ABSTRACT

OTOLITH MORPHOLOGIES IN THE GENUS SINOCYCLOCHEILUS

by
Iqra Iqbal

Sinocyclocheilus is a genus of Cyprinid fish found in southern China. This genus contains 68 species of which 40 species have adaptations for life in cave habitats. A common adaptation seen in fish that live in caves with no light is the loss of vision. Such cavefish must therefore rely on other sensory modalities to capture prey, communicate between conspecifics, and potentially avoid predators. Previous studies have identified sensory adaptations in cavefish, including the increase in size and number of mechanoreceptors. *Sinocyclocheilus* are hearing specialists, and it is possible that cave species of this genus have increased reliance on hearing when compared to their surface-living relatives.

The central hypothesis that motivates this work is that the hearing system of cavefish has adapted for increased hearing sensitivity relative to surface fish, and specifically that their otoliths are larger. Otoliths are functionally important and easy to measure components of fish hearing systems. The size, shape, and volume of otoliths were measured as a first step in identifying and understanding evolutionary changes in hearing related to loss of vision. In general, the findings do not support this hypothesis – no differences in measurements of otoliths across ecotypes were observed.

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by
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Dedicated to:

My late father, for teaching me to never give up on my dreams,
My mother for teaching me strength and compassion,
And to my brothers for their unconditional love and support

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CHAPTER 1

INTRODUCTION

1.1 Sensory Adaptations: Role of Size

The size of sensory organs in an animal often reflects the importance of the sensory system for survival. For example, animals that rely on vision have larger eyes than animals that do not rely on vision. The larger eyes both reflect an increase in the number of sensory cells, the photoreceptors, and increase the amount of light that is captured by the eye. Similarly, animals that rely on hearing to capture prey, such as owls and bats, have larger ears and hearing organs than most other species. Large inner ears reflect increased numbers of hair cells, which detect the vibrations associated with sounds, and the larger pinnae increase the amount of energy that is transmitted to the inner ear.

Animal morphology is generally believed to match the demands of the habitat in which it lives. How morphologic changes occur across evolutionary time is an important question in animal biology. One approach to studying the evolution of morphological specializations is to make comparisons across closely related species that have dramatically different life histories. For example, the genus *Sinocyclocheilus* includes surface-dwelling species, troglophilic species, and blind cavefish species. The surface-dwelling fish can rely on vision, whereas the cavefish do not. As expected, the surface fish have large eyes and the cavefish have reduced or no eyes as adults.

The central hypothesis of this work is that cavefish rely on acoustic information, which has led to a hypertrophy of the auditory system when compared to the surface relatives. To examine this hypothesis, the size and shape of otoliths were measured and compared across thirteen species of cave-dwelling, troglophilic, and surface-dwelling

Sinocyclocheilus. Specifically, this work examined the ***hypothesis that cavefish otoliths are bigger than surface fish otoliths.***

1.2 Hearing in Water

Hair cells, which are specialized sensory receptors for mechanical stimuli including vibrations associated with sound, rely on the relative movement of the stereocilia on its apex relative to the cell body (Figure 1.1). When the stereocilia are deflected, they open channels that cause depolarization, leading to an increase in release of neurotransmitters.

Aquatic organisms and water have almost identical densities. As a result, sound travels through each nearly identically. In general, there is little relative movement caused by sound between materials with similar densities. To increase the relative movement between the stereocilia and the cell bodies of hair cells, fish embed their stereocilia in high-density organs called otoliths (Popper et al. 2005).

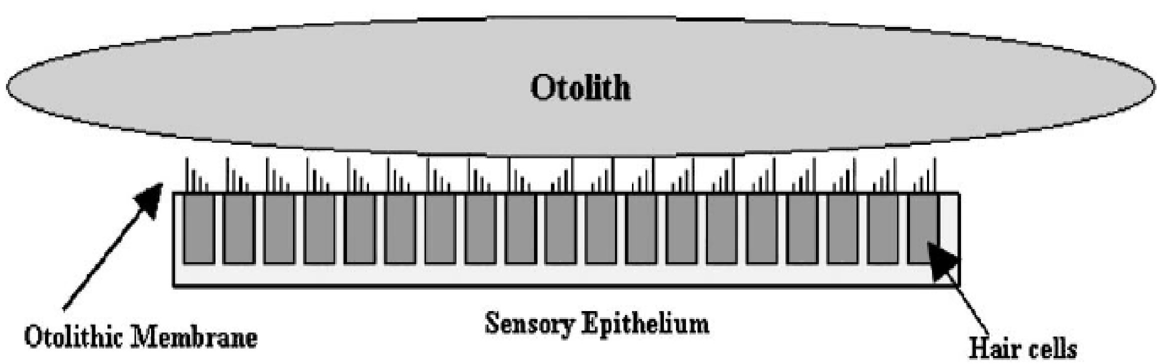


Figure 1.1 Schematic of sound processing by inner in fish; otolith is stimulated by hair cells for sensory transduction.
Source: Arthur N. Popper and Zhongmin Lu (2000).

1.3 Auditory Systems in Fish

There are approximately 35,000 extant species of fish and they inhabit different environmental niches. Audition in fish is a mechanosensory modality and that is mediated by the lateral line, and in some fish, a specialized hearing organ associated with the vestibular system (Coombs 1988). The lateral line is located along both sides of the body of the fish and detects movements and vibrations in the water. The auditory pathway of fish hearing is associated with the vestibular pathway in fish is responsible for balance and spatial orientation in addition to hearing (Popper and Lu 2000). The hearing organs of fish vary in size of their otoliths, shape of sensory epithelia and orientation of sensory hair cells (Figure 1.2) (Schulz-Mirbach et al. 2011).

