Auditory sensitivity of sergeant major damselfish *Abudeqduf saxatilis* from post-settlement juvenile to adult

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ABSTRACT: Recent evidence suggests that pelagic larvae of coral reef fishes are active in their dispersal and return to a reef; however, the mechanisms used to navigate are unknown. Sound is a potentially important cue for organisms in marine environments, especially in noisy environments like coral reefs. The auditory brainstem response (ABR) technique was used to measure hearing of a wide size range of sergeant major damselfish *Abudeqduf saxatilis*. Complete audiograms were measured for 32 fish ranging in size from 11 to 121 mm. Significant effects of standard length on hearing thresholds at 100 and 200 Hz were detected. At these lower frequencies, thresholds increased with an increase in size. All fish were most sensitive to the lower frequencies (100 to 400 Hz). The frequency range over which fish could detect sounds was dependent upon the size of the fish; the larger fish (>50 mm) were more likely to respond to higher frequencies (1000 to 1600 Hz). *A. saxatilis* has poor hearing sensitivity in comparison to other hearing generalists including other species of pomacentrids. In consideration of the high hearing thresholds found in this study in comparison to recorded ambient reef noise, it is unlikely that sound plays a significant role in the navigation of the pelagic larvae of sergeant majors returning to the reef from long distances (>1 km), but it may play a role in short-range orientation (<1 km).

KEY WORDS: Auditory brainstem response · ABR · Hearing sensitivity · Larval settlement

INTRODUCTION

One of the noisiest habitats in the ocean is the coral reef (Cato 1978, Tolimieri et al. 2000). Sounds on or nearby a reef can be abiotic in origin, such as waves crashing on the reef, as well as biotic, short-duration clicks and snaps produced predominantly by snapping shrimp and other invertebrates and fishes (Leis et al. 2002). Numerous species in the large perciform family Pomacentridae (damselfish) are well known sound producers (Allen 1975). One of the most common pomacentrid species is the sergeant major *Abudeqduf saxatilis* (Alshuth et al. 1998). *A. saxatilis* is an Atlantic species found along the western Atlantic coast, on most of the reefs in the Caribbean, around islands of the mid-Atlantic, Cape Verde, and along the tropical coast of western Africa, south to Angola (Allen 1991).

Few studies have been conducted on the ontogenetic changes in hearing sensitivity of fishes (Popper 1971, Kenyon 1996, Wysocki & Ladich 2001, Higgs et al. 2003). Popper (1971) studied 2 different size groups of the goldfish *Carassius auratus*, using a shock-conditioning technique, and found that hearing thresholds were not dependent on size. Kenyon (1996), however, conducted psychophysical experiments, utilizing electric shock, on 4 size groups of the bicolor damselfish *Stegastes partitus*; hearing thresholds decreased exponentially with an increase in size. A study by Wysocki & Ladich (2001) on the croaking gourami *Trichopsis vittata*, a hearing specialist, measured evoked potentials and had findings similar to those of Kenyon (1996).

There is physiological evidence to suggest that ontogenetic auditory changes occur in fishes. Previous
studies indicate that fishes lacking swim bladders have relatively poor hearing in both frequency range and sensitivity (Yan et al. 2000). The closer the swim bladder to the ear, the more sensitive the audition. The swim bladder is thought to act as an amplifier for some fishes by transferring sound pressure into displacement (Yan et al. 2000). Although the role of the swim bladder in hearing generalists is not well studied (Yan et al. 2000), as the fishes grow, their swim bladders presumably increase in size, which could affect audition (Kenyon 1996). A second physiological process that could affect hearing ability is the ongoing addition of inner-ear sensory hair cells (Lanford et al. 1996). Post-embryonic proliferation of hair cells in otolithic endorgans has been seen in various teleosts and elasmobranchs (Popper & Hoxter 1981, Corwin 1983, Lombarte & Popper 1994), but the effect that this increase in hair cells has on hearing is still unclear (Popper & Fay 1999).

