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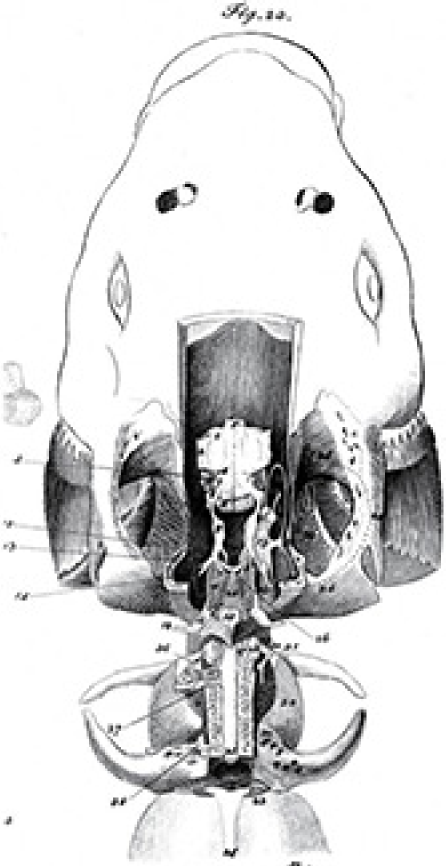
Zebrafish



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Review Article

The Sound World of Zebrafish: A Critical Review of Hearing Assessment

Arthur N. Popper[1] and Joseph A. Sisneros[2]

Abstract

Zebrafish, like all fish species, use sound to learn about their environment. Thus, human-generated (anthropogenic) sound added to the environment has the potential to disrupt the detection of biologically relevant sounds, alter behavior, impact fitness, and produce stress and other effects that can alter the well-being of animals. This review considers the bioacoustics of zebrafish in the laboratory with two goals. First, we discuss zebrafish hearing and the problems and issues that must be considered in any studies to get a clear understanding of hearing capabilities. Second, we focus on the potential effects of sounds in the tank environment and its impact on zebrafish physiology and health. To do this, we discuss underwater acoustics and the very specialized acoustics of fish tanks, in which zebrafish live and are studied. We consider what is known about zebrafish hearing and what is known about the potential impacts of tank acoustics on zebrafish and their well-being. We conclude with suggestions regarding the major gaps in what is known about zebrafish hearing as well as questions that must be explored to better understand how well zebrafish tolerate and deal with the acoustic world they live in within laboratories.

Keywords: hearing, threshold, sound pressure, particle motion, anthropogenic sound, acoustics

Introduction

All animals, from insects[1] to humans[2] including fishes,[3] live in a world where sound in the environment provides a great deal of information about the surrounding world. These sounds, often referred to as the soundscape or acoustic scene, may be produced by natural sources (e.g., water running over rocks, or wind) or anthropogenic (human-made) sources (e.g., traffic, ships, oil, and gas exploration). The soundscape provides animals with an environmental perspective that extends into three dimensions and well beyond the range of any other sense. Also, beyond telling of predators, prey, and serving for communication with conspecifics, the soundscape gives a general environmental sense that helps alert animals to changes around the animal.

The focus of this article is the bioacoustics of zebrafish (*Danio rerio*). While zebrafish are not known to make sounds for communication, they, like all fishes, are likely to use sound to learn about their environment.[4,5] Thus, anything that interferes with the ability of zebrafish to detect these sounds can have a variety of impacts on hearing and behavior.

Perhaps more important than potential effects on behavior, anthropogenic sounds added to the zebrafish tank environment, such as those produced by air stones, pumps, and in-air sounds that get into the water,[6] may have less visible, but equally important consequences on the health and well-being of zebrafish,[7,8] thereby potentially impacting their value in a wide range of studies. Indeed, it is well known that added environmental sounds, such as from traffic or numerous other sources, can have substantial effects on everything from hormone levels to reproduction to sleep, even in humans.[2,9,10]

This article has two goals. The first is to briefly overview what is known about zebrafish hearing and to share critical issues related to how hearing is, and should be, measured in all fishes, including zebrafish. In doing this, we write in general terms, and are not discussing specific aspects of hearing in different genetic strains or during development.

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[1]Department of Biology, University of Maryland, College Park, Maryland, USA.
[2]Department of Psychology, University of Washington, Seattle, Washington, USA.

Our second goal is to consider how the environs in which zebrafish live in research laboratories may affect the fitness and health of the animals. Importantly, while the focus on our review is on postlarval zebrafish, many of the issues raised are also applicable to all stages of zebrafish development.

To accomplish these goals, we first set the stage with a brief discussion of sounds found in the zebrafish laboratory environment. This is followed by an overview of underwater acoustics since it is imperative to understand that sound in water, including in the tanks in which zebrafish live, has characteristics that are different from sound in air, and these characteristics are critical parts of hearing and the acoustic environment of all fishes.

Then, since many workers studying zebrafish focus on hearing (including development and genetics), an area of particular interest to the authors, we provide an *overview* of what is known about the auditory system of zebrafish and about hearing in adult fish. One intent is to demonstrate that relatively little is known about zebrafish hearing. From this, one of the major issues we raise is that there is no ''standard'' way to study hearing in zebrafish, and that reported results of earlier studies very much depend on the acoustic environment and precise way in which questions about hearing are asked.

With this background, we then continue with a discussion of the zebrafish soundscape and consider the potential impacts of this soundscape on fishes used in research. The review concludes with thoughts about questions for future research on zebrafish and sound, directed at the idea of improving the sound world of zebrafish, both for their well-being and their usefulness as research models.

What this article is not, however, is a detailed or comprehensive review of the rather large, and growing, literature on zebrafish hearing, development of hearing, genetics of hearing, and any related topics. Rather, we present a brief overview to put the issues we raise into context. We leave the detailed review to colleagues whose research interests focus on zebrafish, and not, as ours, on broader issues of fish bioacoustics.

