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Auditory Sensitivity of Sergeant Majors (*Abudefduf saxatilis*) from Post-settlement Juvenile to Adult

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Auditory Sensitivity of Sergeant Majors (*Abudefduf saxatilis*) from Post-settlement
Juvenile to Adult

by

Sarah A. Egner

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
College of Marine Science
University of South Florida

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Keywords: damselfish, auditory brainstem response (ABR), hearing sensitivity, larval settlement, coral reef

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AUDITORY SENSITIVITY IN SERGEANT MAJORS (*Abudefduf saxatilis*) FROM POST-SETTLEMENT JUVENILE TO ADULT

Sarah A. Egner

ABSTRACT

There is much evidence supporting the idea that pelagic larvae of coral reef fishes are active participants in their dispersal and return to a reef, however, the mechanisms used to navigate are still uncertain. It has been proposed that sensory cues, such as hearing, play a role. Sound is a potentially important cue for organisms in marine environments, especially in noisy environments like coral reefs. Sensory organs, including otolithic organs, of most coral reef fish form within the first few days of life. The auditory brainstem response (ABR) technique was used to measure hearing on a wide size range of sergeant majors (*Abudefduf saxatilis*). Complete audiograms were measured for 32 fish ranging in size from 11-121 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 (100-400 Hz). The frequency range that fish could detect sounds was dependent upon the size of the fish; the larger fish (>50mm) were more likely to respond to higher frequencies (1000-1600 Hz). *A. saxatilis* have poor hearing sensitivity in comparison to audiograms of other hearing generalists including other species of Pomacentrids. Due to 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 to the return of the reef from large distances.

INTRODUCTION

Most of the ambient noise in the ocean falls between 50-5000 Hz (Cato 1992). One of the noisiest habitats in the ocean is the coral reef. 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 fish (Leis et al. 2002). Fish produce epiphenomenal sounds during sudden movement and when feeding (Colson et al. 1998) and many fish use sound production voluntarily during spawning, schooling and aggressive territorial behaviors (Myrberg and Fuiman 2002). Nocturnal activity by snapping shrimp, fish and urchins creates an ‘evening chorus’ on the reef (Cato 1980; McCauley 1994, 1995).

Numerous species in the large perciform family Pomacentridae (damselfish), represented on reefs worldwide by approximately 320 species, are well-known sound producers (Allen 1975). One of the most common Pomacentrid species is the sergeant major (*Abudefduf 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). Due to their abundance, and the lack of hearing data on this common species, sergeant majors were chosen for this study.

Like most coral reef fishes, sergeant majors have a planktonic larval stage (Alshuth et al. 1998). They deposit demersal adhesive eggs on hard substrates (Alshuth et al. 1998) and the hatching of the larvae occurs four to seven days after fertilization (Alshuth 1998, Foster 1987). The larvae are pelagic for an average of 17-20 days before settling on a reef (Wellington and Victor 1989). Coral reef fish larvae are usually found between 50-100 meters with the highest diversity of taxa in the upper 50 m (Cowen 2002). Pomacentrid larvae are concentrated in the mid depths (20-60m). Newly settled sergeant majors inhabit inshore and offshore coral or rocky reefs and are found at a depth

range of 1-12 m (Allen 1991). Sergeant majors grow to a maximum standard length of 150 mm.

Sergeant majors are not the most vocal of the damselfish but they do produce sound and live in a noisy environment (Santiago and Castro 1997, Tolimieri et al. 2000, Fishelson 1970). There is little published data on sound produced by sergeant majors though damselfish are among the best studied sound-producing fishes (Lobel and Kerr 1999, Spanier 1979, Chen and Mok 1988, Myrberg et al. 1993, Lobel and Mann 1995, Santiago and Castro 1997). A study on an Eastern Atlantic species, *Abudefduf luridus*, found sound production by this fish during some aggressive interactions (Santiago and Castro 1997). Sound was in the frequency range of 50-500 Hz with most of the energy concentrated in the low end of the spectrum. Courtship associated sounds studied in a Pacific species, *Abudefduf sordidus*, were found to be highly variable and of low amplitude in comparison to other Pomacentrids (Lobel and Kerr 1999). Due to the prevalence of sound production in the family, sergeant majors most likely utilize sound in some fashion.