The hearing ability of young coral reef fishes is particularly of interest due to the recent proposals that pelagic larval reef fishes use sounds coming from the reef as a cue to navigate to the reef. Much evidence supports the idea that the pelagic larvae of coral reef fishes actively participate in their dispersal and return to a reef (Stobutzki & Bellwood 1997, 1998, Armsworth 2000). They are strong swimmers, capable of swimming 10s of kilometers to >90 km continuously at speeds fast enough to overcome currents (Stobutzki & Bellwood 1997). Fishes form their sensory organs early in development, usually within the first few days of life (Leis & McCormick 2002, Myrberg & Fuiman 2002).

Because the coral reef is a noisy environment and because sound is used by many coral reef fishes for communication, it is plausible that larval fishes use hearing as a navigational tool (Tolimieri et al. 2000). Otolithic organs are often present at a very early stage in larval development (Leis et al. 1996, Leis & McCormick 2002). In sergeant majors, otoliths have been seen in the auditory vesicles in the eggs (Alshuth et al. 1998). Sound can travel long distances underwater, and is highly directional with little attenuation (Rogers & Cox 1988). Larval fishes are found 10s of meters to 100s of kilometers from a reef (Leis & McCormick 2002), but high ambient noise levels from fishes and invertebrates near reefs may exceed background noise for 10s of kilometers from the source (McCauley 1995). The noise levels are greatest at night, when snapping shrimp are most active, and this is the time reef fishes tend to settle (McCauley 1995).

Studies using light traps that broadcast reef sound sources have shown that some, but not all, species of larval reef fishes are more attracted to ‘noisy’ than to ‘quiet’ traps (Tolimieri et al. 2000, Leis et al. 2003). In addition, the behavior of reef fish larvae differs in response to broadcasted reef sounds rather than broadcasted random sounds (Leis et al. 2002).

In this study, audiograms of 32 sergeant majors ranging in size from 11 to 121 mm were measured to determine any effect size has on auditory sensitivity and the likelihood that the smallest size group of sergeant majors use sound as a navigational tool. Hearing thresholds were measured using the auditory brainstem-response (ABR) technique. ABR is an electrophysiological technique for measuring hearing thresholds in fishes and other vertebrates (Kenyon et al. 1998). Electrodes placed cutaneously or inserted subdermally in proximity to the organism’s brainstem, directly measure nerve impulses created in the VIIIth nerve and brain in response to sounds (Corwin et al. 1982). Signal-averaging is used to filter the evoked potential signal from background noise.

**MATERIALS AND METHODS**

**Fish acquisition and maintenance.** The majority of fish in this study were caught with nets in the Florida Keys by SCUBA divers. The fish were separated into 3 size groups, <30, 30 to 50 and >50 mm). The 12 smallest fish (<30 mm) were collected from lobster traps in water off Long Key, Florida. As the traps were collected every 1 to 3 d, the fish collected were likely to have been newly settled.

All collected fish were held for 1 to 5 d, tested and either euthanized or released. Fish Nos. 1 to 28 were collected near Long Key and were housed in flow-through holding tanks at the Keys Marine Laboratory (KML) in Long Key. ABR tests were performed on these fish on-site at KML. The largest fish (Nos. 29–32) were collected off Tavernier Key, an island about 24 km north of Long Key. These fish were maintained together in a 275 gallon (1040 l) cylindrical tank (salinity, S = 35; temperature, T = 26°C) at the University of South Florida (USF) and fed a few pinches of ‘Tropical Fish Flakes’ twice a day. No bubblers were used in any of the holding tanks. The identical ABR setup, including all instrumentation and test tanks, was used to perform ABRs on the 4 largest fish at the Marine Sensory Laboratory at the USF.

**Experimental setup.** Hearing thresholds were determined for each fish using ABR. An individual fish was secured in a harness constructed from Nitex mesh, fastened with clamps, and suspended from laboratory stands. A custom-made harnesses for each fish restricted movement while allowing normal respiration.