Moreover, it is important to make clear that while we raise several critical issues and suggest what we see as the most important research questions regarding zebrafish hearing and effects of anthropogenic sound, we cannot give solutions that mitigate these issues. Indeed, many of the issues we raise also ''plague'' those of us studying fish hearing more globally, and we have no solutions or even good guidance regarding issues such as tank acoustics and accurate calibration of the sounds to which fish are exposed.

Laboratory Sounds and Zebrafish

With few exceptions for example,[6,8] very little has been written about the sound environment of zebrafish, particularly from the perspective of understanding underwater sound and fish bioacoustics. Indeed, this issue also clearly pertains to any other studies using zebrafish where stress and other affects resulting from increased sound in the environment might affect experimental results. There is evidence that increased sound in the environment can result in hearing as well as hormonal changes in fishes.[8,11–13] Such changes in zebrafish from increased sounds has the potential to alter results in a wide range of experiments related to physiology and behavior.

At the same time, there are some concerns about the potential impacts on zebrafish from sound and vibration, as discussed in AAALAC and RSPCA guidance on zebrafish care and housing by Reed and Jennings.[7] This report states:

> Zebrafish can appear to grow accustomed to their surroundings and as such, may apparently habituate to certain vibrations—from a pump in the room for example. But they can also react strongly to sudden loud noises or novel vibrations so steps should be taken to avoid such disturbances … It has also been suggested that spawning in these fish may be affected if it is very noisy … (p. 22)

However, the guidance gives no information about levels that might have an impact on zebrafish, nor does it discuss anything other than vibrations, such as sounds from the external laboratory environment or sounds produced by pumps and other sources such as music being played in a laboratory or people walking around. Importantly, more recent discussions of the zebrafish laboratory environment have minimal mention of sound or vibration.[14,15]

As discussed later in this review, there have been few studies on the response of zebrafish to sounds in laboratory tanks. Indeed, only one recent study appears to actually address the environment of the zebrafish laboratory sound environment,[16] but it has, as discussed later in the review, substantial methodological problems.

Underwater Sound and the Acoustic Cues Available to Fishes

Although a thorough treatment and discussion of underwater acoustics is beyond the scope of this review, it is necessary to provide a basic overview to appreciate the zebrafish acoustic environment, particularly in fish tanks or other enclosures. Readers who want a better understanding of underwater acoustics are referred to several recent articles[17,18] and to the website of Discovery of Sound in the Sea (DOSITS.ORG) for an authoritative discussion of all aspects of sound in water but written for a lay audience.

Sound can be defined as a mechanical disturbance that propagates as a longitudinal wave through air, water, or any other medium.[19] When a mechanical disturbance occurs (e.g., from a loudspeaker, an automobile, a fish making sounds), energy in the form of both sound pressure and particle motion is radiated away from the sound source. (For an excellent visualization of particle motion vs. sound pressure see https://bit.ly/Particlemotion.)

Sound pressure is a measure of the fluctuations in the density of the medium due to the presence of sound while particle motion is the movement of the particles that make up the media when sound is present. In air, particle motion decays (attenuates) quickly as the sound propagates from the source, whereas in water, particle motion stays a major component of the sound field for very substantial distances from the source. Particle motion is a significant component of sound in water due to the greater density of water as compared to air.

Moreover, because of the higher density of water, the speed of sound in water is about 4.8 times greater than in air (and the wavelength of a sound being much larger in water than in air). Importantly, due to this density difference sound does not transmit well between water and air, Instead, sound is reflected off the air-water interface, just as light reflects off a mirror.[19,20] Therefore, it is impossible to predict levels of sound in one medium based on measures in the other.

The reason for mentioning particle motion as well as sound pressure is that every species of fish (including elasmobranchs), and probably most aquatic invertebrates that detect sound, has an ear that only responds to particle motion and not to sound pressure.[17,21] Indeed, the likely origin of the vertebrate ear is as a biological accelerometer, which only detects particle motion.[22]

Moreover, pressure detection in fishes is a relatively recent adaptation and requires having ancillary structures, such as a swim bladder or other air bubble, that respond to a pressure field. This detector reradiates the energy and puts out a new signal that has both pressure and particle motion. If this source is sufficiently close to the ear, the reradiated particle motion is detectable by the ear.[23,24] In terms of hearing, there is substantial evidence that lower frequency hearing (e.g., perhaps up to 300 or 400 Hz) is primarily driven by the detection of particle motion, and only those species with adaptions that respond to pressure can hear frequencies above perhaps 1 kHz.[25,26]

For the perspective of this review, it is not necessary to go into additional details of the physics of sound pressure and particle motion. However, there are three critical points that must be at a forefront in considering fish, including zebrafish, hearing.

First, particle motion is a fundamental part of underwater sound. Second, both sound pressure and particle must be considered when studying fish hearing, and both are found in places like fish tanks.

Third, zebrafish, like all fishes (including elasmobranchs) detects particle motion, while not all species detect sound pressure. While zebrafish are among those species that also detect sound pressure, it is imperative to keep in mind that they are also detecting particle motion. Thus, studies of zebrafish hearing (larval and adult) must include analysis of detection of particle motion. Moreover, larval zebrafish, before the development of the swim bladder and connection to the ear via the Weberian ossicles, would only detect particle motion!

In addition to sound pressure and particle motion, a third issue, substrate vibration, is getting increased attention by those interested in sound and fishes.[27,28] This interest arises because investigators are becoming more aware that sound from many anthropogenic sources, such as in construction of offshore wind farms and seismic airguns used for underwater exploration for oil and gas, send signals into the substrate. These signals can travel substantial distances before coming out of the substrate as both sound pressure and particle motion are likely detectable by fishes and aquatic invertebrates living on or near the bottom as sound. Of similar concern would be vibrations in the floors and walls of fish tanks and other encloses and the potential effect on fishes and measurement of hearing.