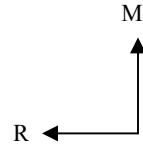
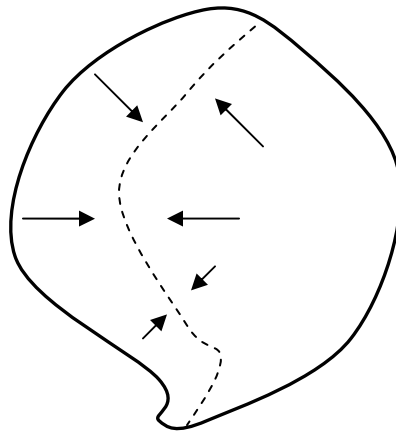
Sound propagates well in water, but the distance it travels while still detectable depends on five main factors: frequency, intensity, water depth, background noise and receiver sensitivity (Kingsford et al. 2002). All other things being equal, lower frequency sounds such as the crash of breaking waves travels further than higher frequencies like a snapping shrimp 'click.' The strength of a sound signal must exceed background noise to be detectable. In coral reef waters, background noise levels are high, and wind can add considerably to the noise (Kingsford 2002). Some biological sound from reefs still exceeds background noise levels at 4-20 km from its source (McCauley 1997).

Fish hearing

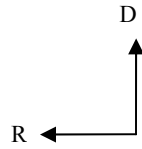
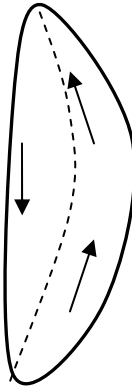
In fish, sound is detected by three otolithic organs, the saccule, utricle and lagena (Popper and Fay 1997). Each of these organs is a fluid filled sac with a sensory epithelium that has numerous sensory hair cells arranged in specific patterns (Figure 1). The hair cells make up a ciliary bundle which consists of a cluster of

Figure 1 Schematic drawings of the sensory hair cell orientation patterns on the otolithic organs. The arrows approximate the orientation of the major portion of the hair cells in each epithelial region. The regions are separated by the dotted lines. The patterns represent the majority of fish species. (D:dorsal, M: mediolateral, R: Rostral) (Redrawn from Popper and Platt 1993).

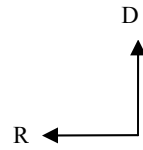
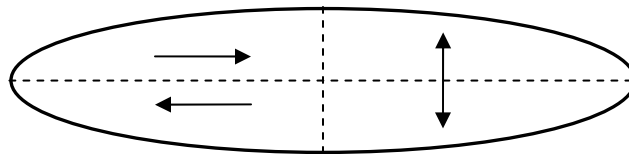
Utricule



Lagena



Sacculle



stereocilia and an eccentrically placed single long kinocilium (Popper and Fay 1999). The base of each ciliary bundle synapses with neurons of the eighth cranial nerve (Myrberg and Fuiman 2002). Depolarization occurs when stereocilia are bent in the direction of the kinocilium. The displacement of the directionally sensitive hair cells relative to the otoliths is the effective auditory stimulus for fish (Montgomery et al. 2001).

Fish are categorized as hearing generalists or specialists depending upon the connection or the proximity of the swim bladder to the ear (Popper and Platt 1993, Yan et al. 2000). Fish with the best hearing sensitivity are those with swim bladder specializations that facilitate the transduction of the pressure component of the receptive field to particle displacement (Montgomery et al 2001). Without some specialization, such as the Weberian ossicle system found in ostariophysans (series of four bones connecting the swim bladder to the ears) (von Frisch 1936), the swimbladder diverticulae in holocentrids (Coombs and Popper 1979), or the gas filled bullae in mormyrids (Stipetic 1939), particle motion from the incident sound wave is thought to be the only stimulus that can be sensed by the fish auditory system (Yan et al. 2000, Kenyon et al. 1998). Reef fish typically do not belong to hearing specialist groups and relatively little is known about their hearing capabilities (Montgomery et al 2001).

Ontogenetic changes in audition

Ontogenetic changes in hearing are well known in mammals (Rübsamen 1992, Zimmerman 1993, Lecanuet and Schaal 1996, McFadden et al. 1996, Reimer 1996) and birds (Gray and Rubel 1985, Golubeva and Tikhonov 1985), but have not been well studied in fish. Most of these mammalian audition studies have had similar results; an increase in size was positively correlated with increased auditory sensitivity (Lecanuet and Schaal 1996, McFadden et al. 1996, Reimer 1996), broadening of hearing range (Rübsamen 1992), especially at higher frequencies, and a shift in the most sensitive frequency of hearing (Golubeva and Tikhonov 1985, Reimer 1996).