The apparatus consisted of a PVC pipe (1.2 m high, 30 cm in diameter), closed at the bottom, and oriented upright. At KML, the test tank was set up in a separate room in which only hearing tests were conducted. At
USF, the test tank was set up in an audiology booth. The PVC container was filled with seawater (S = 35, T = 26°C) to a height of 1.12 m. The fish was suspended 46 cm below the surface and a loudspeaker was placed at the bottom of the PVC pipe.

Subdermal stainless steel needle electrodes (Rochester Electro-Medical) were used for recording the ABR signal. An electrode was inserted about 1 mm into the head, over the medulla region. The reference electrode was placed within the fish’s dorsal musculature and a ground electrode was placed directly in the water in close proximity to the fish.

After a fish had been tested, it was weighed and measured before being returned to the tank. Data from any fish that died or escaped during ABR testing were not used.

**Sound generation and ABR acquisition.** Sound stimuli and ABR waveform recordings were produced with a Tucker-Davis Technologies (TDT) ABR workstation (Fig. 1). TDT SigGen and BioSig software were used to generate the sound stimuli with an RP2.1 enhanced real-time processor, a PA5 programmable attenuator to control sound level, and a power amplifier (Hafler Trans.Ana P1000 110 W professional power amplifier) before being sent to a TST 229 AQUA underwater speaker (Clark Synthesis) where sound was emitted. Stimuli consisted of 20 ms pulsed tones gated with a Hanning window. The phase of the tone was alternated between presentations to minimize electrical artifacts from the recordings.

Acoustic stimuli were calibrated with a Reson hydrophone (sensitivity-212 dB V/1 µPa) connected to the RP2. During calibration, the hydrophone was positioned in the experimental setup in place of the fish, and the sound levels were measured with BioSig, without phase alternation.

During each trial, 8 different frequencies were presented: 100, 200, 400, 800, 1000, 1200, 1400 and 1600 Hz. Sound levels at each frequency were presented at up to 150 dB re 1 µPa and decreased in 6 dB steps until a threshold level was determined.

Evoked potentials recorded by the electrode were fed through an RA16 Medusa amplifier to an RA16 Medusa base station, routed into the computer and averaged by BioSig software. A total of 2000 signal presentations were averaged to measure the evoked response at each level of each frequency.

**Data analysis.** Hearing thresholds were determined using power spectra which were calculated with an 8192-point FFT (fast Fourier transform) for all ABR waveforms and analyzed for the presence of significant peaks (peaks at twice the frequency of the stimulus that were at least 3 dB above background levels). ABR thresholds were defined as the lowest sound level at which significant FFT peaks for the dominant frequency were apparent.

Thresholds were determined for each fish at each frequency and were plotted as a linear regression comparing threshold versus standard length. At each frequency, the $r^2$ values were calculated and slopes of the regression lines were determined. An ANOVA analyzing the overall goodness of fit of the regression line at each frequency was then performed. The highest frequency tested, 1600 Hz, was not included in the regression analysis due to the small number of fish for which a response was detected (n = 6). Because of repetition of statistical tests on the same subjects (ANOVA performed for regression at 7 different frequencies), a Bonferroni correction was used to determine significant values. The alpha level was set at 0.05, and taking the Bonferroni correction into account (0.05/7), slopes were considered significantly different from zero when $p < 0.007$.

**RESULTS**

ABR waveforms indicate that as the sound level of the stimulus decreased, the amplitude of the ABR waveforms decreased (Fig. 2). The dominant frequency in the power spectra was approximately twice the stimulus frequency. Threshold sound levels at each frequency were determined for each fish (Table 1).