Measuring particle motion in a laboratory tank

In this section, we introduce issues about particle motion in laboratory housings of any type from small fish tanks to large aquaculture tanks. It is critical to understand, however, that while we raise very important issues that are as relevant to studies of zebrafish from a tiny Petri dish to a large housing tank, there are yet no easily accessible solutions to predict or measure particle motion in such environments. Indeed, the only tanks in which particle can be effectively controlled and measured are highly specialized ''home-built'' devices that are very expensive and not conducive to the kind(s) of studies being done with zebrafish.[29–31]

Sound pressure in water is measured using a hydrophone. Also, since sound pressure is a scalar quantity, it can be measured as a single number that represents magnitude. In contrast, particle motion is a vector quantity having both magnitude and direction. Because particle motion is a vector quantity, it needs to be measured along three axes (x, y, z), which makes it more difficult to measure than sound pressure.

A critical issue is that instruments to measure underwater particle motion are not readily available and are often custom made, consisting of three velocity transducers or accelerometers aligned in the x, y, and z directions and contained in waterproof housing. Furthermore, the custom-made particle motion sensor needs to be made neutrally buoyant in water so that the sensor moves with the fluid and does not influence the acceleration field.[32,33] Although such custom-made devices are generally not available to the average laboratory scientist, some three-dimensional (3D) velocity transducers or accelerometers can be purchased commercially, but they tend to be very expensive (costing much more than a hydrophone), and they tend to be very large.[32] At the same time, for any serious consideration of zebrafish hearing and the acoustic environment, such data are important to get.[21]

Moreover, measuring particle motion in tanks (e.g., laboratory housing) can be a very complicated issue as the sound fields produced by an underwater loudspeaker in most aquaria are, in general, very complex sound fields unlike biologically relevant sound fields fish would experience in the natural acoustic environment.[32,34,35] Thus, measuring sound in small tanks and other fish aquaria become especially challenging because the relationship between sound pressure and particle motion is significantly altered when close to interfaces with media of different acoustic properties (e.g., an air-water interface or water-glass interface).

In addition, the direction and propagation of particle motion can be affected by the presence of soft and hard surfaces (e.g., the sides of experimental fish tanks) and the size and shape of the tank. In many tanks, when the source sound is in the water, the tank walls act as a pressure release causing the sound pressure to drop close to zero near the walls, bottom, and water surface of the tank while greatly increasing particle motion at those interfaces. Such conditions make it virtually impossible to ever simulate the acoustic conditions that a fish would experience in the natural environment.

For a more detailed discussion of particle motion and sound pressure in tanks, the reader is encouraged to read the articles by Parvulescu[20] and Rogers *et al.*[34]

Issues and Caveats in Understanding Fish Hearing Data

Since part of many studies of hearing in zebrafish of any age incorporate determining hearing bandwidth and sensitivity, it is critically important for investigators to appreciate basic issues in how to study hearing in fishes as well as the complex acoustics of the tanks in which studies are done. As pointed out by Popper and Hawkins[36] in greater detail, hearing studies in fishes are rather more complex to do than for any other vertebrate group. This is primarily because of several methodological issues that are, only now, coming into the forefront of fish hearing studies and which are critical for understanding the data to date on zebrafish hearing. Indeed, these caveats are even more critical to deal with as we move forward in using zebrafish as model systems for the study of the genetics and development of hearing.

What stimuli are zebrafish responding to?

Because of the complexity of the sound field in a tank, as discussed above, it is generally impossible to know the nature of the stimulus to which fish are responding when referring to ''hearing.'' Thus, an investigator may have measured sound pressure in a tank or substrate vibration, but the actual stimulus to which the fish is responding is particle motion. It should be noted that some investigators have tried to approximate particle motion based on measures of sound pressure, but the methods used, and the equations developed to do this, are based on sounds in a free field, such as the deep ocean and cannot be applied to shallow water or to enclosed environments such as tanks.

As a consequence, even when an experiment reports hearing sensitivity and bandwidth in terms of sound pressure, we have no way of knowing if the fish was responding to the pressure or to the uncalibrated particle motion associated with the pressure in a particular tank configuration. This is a particular problem for species that primarily detect particle motion when the only sound levels measured are pressure (e.g., salmon, sharks, flatfish, tuna).

Similarly, when data are reported for hearing in zebrafish, or other species where the swim bladder is involved in hearing, one may be able to get a reasonable approximation of sensitivity at higher frequencies where hearing is probably related to sound pressure. However, at lower frequencies, perhaps 300 or 400 Hz and below, where all species heavily depend on particle motion for hearing, it becomes impossible to get accurate hearing thresholds.

Methodologies

A limited number of studies of fish hearing, including that of the zebrafish, used operant or classical conditioning paradigms where the fish responded in a discrete way when they detected a sound.[37–42] The use of unlearned behavioral responses (innate reflexes) has also been applied in psychoacoustic studies to characterize the hearing capabilities of larval zebrafish.[43] Innate reflex responses are used in hearing assays such as the acoustic startle response (ASR) and in prepulse inhibition (PPI). The ASR has been prominently used in zebrafish studies of hearing development to test whether or not the auditory system is functional for example,[44–46] while use of PPI has been used to characterize hearing sensitivity in larval zebrafish.[47,48]

While the behavioral studies give important data, most recent studies tend to use electrophysiological methods that involve recording auditory brainstem responses (ABR) or auditory evoked potentials (AEP) from the ear or brainstem.[49–52] The reason for doing this is that these methods do not involve training and are fast and many fish can be tested over a short period of time.

The problem is that while investigators using ABR and AEP often say that they are determining hearing sensitivity and bandwidth, Popper and Hawkins[36] strongly argue that these and other electrophysiological methods are not measuring ''hearing''—but rather only the auditory physiological response of the ear and perhaps the lower end of the brain (also see Ladich and Fay[53] and Sisneros *et al.*[54]). Indeed, electrophysiological responses do not represent the full perception of sound that constitutes hearing as hearing is a precept that can only be measured by a behavioral assay[55] where fishes are ''asked'' what they hear by training them to do a behavioral task to indicate a response (see also Ladich and Fay[53] and Sisneros *et al.*[54]).

