Revisiting Psychoacoustic Methods for the Assessment of Fish Hearing

Ashwin A. Bhandiwad and Joseph A. Sisneros

Abstract Behavioral methods have been critical in the study of auditory perception and discrimination in fishes. In this chapter, we review some of the common methods used in fish psychoacoustics. We discuss associative methods, such as operant, avoidance, and classical conditioning, and their use in constructing audiograms, measuring frequency selectivity, and auditory stream segregation. We also discuss the measurement of innate behavioral responses, such as the acoustic startle response (ASR), prepulse inhibition (PPI), and phonotaxis, and their use in the assessment of fish hearing to determine auditory thresholds and in the testing of mechanisms for sound source localization. For each psychoacoustic method, we provide examples of their use and discuss the parameters and situations where such methods can be best utilized. In the case of the ASR, we show how this method can be used to construct and compare audiograms between two species of larval fishes, the three-spined stickleback (*Gasterosteus aculeatus*) and the zebrafish (*Danio rerio*). We also discuss considerations for experimental design with respect to stimulus presentation and threshold criteria and how these techniques can be used in future studies to investigate auditory perception in fishes.

Keywords Behavior • Psychoacoustics • Conditioning • Reflex responses • Stimulus presentation

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1 Introduction

Interest in teleost audition dates back to Aristotle's observation that "fishes undoubtedly hear…For they are observed to run away from any loud noise, such as would be made by the rowing of a galley, so as to become easy of capture in their holes; for though a sound be very slight in the open air, it has a loud and alarming resonance to creatures that hear under water" (for translation of Aristotle's original text see Barnes 1984). However, the formal study of hearing in fishes began at the turn of the twentieth century after Retzius (1881) published his study of the structure of inner ears of 48 species of fishes, which spawned interests in studying the mechanisms and processes underlying fish hearing. Parker (1903) was the first to quantitatively show that these inner ears were functional and that fish possess a sense of hearing. Working with the killifish (*Fundulus heteroclitus*), Parker performed a relatively simple experiment in which he attached a viol string to an aquarium wall (Fig. 1), vibrated the string, and observed that fish responded to acoustic stimuli by moving their pectoral fins in 96 % of the trials. These movements were also observed when the lateral line nerves were cut (94 %), but rarely when the acoustic division of the VIIIth nerve was cut (18 %). Parker then replaced the viol string with a 128 Hz tuning fork and observed the same pectoral fin movements in response to acoustic stimuli, but only when the fork was both in motion and touching the aquarium wall. Since Parker's first experiments, behavioral methods have become a fundamental tool used to study the bioacoustics and hearing of fishes.

Because they are not invasive, behavioral methods are often a preferred method used to measure auditory capabilities in fishes. In some cases, behavioral methods

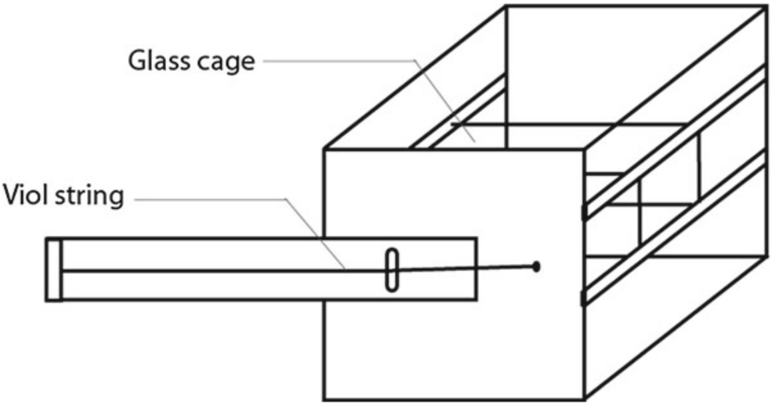

Fig. 1 Diagram of the experimental tank and testing apparatus used by Parker (1903) to test whether the killifish (*Fundulus heteroclitus*) could detect acoustic stimuli. A wooden board with an attached viol string was secured to one end of the tank. The fish was suspended in the center of a tank in a glass cage in order to stabilize it; during the experiment the viol string was plucked (F_0: 40 Hz) and the presence of a suite of behaviors was measured (most commonly pectoral fin movement) to determine whether the fish detected the acoustic stimulus. Adapted from Parker (1903)

may be the only means available to study hearing because alternative methods such as auditory electrophysiology require surgical preparation; this preparation prevents the use of many species that are sensitive and less stress tolerant to the surgery required for invasive auditory physiology experiments. Another advantage of using, noninvasive behavioral methods is that they can be used to test hearing capabilities of fish in longitudinal studies, which is useful in determining the onset and development of hearing in a given species. Finally, auditory evoked behaviors require the integration of multiple circuits and higher order auditory processing to produce a reliable and behaviorally relevant response. Thus, the use of auditory evoked behaviors provides an inherently sensitive way to assess hearing.

Why are behavioral methods important in understanding auditory function in fishes? Because hearing capability is often directly related to a behaviorally relevant function of an animal, it should follow that any stimulus that can evoke a behavioral change is a "relevant" stimulus to that animal. When considering the use of behaviorally relevant stimuli, researchers must use auditory stimulus parameters that take into account the hearing range of the species of interest. For example, in determining an audiogram for a fish species with no known hearing specializations, the use of stimulus frequencies greater than 2 kHz (roughly the upper frequency limit of sound–pressure sensitive fish) would often be superfluous, except in cases where fishes might be sensitive to ultrasound (>20 kHz). However, researchers should also be cautious to keep stimulus parameters relatively broad in order to conservatively assess the complete range of auditory capability in a given species. As Popper and Fay (1993) stated in their influential review on sound detection and processing in fish "…we could say that all objects that may produce or scatter sound simultaneously are equally 'biologically significant', in the sense that no source can be identified or localized without significant processing of the simultaneous sounds from the other sources."

In general, electrophysiology has been the most commonly used technique during the past 30 years to assess the auditory capabilities of fishes. Techniques such as single neuron recordings, auditory evoked potential (AEPs, also referred to in previous literature as the auditory brainstem response, or ABR), and microphonic potential recordings have been instrumental in understanding various auditory capabilities of fishes including temporal encoding (Fay 1978a; Fay and Coombs 1983; Bodnar and Bass 1997; Kozloski and Crawford 2000), frequency selectivity (Fay and Edds-Walton 1997; Weeg et al. 2002), auditory plasticity (Sisneros and Bass 2003; Sisneros 2009), directional sensitivity (Enger et al. 1973; Lu et al. 1996; Fay and Edds-Walton 2000; Edds-Walton and Fay 2003), and the role of inhibition in shaping frequency tuning properties and phase locking ability (Fay 1978b; Fay 1995; Kawasaki and Guo 1998; McKibben and Bass 1999; Maruska and Tricas 2009). These methods are rapid and precise compared to behavioral methods, and as a result, they have been the technique of choice to investigate the hearing abilities of fish since the early 1960s (Enger 1963; Furukawa and Ishii 1967).