Mean thresholds for all fish suggest that they are most sensitive at the lower frequencies tested (100, 200, 400 Hz) and require loud sound levels (>140 dB sound pressure level, SPL) to detect tones at higher frequencies (800 to 1600 Hz) (Fig. 3). The most sensitive frequency was 100 Hz (mean = 118 dB, SD = 10.9).

All size groups had the lowest thresholds at the lower frequencies (100 to 400 Hz) ranging from 112 to
The most sensitive frequency was 100 Hz for all 3 size groups. Threshold levels greatly increased for all size groups at 800 Hz.

The degree of variability between individual fish was large at each frequency (Fig. 5). The r² values were low for all the regressions; however, the slopes for all frequencies that were detected by more than 1 size group were positive. The regression lines for 100 Hz (p = 0.001) and 200 Hz (p = 0.006) were significant, indicating a true effect between size and threshold at these frequencies. At 400 Hz, p was 0.022, but because of the Bonferroni correction the regression is not considered significant. The p-values for the regressions at 800, 1000, 1200 and 1400 Hz were not significant (p = 0.451, 0.458, 0.530, 0.176, respectively).

The frequency range detected by the fish at the maximum sound level also appears to be a function of fish length (Fig. 6). The larger fish were more likely to respond to higher-frequency sounds. Almost 100% of the tested fish responded to sound presented from 100 to 800 Hz. As the frequency increased above 800 Hz, the number of smaller fish with a response decreased. A test for significance of differences between the proportion of responses among the 3 groups at the loudest sound level presented at each frequency found significant differences at frequencies of 1000 to 1600 Hz (Table 2).
Fig. 3. *Abudefduf saxatilis*. Mean (±SD) audiogram for all fish (combined data) at each tested frequency (n = 32). SPL: sound pressure level.

Fig. 4. *Abudefduf saxatilis*. Mean (±SD) audiograms for the 3 size groups at each tested frequency. SPL: sound pressure level.

Fig. 5. *Abudefduf saxatilis*. Regressions of standard length on auditory sensitivity for all frequencies tested except 1600 Hz, to which only a small percentage of fish responded.
DISCUSSION

Auditory sensitivity

Overall, sergeant majors have poor hearing sensitivity and would be classified as hearing generalists. This classification was expected because, although this fish has a swim bladder, a connection between the bladder and the auditory endorgans or any other accessory auditory structure has not been found in pomacentrids (Myrberg & Spires 1980, Myrberg et al. 1986).

Fig. 7 shows the audiogram produced for the sergeant majors along with those for other adult teleost species of the coral reef classified as hearing generalists. The ABR is considered more conservative in judging threshold levels than the classical behavioral approaches used to create the other audiograms (Kenyon et al. 1998). ABR is a relatively new technique for determining thresholds for fishes; however, work with humans has demonstrated that auditory thresholds determined using tone-burst ABR are generally higher, by 10 to 20 dB, than those obtained using behavioral methods (Gorga et al. 1988, Kenyon et al. 1998). Kenyon et al. (1998) found no statistically significant difference in thresholds determined using ABR and those found with behavioral methods, but they did find that ABR thresholds were generally higher than behavioral values at frequencies below 1500 Hz.

The threshold at the most sensitive frequency of the sergeant major (118 dB, 100 Hz) is above those of the majority of the other species: Stegastes (average of 6 species) = 82 dB, 500 Hz; Adioryx vexillarius = 90 dB, 600 Hz; Haemulon sciurus = 80 dB, 100 Hz; Epinephelus guttatus = 90 dB, 200 Hz; Thalassoma bifasciatum = 107 dB, 500 Hz; Lutjanus apodus = 110 dB, 300 Hz (Stegastes spp.: Myrberg & Spires 1980; all other species: Tavolga & Wodinsky 1963). Although the frequency range detected by the sergeant major is greater than that for most of the other species represented, this most probably reflects a limitation of the maximum sound levels used in the other studies.