Variability in results across studies

One of the real problems in understanding fish hearing in general is that there is very substantial variability in results on the same species from different laboratories that use different acoustic environments and ask questions with different methods.[53] This variability is best seen from studies with the ''white rat'' of fish hearing research, the goldfish (*Carassius auratus*), a species in the same taxonomic family as zebrafish. Goldfish hearing has been investigated using behavioral methods at least six times since the first studies in the late 1960's[56,57] and at least 13 times using AEP. The results from these studies vary 20–30 dB at different frequencies,[36,53,54] resulting in the problem that we really do not know the hearing sensitivity for goldfish, and the same concern arises for zebrafish.

Of course, there are some cases where hearing data can be compared. This is when the tests are done under identical conditions, including in the same acoustic environment and in the same laboratory and using the same methodology. The critical thing is that the comparisons can only be done within the fish tested under the same conditions, and not between experiments done in different laboratories as the environmental and experimental acoustic conditions inevitably vary across laboratories.[36]

Is measuring bandwidth enough?

The final issue is that most of the focus on hearing in zebrafish and other species has been on bandwidth (the range of frequencies detectable) and sensitivity (or threshold, the lowest sound level detectable). These, however, are not the most important aspects of hearing in any animal. Instead, it is much more important for an animal to be able to detect biologically relevant signals in the presence of other masking sounds to determine the direction of a sound source such as a predator or prey, and to discriminate between sounds such as those of conspecifics versus other species or other sounds in the environment that are part of the acoustic scene.[58] With the exception of one study,[40] nothing is known about any of these functions in zebrafish.

In fact, the only species for which we have any substantial data are the goldfish[59] and the Atlantic cod (*Gadus morhua*).[60] This leads to the question of whether some of the genetic studies of hearing in zebrafish could be asking the wrong question about hearing when they only focus on bandwidth and sensitivity, while more critical parts of hearing may be associated with discrimination or other auditory functions that are not examined!

The conclusion one must reach from these caveats is that determining hearing capabilities in zebrafish (and all other fishes) is not nearly as easy as measuring hearing in mammals in air. There are issues and parameters that must be considered if we are ever to get a real understanding of the range of sounds that zebrafish hear, their sensitivity, and the additional aspects of sound detection that are far more important than these simple parameters. Indeed, without knowing what zebrafish detect in terms of particle motion, investigators may be missing potential genetic or developmental effects that relate to inner ear function as an accelerometer!

Zebrafish Hearing

The inner ear

The inner ear of zebrafish (like other fishes) has three semicircular canals and three otolith organs, the saccule, lagena, and utricle (Fig. 1). While there are some data on the anatomy of the ear in zebrafish,[61,62] much of what we know of structure and function of the ear comes from studies of other species in the same taxonomic group, the otophysans, and most notably the closely related goldfish.[61,63]

Each otolith organs in fishes has a sensory epithelium (or macula) that contains sensory hair cells that are very similar to those found in the ears of other vertebrates.[64] The otolith organs also contains a single calcium carbonate structure, the otolith which is held closely to the macula via an otolith membrane.[65] Since the otolith is about three times denser than the rest of the fish's body, it lags behind when the rest of the body, which is about the same density as water, is set in motion by an impinging sound. This results in relative motion between the epithelium and otolith, and in bending of the ciliary bundles on the sensory cells along the axis of stimulation. In effect, the otolith organs act as accelerometers for detection of particle motion.[21,66,67]

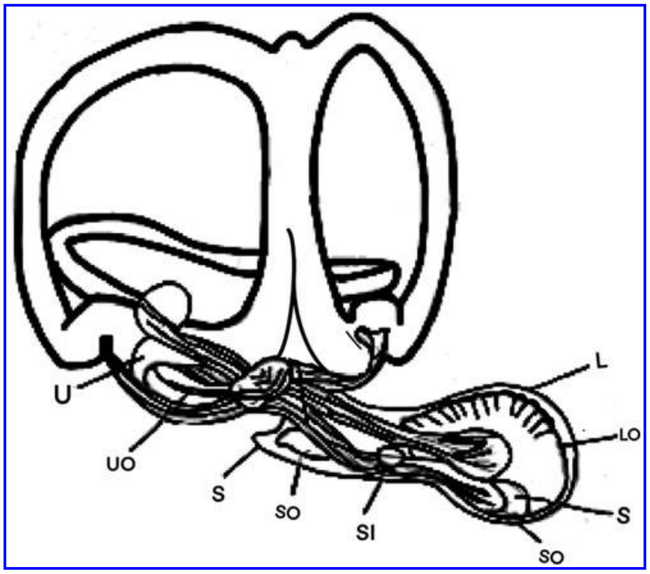

FIG. 1. Drawing of the right inner ear of typical cyprinid fish, such as zebrafish or goldfish. Anterior to the left, dorsal to the top. The sensory epithelia (maculae) are not shown but lie along the otoliths. The rostral most macula and otolith are those of the utricle. The ear also has three semicircular canals and is innervated by the eighth cranial nerve. L, lagena; LO, lagena otolith; S, saccule; SI, sinus impar connecting the two saccules; SO, saccular otolith; U, utricle; UO, utricular otolith.

Detection of sound pressure

Detection of particle motion enables fishes to hear sounds from low frequencies (below 50 Hz) to perhaps 300–400 Hz, and in some species possibly up 500–1000 Hz.[21] Detection of lower intensity sounds above about 300 Hz, and all sounds above 500–1000 Hz, involves detection of sound pressure.[25,26] This happens due to the presence of some air-filled chamber, most notably the swim bladder, that responds to pressure and then converts it to particle motion which is reradiated to the inner ear. The amount of particle motion energy that gets to the ear is related to the distance between the swim bladder and the ear.[23] Indeed, in many species, even with the presence of a swim bladder (e.g., salmon, tuna), fishes do not detect sound pressure.[26,68]

One way to enhance detection of sound pressure is to bring the swim bladder or other air bubble closer to the ear. While there are many such examples (reviewed in Popper and Hawkins[18]), perhaps the most unique are a series of modified vertebrate found in all adult otophysan fishes that were first described by Weber in 1820, and now known as the Weberian ossicles[69] (Fig. 2) (also see Alexander[70]). These most posterior of the ossicles attach to the walls of the swim bladder, while the most anterior makes up a wall of a fluid-filled chamber that leads into the inner ear.

