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Research paper

Otolith morphology and hearing abilities in cave- and surface-dwelling ecotypes of the Atlantic molly, *Poecilia mexicana* (Teleostei: Poeciliidae)

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ABSTRACT

Cave fish have rarely been investigated with regard to their inner ear morphology, hearing abilities, and acoustic communication. Based on a previous study that revealed morphological differences in the saccular otolith between a cave and two surface populations of *Poecilia mexicana*, we checked for additional differences in utricular and lagenar otoliths and tested whether different populations have similar hearing sensitivities. We found pronounced differences in the shape of all three otoliths. Otoliths of the saccule and lagena from cave fish differed from those of surface fish in the features of the face oriented towards the sensory epithelium. In addition, otoliths of the utricle and lagena were significantly heavier in cave fish. Auditory sensitivities were measured between 100 and 1500 Hz, utilizing the auditory evoked potential recording technique. We found similar hearing abilities in cave and surface fish, with greatest sensitivity between 200 and 300 Hz. An acoustic survey revealed that neither ecotype produced species-specific sounds. Our data indicate that cave dwelling altered the otolith morphology in Atlantic mollies, probably due to metabolic differences. Different otolith morphology, however, did not affect general auditory sensitivity or acoustic behavior.

1. Introduction

Unlike in most other vertebrates that possess numerous tiny otoconia in their inner ears, modern bony fishes (Teleostei) have a single massive calcareous concretion – the so-called otolith – in all three end organs. Fish otoliths are composed of calcium carbonate crystals suspended in a protein matrix. Calcium carbonate is usually deposited as aragonite in the otolith of the saccule (sagitta) and utricle (lapillus) and in a crystallized, less dense form termed vaterite in the otolith of the lagena (asteriscus) (Oliveira and Farina, 1996; Falini et al., 2005). These otoliths, especially the saccular otolith, show a (species-)specific morphology (Nolf, 1985). In examining the diversity of otolith morphologies and its implications for physiological functions in teleost fishes, one interesting question at the interface between otolith research and studies on inner ear physiology is whether and how otolith morphology may be related to inner ear physiology, such as hearing sensitivities (Popper et al., 2005; Popper and Schilt, 2008). Oxman et al. (2007), for example, reported that juvenile Chinook salmon (*Oncorhynchus tshawytscha*) with aberrant (vateritic) sagittae displayed significantly poorer auditory sensitivity than individuals with normally developed (aragonitic) sagittae. Ramcharitar et al. (2004) showed that the sciaenid species *Bairdiella chrysoura* has a unique otolith morphology, i.e. thick, large otoliths (asteriscus and lapillus) and a sagitta with a conspicuously deep sulcus acusticus which may be linked to better hearing abilities compared to other members of the family Sciaenidae. In a preceding comparative study (Schulz-Mirbach et al., 2008) on the sagittae of different ecotypes of the Atlantic molly (*Poecilia mexicana*), we found pronounced contour differences in cave- versus surface-dwelling populations. Moreover, sagittae of cave mollies often had a deep sulcus, lacking in surface-dwellers. We assumed that these differences might be related to hearing ability. In this study, we therefore examine whether otolith morphology reflects inner ear physiology (i.e., hearing sensitivities). Popper (1970)

Abbreviations: a, anterior; AEP, auditory evoked potential; c, caudal; d, dorsal; DFA, discriminant function analysis; FD, Fourier descriptor; GLM, general linear model; l, lateral; m, medial; Mag *a*, magnitude level of particle acceleration; PC, principal component; PCA, principal component analysis; r, rostral; rmGLM, general linear model for repeated measurements; s.d., standard deviation; s.e.m., standard error of mean; SEM, scanning electron microscopy; SL, standard length; SPL, sound pressure level; TDT, Tucker-Davis Technologies.

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found similar hearing sensitivities in the blind, cave-dwelling Mexican tetra *Astyanax mexicanus* (Characidae) and its surface-dwelling conspecifics, but did not investigate otolith morphology. We therefore tested whether (1) the cave form of *P. mexicana* and its surface-dwelling relatives have similar hearing sensitivities (as shown for the Mexican tetra) or (2) whether they have different hearing sensitivities that are reflected by the changed sagitta morphology.

Cave fishes are under strong selection pressure to develop and enhance non-visual communication channels (Burt de Perera, 2004; Montgomery et al., 2001; Parzefall, 1970, 2001). To compensate for the lack of visual input, they have evolved several modifications of their sensory systems such as a well-developed lateral line system (Burt de Perera, 2004; Montgomery et al., 2001) or improved the senses of taste and touch (Parzefall, 1970, 2001). A potentially altered sense of hearing and acoustic communication has received little attention. No data are available on these aspects in poeciliids in general. We therefore studied cave and surface populations of *P. mexicana* with respect to hearing abilities and acoustical signaling. Cave and surface populations of the Atlantic molly differ in their sagitta morphology, making them perfect model organisms to investigate the potential relationship between otolith morphology and hearing sensitivities.