Effect of size on auditory sensitivity

Size of the fish significantly affected the auditory sensitivity and the frequency range the fish was able to detect. At the lower frequencies (100 to 200 Hz), thresholds increased with an increase in length. In addition, the larger fish more readily responded to the higher frequencies (800 to 1600 Hz) at a significant level. However, the most sensitive frequency was 100 Hz for each size group.

Surprisingly, threshold levels actually increased with increasing fish size at the lower frequencies. The closer proximity of the swim bladder to the otolith in a smaller...
fish may allow the bladder to act as a more pronounced amplifier at certain frequencies. The decrease in hearing sensitivity with age could also be explained by the life history of the fish. Perhaps the change in habitat from pelagic waters to the reef requires less sensitive audition. Settled sergeant majors are thought to produce sound occasionally as a form of communication, primarily during spawning, but the fish are generally in close proximity (Prappas et al. 1991, Lobel & Kerr 1999). The threshold levels of this species are probably low enough to detect sounds produced by neighboring fish.

Alternatively, the lower thresholds exhibited by the juvenile fish could also be explained by variation in electrode placement. In the attempt to be as consistent as possible with every trial, the electrode was placed about 1 mm below the surface of the head of every fish. In smaller fish, therefore, the electrode was probably closer to the brain, which may have resulted in a larger ABR response in some of the smaller fish compared to larger fish. If the ABR response was indeed lower for the larger fish, this could have resulted in slightly higher thresholds for the adult fish than was actually the case.

The results from this study are in contrast to a study on the bicolor damselfish Stegastes partitus, which found an exponential decrease in threshold with increasing size (Kenyon 1996). All size groups in Kenyon’s (1996) study responded to sounds in the frequency range of 300 to 1500 Hz. The most sensitive frequency at 500 Hz remained the same in each size group. Popper (1971) found that there was no difference in audition between 2 size groups of goldfish Carassius auratus. Kenyon (1996) suggested that the difference in results might be due to the physiological differences between the hearing specialist, goldfish, and the hearing generalist, bicolor damselfish.

It is important to note that the methods used by Popper (1971), Kenyon (1996) and this study differed. Popper (1971) used a behavioral shock-conditioning technique, whereby the fish were shocked if they did not swim across a barrier in response to a sound. Similarly, Kenyon (1996) conducted psychophysical experiments, whereby the fish were trained to associate sound with an electric shock; the fish eventually showed an avoidance response with the onset of detectable sound.

In addition to the different methods, the differing results between this damselfish study and the study of Kenyon (1996) could be due to the sound-production habits of the 2 species of fish. Sergeant majors are not as vocal as many other members of the pomacentrid family including Stegastes spp. Territoriality, a behavior which incorporates the production of sound in many pomacentrid species (Myrberg 1997), is usually seen in sergeant majors only during reproductive periods (Fishelson 1970). Additionally, a study on the blackspot sergeant Abudefduf sordidus found that this close relative of the sergeant major does not rely on sound during courtship as much as other pomacentrids such as Stegastes spp. (Lobel & Kerr 1999). Sound production most probably plays an important role in the hearing ability of each species and may account for the more sensitive hearing of the adult fish in Kenyon’s (1996) study.

Although Popper (1971) and Kenyon (1996) did not see an increase in frequency range with an increase in size, a similar result was found in an ontogenetic study utilizing ABR on the audition of the hearing specialist, croaking gourami Trichopsis vittata (Wysocki & Ladich 2001). The increased frequency range for this species, however, is attributed to the resonance of the air-filled suprabranchial chamber (SBC), which is utilized for air-breathing and also acts as an accessory hearing structure. The sergeant major has no known accessory hearing structure.

Hearing generalists rely on particle velocity detected by sensory hair cells (kinocilium and stereocilia) on the otolith. Kinocilium length generally corresponds with the different frequencies at which hair cells are stimulated by incoming sound (Platt & Popper 1984). Regions of the sensory epithelium in goldfish with longer kinocilia were considered responsive to lower frequencies while those with shorter kinocilia were considered responsive to higher frequencies (Platt & Popper 1984). Perhaps the ongoing addition of the sensory hair cells to the sensory epithelium of the otolithic organs and the placement of the new sensory hair bundles play a role in the frequencies that hearing generalists can detect.