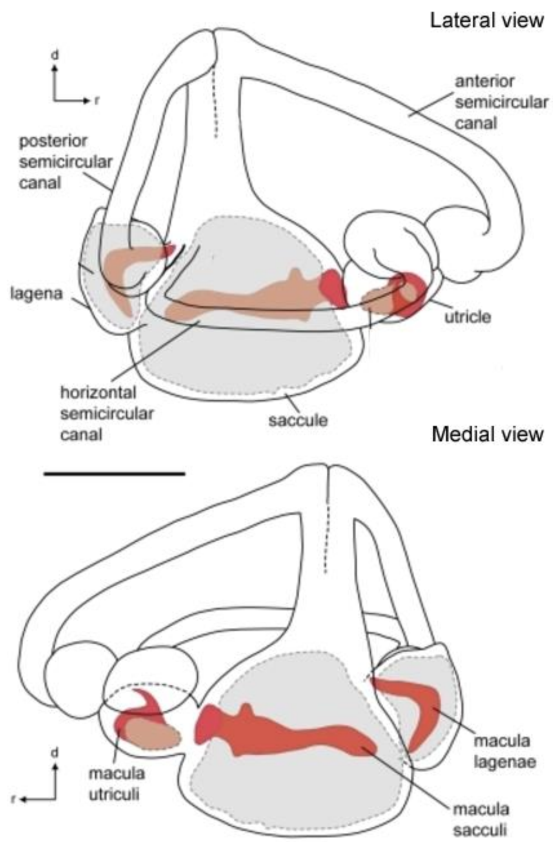


Figure 1.2 Right inner ear with semicircular canals and end organs of a generic fish inner ear (constructed from the small Molly, *Poecillia reticulata*). Otoliths are shown in gray and sensory epithelium is shown in red in lateral view (top) and in medial view (bottom). Note the difference in saccule morphology in the Molly (Cyprinodontiformes) and *Sinocyclocheilus* (Cypriniformes) is not uncommon in teleosts. Scale bar=1 mm. Source: Schulz-Mirbach et al. (2011).

Cyprinids are among fish species that are categorized as hearing specialists. In these fish, sound waves vibrate the gas-filled swim bladder, which is a mechanical amplifier of acoustic vibrations. The vibration of the swim bladder is communicated to the hearing sensors via four bones on the anterior end of the swim bladder known as ian ossicles. Vibrations of the Weberian ossicles induce vibrations of the otoliths, which then cause shearing motions across the stereocilia of the hair cells. The differential movement between hair cells and otolith, which are connected via a structure known as the tectorial membrane, induces neural responses.

Sinocyclocheilus have three otoliths (in the fluid filled cavities sagitta, lagena and saccule; Figure 1.3) that vary in size and shape across species of fishes (Paxton 2000). Some species of sound producing fish appear to have larger otoliths, but the relation between otolith size and function is not well established (Paxton 2000).

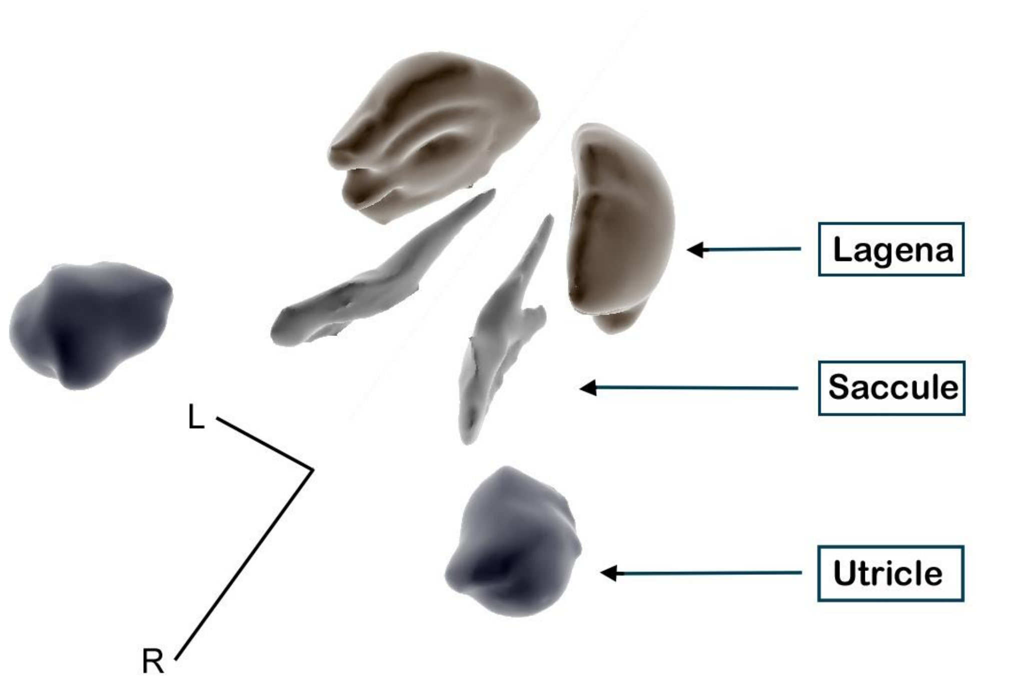


Figure 1.3 The three otoliths in the inner ear of *Sinocyclocheilus* genus. Scale 1 mm.

In fish, otoliths are located towards the caudal aspect of the skull (Figure 1.4). In many species, the sizes of ears, swim bladders and the lateral lines are correlated (Popper and Fay 1993).

