In a follow-up study, Schulz-Mirbach et al. (2010) examined the morphology of all three inner ear otoliths (sacculle, utricle, and lagena) and hearing sensitivities between cave and surface ecotypes and found differences in shape of all three otoliths between the cave and surface form, while the otoliths of the lagena and utricle were heavier in the cave form. The first microanatomical study of the inner ear by Schulz-Mirbach et al. (2011a) using a combination of micro-CT analyses, scanning electron microscopy and immunocytochemical methods also revealed differences between cave and surface ecotypes, including the shape and curvature of the macula lagenae, curvature of the macula sacculi, and a much thicker otolith membrane housed in a deep sulcus of the sagittae. However, hearing sensitivities,

based on auditory evoked potentials (AEPs), were similar between the two ecotypes with greatest sensitivities between 200 and 300 Hz. The authors found no evidence for intra-specific acoustic communication in both cave and surface ecotypes. Differences in otolith morphology between ecotypes may reflect metabolic differences but had minimal influence on hearing sensitivity or acoustic behavior (Schulz-Mirbach et al. 2010).

Schulz-Mirbach et al. (2011b) compared otolith morphology of several locally adapted populations of *P. mexicana* living in surface and cave habitats that differed in levels of hydrogen sulfide (H<sub>2</sub>S) and darkness. Asterisci, lapilli, and sagittae from a non-sulfidic cave were larger than those from the sulfidic cave, and generally larger than otoliths from surface habitats (sulfidic and non-sulfidic). As noted previously, cavefish had thicker otoliths with deep furrows housing the sensory epithelium. Schulz-Mirbach et al. (2011b) also examined inner ear size and brain morphology. The length and width of the optic tectum were smaller in fish from the two cave populations compared to fish from surface habitats. However, inner ear size and length of the sacculus were similar between cave and surface forms. Although the populations studied showed clear differentiation in otolith morphologies, no clear directional pattern of trait divergence along the two environmental gradients (darkness and hydrogen sulfide concentration) was discernible. Similar hearing sensitivities despite differences in otolith morphology between cave and surface fish may reflect the role of hearing for orientation (Popper et al. 2005; Popper and Schilt 2008) or other structures, such as the sensory epithelia, may have co-evolved with otolith changes to maintain inner ear function (Schulz-Mirbach et al. 2011b).

Our own studies have examined hearing ability between related cave and surface fishes in the family Amblyopsidae (Niemiller et al. 2013) (Fig. 2). Specifically, we compared hearing sensitivities between the related, surface-dwelling *Forbesichthys agassizii* and cave-dwelling *Typhlichthys subterraneus* and *Amblyopsis spelaea*. *Forbesichthys* is the sister group to a clade of subterranean genera, including *Amblyopsis*, *Typhlichthys* and *Speoplatyrhinus* (Niemiller et al. 2013). We used AEPs and showed that all three species exhibited similar hearing sensitivities at frequencies lower than 800 Hz, consistent with previous studies in other cavefishes (Popper 1970; Schulz-Mirbach et al. 2010). Unexpectedly, the two cave species were unable to hear above 800 Hz, whereas surface-dwelling *F. agassizii* exhibited a response up to 2 kHz, the maximum frequency tested in our experimental design. Eigenmann and Yoder (1899) noted no gross anatomical changes in the inner ear of *Amblyopsis*; however, we noted significant differences in saccular hair cell densities. The cave species, *Typhlichthys subterraneus* and *A. spelaea*, had 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* to our knowledge represents the first report of regressive evolution of hearing in a subterranean organism. In addition to testing hearing ability, we characterized aquatic environmental sound profiles in cave and surface habitats inhabited by each amblyopsid cavefish and the surface *F. agassizii* (Niemiller et al. 2013). Audio recordings from



**Fig. 2** Auditory thresholds of amblyopsid fishes. Values are means  $\pm$  standard errors. The surface fish *Forbesichthys* (blue) reaches up to 2 kHz while the cave fish *Typhlichthys* (1) and *Amblyopsis* (2) are limited to 1 kHz. Fast Fourier Transformation (FFT, green line) of sound recorded in a cave pool, Pulaski Co., Kentucky, USA. The pool was carved in bedrock by a small stream. The recording was made 0.5 m deep and approximately 1 m from a small waterfall. The ceiling of the cave was also dripping into the pool. *Insert*: Auditory evoked potential traces of all species to a 400 Hz tone burst at 60 dB. Modified from Niemiller et al. (2013)

native cave habitats revealed that riffles in cave streams and water droplets dripping from the ceiling into pools create loud high-frequency background noise generally above 800 Hz; such background noise was absent in surface recordings. We therefore hypothesized that cave amblyopsids may have lost hearing at high frequencies in response to living in loud 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.