It is thought that motion of the swim bladder wall in response to sound results in movement of the Weberian ossicles and results in fluid movement within the ear for fascinating perspectives (see Refs.[71–75]). However, we should note that the Weberian apparatus is only functional in juvenile zebrafish after they are fully formed by ~56 days postfertilization (dpf; ~2 months of age) or ~20 mm total length (TL).[76]

Because of this connection, all movements of the swim bladder resulting from impinging pressure signals are carried to the ear without attenuation. This serves to increase sensitivity to sound, thereby lowering hearing thresholds and broadening the bandwidth of hearing so that most otophysans, including zebrafish, can detect sound up to 3–4 kHz.[25,75,77]

Hearing sensitivity

As we discuss what is known about zebrafish hearing, it is important to keep the caveats discussed in the previous section in mind since virtually none of the earlier work adhered to some, or all, of the points raised. Thus, it is critical to understand that virtually all zebrafish data on hearing capabilities, changes with age, sensitivity, and bandwidth must be viewed with caution.

There are a number of studies that report hearing thresholds and bandwidth for zebrafish as adults[40–42,52,78–80] (Fig. 3) and during development (Fig. 4).[47,76,79,81,82] However, from reviewing the literature, there has been no single study that has examined the whole range of hearing based on a behavioral assay for adult zebrafish, nor has there been comprehensive analysis of thresholds based on the auditory physiological response of the zebrafish ear and auditory central nervous system (CNS).

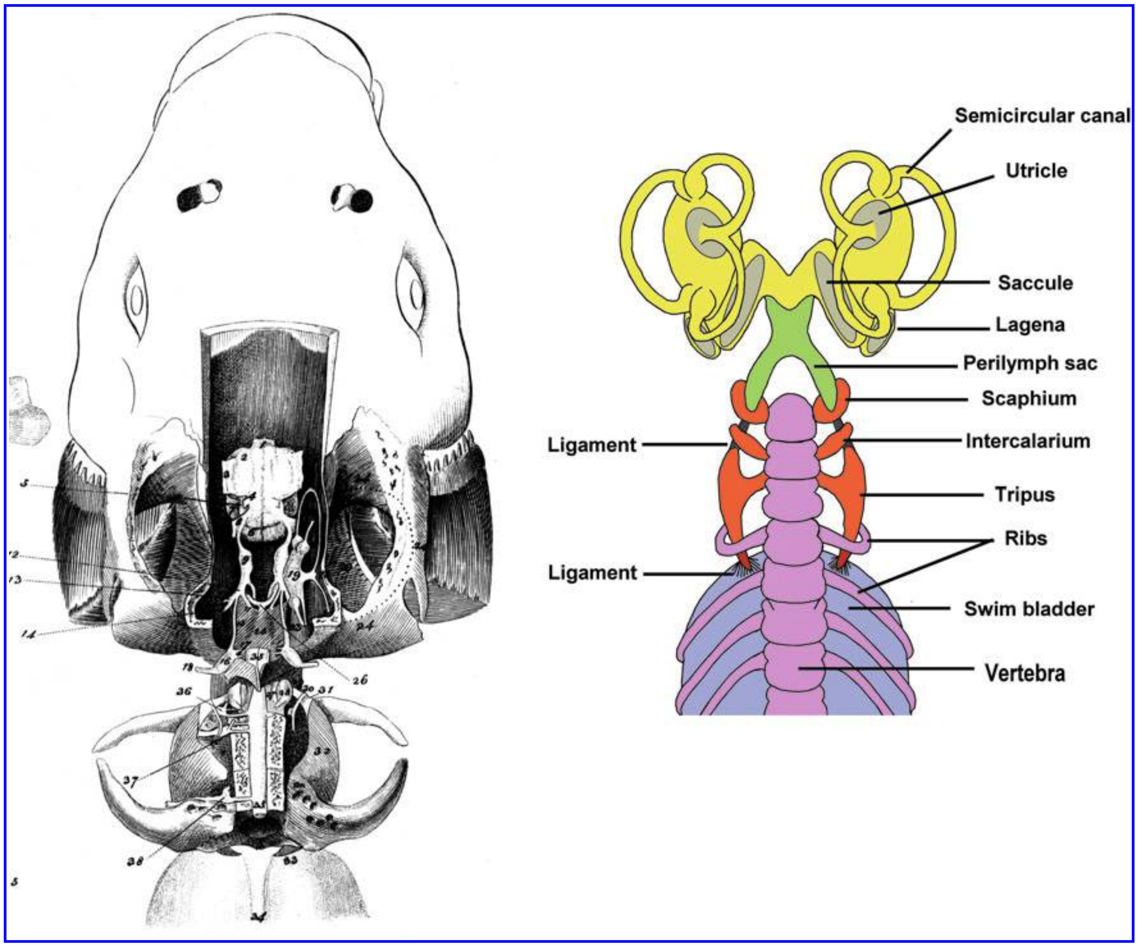

FIG. 2. The Weberian ossicles. *Left* is a portion of plate 20 from the original description of the bones by Weber.[69] The illustration shows a dorsal view of a carp (*Cyprinus carpio*), a close zebrafish relative, and illustrates the Weberian ossicles (30, 31, 32), the inner ear (19), and the swim bladder (33). *Right* is a drawing showing the details of the ossicles and their connection to the swim bladder an inner ear typical of most cyprinids, including zebrafish. The ossicles (tripus, intercalarium, and scaphium) are set into motion when the swim bladder walls move in response to sound pressure. The rostral-most ossicle, the scaphium, makes up the outer walls of a fluid-filled perilymphatic sac. Fluid movements connect to a transverse canal that connects the left and right saccules at the sinus impar (Fig. 1). Movement of the fluid causes direct movement of the saccular otoliths and stimulation of the sensory hair cells on the epithelium. Figure © 2021 Anthony D. Hawkins, all rights reserved. Adapted from Popper and Hawkins.[108] Color images are available online.