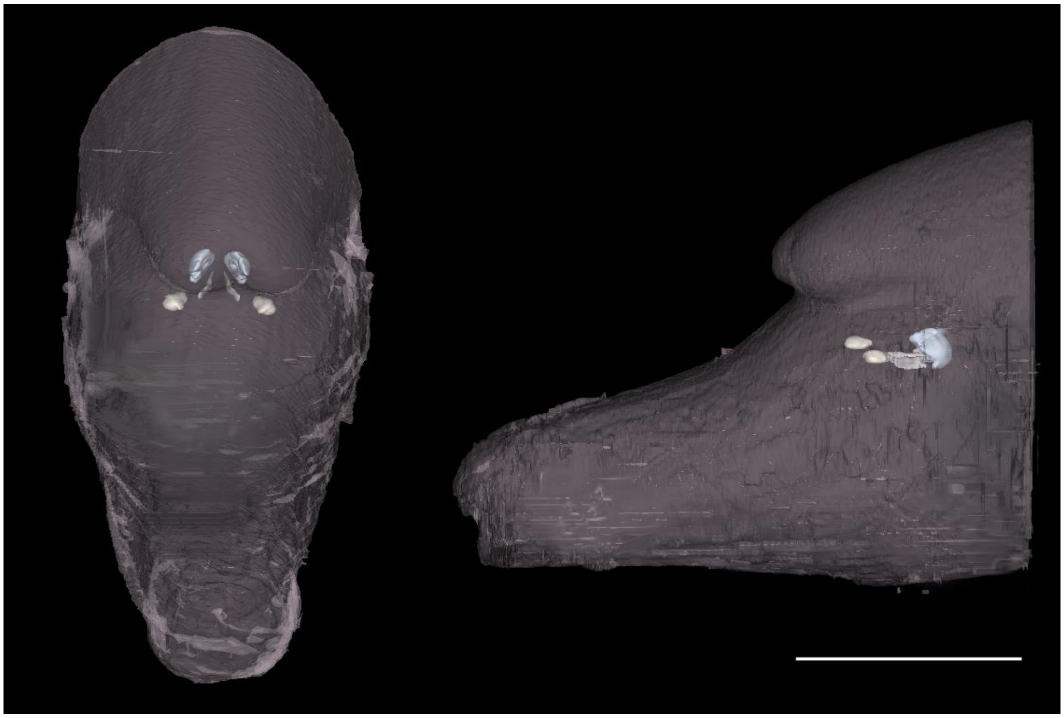


Figure 1.4 Placement of otoliths in the skull of a *Sinocyclocheilus* species. Scale 1 cm.

Three studies have examined auditory responses in cavefishes. Popper (1970) studied *Astyanax mexicanus* using behavioral methods and found no differences in the cavefish vs. surface fish. Schulz-Mirbach et al. (2010) observed that cave and surface fish had similar hearing performance, with the sensitivity being the greatest between 200 and 300 Hz. Nevertheless, Schulz-Mirbach et al. (2010) found significant differences in the shape of each of the three otoliths. Finally, Niemiller et al. (2013) compared hearing characteristics in surface and cavefish in the amblyopsid species and found that cavefish had higher acoustic thresholds than the surface fish.

1.4 Cavefishes

Organisms that spend their entire lives in subterranean habitats are known as troglobites. Caves host various bacteria, fungi, and a few vertebrate taxa. The most common vertebrate group found in caves is fish. There is over 170 species that have been identified to live in freshwater caves (Soares and Niemiller 2013). Cavefish species are found every continent except Europe and Antarctica (Soares et al. 2016). All of these species are fresh water fishes except for one species, lives in saltwater and can be found in the Bahamas (*Lucifuga speleotes*) (García-Machado 2011).

Caves often include regions that have no light, which leads to lower productivity and less availability of nutrients (Yoshizwa 2015; Niemiller and Soares 2015). Animals that live in the regions of caves often exhibit morphological features that include loss of pigmentation, slower metabolism, increased life spans, and eye degeneration (Jeffery 2001; Schulz-Mirbach et al. 2010, Soares et al. 2016). An important question is how these organisms have adapted and survived without eyesight.

CHAPTER 2

Materials and Methods

2.1 Purpose

We measured otolith morphology for three reasons. First, otoliths are critical for hearing in fish. Otoliths have much higher-density than the tissue surrounding them, leading to differential motion induced by vibratory stimuli. The movement of hair cells, which encode vibration information, detects this differential motion. Second, changes in otolith size may reflect functional changes in hearing efficiency. Third, because otoliths are dense, they are easy to study in micro CT scans.

The swim bladder, which also may be modified to enhance the efficiency of hearing, was not measured in this study because the available micro CT scans only included the skull of the fish and did not include any portions of the swim bladder.

2.2 Anatomy

This study was conducted on thirteen species of the genus *Sinocyclocheilus*. All fish samples belong to the Beijing Museum of Natural History. The fish heads were scanned using an Xradia Micro XCT-400 (Carl Zeiss X- ray Microscopy, USA) at the Institute of Zoology of the Chinese Academy of Sciences, Beijing, China. The thirteen individuals that were used in this study are shown in Table 2.1.

The skull and otoliths were reconstructed and analyzed in 3-D images using the software Mimics 18.0 (Materialize, USA). Each reconstruction of the skull included pairs of otoliths that are in the fluid filled sacs saccule, lagena and utricle (Fig 1.3). For each fish, the gray scale threshold was optimized to see the ossified structures clearly.

2.3 Otolith Measurements

The measurements for each otolith consisted of height (ventral-dorsal), length (rostral-caudal) and width (medial-lateral) and its volume using the software Mimics v18 (Materialize MI, USA). All of the measurements were organized in an Excel (Microsoft, USA) and descriptive statistics were done with XLMiner Analysis toolpak and further validated in Matlab (Mathworks, USA).

Table 2.1 Measurements of Sinocyclocheilus along with their environments and their relative length (cm). Note: *Pictures are not to scale – all pictures obtained from collaborators in China.