A few studies have been conducted on the ontogenetic changes in hearing sensitivity for fishes (Popper 1971, Kenyon 1996, Wysocki and Ladich 2001, Higgs et al.

2003). Popper studied two different size groups of goldfish, using a shock conditioning technique, and found that hearing thresholds were not dependent on size (Popper 1971). The two size groups used had similar audiograms. Kenyon, however, conducted psychophysical experiments, utilizing electric shock, on four size groups of the bicolor damselfish (*Stegastes partitus*). Hearing thresholds decreased exponentially with an increase in size (Kenyon 1996). A study on the croaking gourami (*Trichopsis vittata*), a hearing specialist, measured evoked potentials and had findings similar to Kenyon (Wysocki and Ladich 2001). The authors of the latter two studies suggest the use of only two size groups in Popper's study swayed the results (Kenyon 1996, Wysocki and Ladich 2001). In addition, goldfish are ostariophysan fishes, which have a fundamentally different peripheral ear morphology in comparison to damselfish and gouramis.

There is physiological evidence to suggest that ontogenetic auditory changes occur in fish. Previous studies indicate fish lacking swim bladders have relatively poor hearing in both frequency range and sensitivity (Yan et al. 2000). The closer the swim bladder is to the ear, the more sensitive the audition. The swim bladder is thought to act as an amplifier for some fish by transferring sound pressure into displacement (Yan et al 2000). Though the role of the swim bladder in hearing generalists is not well studied (Yan et al. 2000), as the fish grow, the swim bladder presumably increases 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 elasmobranches (Corwin 1983, Popper and Hoxter 1984, Lombarte and Popper 1994), but the effect this increase in hair cells has on hearing is still unclear (Popper and Fay 1999).

Settlement of pelagic larval fish

Most coral reef fishes have a bipartite life cycle where they spend days to months in the open ocean as developing larvae until the fish eventually settle on the reef as juveniles (Leis 1991). Sergeant majors have a larval stage approximately 17-20 days in

duration (Wellington and Victor 1989). One question in coral reef fish ecology that has become increasingly important is how pelagic larval coral reef fish navigate their return to the reef.

There is much recent evidence supporting the idea that the pelagic larvae of coral reef fish are active participants in their dispersal and return to a reef (Stobutzki and Bellwood 1997, 1998, Armsworth 2000). They are strong swimmers, capable of swimming tens of kilometers to greater than 90 kilometers non-stop at speeds fast enough to overcome currents (Stobutzki and Bellwood 1997). The methods used to navigate, however, are still uncertain. Fish form their sensory organs early in development, usually within the first few days of life (Leis and McCormick 2002, Myrberg and Fuiman 2002); the use of senses such as vision, olfaction, and hearing have been proposed in literature.

Because the coral reef is a noisy environment and because sound is used by many coral reef fish in communication, it is plausible that larval fish use hearing as a navigational tool (Tolimieri et al. 2000). Otolithic organs are often present at a very early stage in larval development (Cato 1978, Leis et al 1996, Leis and 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, is highly directional, and has little attenuation (Rogers and Cox 1988). Larval fish are found tens of meters to hundreds of kilometers from a reef (Leis and McCormick 2002) but high ambient noise levels from fish and invertebrates near reefs may exceed background noise for tens of kilometers from the source (McCauley 1995). The noise levels are greatest at night, when snapping shrimp are most active, which is the time reef fishes tend to settle (McCauley 1995).

Studies using light traps that broadcast reef sounds have shown some, but not all, species of larval reef fish to be more attracted to the “noisy traps” than “quiet traps” (Tolimieri et al. 2000, Leis et al. 2003). In addition, the behavior of reef fish larvae differ in response to broadcasted reef sounds rather than broadcasted random sounds (Leis et al. 2002). These studies show that the larval fishes can detect reef sounds and can differentiate between reef sounds and random noise, but the frequency range detected, the

distance the sounds can be perceived and the ability to actually localize the sound all require further study.

Auditory brainstem response

The auditory brainstem response (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 eighth nerve and brain in response to sounds (Corwin et al. 1982). Signal averaging is used to pull the evoked potential signal out of background noise. The benefits of ABR in comparison to traditional behavioral tests include prompt evaluation of hearing, ability to repeatedly test animals and a minimization of overall physiological strain on the test subjects (Kenyon et al. 1998; Yan and Popper 1991).