Furthermore, at least one study has reported that auditory sensitivity based on the physiological responses in adult zebrafish differs across various zebrafish lines, and perhaps even within lines (e.g., AB, WIK, and TL zebrafish strains) used in auditory research.[80] However, these differences may not be genetic *per se*, since there are reports of differences in hearing abilities between different groups of the same batch of hatchery-raised rainbow trout (*Oncorhynchus mykiss*) that had slightly different histories when fertilized or start-times for development.[83]

So, what can we surmise from the data that we do have, and from data from related species? While there have been no complete audiograms for zebrafish, the data suggest that larvae (4–21 dpf) can hear up to ~1200 Hz (Fig. 4)[46,47,84] while adults can hear sound up to 3–4 kHz,[42,76] which is generally comparable to the upper frequency for most otophysans.[53,85]

A few recent studies have reported that larval zebrafish can detect frequencies up to 4 kHz[86] and adults up to 12 kHz.[81] Poulsen *et al.*[86] used whole-brain calcium imaging in larval zebrafish to characterize the sensitivity of auditory neurons in the CNS to auditory stimuli delivered by a mini-speaker directly attached to the coverslip wall of a 3D printed imaging chamber (it was noted that particle motion was calibrated using only a single-axis accelerometer). Wang *et al.*[81] examined the AEP thresholds of zebrafish during ontogeny from juveniles to adults using an underwater speaker coupled to the bottom of the test tank (calibrations of sound pressure measurements were conducted, but particle motion were not).

Based on the caveats in doing hearing studies in fishes discussed earlier, these studies must be viewed with caution, especially about the reported wide bandwidths. This is suggested since there is no evidence that the investigators characterized possible contaminant, low frequency vibrational stimuli (i.e., in three dimensions: *x*, *y*, and *z*), which may have potentially stimulated the zebrafish inner ear, and was likely coincident with the high amplitude and high frequency stimulus delivery in those studies. Interestingly, the

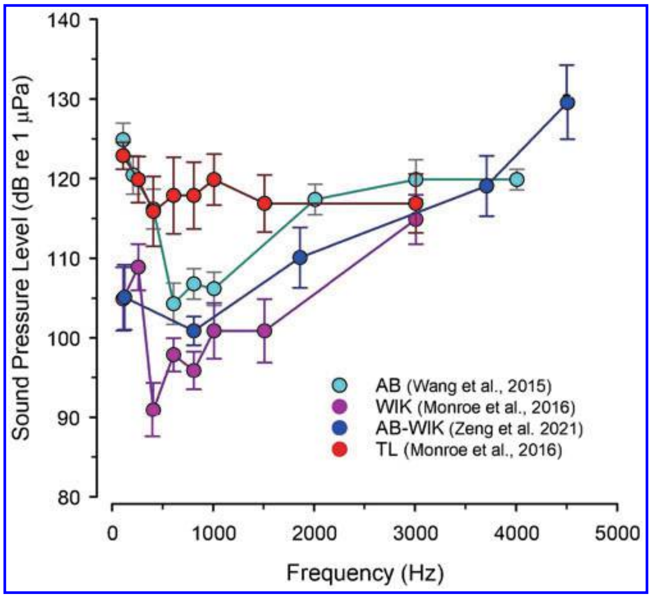

FIG. 3. AEP tuning curves for adult zebrafish based on sound pressure level (dB re: 1 μPa) for four different zebrafish strains. The sound pressure sensitivities of the different strains are summarized from the following AEP studies: AB,[81] WIK,[80] AB-WIK,[52] and TL.[80] All data are plotted as mean ± standard error. AEP, auditory evoked potential; TL, Tupfel long fin. Color images are available online.

higher frequency hearing (>1.2 kHz) in zebrafish does not show up until the Weberian ossicles are fully formed at ~56 dpf or about 20 mm TL,[76,87] supporting the finding of others that signals reradiated from the swim bladder are necessary for hearing above perhaps 800–1000 Hz in fishes.[26,75]

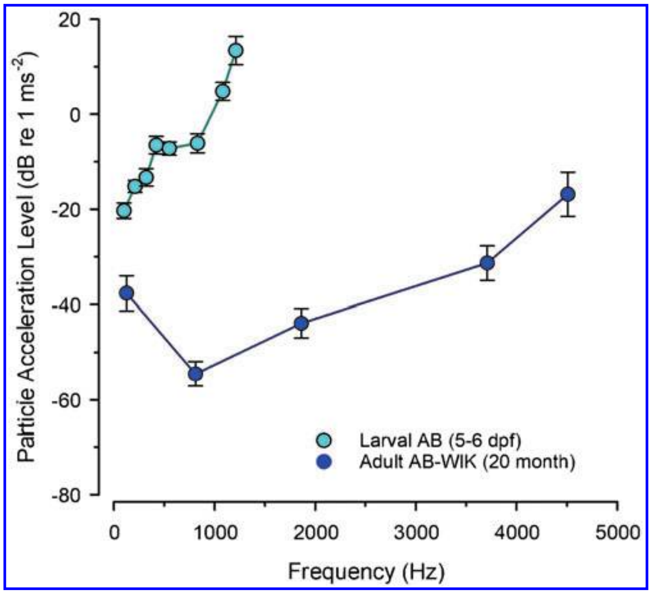

FIG. 4. Auditory threshold tuning curves for larval and adult zebrafish based on particle acceleration level (dB re: 1 ms^{-2}). The particle acceleration sensitivity was replotted for 5–6 days postfertilization AB larval zebrafish based on a behavioral prepulse inhibition assay[47] and for 20-month-old AB-WIK adult zebrafish based on AEP recordings.[52] All data are plotted as mean ± standard error. Color images are available online.