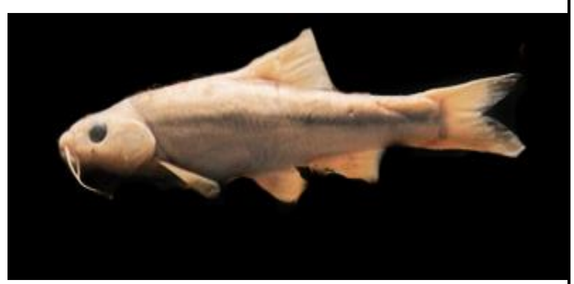
Sample	Environment	Length (cm)	Morphology
S. angustiprous	Troglophile	7.9	 *Y. Dante
S. angularis	Troglophile	8.4	 *Y. Yahui

Table 2.1 (continued)

S. anshuiensis	Cave	8.4	 *Y. Yang
S. bicornuts	Cave	10.4	 *Y. Dante
S. cyphotergous	Cave	7.2	 *Y. Yahui
S. flexuodorsalis	Cave	n/a	 *Y. Zhu

Table 2.1 (continued)

S. furcodorsalis	Cave	7	 *Y. Yahui
S. jii	Surface	6.1	 *Y. Dante
S. lateristritus	Trogophile	12	 *Y. Yahui
S. rhinocerous	Cave	6.3	 *Y. Yahui

Table 2.1 (continued)

S. tainlinesis	Cave	9.3	 *Y. Yahui
S. tilehornes	Cave	6.9	 *Y. Dante
S. quibenisis	Trogophile	9.9	 *Y.Yahui

CHAPTER 3

RESULTS

3.1 Volume of Otoliths

There was no significant difference in the volumes of the right and left otoliths (paired Two Sample T-test for means, p=0.9, t= -0.14, alpha = 0.05), saccule volumes (p=0.6, t= -0.5, alpha = 0.05) and utricle volumes (p=0.17, t= -1.45, alpha = 0.05). The linear regression was y = 1.00 x + 27.10 giving the value of r^2 =0.96. The slope of the relation between left and right volumes is not significantly different from unity (Fig. 3.1), which indicates that the volumes for the right and left sides are symmetric. Therefore, the average of both otoliths, one from each side, were used in all subsequent analyses.

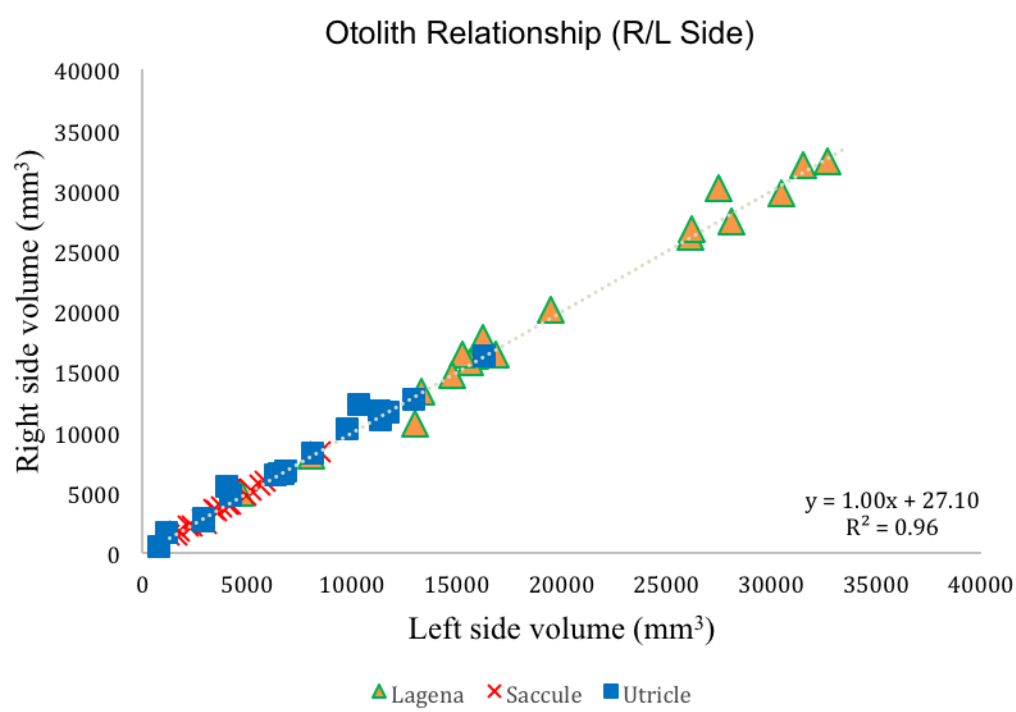


Figure 3.1 The relationship between right and left otoliths. The right and left otoliths are similar in volume in all of the measured species of *Sinocyclocheilus*.

3.2 Dimensions of Otoliths

The dimensions of each otolith were measured to determine if there is a pattern related to the ecotype. Each otolith was measured in height, length and width and compared among ecotypes.