Although electrophysiological methods are critical in the study of fish hearing, they do have some limitations. First, some methods, like single auditory neuron and microphonic potential recordings, are technically difficult to perform, can involve invasive surgeries, and require physical restraint of the animal. Even noninvasive methods, such as the AEP recording technique, which has been used in over 100 fish species (Ladich and Fay 2013), require the animal to be physically restrained. Unfortunately, some fish species are difficult to test using electrophysiological methods due to a reduced tolerance for restraint, surgery, or other invasive methods. Second, electrophysiological methods are often difficult to perform on small animals, especially those that are early in development and less stress-tolerant. Although microphonic potentials have been recorded from larval fish (Lu and DeSmidt 2013; Inoue et al. 2013), most other electrophysiological recording methods are technically challenging to perform on embryonic and larval fishes. This limitation reduces the ontogenetic stages and the age/size ranges that can be compared and makes developmental physiology studies more difficult to perform. Third, electrophysiological methods such as the AEP recording technique that are used to measure auditory thresholds are difficult to compare to behavioral measures of hearing and are even more difficult to interpret in the context of natural auditory driven behaviors (Ladich and Fay 2013; Sisneros et al. 2015). Microphonic potentials of the fish inner ear, for example, can only inform us of hair cell activity, but not whether this activity results in an auditory percept for these fishes. Thus, there is a gap in our understanding of the relationship between behavioral and electrophysiological thresholds; although some correlations have been described for electrophysiological and behavioral thresholds, these correlations have only been made for goldfish (*Carassius auratus*) with no single representative relation between behavioral and physiological measures of auditory sensitivity (Ladich and Fay 2013). Variation of auditory thresholds obtained by electrophysiological measures can often be related to such factors such as electrode placement, morphology of the inner ear and skull, and the threshold criteria used.

This review primarily focuses on behavioral methods that are commonly used to assess the hearing capabilities of fishes. We discuss the use of associative (conditioning) methods and reflex (innate) responses in psychoacoustic studies of fish hearing. While many variants of these methods exist, we wish to convey the general techniques used to assess hearing in fishes and highlight the principles underlying these techniques. We also discuss the benefits and limitations of various psychoacoustic methods used to assess fish hearing and emphasize the techniques that are appropriate for investigating fundamental processes related to the sense of hearing in fishes.

2 Associative Methods

2.1 Operant Conditioning

Operant conditioning methods were used to study audition in fishes soon after Parker (1903) published his findings that fish could detect acoustic stimuli. Operant conditioning (also called instrumental conditioning) is a technique by which a behavior is either enhanced (through reinforcement) or suppressed (through punishment), before and/or after a cued stimulus. This form of conditioning can either enhance or suppress voluntary behaviors; continued behavioral reinforcement eventually leads to an association of the behavior and a consequence for that behavior. A similar apparatus to Parker (1903) was used to show that minnows (*Pimepheles notatus*; McDonald 1922), mudminnows (*Umbia limi*; Westerfield 1922), and wrasses (*Crenilabrus melops*; Bull 1928) could be conditioned using an auditory stimulus, and therefore demonstrated that these fishes could detect auditory stimuli and thus possessed a sense of hearing. Operant conditioning methods were later used by von Frisch (1936) in an attempt to train minnows to localize sound for a food reward and thereby establish whether fish could localize sound sources. Von Frisch tried to modify the "feeding reflex" of European minnows using an operant conditioning paradigm wherein minnows were trained to approach one of multiple available feeding stations when they heard a loud sound (the conditioned stimulus) that was produced by an underwater horn positioned under one of the feeding stations. Von Frisch was unsuccessful in his training of the fish and eventually he (incorrectly) concluded that fish could not localize sound sources, but the use of operant conditioning methods persisted and were later successfully used in subsequent fish hearing studies. For example, Schuijf (1975) used a forced choice conditioning method to successfully show that acoustic directional sensitivity in fish was mediated by the inner ear and not the lateral line. For a more complete review of the behavioral experiments used to investigate directional hearing and sound source localization in fishes see Hawkins (1981) and Sisneros et al. (in this volume).

Operant conditioning methods using feeding (positive) reinforcement have been successfully used in the studies of vision in fish (Yager and Thorpe 1970; Allen and Fernald 1985), but these methods have not been extensively used in the study of fish hearing. One excellent use of an operant conditioning paradigm was that used by Yan and Popper (1991), who developed an automated positive reinforcement system in which fish could be trained to respond to a piezo-pressure paddle via a feeding reflex when it heard a particular sound (Fig. 2). Goldfish (*C. auratus*) were trained to strike an observation paddle to initiate the trial after which they would strike a second "report" paddle if they heard a sound. Correct responses were rewarded with food, whereas false positive responses resulted in a punishment with the lights being turned off removing any possibility of successful prey capture. During the testing phase, sounds of varying frequencies and intensities were played, and responses were recorded, but not rewarded/punished. This paradigm was used to measure audiograms in goldfish, and was later used for intensity discrimination (Yan and Popper 1993), and in measuring audiograms in the cichlid Oscar (*Astronotus ocellatus*; Yan and Popper 1992).

There are three primary reasons why operant conditioning methods are rarely used in fish hearing studies. First, operant condition methods require the fish to be unrestricted and free-swimming. Most studies use a speaker to deliver acoustic stimuli, and a freely moving fish would likely encounter different aspects of the sound field at different points within the testing arena or apparatus, which makes it

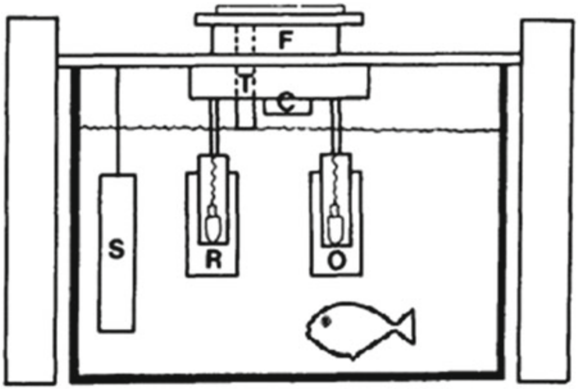

Fig. 2 Cross-section diagram of the apparatus used for operant conditioning by Yan and Popper (1992) to determine the hearing capability of the oscar (*Astronotus ocellatus*). Fish were trained to press the observation paddle (O) and attend to a sound played through an underwater speaker (S). If they perceived a sound stimulus, they were trained to press the response paddle (R). Correct responses were rewarded with a food pellet delivered from an automatic feeder (F) through a tube (T). Incorrect responses resulted in the ceiling light (C) being switched off. Modified from Yan and Popper (1992)

difficult for the researcher to control the playback stimuli. This is a major problem when trying to determine auditory thresholds to acoustic playback stimuli or in behavioral tasks that involve sound-level discrimination. Second, operant conditioning methods require significant motivation of the animal being tested; feeding assays are only effective in cases where the reinforced reward has a strong biological significance to the animal (e.g., a food reward to a food-restricted animal). This may significantly impact the training time required for learning. For example, using this method, goldfish can be trained within 1–2 days, whereas oscars (*A. ocellatus*) can take 12–14 days to train (Yan and Popper 1992). Finally, the operant conditioning paradigm requires the use of an unconditioned stimulus that is biological relevant to the animal's unconditioned response. In the case of a food reward, the unconditioned stimulus would require modification of a feeding reflex. In the Yan and Popper (1991) conditioning paradigm, this required a species specialized for strike-feeding with the striking motion being conditioned to the auditory stimulus. Since not all fish have the same mode of prey capture, this may make a similar operant conditioning paradigm difficult to use in comparative studies.

2.1.1 Avoidance Conditioning

Avoidance conditioning is a variant of the operant conditioning paradigm that has been successfully used to determine the audiograms of a number of fish species. Tavolga and Wodinsky (1963) were the first to perform an exhaustive comparative study of the auditory capacities of nine species of fishes using avoidance conditioning. In this study, the authors trained fish to cross a barrier upon hearing a tone that was quickly followed by an electric shock (Fig. 3). The unconditioned stimulus was the electric shock that provided negative reinforcement and a successful barrier crossing resulted in a cessation of that shock. After training, the fish crossed the barrier very quickly after hearing the sound stimulus to avoid the unconditioned shock. Using this paradigm, the authors were able to determine auditory thresholds at various frequencies and construct audiograms for the nine species of fish. This method, though useful, requires a very long training period, up to 30 days in the case of the cichlid *Tilapia macrocephala* (Tavolga 1974).