## 2 Lateral Line in Cavefishes

Recently, Higgs and Radford (2012) showed that the lateral line contributes to AEPs of the brainstem of fishes. Their results suggest that auditory responses to sound should not be considered as a measurement of hearing ability alone but rather a

multimodal mechanosensory response driven by both the ear and the lateral line system. With this in mind, it is interesting to re-examine the body of literature of cavefish mechanosensation. Most of our knowledge of cavefish lateral line systems comes from studies of the Mexican cavefish *Astyanax mexicanus*, and this species has made important contributions to understanding lateral line systems in general (Montgomery et al. 2001). The cave forms of *Astyanax* not only have many more neuromasts than their surface counterparts (Montgomery et al. 2001; Windsor et al. 2008; Yoshizawa et al. 2010) but also have enhanced behavioral sensitivity in feeding and navigation tasks (Sharma et al. 2009; Yoshizawa et al. 2010). *Astyanax* has also evolved a form of active sensing in which they use 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* use the distortions of nearby obstacles in the self-generated flow field and spend more time gliding when investigating a new object (Patton et al. 2010). Cave amblyopsid species, the second most studied cavefishes, also have more superficial neuromasts that are larger with longer cupulae than their surface relatives, which allow the detection of moving prey at a greater distance compared their surface relatives (Poulson 1963; Niemiller and Poulson 2010). Thus, the mechanosensory neuromasts have also undergone adaptive evolution, and, in conjunction with auditory hair cells, allow enhanced non-visual detection of prey, predators, and obstacles in their natural environment.

### 3 Influence of Arthur Popper and Richard Fay on Cavefish Bioacoustics

While Arthur Popper obviously influenced this specific research topic by being the first to study hearing in cavefish (Popper 1970), the influence of both Popper and Fay goes well beyond any individual research papers. The entire question of for what, precisely, fish are using their sense of hearing (i.e., what fish are listening to) remains largely unsolved, and Popper and Fay have repeatedly pushed the field to properly address this question (Popper and Fay 1973, 1993, 1997; Fay and Popper 2012). Their hypothesis of the ear evolving in response to “auditory scene analysis,” while largely untested, was a central reason for us to test the acoustic soundscape of the cave and surface environments in our work and formed the underpinnings of much of what we were trying to assess. In addition, their long-standing interest in the evolution of fish hearing greatly influenced all three of us to use the cave- and surface-dwelling species as a natural experiment to test hypotheses first laid out in the seminal papers of these two senior bioacousticians (Fay and Popper 2012). Both have also served a personal mentoring role for two of the authors of this current work, as they have done for the vast majority of researchers in this field.

## 4 Future Directions

Cavefishes have evolved an integrated suite of behavioral, morphological, and physiological adaptations to cope with the abiotic and biotic challenges associated with subterranean habitats. The close similarity of phenotypes among diverse fish taxa around the world suggests that cave adaptation may be a general process resulting from similar selective pressures. Some of the more notable adaptations include the degeneration of eyes, reduction in pigmentation, enhancement of mechanoreception, lower metabolism, and increased longevity. However, our understanding of sensory biology, including hearing, and ecology of cavefishes is largely limited to just a handful of species, particularly the characid *Astyanax* and amblyopsid cavefishes. While these groups have been invaluable models in the study of hearing in cavefishes, several important questions remain. For example, have different cavefish lineages evolved similar if not the same adaptive strategies to hearing specialization or regression? Hearing ability has only been examined in four of the over 170 cave obligate fishes known globally. Two species show no differences in hearing ability relative to their surface relatives, while the other two species exhibit regression.

Another important question is whether any cavefishes exploit acoustic communication, which plays an important role in agonistic and mating behaviors in many fishes. Acoustic communication may be important in the darkness of caves in some species, but the only study to examine acoustic communication in cave taxa found no evidence for the production of species-specific sounds in lab-reared cave- or surface-dwelling molly *Poecilia mexicana* (Schulz-Mirbach et al. 2010). The authors hypothesized that the 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. Evidence for acoustic communication in cavefishes remains to be found.

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