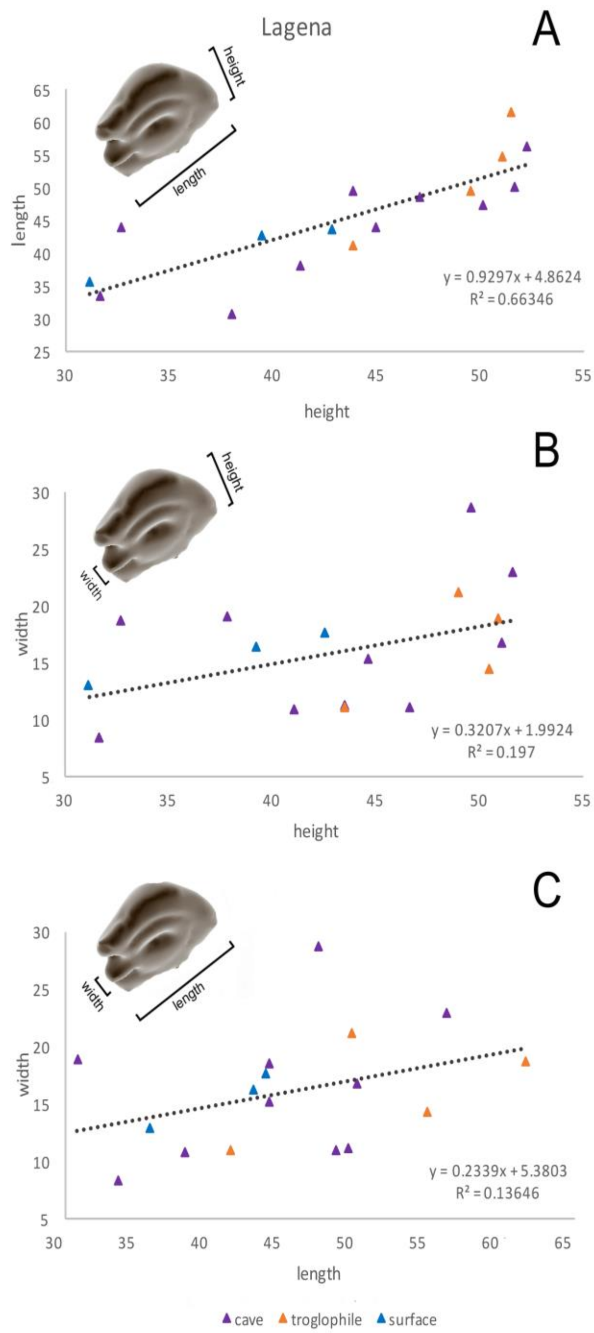


Figure 3.2 Lagena measurements. A) Height vs. Length, B) Height vs. Width, C) Length vs. Width. All measurements in mm*10.

The lagena showed a positive relationship between height and length among species, with a regression line with a slope of ≈ 0.93 (Figure 3.2 A). The relationship between height and width was almost flat, with a slope of 0.32, the results we also more variable with a low fit ($R^2 \approx 0.20$; Figure 3.2 B). Length and width relationship was similar to the height and width relationship, and the regression line had a slope of 0.23 ($R^2 \approx 0.14$; Figure 3.2 C).

The structure of the lagena is round in the dorsal ventral dimension (height) and rostral caudal dimensions (length), but flat in the medial lateral dimension (width). The structure of the lagena varied among species so that as it became taller, it became slightly wider and longer. This was consistent among all cave and troglophile species of *Sinocyclocheilus*, while the surface counterpart tended to be shorter and skinnier.

As the saccule got taller in the dorsal-ventral dimension it also got longer in the rostral-caudal dimension across species. A regression line of a slope of ≈1.44 had a low fit of ≈ 0.24 (Figure 3.3 A). The height vs. width correlation was also fairly flat with a regression line of slope of ≈0.27 ($R^2 \approx 0.48$; Figure 3.3 B). Width did not vary with length in the saccule among species, represented by a regression line of 0.05 ($R^2 \approx 0.17$; Figure 3.3 C).

When comparing species, the height of the saccule increased with length: As the saccule is longer, it gets taller at a ratio of 1.5. This variation could be due to slight changes in shape, which were not analyzed in detail in this study. The saccule did not get wider with length, but did vary in height. It seems that the most plastic dimension is the dorsal-ventral axis, with a potential constraint on width. More specifically the troglophiles had longer and taller saccules while surface fishes had shorter length and height. Surface fishes and troglophiles saccule morphology overlapped cavefish in length and width parameters.