The present study focuses on three aspects. First, we tested for potential differences between the two ecotypes in the morphology of all three otolith types (asterisci, sagittae, lapilli), with special emphasis on details of the sulcus region of the sagittae and on the overall morphology of asterisci and lapilli. Second, we tested whether cave and surface-dwelling fish show similar hearing sensitivities, or whether the former display changed hearing sensitivities as an adaptation to perpetual darkness. Third, we conducted an acoustic survey to determine whether *P. mexicana* communicates acoustically.

2. Materials and methods

2.1. Study system and animals

The Atlantic molly, *P. mexicana* Steindachner, 1863 (Poeciliidae) is widespread in freshwater surface habitats along the Atlantic versant of Central America (Miller, 2005). The cave form of *P. mexicana* inhabits the Cueva del Azufre cave system, which is divided into 13 interconnected cave chambers (Gordon and Rosen, 1962). A creek flows through the cave, forming several shallow pools that are partially divided by riffle passages. While the front cave chambers receive some dim light, the inner parts of the cave are lightless, and the molly population from the innermost cave chamber XIII (Gordon and Rosen, 1962) permanently lives in the dark. With the exception of chamber XIII, the water in all cave chambers is characterized by medium to high concentrations (up to 300 μM/L) of naturally occurring hydrogen sulfide (Tobler et al., 2006, 2009).

2.1.1. Otolith morphology and hearing sensitivity

A total of 46 fish from the cave form and two surface populations of *P. mexicana* were investigated with regard to their otolith morphology and hearing sensitivity (Table 1). One of the surface populations originated from the Río Oxolotán, a river with sulfide-free water near the cave (Tobler et al., 2006). The second population of surface-dwelling fish came from brackish coastal waters near Tampico (Tamaulipas, eastern Mexico). Large, randomly outbred stocks of the cave population and from Río Oxolotán have been maintained in 200-l aquaria at the University of Potsdam since 2004; stocks from Tampico were founded using wild-caught fish in 1995. Fish were transferred to the University of Vienna in December 2008 and February 2009 for the auditory analyses. They were kept in 120 to 160-l aquaria, which were equipped with a sand bottom, halved flower pots as hiding places, and external filters. No internal filters or air stones were used in order to create a quiet acoustic environment for the test fish. Fish were kept under a 12:12 h L:D cycle at 25 ± 1 °C and were fed once daily with commercial flake food. The conditions were comparable to those at the University of Potsdam. Fish were given a habituation period of two to three days prior to the auditory experiments. All hearing experiments were performed with the permission of the Austrian Federal Ministry of Science and Research (GZ 66.006/0023-II/10b/2008).

2.1.2. Acoustic survey

Cave fish for the survey of the acoustical behavior originated from chamber V of the Cueva del Azufre (Gordon and Rosen, 1962), and surface fish came from the Río Amatan, another sulfide-free river that merges with the Río Oxolotán downstream, but close to the cave (Tobler et al., 2008). Both populations were a mix of wild-caught and first generation laboratory-reared fish, originally collected and established in January 2009. Fish were maintained at the University of Oklahoma under 12:12 h L:D cycles, kept in 160-l tanks at 26 °C with gravel bottom and internal filters, and were fed once daily with either commercially available flake food or mosquito larvae (bloodworms).

2.2. Otolith dissection, otolith measurements and shape analysis

Following the measurements of hearing sensitivities, the standard length of the fish was measured to the nearest millimeter and animals were decapitated. Sex was determined by inspection of the gonopodium (transformed anal fin) of the males and by dissection of the ovary of the females. The three otolith types – lapillus, sagitta, and asteriscus – were dissected from the left membranous labyrinth, cleaned of organic residues with 1% potassium hydroxide solution for 4–6 h and rinsed several times in distilled water. After cleaning, otoliths were stored dry at room temperature in small plastic cells (Krantz®-cells). Fresh otoliths showed the same morphological features, e.g. development of the sulcus center in sagittae, as those after cleaning. No cracks or artificial crystallization were observed on the surface of the dried otoliths. Otolith

Table 1
Populations, number of specimens, and size ranges of *Poecilia mexicana* used for auditory measurements and otolith analyses. BW, body weight; f, female; m, male; N, number of specimens; SL, standard length.

Population	Auditory measurements			Otolith analyses	
	N [f/m]	*SL* (mm)	*BW* (g)	*N* [f/m]	*SL* (mm)
Cueva del Azufre	13 [8/5]	f: 35–43; m: 33–40	f: 0.8–1.2; m: 0.7–1.0	19 [12/7]	f: 35–55; m: 32–40
Tampico	14 [11/3]	f: 30–52; m: 28–32	f: 0.6–2.9; m: 0.4–0.8	20 [15/5]	f: 30–54; m: 28–34
Río Oxolotán	3 [2/1]	f: 35; m: 44	f: 0.8–1.1; m: 2.4	3 [2/1]	f: 35; m: 44

The first numeral given in brackets indicates the number of females; the second numeral represents the number of males.