There are few data on hearing sensitivity in adult zebrafish derived from behavioral assays and particle motion sensitivity. One behavioral study of adult zebrafish by Cervi *et al.*[40] reported best hearing from 400 to 600 Hz with sound pressure thresholds of ~118 dB re: 1 μPa, which is higher than thresholds based on the AEP physiological assay. A more recent behavioral study[42] showed best hearing at 800 Hz in adult zebrafish, but with substantially lower thresholds than reported by Cervi *et al.*[40]

Best frequency sensitivity for adults based on AEP recordings has been reported to be 600–800 Hz at sound pressure thresholds ranging from 102–104 dB re: 1 μPa.[52,81] Furthermore, only one study has reported an AEP audiogram for adults based on particle acceleration with maximal sensitivity at 800 Hz and corresponding particle acceleration thresholds of −54 dB re: 1 ms^{-2}.[52]

Clearly, the paucity of such hearing studies on adults, and the variability in results, particularly for lowest sound levels detectable, clearly warrants future investigations. If nothing else, it is imperative to have a valid and reliable baseline for wild-type zebrafish, against which to compare developmental and genetic changes.

Why do zebrafish (and other otophysans) hear sounds >2 kHz?

Zebrafish, as most of the ~10,000 known species of Ostariophysans, evolved, and still live, in fresh water, inhabiting rivers, streams, and the like around the world.[88,89] The presence of the Weberian ossicles is diagnostic of Ostariophysans, and all but a few species detect sounds to over 3 kHz.[53,85] The one known exception is the marine hardhead catfish *Ariopsis felis* (family Siluridae) which only hears up to about 1 kHz, but has exceptional hearing sensitivity at low frequencies due to special adaptations in the utricle.[90]

The question is why this taxonomic group evolved higher frequency hearing. One reason may be that otophysans are likely to have evolved in relatively shallow freshwater,[88,89,91] an environment where sound propagation at lower frequencies decreases as water gets shallower.[92,93] As pointed out by Rogers and Cox,[92] in shallow water, low frequencies only propagate very short distances from a source, while higher frequencies travel far greater distances before attenuating.

The shallow water will thereby limit the propagation and detection of biologically relevant, low frequency acoustic stimuli associated with a predatory strike or event produced by a large fish predator or by a fishing bird striking the water surface with its beak in shallow water. Such predatory events are often associated with acoustic and vibrational stimuli that can elicit a characteristic startle response (C-start) that results in the fish swimming away from the acoustic source.[94]

Thus, if a fish living in such an environment is using sound to listen for predators or gleaning information from the auditory scene, there could have been strong selective pressures to detect higher frequencies to get information from greater distances.[85,93]

Anthropogenic Sound and Zebrafish

Sound levels in the laboratory environment

The natural habitats of zebrafish are rivers and streams in Southeast Asia. In an excellent and comprehensive study, Lara and Vasconcelos[6] examined the sound pressure component of natural soundscape in several streams inhabited by wild zebrafish and compared the sound pressure levels to those in five different zebrafish laboratory environments. They found that the bulk of the sound pressure energy in the wild was below 3 kHz with sound levels ranging between 96 and 126 dB (re: 1 μPa). In comparison, the sound pressure levels in the laboratory environments were primarily below 1 kHz, and sound pressure levels were, on average, 20–30 dB higher than in the field. The investigators did not examine particle motion or vibratory signals in field or laboratory.

The investigators suggest that the sound pressure levels recorded in laboratory zebrafish housing are sufficiently high to potentially mask detection of biologically important sounds. While this is quite a valid observation, it is not clear what such biologically relevant sounds might be. However, the very fact that there is a level of background sound in the laboratory tanks suggests the potential for increased stress and a myriad of other physiological responses that could harm the well-being of the animals in their housing,[6] just as increased environmental sounds can have significant physiological effects on humans and other animals.[2]

Indeed, the characterization of the ''sound world'' or soundscapes for zebrafish is critical for us to understand what zebrafish listen to in their natural environment and what are the ambient levels of the zebrafish's sound world? Zebrafish researchers are beginning to address these questions but still many questions remain. For example, what about the natural acoustic soundscapes of zebrafish in terms of particle motion and vibrational stimuli? Moreover, how might ambient vibrational information from the substrate play a role in how zebrafish perceive their environment?

While we will not review in any depth here, it is clear that an adverse sound environment has the potential for impacting both adult and larval zebrafish and their development.[8] This is not dissimilar to results on goldfish.[95] As an aside, however, while it is quite reasonable to extrapolate data from goldfish to zebrafish, care must be taken in extrapolation from data on effects of sound on other species outside of the Otophysi that do not have adaptations that result in a wide hearing bandwidth and low thresholds.

Effects of anthropogenic sound on zebrafish

Few studies have examined the potential effects of human-generated sounds on larval and adult zebrafish. One such study has examined the effects of noise exposure on the hearing sensitivity and the acoustic startle behavior of larval (5–6 dpf) zebrafish.[48] Bhandiwad *et al.*[48] showed that after 18 h of flat-spectrum noise exposure at 20 dB re: 1 ms^{-2}, larval zebrafish exhibit a temporary 10–15 dB threshold decrease (i.e., decrease in sensitivity) to startle stimuli and reduced habituation to startle-inducing stimuli. Interestingly, the noise-induced sensitization was not due to changes in overall hearing sensitivity, which suggests a complex effect of noise exposure on the auditory-evoked behaviors of larval zebrafish.

Overexposure to moderate noise levels (sound pressure levels of 112 dB re 1 μPa) in adult zebrafish is known to affect their swimming behavior by changing their group cohesion, swimming speed, and swimming height in the water column.[96] Furthermore, Shafiei Sabet *et al.*[97] showed that adult zebrafish exposed to higher anthropogenic sound levels (122 dB re 1 μPa) exhibit startle responses at the onset of sound exposure followed by a general slow-down and sometimes freezing behavior. These investigators suggested that such exposure to noise can produce anxiety-related behaviors in captive zebrafish.