Use of reef sound as a navigational cue for pelagic larvae

To determine the likelihood of the use of sound as a navigational cue for pelagic larval sergeant majors, the audiogram of the newly settled sergeant majors is compared to sound levels of recorded reef noise in Fig. 8. Since the audiogram measures hearing at 1 frequency while the reef generates a broad-band sound spectrum, the audiogram was adjusted to estimate the ability to detect a broad-band signal. This was done by lowering the audiogram thresholds. The audiogram has been adjusted by an estimated critical bandwidth that is assumed to be 10% of the center frequency, i.e. the threshold has been lowered by 10 log (critical bandwidth) (Yost 2000). If one assumes that the hearing abilities of the newly settled juveniles are similar to larval audition, then it is unlikely that larval sergeant majors use coral reef noise as a sole navigational cue from great distances.
Although Kenyon’s (1996) study on the hearing of damselfish juveniles supports the idea that the larvae cannot use sound as a navigational cue over long distances, studies have been conducted which suggest a role of audition in settlement: Tolimieri et al. (2000) and Leis et al. (2002, 2003) found that significantly more larvae of specific taxa (Tripterygiidae: Tolimieri et al. 2000; Apogonidae, Mullidae, Pomacentridae, Serranidae, Sphyraenidae: Leis et al. 2003) were caught in light traps broadcasting recorded reef sounds than in ‘silent’ traps. However, the distance that the larvae had travelled was not known in either study.

McCauley’s (1997) data of a reef fish chorus measured 4.3 km from the Great Barrier Reef was used to estimate the farthest distance at which juvenile sergeant majors could detect reef sounds (Fig. 9). Assuming spherical spreading loss (transmission loss = 20 log r [where, r = distance]), the farthest distance from which sergeant major juveniles could detect a reef chorus, on average, was calculated to be about 0.54 km from the reef. However, the hearing sensitivity of 1 juvenile fish in the present study would allow detection of this reef chorus at 2.15 km. These calculations assume that the ABR thresholds are the same as behavioral thresholds; if behavioral thresholds were lower, then the fish would be able to detect the reef from greater distances. This also assumes that background noise from other sources, such as surface waves, does not mask the noise from the reefs.

In addition to distance, another consideration when studying sound detection as a navigational cue is the ability of the fish to localize the sound. Even if the fish do have the ability to detect sounds from the pelagic environment, they must be able to determine the direction from which the sounds are coming. Although how localization occurs is still being investigated, the ability to determine the direction of sound has been demonstrated in some adult fishes including pomacentrids (Myrberg & Spires 1980, Myrberg et al. 1986).

A study on the black axil chromis Chromis atripectoralis was conducted in which reefs sounds were broadcasted to larvae released 50 to 100 m from the loudspeaker (Leis et al. 2002). It was found that the larval fish responded to the sound and had a different behavioral response when played random noise as opposed to recorded reef sounds. The response of the larval fish to reef sounds, however, was to swim faster and in random directions, so there was no indication that they had the ability to localize the sound. A more recent study using traps with a radius of attraction of sound greater than that of light showed that the larvae of some fishes, primarily pomacentrids and apogonids, can use reef sound to navigate at 30 to 65 m distance from the sound (Leis et al. 2003).

Rather than sound from the reef being the sole cue for pelagic larval fishes to follow, it is more likely that larvae use a combination of sensory organs to navigate their return to the reef (Kingsford et al. 2002). Vision is obviously important over small scales (5 to 15 m) (Leis & Carson-Ewart 1998, 1999, 2002), and olfaction has been shown to play a role in some pomacentrid and apogonid species at distances of a few to 10s of meters.
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