Avoidance conditioning has been used very effectively in studies of frequency selectivity using maskers. McCormick and Popper (1984) used avoidance conditioning with maskers to determine the auditory thresholds of elephant nose fish (*Gnathonemus petersii*), and conditioned fish to avoid a 500 Hz test tone. The authors presented the test tone in the presence of an acoustic masker that varied in frequency between 100 and 800 Hz and then were able to measure the animal's frequency tuning and its tuning sharpness or Q_{10}, the ratio of test frequency to the bandwidth 10 dB above threshold. This application of the avoidance conditioning technique demonstrated that *G. petersii* had a tuning curve with a Q_{10} similar to the filter shape in goldfish. Avoidance conditioning continues to be a promising method that can be used to study similar questions about directional hearing, frequency selectivity, and masking in future studies of fish hearing.

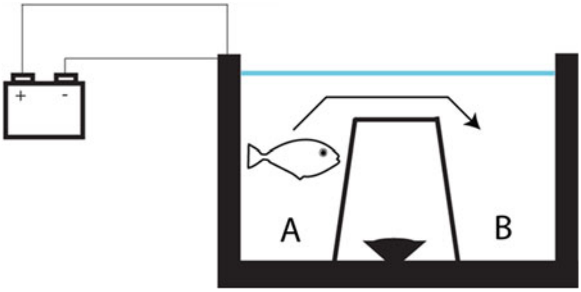

Fig. 3 Cross-section diagram of the apparatus used by Tavolga and Wodinsky (1963) to test the hearing ability of fishes using avoidance conditioning. Fish were initially placed in the left compartment of the chamber (A) separated from right compartment (B) by a raised barrier in the center of the tank. Fish were conditioned to cross from one compartment (A) into the other compartment (B) when they heard a tone that was followed quickly by an electric shock. Acoustic stimuli were played via a speaker (placed below the barrier) and the electric shock was produced by a DC battery that had terminal contacts embedded in the tank wall. Correct responses consisted of the fish crossing over the barrier into the other compartment of the tank. After conditioning, fish crossed from (A) to (B) upon hearing the sound stimulus alone

2.2 Classical Conditioning

Classical conditioning is the most commonly used technique in the study of fish hearing. Classical conditioning, also called Pavlovian or respondent conditioning, is an associative learning paradigm in which a conditioned stimulus (CS, e.g. tone) is paired with an unconditioned stimulus (US, e.g. taste of food) and an unconditioned response (UR, e.g. salivation); after repeated pairings, the CS (tone) alone can invoke the UR (salivation). This technique has been used in fish hearing studies to determine audiograms (Buerkle 1967; Fay 1969; Popper et al. 1973), frequency discrimination (Fay 1970; Chapman and Johnstone 1974), sound source discrimination (Buwalda et al. 1983; Lu et al. 1996), and for auditory scene analysis (Fay 1992, 1998). This form of conditioning has become a powerful tool because it uses an innate response to a biologically potent stimulus that does not require voluntary movement. After Bull (1928) showed that eels (*Anguilla vulgaris*) could be conditioned to vibratory stimuli using electric shock, many other studies showed that this type of conditioning was generalizable. All of these methods use a form of a reflexive response, such as a defense response (Kenyon et al. 1998) or a suppression of ventilation or cardiac activity (Fay 1969; Buwalda et al. 1983; Lu et al. 1996). In the case of ventilation suppression (measured as a suppression of mouth or opercular movements), a tone–shock paradigm is used with the unconditioned response measured as a temporary reduction in the frequency of opercular ventilation movements; this response is robust and easy to measure in most fish, especially goldfish (Otis et al. 1957; Fay 1972, 1988, 1998). During the conditioning period, the fish begins to associate the tone with a shock and in subsequent trials starts to suppress ventilatory movements in the anticipation of the shock when the tone is heard. After conditioning, the fish will suppress its ventilatory movements upon hearing the tone alone, even in the absence of a shock. The stimulus tone can be altered with respect to frequency or intensity to determine the fish's frequency selectivity or absolute hearing threshold.

In almost all species tested, classical conditioning methods have yielded the lowest auditory thresholds compared to other methods that measure auditory thresholds including auditory physiology. The lower auditory threshold measures produced by behavioral methods are, in part, likely due to higher order processing and integration of auditory information required for whole animal behaviors. However, classical conditioning methods also have some potential limitations. Although training time is relatively short compared to the operant and avoidance conditioning paradigms, initial classical conditioning can still take ~40–50 trials for some fish species (Fay 2009). Furthermore, because the unconditioned stimulus is often an electrical shock, this might preclude the use of particular species that are sensitive to stressors, like certain cichlid species (Tavolga 1974; Allen and Fernald 1985). Classical conditioning also requires constant retraining during the testing phase. Finally, animals can only be conditioned a limited number of times, which then reduces the number of stimulus parameters that can be investigated, and subsequent stimulus parameter training requires additional subjects and/or longer test times.

Perhaps the most powerful use of classical conditioning methods has been in the study of auditory scene analysis in goldfish (*C. auratus*), in particular auditory stream segregation. Utilizing a ventilation suppression reflex, Fay (1998) trained goldfish to suppress ventilation when presented with a complex acoustic stimulus that was paired with a mild electric shock. During conditioning, the goldfish learned to anticipate the shock paired with the acoustic stimulus by suppressing their ventilatory movements, functionally measured as the rate of mouth openings (Otis et al. 1957). The pairing of the conditioned acoustic stimulus and an unconditioned shock stimulus led to an association of the conditioned stimulus with an unconditioned response, the involuntary suppression of ventilatory movements of the goldfish, within 40 trials. Fish were placed in the training apparatus (Fig. 4) and conditioned to suppress their ventilation to a complex stimulus of pulse trains of two separable frequencies presented at two discernibly different rates. Each pulse train was independently played back and the ventilation rate was measured. Ventilation was suppressed when each pulse train was presented at the same frequency and pulse period as it was during the training phase. Furthermore, fish suppressed ventilation to single components of the complex stimulus, indicating that the two components of the complex conditioning stimulus were analyzed independently, as if they were from two separable sources and suggested that the fish were capable of auditory

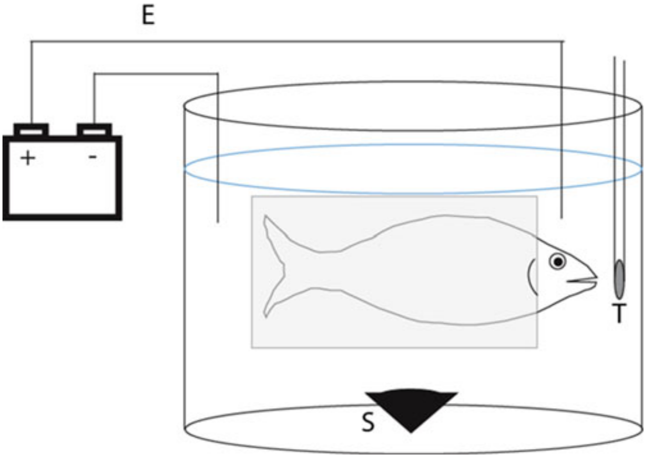

Fig. 4 Diagram of the apparatus described by Fay (1995) to measure frequency discrimination thresholds in goldfish (*Carassius auratus*) using classical conditioning. Fish were restrained in a cloth bag with their mouth and gills exposed and positioned 2 cm below the surface of the water in the center of the tank. Sound was produced by an underwater speaker (S) on the bottom of the tank and paired with a shock delivered through electrical leads positioned near the head and tail of the animal (E). Changes in ventilatory movements of the fish were measured using a thermistor (T), which registered a change in temperature when water was moved past the sensor

stream segregation. Similar conditioning protocols using a complex stimulus and analysis of the components have also been used to study pitch perception in goldfish (Fay 1995).