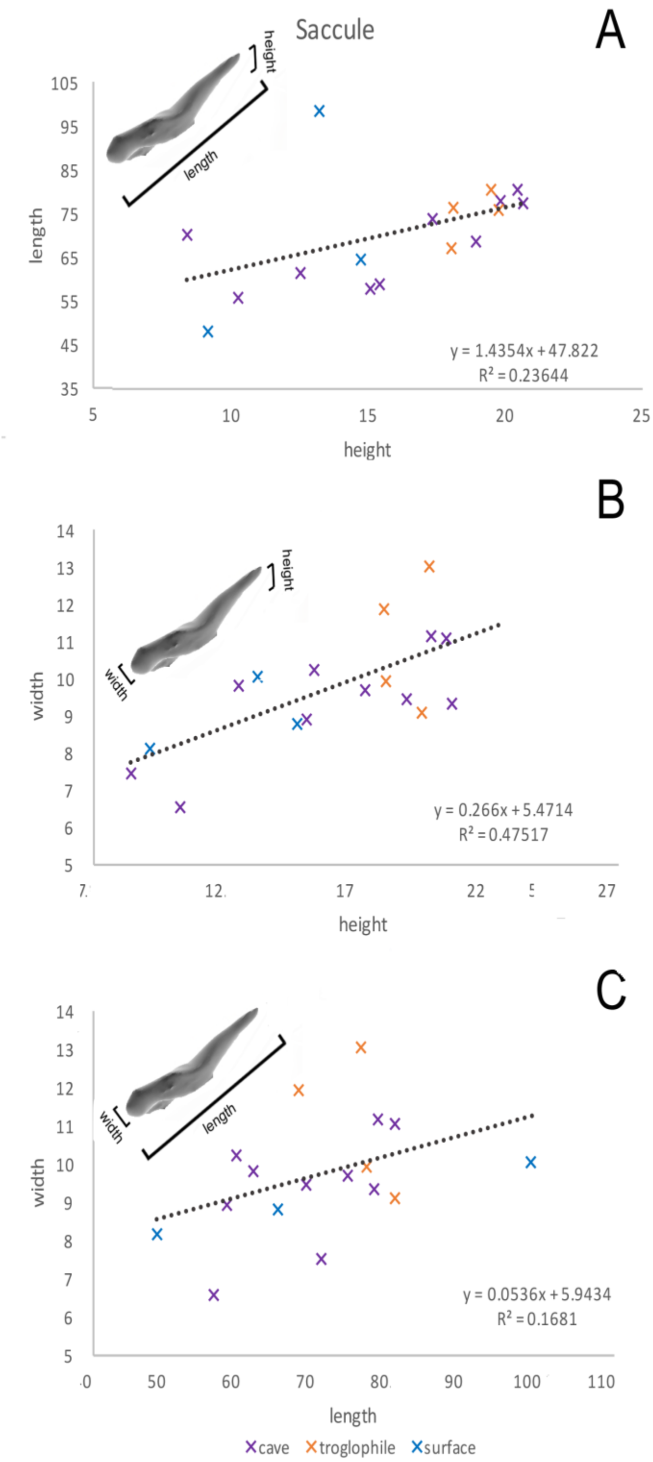


Figure 3.3 Saccule measurements. A) Height vs. Length, B) Height vs. Width, C) Length vs. Width. All measurements in mm*10

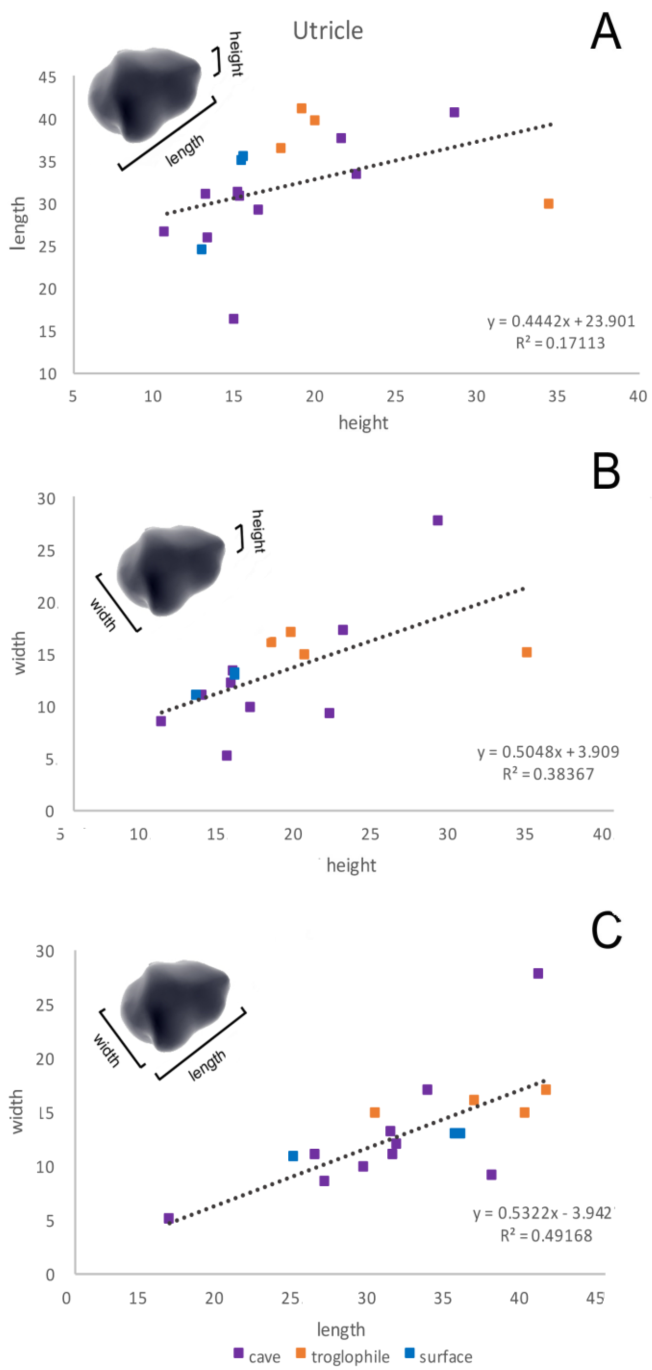


Figure 3.4 Utricle measurements. A) Height vs. Length, B) Height vs. Width, C) Length vs. Width. All measurements in mm*10.

Longer utricles (increasing length) across species were associated with even greater heights: the height grew at a faster rate (regression line (R^2= 0.17) showed a slope of 0.44 of height versus length) (Figure 3.4 A). The same trend was seen in width compared with height, with the otolith width increasing at a rate of 0.50 (R^2= .38; Figure 3.4 B). Width and length were also positively correlated, with a regression line slope of ≈0.50 and R^2= 0.49 (Figure 3.4 C).

The utricle was the roundest otolith in all dimensions for the included species of *Sinocyclocheilus*. It changed half as much in length and width with height. Troglophile utricles tended to be longer, taller and wider, which was the opposite of surface fish. Troglophile morphological parameters did not overlap with surface fishes in length, height and width but the cavefish species overlapped all the fishes. There was no significant difference in cavefish otoliths in relation to ecotypes.

The sample sizes and the differences in morphologic measurements for each ecotype in this study were not large enough to produce statistically significant differences. Nevertheless, there are some trends in the data that may reflect real differences between species. In six of the nine measurements of otoliths, troglophiles had larger measures than surface fish (compare orange versus blue symbols).