Purpose

The purpose of this study was to measure audiograms for sergeant majors ranging in age from newly settled juveniles to adults using the auditory brainstem response technique. Though the reef is one of the noisiest habitats in the sea, coral reef fishes are not well studied in regards to hearing (Montgomery et al. 2001). Audiograms have yet to be measured for sergeant majors and few studies have succeeded in determining hearing thresholds for juvenile fish (Kenyon 1996, Popper 1971, Wysocki and Ladich 2001).

The audiograms were used to provide information on three aspects of sergeant major audition. First, the audiograms were analyzed to determine the frequency range each fish could detect and auditory thresholds at each of the frequencies for which there was a response. The audiograms were also examined to ascertain whether size is a factor in the hearing sensitivity of the sergeant major. The thresholds were compared to determine how hearing changed as the fish increased in length. Lastly, the audiograms of the smallest fish were analyzed to determine whether it is feasible for pelagic larval sergeant majors to use sound as a navigational tool.

METHODS

Fish acquisition and maintenance

Audiograms were measured for 32 sergeant majors ranging in size from 11.1 mm to 121 mm (Table 1). The majority of the fish in this study were caught with nets in the Florida Keys by divers on SCUBA. The 12 smallest fish (<30 mm) were collected from lobster traps set up in water off of Long Key, FL. The traps were collected every 1-3 days so the fish collected were likely newly settled.

All fish were collected, held for 1-5 days, tested and either euthanized or released. Fish #1-28 were collected near Long Key and were housed in flow-through holding tanks at the Keys Marine Lab (KML) in Long Key, FL. Auditory brainstem response hearing tests (ABRs) were performed on these fish on-site at the Keys Marine Lab. The larger fish were collected off of Tavernier, Florida Keys, maintained together in a 275 gallon cylindrical tank (S=35, T=26°C) at the University of South Florida and fed a few pinches of Tropical Fish Flakes twice a day. The identical ABR setup, including all instrumentation and test tank, was used to perform ABRs on the six largest fish at the Marine Sensory Lab at USF. All procedures were approved by the University of South Florida Institutional Animal Care and Use Committee.

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. Harnesses were custom made for each animal so that movement was restricted while allowing respiration to occur normally.

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

Table 1 Standard length and weight for each fish. Group 1 (n=12) included fishes less than 30 mm, Group 2 (n=10) included fishes 30-50 mm and Group 3 (n=10) included fishes greater than 50 mm.

GROUP 1			GROUP 2			GROUP 3		
fish #	SL (mm)	weight (g)	fish #	SL (mm)	weight (g)	fish #	SL (mm)	weight (g)
1	11.1	0.08	13	31.7	1.73	23	51.4	8.53
2	11.7	0.08	14	34.8	2.35	24	52.6	9.38
3	12.7	0.16	15	37.5	2.37	25	57.4	10.92
4	12.8	0.13	16	37.8	2.82	26	89.6	47.37
5	13.2	0.15	17	38.8	2.68	27	105	63.23
6	19.1	0.76	18	38.9	3.34	28	108	72.5
7	19.8	0.40	19	39.1	3.57	29	115	80.48
8	21.4	0.70	20	46.1	5.73	30	116	75.32
9	21.6	0.69	21	47.3	6.29	31	120	77.34
10	24.0	0.72	22	48.7	6.41	32	121	75.93
11	27.3	1.11						
12	28.0	1.23						

audiology booth. The PVC container was filled with sea water (S=35, T=26°C) to a height of 1.12 m. The fish was suspended 46 cm below the surface and a speaker 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 animal.