Indeed, overexposure to moderate and high levels of noise can have acute effects on the behavior and well-being of adult zebrafish. However, what about long-term or chronic exposure of adult zebrafish to low-moderate levels of anthropogenic sounds in captive environments? Lara and Vasconcelos[6] reported that ambient sound pressure levels in zebrafish housing systems can vary from ~120 to 147 dB re 1 μPa due to the tank's pump, filtration, and aeration systems. The spectral energy of the fish housing systems reported[6] were up to ~22 dB above the previously reported best auditory thresholds in adults.[52,78,80,81] Such noise exposure levels in zebrafish housing systems may potentially have long-term effects on the health, behavior, and hearing of zebrafish; however, there is currently no guidance whatsoever as to what sound or vibration levels might impact the fish.[7,14,15]

The potential impact of long-term exposure to sounds at similar levels (130 and 150 dB re 1 μPa) has shown various physiological and behavioral effects in larval zebrafish, including damage to sensory cells in the saccular epithelium.[8,98] These included changes in mortality, cardiac rates, behavior, and cortisol levels in different aged larvae, suggesting that, at least for larvae, anthropogenic sounds introduced into rearing tanks could have an impact on fish as they developed.

It has also been shown that zebrafish, like other otophysans, exhibit detrimental effects of noise on fish hearing that include noise-induced auditory threshold shifts and hearing loss. Breitzler *et al.*[99] found that exposure of adult zebrafish to 24 h of white noise resulted in a temporary threshold shift (TTS) that lasted for up to 14 days when the noise was 150 dB re 1 μPa. In a different study, Han *et al.*[100] also found TTS in adult zebrafish to white noise from 200 to 1000 Hz and also to pure tones at 200 and 1000 Hz.

This study showed that there was some hair cell damage because of sound exposure. Interestingly, the greatest hair cell damage in response to 200 Hz exposure was at the caudal end of the saccular sensory epithelium, whereas most damage was at the rostral end for the 1000 Hz exposure. These results suggest that different saccular regions are most sensitive to different frequencies, a finding that was first reported in an otophysan, the goldfish, by Furukawa and Ishii.[101]

Zebrafish TTS results are further supported by work on other otophysans. Gutscher *et al.*[95] showed that overexposure to noise of 119 dB re 1 μPa produced from external tank filters could induce auditory threshold shifts up to 15–19 dB in the goldfish. Similarly, Wysocki and Ladich[102] showed that exposure to continuous white noise of 110 dB root mean square (RMS) re 1 μPa induced threshold shifts by 15–20 dB in goldfish and 4–22 dB in the Raphael catfish (*Platydoras costatus*), while exposure to white noise of 130 dB RMS re 1 μPa resulted in threshold shifts of 23–44 dB in both species.

Exposure to even louder noise levels (158–170 dB re 1 μPa) for 12–24 h can result in similar auditory threshold shifts up to 26–28 dB in goldfish and 32 dB in the vocalizing catfish (*Pimelodus pictus*).[103,104] In addition, such intense noise exposure can result in elevated levels of plasma cortisol and hearing loss that can last up to 14 days in goldfish.[104] Future studies are needed to further determine the long-term consequences of noise exposure in zebrafish and other fishes.

Finally, one recent study tries to address the environment of the zebrafish laboratory sound environment[16] and suggests the use of music to calm zebrafish. However, this study reflects a clear lack of understanding of basic issues regarding acoustics and bioacoustics. For example, the study played sounds outside of the tanks without consideration that such sounds are attenuated substantially when crossing the air-water interface and that the sound levels in the tank are likely well below the hearing thresholds of zebrafish.

Knowledge Gaps

From this review, it should be clear that we know very little about hearing and the role of sound in the lives of zebrafish. Indeed, this is not surprising since there are very substantial gaps in our understanding of all aspects of fish hearing. This was pointed out in a detailed review by Hawkins *et al.*[105] and also more recently by Popper *et al.*[106] Most of the gaps raised have not been filled, even today.

Of course, not all of the gaps noted by Hawkins *et al.*[105] are relevant to zebrafish, particularly from the perspective of the use of the species in research on genetics and development of hearing. The following suggestions, therefore, are our perspective as to the most important questions on zebrafish that would further their use in research.

(1) Basic hearing capabilities:
  - Studies are needed under acoustic conditions that allow for understanding both hearing bandwidth and sensitivity to sound pressure, particle motion, and vibration. Ideally, such studies would be performed in environments that are acoustically similar to the environments inhabited by wild-type fish. Furthermore, to understand ''hearing,'' investigations must use behavioral approaches to ''ask'' fish what they detect as opposed to studies that only measure the physiological response of the ear or regions of the brain.[36]
  - Development of ''standard procedures'' for measuring hearing in zebrafish so that data can be compared between different strains and developmental stages between laboratories. In addition, develop an acceptable base audiogram for wild-type zebrafish against which various genetic strains can be compared. This baseline will allow comparison against a ''standard'' rather than comparing suggesting differences in hearing between strains.
  - Investigations of more important aspects of hearing such as discrimination, detection of signals in the presence of a masker, and sound source localization. Such studies are needed to understand the genetics of hearing and the ear since it is possible that genetic changes will not be manifest in bandwidth or threshold, but in the more important roles of the hearing. At the same time, some of these data may be extrapolated from the extensive body of data on the goldfish.[59]
  - Developmental studies of hearing that parallel the studies on adults. These studies should also look at the development of the contributions of the swim bladder and Weberian ossicles to hearing in fishes.

(2) Effects of the laboratory environment on zebrafish:
  - What is the acoustic environment of zebrafish under different laboratory environments? Focus is needed on sound pressure, particle motion, and on substrate vibration and includes an understanding of sound and vibration levels in tanks used for housing and experiments on the fish.
  - How do different laboratory acoustic environments affect hearing during development and in adults, including the potential for temporary hearing loss.[107]
  - What is the potential impact of different acoustic environments on zebrafish behavior?
  - What is the potential impact of different acoustic environments on zebrafish physiology (e.g., endocrine responses)?

(3) Mitigation:
  - What can be done to mitigate any effects of laboratory acoustic environment on zebrafish?

Ethical Statement

No animals used in this review

Authors' Contributions

Both authors contributed equally to writing this review.

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Address correspondence to:
Arthur N. Popper, PhD
Department of Biology
University of Maryland
College Park, MD 20742
USA

E-mail: apopper@umd.edu