3 Use of Innate Behavioral Responses in Psychoacoustic Studies of Fish Hearing

Many psychoacoustic studies of fish hearing have taken advantage of using innate behavioral responses to assess the auditory capabilities of fishes. Innate behavioral responses are regarded as genetically programmed responses to external stimuli. These stereotyped innate responses can often be used to probe an animal's perceptual world or "umwelt" and characterize an animal's sensory capabilities. Simple reflex responses and the more coordinated movement of reflexive locomotion are two broad categories of innate behavioral responses that have been successfully used to assess hearing in fishes.

3.1 Reflex Responses

Reflexes are involuntary movements in response to a sensory stimulus. Innate reflex responses are often used in psychoacoustic studies of hearing because they are stereotyped, repeatable and do not require conditional behavioral training in order to evoke them. Furthermore, these conserved innate responses serve a behaviorally relevant function, and are therefore robust and can be elicited easily. This allows for very fast and efficient measures of auditory capability.

3.1.1 Acoustic Startle Response

The most common reflex response described across multiple species is the auditory startle response (ASR). Although variants of the ASR have been described since Aristotle, Wilson (1959) was the first to show that the "tail-flip" startle response in fish was driven by Mauthner cells (M-cells), giant neurons found in the fourth segment of the reticulospinal formation of the hindbrain (R4). This stereotyped startle response is described in mammals (Parham and Willott 1988), anurans (Cioni et al. 1989), and urodeles (Marini et al. 1991). In fish, as well as anurans and urodeles, the M-cell circuitry is relatively simple (Zottoli and Faber 2000). Briefly, afferent neurons of the VIIIth nerve synapse onto the lateral dendrite of the M-cell. The M-cells cross-over and innervate the motor neurons on the contralateral side of the fish. When activated, an M-cell fires a single spike that activates all the motor neurons on the contralateral side of the fish, causing the fish to bend and accelerate away from the direction of the stimulus. The startle-escape response was later formalized as the

"C-start" response, so called because of the conformation of the body to from a "C" at the apex of the response when all the muscles of that side are contracted (Kimmel et al. 1974). The authors used an experimental paradigm in which they dropped a metal ball into the tank containing zebrafish (*Danio rerio*) from varying heights (a greater height would correspond to a larger intensity) and recorded the startle behavior of the fish using a video camera. Using this, they were able to show that the startle response is present in both larval and adult zebrafish, it could be elicited with auditory or tactile stimuli, and it could be described using a psychometric function. The latter finding is important because it shows that the M-cells have intensity-dependent firing probability (Neumeister et al. 2008). This property allows for model fitting of this response to a psychometric function, and allows for interpolation of threshold from discrete responses.

The ASR has been used most prominently in studies of the development of hearing in larval zebrafish (Kimmel et al. 1980; Zeddies and Fay 2005), but in most other studies it has served only as a test to determine whether or not the auditory system is functional. Zeddies and Fay (2005) were the first to use acoustic startle-like responses to construct audiograms in larval zebrafish. In this study, the authors stimulated larval zebrafish aged 5 days post-fertilization (dpf) using a one-dimensional shaker (Fig. 5) and measured responses using a standard video camera. Using the shaker, the authors were able to provide pure-tone particle motion stimuli and measure the acoustically evoked behavioral responses (AEBR) to the particle motion stimuli. The AEBRs were defined as any acoustically mediated event that resulted in the movement of the fish and a difference in pixel distribution after frame subtraction in two consecutive video frames; if the number of differing pixels was two standard deviations above pixel differences during a no-stimulus trial, the fish

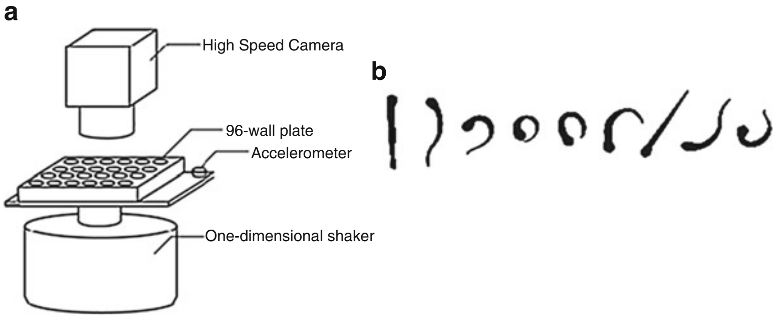

Fig. 5 (**a**) Apparatus used to measure auditory thresholds in larval zebrafish (*Danio rerio*) and sticklebacks (*Gasterosteus aculeatus*) using acoustic startle response and prepulse inhibition assays, as used in Bhandiwad et al. (2013). A 96-well plate was mounted on an acrylic plate attached to a one-dimensional shaker. Particle motion stimuli were delivered through the shaker to larvae placed in individual wells of the 96-well plate. An accelerometer measured stimulus level and the resulting ASRs were recorded using a high-speed video camera at 1000 frames per second. (**b**) Diagram of representative Mauthner-cell mediated ASR, digitized from data. Successive frames are 4 ms apart. Note that the characteristic "C" shape of the startle response can be seen in panel 4

was determined to have performed an AEBR. AEBRs served as proxies for the ASR when using a non-high-speed camera to record the responses because the ASR occurs on the timescale of ~5–10 ms and standard (30 frames per second) cameras have a temporal resolution of ~33 ms. The authors were able to use this technique to show group-level absolute thresholds for larval zebrafish in early development (from 5 to 26 days dpf).

We have used the ASR to determine how auditory thresholds differ during early development in two distantly related species of fish. Zebrafish (*D. rerio*, order Cypriniformes) have specialized accessory structures for hearing, whereas the three-spined sticklebacks (*Gasterosteus aculeatus*, order Gasterosteiformes) are non-specialists that lack specialized accessory hearing structures. Fishes with hearing specializations generally have greater auditory sensitivity and frequency bandwidth detection than fish species that do not (Popper and Fay 2011). However, whether these differences are present before the development of hearing specializations is not known. The ASR is a useful method to probe an animal's auditory system in terms of auditory sensitivity and frequency detection capability at hearing onset because it is rapid and can be retested over time.

Using the apparatus described by Bhandiwad et al. (2013), we presented pure tone stimuli to larval three-spined sticklebacks and zebrafish. We first used the kinematics of the startle response to determine whether startle responses of larval sticklebacks were similar to those found in larval zebrafish (Fig. 6). Sticklebacks that were tested consisted of Japan Pacific, Paxton Lake Limnetic, and Paxton Lake Benthic species and were chosen due to their morphological differences in lateral line and therefore represented the diversity of stickleback species (Wark and Peichel 2010). We show that the kinematics of the startle response in both zebrafish and sticklebacks are essentially the same, although the maximal bend angle is much smaller in sticklebacks (Fig. 7). Because both species' startle responses are on the same timescale, we posited that they are both mediated by the M-cell pathway.

Next, we tested groups of larval stickleback fish daily from the day they became free swimming until they exhibited the ASR. In zebrafish, the onset of the ASR to pure tones is 5 dpf (Zeddies and Fay 2005; Bhandiwad et al. 2013), but ASRs can be evoked earlier by an acoustic broadband stimulus at 4 dpf (Fig. 8). In contrast, sticklebacks begin to exhibit ASRs to broadband acoustic stimuli at 9 days post hatch (dph) and showed frequency-specific differences in ASR onset. Post-hatch days were more accurate measures of development in sticklebacks due to the long duration and variability of the embryonic period; in comparison, all zebrafish larvae hatched at 3 dpf. Low frequency stimuli (e.g., 45 Hz) evoked ASRs in sticklebacks at 12 ± 2 dph (mean ± SD), but higher frequency stimuli (e.g., 90 Hz) did not evoke ASRs until 15 ± 1 dph. These data suggest that there may be an ontogenetic change in frequency sensitivity of larval sticklebacks during early development from 9 to 16 dph. ASRs evoked by either pure tones or broadband stimuli were all-or-none response in both zebrafish and sticklebacks.