CHAPTER 4

DISCUSSION

4.1 Summary of Results

There was no significant asymmetry between right and left otoliths (Lychakok and Rebane 2005). Further, otolith sizes in cavefish are not statistically different from those in surface fishes. There was, however, a potential trend in the data between surface fish and troglophiles. Troglophiles appeared to have larger otoliths, generally speaking, than surface fishes. This result could be a result of systematic size differences – for example, the troglophiles may be larger than surface fish. If all of these fish species were around the same size, then this result would suggest that there might be a selective pressure for increased otolith size in troglophiles. The actual mean ecotype for surface fish species 8.7 cm, cavefish 8.1 cm and troglophiles 7.4 cm. Finally, the distribution of otolith size in cavefish is wider than the distributions of either surface fish or troglophiles – cavefish sizes span across both distributions.

4.2 Otoliths in Cavefish

In all fish, larger otoliths are associated with increased numbers of stereocilia, which can affect hearing (Popper and Lu 2000). This relationship does not always obtain, however, as a study using mollies showed that there were no significant differences in the hearing sensitivity in relation to otolith size (Schulz-Mirbach 2010). Although there were no differences in the otolith sizes in cavefish, hearing in cavefish may yet exhibit interspecific differences related to loss of sight. But, Lombarte and Lleonart (1993) reported that otolith size can be dependent not only on genetic factors but also environmental factors. They found that cold water leads to smaller otoliths, and warmer water leads to larger otoliths. These authors argue that temperature regulates the amount of carbonate material deposited during the formation of the otoliths. Future studies should include more functional analyses, such as behavior and electrophysiology, to resolve therelations between otolith size and function.

4.3 Future Directions

There are two types of experiments that can be pursued that can provide additional information on the inner ears in cavefish.

4.3.1 Behavior*:* Behavioral experiments can be used to elucidate the hearing ranges of *Sinocyclocheilus*. As there were no visible differences in morphology between cave and surface types of *Sinocyclocheilus* otoliths, functional differences may manifest in other locations in the auditory pathway. For example, there could be changes in the density of hair cells, which has been observed in amblyopsids (Niemiller et al. 2013). The first step to understand behavioral differences in hearing would be to measure if there are various thresholds or auditory ranges in these fishes.

One experimental approach that could be used to examine behavioral responses to different acoustic environments is to vary the amplitude of a projecting sound. The various amplitudes need to be randomized to allow the fish to have an acoustic startle response. The acoustic startle response will allow observation for different intensities (soft or loud). Although, the otolith morphology did not show much significant difference among ecotypes, we assume that the cavefish will hear lower thresholds allowing them to hear softer sounds than their surface relatives.

4.3.2 Physiology: Behavioral responses can vary dramatically depending on the state of the animal and other factors that can be difficult to control experimentally. Another approach to understanding the hearing function of an organism that does not rely on behavioral output is to record the electrical activity of the auditory system. A common approach is known as Auditory brainstem recordings (ABR) that detects activity in VIIIth cranial nerve that carries auditory information from the ear to the central nervous system (Wysocki and Ladich 2002). ABR activity can provide insights into the different sensitivities and frequency ranges of animal ears.

An experimental approach to use ABR to measure auditory thresholds and ABR waveforms in fish is by connecting electrodes to the skull of the fish, while still submerged underwater and applying a stimuli. ABR recordings provide a response to the stimulus frequency to obtain audiograms. The audiograms demonstrate the hearing range and threshold for the fish. It can be assumed that if audiograms were created for thirteen species of *Sinocyclocheilus*, the cavefish hearing will be lower in threshold and broader in frequency, allowing them to hear softer sounds over a greater range.

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APPENDIX A

Annotated Biography for

OTOLITHS MORPHOLOGIES IN THE GENUS SINOCYCLOCHEILUS

Cruz, A., and Antoni Lombarte (2004). "Otolith size and its relationship with colour patterns and sound production." ***Journal of Fish Biology*** **65.6: 1512-1525.**

The researchers confirmed their hypothesis that the relative otolith size improves hearing capabilities related to sound perception is correct. Their results showed statistical differences between the sizes in various fish species in families separated by body color.

Fay, Richard R., and Arthur N. Popper (2000). "Evolution of hearing in vertebrates: the inner ears and processing." ***Hearing research*** **149.1: 1-10.**

This paper reviews the evolution of the vertebrate auditory system. The authors describe how the auditory system conducts basic task including sound localization, acoustic feature discrimination and frequency analysis.

Ladich, Friedrich (2014). "Fish bioacoustics." ***Current opinion in neurobiology*** **28: 121-127.**

Fish bioacoustics includes sound detection and production. Ladich combines the ideas that sound generation and detection work together provide fish with a greater advantage in their marine habitats. Fish gain advantages from listing to their surroundings to avoid danger and find suitable living habitats. The fish are well adapted to surrounding noise within their habitats and any outside noise can delay prey detection and acoustic communication.

Ladich, F., and T. Schulz-Mirbach (2016). "Diversity in Fish Auditory Systems: One of the Riddles of Sensory Biology." Front. Ecol. Evol. 4: 28. doi: 10.3389.

This review discusses the diversity that exists in fish inner ears, auditory accessories and sensitivities. The authors categorize morphology of the inner ear in terms of diversity by (1) size of ears, fish and the brain, (2) amount of bones within the skull (3) distance between right and left ears (4) position of ears for example the position of the utricle compared to saccule and lagena (5) the size and diameter of semicircular canals (6) ratio of utricle, saccule and lagena (7) number of pouches formed by saccule and lagena. They conclude that the diversity of inner ears still needs to be further researched and a possibility of their diversity could be to ecological adaptations.

Lombarte, Antoni (1992). "Changes in otolith area: sensory area ratio with body size and depth." *Environmental Biology of Fishes* 33.4: 405-410.

Lombarte examined the relationship between the sensory areas in the ear to body size and depth in the genus *Merluccius*. He examined areas in the sulcus acusticus and the sagittal otolith. His results indicated that the sagittal growth was negatively related with respect to the fish size, while the sulcus acusticus increases with fish size.