After a fish was 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

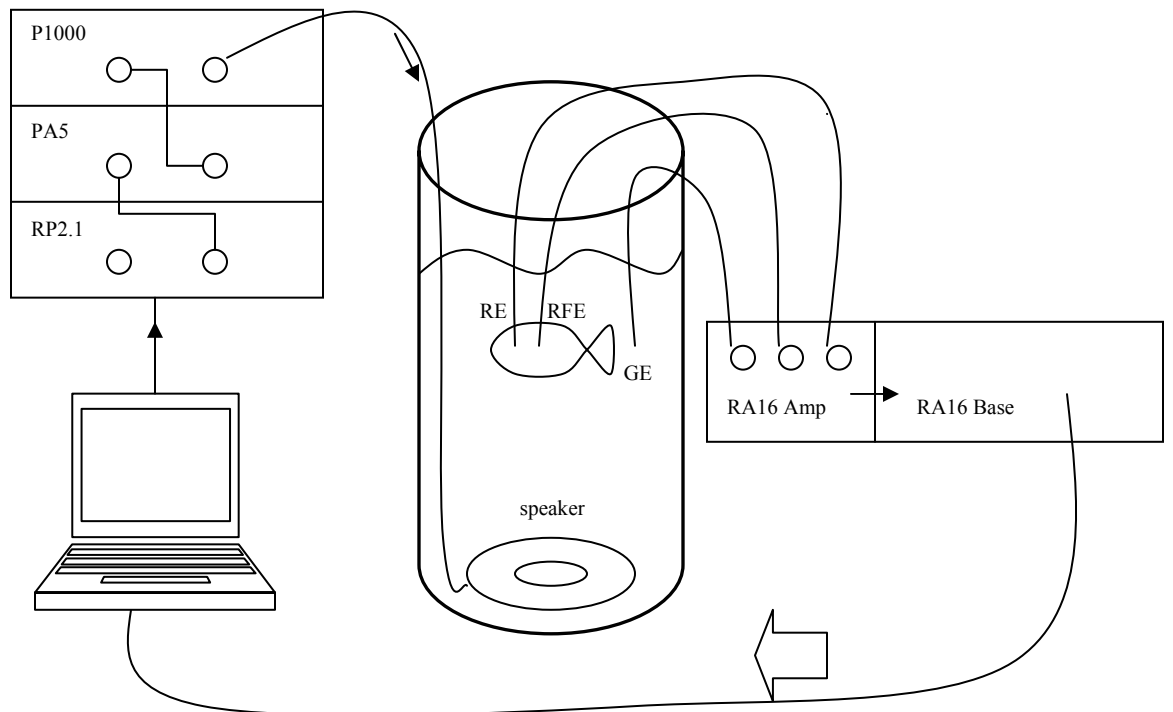
Sound stimuli and ABR waveform recordings were produced with a Tucker-Davis Technologies (TDT) ABR workstation (Figure 2). TDT SigGen and BioSig software was used to generate the sound stimuli, with an RP2.1 Enhanced Real-Time Processor (digital to analog converter), a PA5 Programmable Attenuator and a power amplifier (Hafler Trans.Ana P1000 110 Watt Professional Power Amplifier) before being sent to the TST 229 AQUA (Clark Synthesis) speaker where sound was emitted. Stimuli consisted of 20 ms pulsed tones gated with a Hanning window to improve resolution of peaks. 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 μ Pa) 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.

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

Evoked potentials recorded by the electrode were fed through the RA16 Medusa Amplifier to the RA16 Medusa Base Station (analog to digital converter), routed into the

Figure 2 Diagram of ABR-recording setup. (RP2.1 Enhanced Real-Time Processor, PA4 Programmable Attenuator, P1000 110 Watt Professional Power Amplifier, RE: Recording Electrode, RFE: Reference Electrode, GE: Ground Electrode, RA16 Medusa Amplifier, RA16 Medusa Base Station).



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 by 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). The FFT converts a signal in the time domain into the frequency domain allowing the viewer to determine if there is a signal in response to the frequency tested. Analyses of significant peaks was done using visual inspection, which is the traditional means of determining thresholds in ABR audiometry (Kenyon et al. 1998, Jacobsen 1985, Kileny and Shea 1986, Gorga et al. 1988; Hall, 1992; Song and Schacht 1996). ABR thresholds were defined as the lowest sound level where 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 ($n=6$). Due to repetition of statistical tests on the same subjects (ANOVA performed for regression line at seven different frequencies) a Bonferroni correction was used to determine significant values. The alpha level was set at 0.05 so taking the Bonferroni correction into account ($0.05/7$), values were considered significant when $p<0.007$.

Thresholds of every fish were averaged to form one audiogram as representative for all 32 fish. Fish were then separated into three size groups (11-30mm, 31-50mm, >50mm), thresholds were averaged for each group and an audiogram was produced for each group.

RESULTS

ABR waveforms in the time (nV/ms) and frequency domain (FFT) show that as sound level of the stimulus decreased, the amplitude of the ABR waveforms decreased, as well as the ABR voltages measured by the electrode (Figure 3). The dominant frequency in the power spectra was approximately twice the stimulus frequency.