Auditory thresholds on the day of hearing onset also differed between larval zebrafish and sticklebacks. Because there were frequency-dependent differences in ASR onset, sticklebacks were tested between 24 and 31 dph. No significant differ-

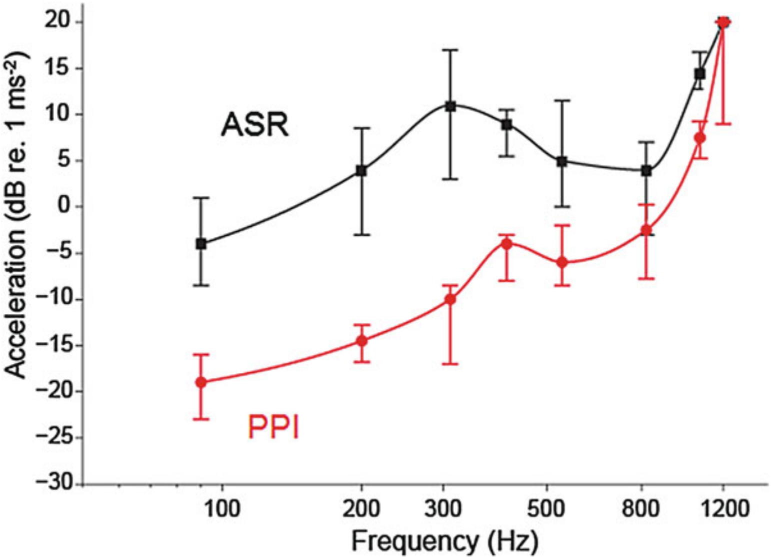

Fig. 6 Comparison of ASR (*black squares*) and PPI (*red circles*) audiograms in 5 day post-fertilization zebrafish (*Danio rerio*) to particle motion stimuli. Threshold is defined as a 5 % probability of startle for ASR assay or 5 % inhibition of startle from the paired catch trials in the PPI assay. Both studies were performed on the same population of fish ($n = 10$ plates of 24 fish for both assays). Data presented as median ± 1 quartile and lower numbers indicate higher sensitivities. The results show that auditory sensitivity determined using the PPI method are much lower than ASR thresholds

ences were observed in the thresholds across 24–31 dph; therefore, the thresholds were pooled for all sticklebacks tested. Zebrafish were tested at 5 dpf. At the onset of the auditory startle response, sticklebacks had much higher startle thresholds (up to 25 dB at 30 Hz) than larval zebrafish (Fig. 9). Furthermore, zebrafish exhibited ASRs to acoustic frequencies as high as 320 Hz, whereas sticklebacks only showed ASR to frequencies ≤180 Hz (Fig. 9).

Reflex responses like the ASR also have certain limitations. The first, and most prominent, is the habituation of the reflex response to repeated auditory stimuli. Habituation is a non-associative learning process by which an organism decreases the strength of its response after repeated presentations. Although the effects of habituation can be reduced by increasing the inter-stimulus interval between stimulus presentations (Zeddies and Fay 2005), there is an upper limit of approximately 15 stimulus presentations before the response rate is degraded (Roberts et al. 2011; Bhandiwad et al. 2013). Another potential problem with the ASR assay is that a very intense acoustic stimulus is required to facilitate the startle response. The threshold for the ASR is very high because it is often evoked by a predatory attack and requires a large energetic output for the response. Thus, repeated presentations of high intensity stimuli can often lead to habituation very quickly. Furthermore, the sound levels necessary to evoke the ASR are significantly higher than auditory

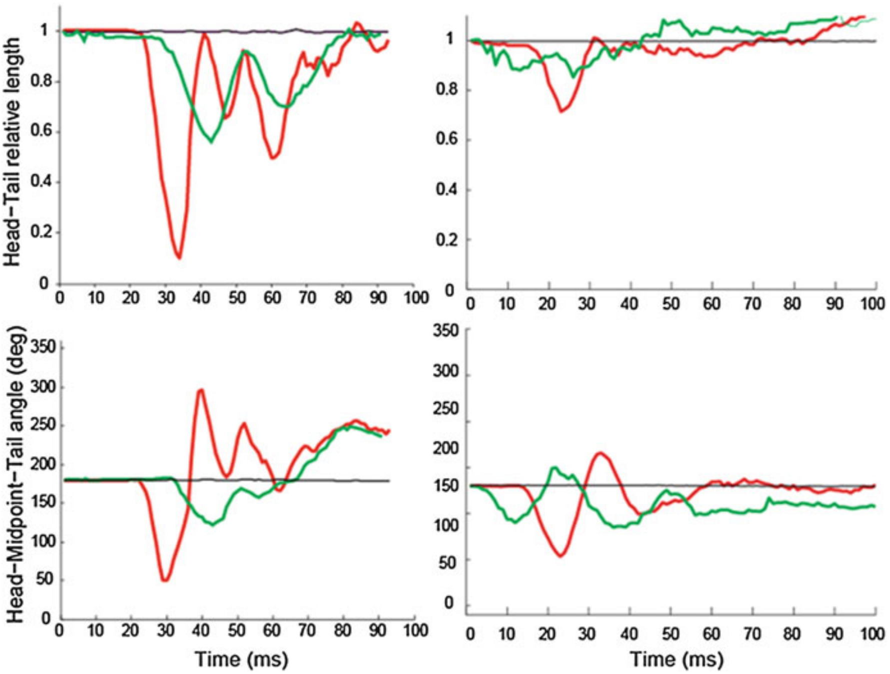

Fig. 7 Comparison of representative kinematics of the ASR as measured by the head-to-tail Euclidean distance (*top*) and the head-midpoint-tail angle (*bottom*) in 5- to 7-day-old zebrafish larvae (*Danio rerio*; *left*) and 17- to 27-day-old stickleback larvae (*Gasterosteus aculeatus*; *right*). M-cell based acoustic startle responses are uniquely identifiable (*red trace*) when compared to non-startle motion (*green trace*) and non-response (*black trace*) in both species analyzed. In all four panels, onset of startle response is highly correlated ($r = 0.78$) and is characterized by a large, rapid bend in one direction, followed by a series of refractory bends. Though the magnitude of the bends are different between zebrafish and stickleback larvae, the time course and magnitude relative to non-startle bends is conserved

thresholds obtained from electrophysiological measures, such as microphonic potentials or AEPs, suggesting that it greatly underestimates the hearing ability of fishes. Finally, although startle responses can occur with other reticulospinal neural networks, an M-cell pathway is necessary for the "fast-startle" responses currently described. Therefore, the ASR can only be reliably tested in fish species that exhibit the characteristic fast "C-start" startle response mediated by the M-cell pathway.

3.1.2 Prepulse Inhibition

One variant of the ASR paradigm is the use of reflex suppression in order to determine auditory sensitivity. Reflex suppression has been used to determine sensitivity of sensory systems since the early twentieth century when Yerkes (1903) used it to