Lombarte, A., and Jordi Lleonart (1993) "Otolith size changes related with bodygrowth, habitat depth and temperature." *Environmental biology of fishes* 37.3 297-306.

This paper examined the relationship between otolith size and various environmental parameters. They proposed that otolith size is dependent on genetic and environmental factors, so that cold waters lead to smaller otoliths. That would be because temperature regulates the amount of carbonate material deposited during the formation of the otolith.

Lu, Zhongmin (2004). "Neural mechanisms of hearing in fishes." *The Senses of Fish*. Springer Netherlands, 147-172

There are multiple neural mechanisms that take place to allow fish to hear. Neural circuits respond to sound pressure and sound intensity, which are important for temporal responses, localization of sound sources and directional information. A fish's hearing response is dependent on the characteristic frequency, sensitivity and tuning of sound waves.

Lychakov, D. V., and Yu T. Rebane (2000). "Otolith regularities." *Hearing research* 143.1: 83-102.

This paper examined 15 different species of fish from the Black Sea. They explained otolith regularities in four rules. The first was that the otoliths growth is isometric to the fish growth. The second is that the ratio of the sacculus and lagena or sacculus and utricle that is not dependent or does not change with the growth of the fish. The third rule is an equation that represents the otolith mass and area. The fourth rule is the ratio between the otolith and the macula that does not depend or change based on the fish size. Their research shows that the greater the otolith mass the higher acoustic sensitivity. The saccular and lagenar otoliths maintain a constant distance throughout the growth of the fish.

Niemiller, Matthew L., and Daphne Soares (2015). "Cave Environments." *Extremophile Fishes*. Springer International Publishing, 161-191.

Caves are one of the most challenging habitats, which result in a wide range of species. Species such as cavefish have gone through evolutionary development to adapt to their extreme environments. Their resource-limited environment includes absence of light and scarcity of food. In these caves there is minimal genetic variation among the species due to the resource-limited environment.

Paxton, John R (2000). "Fish otoliths: do sizes correlate with taxonomic group, habitat and/or luminescence?" Philosophical Transactions of the Royal Society of London B: Biological Sciences 355.1401: 1299-1303.

Paxton researches otolith size with different taxonomic groups, habitat and luminescence. Out of 247 species he analyzed he was able to find a correlation between a few taxonomic groups and the size of the sagitta being larger. His study also finds that there is a correlation with smaller sized otoliths within the epipelagic habitat. Lastly, he also finds a correlation between luminous fishes having larger otoliths. The luminescent fishes are found in environments where there is an absence of sunlight.

Popper, A.N., Ramcharitar J., and Steven E. Campana (2005). "Why otoliths? Insights from inner ear physiology and fisheries biology." *Marine and freshwater Research* 56.5: 497-504.

This group of investigators provides insight and further question on to why otoliths are important aspect of the inner ear. They provide ample background information on the size, shape and growth of otoliths. The researchers conclude the review paper by setting up questions to further examine the relationship of different otoliths in respect to the ear function in fish.

Popper, A. N., and Richard R. Fay (2011). "Rethinking sound detection by fishes." *Hearing research* 273.1: 25-36.

The investigators in this paper attempt to reevaluate the terms “hearing specialist” or “hearing generalist”. They argue that some fish species are frequency dependent and are sensitive to pressure and motion, therefore they would not fall under either classification. They also propose that the term “specialization” be limited to anatomical structures that are involved in enhancing sensitive to sound pressure. Instead, they propose to use the term “motion sensitive” for fish without any pressure sensitivity.

Popper, A. N., and Zhongmin Lu (2000). "Structure–function relationships in fish otolith organs." *Fisheries research* 46.1: 15-25.

The investigators examined the basic structure of the auditory system in teleost fish and describe their hearing in detail. Their results showed that there are significant differences in frequency range of sounds and the sensitivity to the sounds that fish hear. Popper et al conclude that the inner ear is the most vital organ of the sensory system to detect distant sources and provide the fish with a “general impression” of their surrounding environments.

Schulz-Mirbach, Tanja, et al (2010). "Otolith morphology and hearing abilities in cave-and surface-dwelling ecotypes of the Atlantic molly, Poecilia mexicana (Teleostei: Poeciliidae)." ***Hearing research*** **267.1: 137-148.**

In this paper the investigators have conducted a research study on the Atlantic molly (Poecilia Mexicana). They conducted an acoustic survey on two ecotypes in the cave form and the surface populations. The researchers also looked into detail if the otolith morphology is reflected by the inner ear physiology. They divided their research down into three components. The first was to see if there are potential differences between two ecotypes in the morphology of all three otoliths (lagena, sagitta and utricule). The second was to see if hearing sensitivities are similar between cavefish and surface fish and the third concluded with an acoustic survey to determine whether the species P. *mexicana* communicates acoustically.

Soares, Daphne, Matthew L. Niemiller, and Dennis M. Higgs (2016). "Hearing in Cavefishes." ***Fish Hearing and Bioacoustics*****. Springer International Publishing,. 187-195.**

The investigators provide a review on hearing in cavefish that have thrived in resource-limited environments. They discuss the influence Popper and Fay on cavefish bioacoustics and support their research by discussing their own work on Amblyopsid cavefish.

Yoshizawa, Masato (2015). "Behaviors of cavefish offer insight into developmental evolution." ***Molecular reproduction and development*** **82.4: 268-280**

Yoshizawa provides an insight into developmental evolution by studying the behaviors of cavefish. In this review article cavefish are grouped together based on their convergent morphologies. He also emphasizes that cavefish are a model organism to study developmental evolution. The focus of this paper is on *Astyanax mexicanus* and their physical traits and ecosystem. The restricted environment of a cavefish such as absence of light and sparse food has provided evidence that many morphological traits such loss of pigmentation and behavioral traits such has advanced prey capture and feeding angle has evolved.