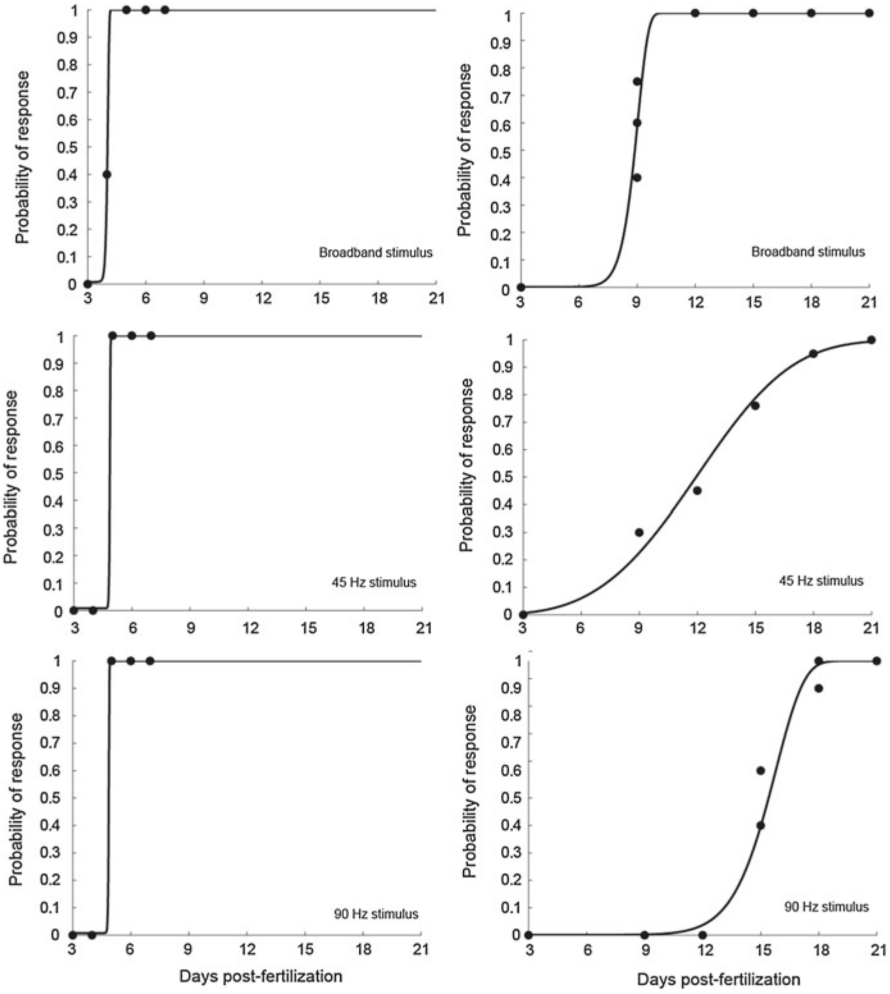

Fig. 8 Ontogeny of the startle response for larval zebrafish (*Danio rerio*; *N*=24; *left*) and larval sticklebacks (*Gasterosteus aculeatus*; *N*=11; *right*). The probability of observing a startle response for a cohort of fish was tested using a repeated measures design. Fish were tested daily after hatching for the presence of the ASR to broadband acoustic stimuli (*top*) and pure tone stimuli of 45 Hz (*middle*), and 90 Hz (*bottom*). Zebrafish had a rapid onset of ASR at ~5 dpf. However, sticklebacks did not show the ASR until 8–13 dpf with frequency-specific differences in ASR onset

study the effect of touch on tactile reflexes. Reflex suppression was later applied to study the sensitivity of the mammalian auditory system by Russo (1979). Conditioning paradigms in combination with the suppression of ventilation and cardiac reflexes have also been successfully used in fishes to determine auditory sensitivity. Recently the suppression of the ASR using a prepulse inhibition (PPI) paradigm was shown to yield lower auditory thresholds than the ASR assay alone (Bhandiwad et al. 2013; Fig. 6).

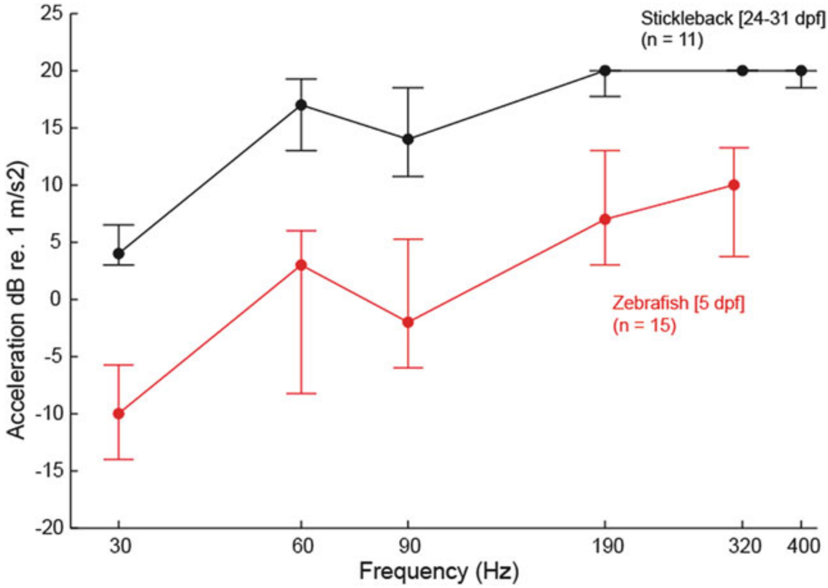

Fig. 9 ASR thresholds for 5-day-old zebrafish (*Danio rerio*; $N = 8$ groups of 24 fish) and 24 to 31-day-old sticklebacks ($N = 11$ groups of 9 fish) to particle motion stimuli. Thresholds were defined as a 5 % probability of startle. *Note*: data are presented as median ± 1 quartile, and lower threshold values indicate higher sensitivity. At all frequencies tested, larval zebrafish have significantly greater sensitivity to particle motion stimuli than larval sticklebacks, with the greatest difference at low frequencies (~16 dB difference at 30 Hz)

PPI is a phenomenon by which the response evoked by a high intensity stimulus can be suppressed by the prior presentation of a lower intensity stimulus (the prepulse). In the case of inhibiting the ASR, the prepulse can be operationalized as a low intensity tone presented prior to a startle-inducing tone, which then acts to reduce the probability of the ASR. PPI has been used to study auditory sensitivity in rodents (Willott et al. 1994; McCaughran et al. 1999), but only recently it has been use to characterize the sensitivity of the teleost auditory system. The circuitry for PPI in teleosts has also been well studied (Neumeister et al. 2008; Weiss et al. 2006; Korn and Faber 2005; Burgess and Granato 2007; Kohashi et al. 2012). Briefly, the M-cells are inhibited not only by the contralateral M-cells but also by a set of neurons near the body and axon hillock of the M-cells. The most prominent of these is the passive hyperpolarization (PHP) cell, which also receives input from the VIIIth nerve afferents and hyperpolarizes the axon hillock of the M-cell, leading to a suppression of firing probability in the M-cell (Medan and Preuss 2014). This suppression happens at stimulus intensities too low to activate the M-cell, and is presumably a mechanism that prevents unnecessary firing of the M-cell.

The use of PPI to determine auditory thresholds has recently been used in larval zebrafish (Bhandiwad et al. 2013). In this study, the authors determined that an acoustic stimulus of 820 Hz at 20 dB re 1 m/s^2 was a reliable startle stimulus and sufficient to produce an 80–90 % startle response probability for a plate of 24 larval zebrafish mounted on a shaker system (Fig. 5). After determining the baseline startle response probability, a set of acoustic stimuli ranging from 90 to 1200 Hz at sub-startle threshold levels were used as prepulse stimuli. Startle response probabilities were measured, and the change in probability of evoking the ASR was quantified. Auditory threshold was determined as the prepulse sound level that effectively reduced the ASR probability by 5 % from the baseline response probability. Using this method, the authors were able to show that auditory thresholds in 5 dpf zebrafish were 10–15 dB (re. 1 m/s^2) lower than was previously shown by just using an ASR assay (Fig. 6).

The PPI assay has similar limitations to ASR assays. Habituation to PPI can be reduced, but it still imposes an upper limit on the number of stimulus presentations that can be effectively performed. The use of PPI also requires the presence of the M-cell circuitry and the associated PHP cells, which are known to occur in zebrafish and goldfish (Neumeister et al. 2008; Medan and Preuss 2011). Future work in this field should investigate whether the PPI of the startle response can be implemented to study of auditory function in species that lack Mauthner cells.

PPI is potentially a powerful tool to study ontogenetic changes in auditory sensitivity, particularly in larval fishes. In this context, electrophysiological methods can be too invasive, conditioning methods can be too time-consuming, and ASR assays are not sensitive enough to study the auditory capabilities of larval fishes. PPI assays are sensitive enough to determine changes in auditory sensitivity of the animal during development and the same animals can be tested again at different stages of development. Furthermore, the acquired threshold estimates can be just as or more sensitive than those derived from electrophysiological methods. Studies using the Mongolian gerbil (*Meriones unguiculatus*) have shown that auditory thresholds determined by PPI are equivalent to thresholds determined using an ABR approach (Walter et al. 2012). Future work on PPI will inform us whether this finding can be generalized in larval and adult teleosts.

3.2 Phonotaxis

Phonotaxis is a reflexive locomotor response toward (positive) or away from (negative) an external auditory stimulus. This innate response is associated with a biologically relevant function, and as a result can only be elicited with a specific stimulus. Stimuli that “release” phonotaxis can be biogenic (in the case of conspecific advertisement calls) or produced by physical features of the environment (e.g., reef sounds that attract pelagic fish larvae; Pijanowski et al. 2011). Such acoustic “sign” stimuli are known to have unique spectral and temporal features (Amorim 2006) that are strongly stereotyped, complex, and behaviorally relevant to the animal. Many fish species have inherent behaviors that are evoked by specific acoustic stimuli including acoustic signals used for communication. Courtship and agonistic/distress sounds have been recorded in mormyrid fish (*Gnathonemus petersii*; Rigley and Marshall 1973), cod (*Gadus morhua*; Hawkins and Chapman 1966), toadfishes (family Batrachoididae; Gray and Winn 1961; Cohen and Winn 1967), searobins (*Prionotus carolinus*; Fish 1954), cichlids (family Cichlidae; Lobel 2001), damselfishes (*Chromis viridis*; Amorim 1996), gobies (family Gobiidae; Fish and Mowbray 1970), and catfishes (families Pimelodidae, Mochokidae, and Doradidae; Ladich 1997). Behaviors that are evoked by particular acoustic signals can be in the context of aggression (Myrberg 1981; Ladich 1997) or affiliative behavior (Fine 1978; Brantley and Bass 1994). These characterized behaviors to specific stimuli can be used to measure properties of the auditory system for these species. For example, behavioral studies of female midshipman fish (*Porichthys notatus*) have used the advertisement call of males to investigate the salient acoustic cues (e.g., pressure and particle motion) used in sound source localization by females (Zeddies et al. 2010, 2012). In these studies, the authors played an acoustic stimulus similar to the fundamental frequency of the male midshipman's advertisement call to females released approximately 100 cm from the sound source and then tracked the females' paths to the source. The authors showed that midshipman use particle motion cues to localize sound sources. Using this method, the same research group was able to determine in subsequent studies (Coffin et al. 2014) that pressure reception via the swim bladder is likely important for sound source localization but the lateral line system may not be required for localizing sound sources.

Because positive phonotaxis is robust in gravid female midshipman, the use of this behavior has become a powerful tool to understand how fish localize simple and complex sound sources. However, these phonotaxis behaviors are often diverse and species-specific and therefore may not be useful for all fish species.

4 Experimental Design Considerations for Psychoacoustics Experiments

The experimental design and the appropriate use of behavioral methods are important considerations in the study of fish audition. Conditioning and PPI are two of the most sensitive behavioral methods that have been used to investigate fish hearing, but both of these methods come with certain advantages and disadvantages as mentioned previously. Conditioning methods have been very effective in determining absolute hearing thresholds and frequency discrimination in a number of fish species. Both conditioning and PPI are useful in studies of noise exposure and comparative hearing because they are precise and such mechanisms are conserved across taxa. However, one must also consider how acoustic stimuli are presented during behavioral experiments. Issues of tank acoustics, sound generation, and the characterization of pressure/particle motion stimuli have been discussed elsewhere (Popper and Fay 1973, 1993), but how acoustic stimuli are presented has been and continues to be a very important consideration (reviewed in Hawkins 1981).

4.1 Stimulus Presentation Considerations

The stimulus presentation protocol is an important experimental design consideration in determining the appropriate techniques to be used to assess fish hearing. The number of stimulus presentations required to accurately determine thresholds or positive responses differs between stimulus presentation methods. Unlike electrophysiological methods such as the AEP recording technique, which can show changes in response amplitude or rate, behavioral methods often have binomial outcomes (response or no response). Therefore, the appropriate stimulus presentation method used will be important in order to minimize the number of trials required to accurately estimate the parameters of interest (e.g., auditory threshold). The three major classes of stimulus presentation methods are the method of constant stimuli, the method of limits, and adaptive procedures (Gescheider 1997). Although the theory behind these techniques is beyond the scope of this chapter, we invite the reader to consider the underlying assumptions of these procedures before implementation (Jesteadt 1980; Fay 1988).

4.2 Method of Constant Stimuli

The oldest and most common presentation paradigm currently used in fish hearing studies is the method of constant stimuli, characterized by a randomized set of parameters (in the case of absolute thresholds, these would be sound level and frequency). This method helps span the entire range of testable parameters and the full range of the psychometric function required to estimate auditory threshold, but unfortunately this method often requires a large number of stimulus presentations. In the larval zebrafish ASR and PPI experiments, the method of constant stimuli has been used to explore the entire frequency detection bandwidth (Zeddies and Fay 2005; Bhandiwad et al. 2013). This method works because the large number of fish (replicates) being tested at one time reduces the number of total trials required to determine the probability of an evoked response, but requires a relatively large number of individuals. This method has also been used in studies of frequency discrimination (Fay 1992). For example, fish conditioned to one frequency can be given a randomized frequency from a set of test frequencies and the probability of observing the unconditioned response can be modeled using a psychometric function.

4.3 Method of Limits

The method of limits is a paradigm by which stimuli are presented in an ascending or descending order until the behavior of interest is either present or absent, respectively. This procedure is repeated multiple times and the derived thresholds are averaged to estimate the true absolute threshold. The method of limits has been used to assess fish hearing in psychoacoustic studies that use cardiac conditioning (Fay 1970), avoidance conditioning (Tavolga and Wodinsky 1963), and operant conditioning (Casper et al. 2003). The advantage of using this stimulus presentation method over the method of constant stimuli is that only a subset of stimuli is required to determine threshold. However, in psychoacoustic studies using the ASR where habituation is an issue, the method of limits is problematic. In the descending method of limits, repeated presentations of the same stimulus can lead to habituation with an earlier than expected cessation of response, which can lead to an incorrect estimation of auditory threshold.

4.4 Adaptive Procedures

Adaptive procedures are experimental stimulus presentation paradigms where the stimulus parameter presented is dependent on the response of the animal being tested to the previous stimulus. Unlike the methods of limits and constant stimuli, adaptive procedures allow for testing of a range of parameters (e.g., frequency and sound level) that has not previously been specified. For example, in the case of absolute threshold determination, the experimenter can begin the testing procedure at any sound level and converge onto a threshold. Adaptive procedures are also robust and less susceptible to sequential errors that occur with the method of constant stimuli or the method of limits (Woodworth and Schlosberg 1972). Sequential errors, or errors of perseverance, are errors committed by over-responding to a sequence of stimuli. For example, using the descending method of limits may result in some false positive sub-threshold responses simply because of repeated responses to previous supra-threshold stimuli.

4.4.1 Staircase Method

The staircase method has been a stimulus presentation method in use in fish studies since the 1960s (e.g., see Tavolga and Wodinsky 1963), but is used less often than the methods of limits and constant stimuli. The staircase method was developed for audiometric testing in humans (Békésy 1947) and has been adapted for use with fish to determine auditory thresholds (Popper 1972), frequency discrimination (Jacobs and Tavolga 1968), and auditory masking (Popper and Clarke 1979).

The procedure begins with a presentation of a high intensity stimulus, similar to the descending method of limits. The stimulus intensity is lowered until the evoked behavioral response ceases, at which point the stimulus intensity reverts to the previous stimulus intensity presented (reversal of staircase). Staircases are reversed each time there is a shift from a series of evoked responses to a non-response. There are multiple variants of the staircase procedure that determine when to reverse stimuli. These follow an "*N* up, one down" framework, where *N* is the number of correct responses in a row which makes the stimulus less detectable and the one non-response makes the stimulus more detectable. It is important to determine the most appropriate framework with respect to the number of presented trials (fixed or variable), threshold characterization (discussed later in this chapter), and psychometric model used to estimate threshold (García-Pérez 1998). The staircase methods can be a very useful tool in behavioral methods in cases where trial number is restricted because the adaptive procedure mainly samples at or near threshold. This allows for the greatest confidence around the threshold estimate and reduces the number of trials by down sampling at the tails of the psychometric function. The staircase method is only effective for binomial response choices and is problematic in use for multinomial responses (for example, in operant feeding assays with multiple locations). Furthermore, real-time feedback and analysis about behavioral outputs are required for adaptive tracking. That is, experimenters are required to analyze whether the response outcome is positive or negative in the time between stimulus presentations.

4.4.2 Other Adaptive Procedures

Other adaptive stimulus presentation procedures like QUEST (Watson and Pelli 1983) and ML-PEST (Harvey 1996) can also be used in behavioral testing (as reviewed by Treutwein 1995). Unlike the previously discussed methods, these procedures rely on a Bayesian framework and use an estimation of threshold (from electrophysiological data, for example) as a prior probability of stimulus detection and weight stimulus presentations around the previously estimated threshold. These adaptive procedures could be useful in behavioral studies with fish species that have low evoked response rates to acoustic stimuli.

4.5 Threshold Criteria

Auditory threshold criteria can vary widely across behavioral studies and are an important consideration when comparing thresholds between different species or conditions (Hawkins 1981). Because there is a continuous distribution of response probability, accurate characterization of a threshold is important to understanding the auditory sensitivity and capability of animals. However, because we do not fully understand the mechanisms underlying how the probability of auditory evoked responses changes across animals, auditory thresholds determined using different criteria are very difficult to compare within and across species.

Auditory thresholds obtained using the method of constant stimuli often have the greatest variability and are least accurate in threshold characterization (Fay 1974; Lu et al. 1996; Yan and Popper 1993; Zeddies and Fay 2005). Thresholds obtained by this method are often defined as a 0.5 probability of response, a positive response greater than three standard deviations above the mean expected response probability in the absence of a stimulus (Zeddies and Fay 2005), or as a probability of response that results in a repeatable non-zero probability of response (Bhandiwad et al. 2013).

Auditory threshold criteria used with the method of limits are more well defined than threshold criteria for the method of constant stimuli. Because the method of limits uses multiple stimulus presentation trials to sequentially reduce stimulus parameters, each trial ends at threshold estimation. By definition, the threshold is described as the point at which the animal changes its pattern of response to the delivered stimulus. The estimated threshold for the subject population is determined by averaging the thresholds of multiple stimulus presentation sequences. The only free parameter between experimenters is whether the ascending method of limits (each stimulus step increases the intensity level until the animal responds once) or the descending method (each stimulus step decreases intensity level until the animal ceases to respond) is used. Although we assume thresholds obtained using these methods should be identical, there is no a priori reason that establishes equivalency between these two methods.

Auditory threshold criteria used with the staircase method are more well defined than the stimulus presentation parameters discussed above. The adaptive quality of stimulus presentation procedure allows for a greater sampling at or near threshold, which can be mathematically determined. Earlier procedures defined threshold similar to the method of limits, where the threshold was estimated from the averaged samples taken at the “reversal,” i.e., the sound level where the increasing or decreasing stimulus level was reversed to estimate threshold (Jesteadt 1980). However, this procedure is no longer used as the best measure for threshold determination (García-Pérez 1998). The total number of reversals, however, is still used as a stopping condition. Threshold for an “*N*-down, one up” protocol is defined as the probability of getting *N* trials correct by chance. For example, in a protocol where two “correct” answers result in the stimulus parameter becoming more difficult; threshold is defined as the point at which the probability of getting two correct answers in a row is 0.5. Threshold is therefore the square root of 0.5, or 0.71 (71 % correct). In the case of a three down, one up protocol, threshold is defined as the cube root of 0.5, or 79 % correct. In the mathematical limit (i.e. as the number of presentations approach infinity), threshold can be conceptualized as the point at which the adaptive procedure is equally likely to increase or decrease after each trial.

5 Conclusion and Future directions

Behavioral measures of hearing remain the best methods to investigate the perceptual hearing ability of an organism (Ladich and Fay 2013). Conditioning methods in particular are powerful tools and should continue to be used in studies of fish hearing. However, we suggest that more studies should utilize innate behavioral responses where appropriate, particularly the use of PPI assays to determine auditory sensitivity. Although this method has been used for larval fish, it can easily be adapted for adult fish and juveniles, allowing for the better understanding of how auditory capabilities change during ontogeny. In addition, the PPI assay is also potentially useful in comparative hearing studies that examine closely related species and species that possess hearing specializations. We suggest that future researchers explore the use of psychoacoustic methods to assess fish hearing and consider the following areas of research mentioned briefly below.

1. In order to maximize the efficiency and accuracy of fish psychoacoustic studies, we suggest researchers should carefully consider the most appropriate associative (conditioning) method and stimulus presentation method available in order to assess the hearing capability of a given fish species. We also highly recommend the use of adaptive stimulus presentation methods, such as the staircase method, whenever possible to determine auditory threshold estimations, in order to minimize the number of stimulus presentations and reduce habituation of the measured behavioral response.
2. Future psychoacoustic fish studies should incorporate the latest automated methods to measure and analyze the movements/responses of animals. Video-based automation and tracking have been utilized to measure long-term locomotor behavior in rodents (Noldus et al. 2001), *Drosophila* (Branson et al. 2009), and larval zebrafish (Fontaine et al. 2008). Application of these tracking methods and their use with behavioral psychoacoustic paradigms to assess fish hearing can provide rapid data collection, analysis, and allow for more dynamic behavioral experiments that may provide a better understanding of auditory capabilities and perception in fishes.
3. Our psychoacoustic data from larval *D. rerio* and *G. aculeatus* suggest that auditory sensitivity is different across taxa, even before the development of accessory auditory structures in species that are "hearing specialists." The use of behavioral methods, particularly PPI assays can be useful in understanding how development of the auditory system and perceptual ability differ within and across species. Many species of larval fish use sound as a settlement cue (Simpson et al. 2004), but the hearing ability of such species has not been well characterized; the PPI assay could be a potential tool used to determine which pelagic larval fish are capable of hearing abiotic and biotic sound cues and provide a way to characterize the auditory sensitivity of various pelagic larval fishes.
4. Behavioral methods can inform us about auditory perception in fish species that are too delicate to investigate by other methods such as electrophysiology. In addition, psychoacoustic methods can be applied and used in longitudinal studies to investigate the effects of seasonal differences in auditory perception, discrimination of relevant vs. non-relevant stimuli, and auditory stream segregation. Longitudinal studies can also be used to assess auditory development and sensitivity within the same individuals using the same technique.
5. A prominent question in fish hearing is the effect of anthropogenic sound on fishes. Anthropogenic sounds can act as maskers, which may change the perceptual environment of fishes, or can be loud enough to have profound short- and long-term effects on the auditory systems such as causing short-term temporary

threshold shifts. The extent to which these processes affect changes in auditory perception in fishes is not known; behavioral methods can be used to investigate such short- and long-term effects.

6. Top-down auditory attention effect on hearing (defined as an endogenous and selective concentration on one stimulus) in fishes remains largely unexplored. How does attention affect the perception of sound in fishes? Although many species attend to conspecific vocalizations, a generalized framework for auditory attention and mechanisms underlying it have not been investigated.

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