Figure 4 shows the ABR output response from one individual fish representative of Group 1 (A) and one fish from Group 3 (B). For both fish, the greatest output resulted from tones played at 100 and 400 Hz. The ABR voltage is slightly greater for the larger fish. Thresholds and frequency range detected were determined for all fish (Table 2).

Mean thresholds for all fish suggest the fish are most sensitive at the lower frequencies tested (100, 200, 400 Hz) and require loud sound levels (>140 dB SPL) to detect tones at higher frequencies (800-1600 Hz) (Figure 5). The most sensitive frequency was 100 Hz (118 dB, SD=10.9).

Audiograms were produced for three size groups (Figure 6). All size groups had the lowest thresholds at the lower frequencies (100-400 Hz) ranging from 112-133 dB. The most sensitive frequency was 100 Hz for all three size groups. Threshold levels greatly increased for every size group at 800 Hz. The frequency step from 400 Hz to 800 Hz caused about a 15 dB change in threshold for all size groups.

The degree of variability between individual fish was large at each frequency (Figure 7). The R^2 values are very low for all of the regressions but the slopes for all frequencies that were detected by more than one size group are positive, indicating threshold levels increase as the fish increases in size. The regression lines for 100 Hz ($p=0.001$) and 200 Hz (0.006) were found to be significant, indicating a true effect between size and threshold at these frequencies (Table 3). For 800 Hz, 1000 Hz and 1200 Hz the p values were much larger ($p=0.451$, 0.458, 0.530, respectively). The p

Figure 3 Example of an ABR response from fish #1 (11.1 mm) when played tone at 800 Hz. **A)** Response in time domain. **B)** Response in frequency domain (FFT).

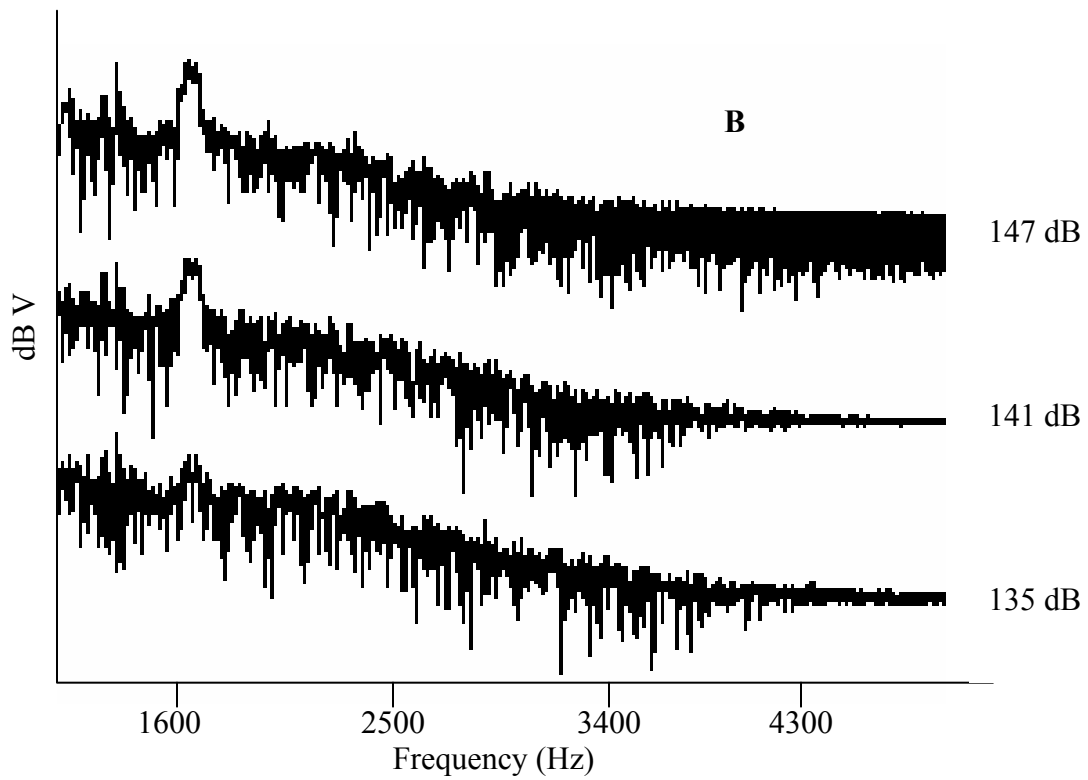
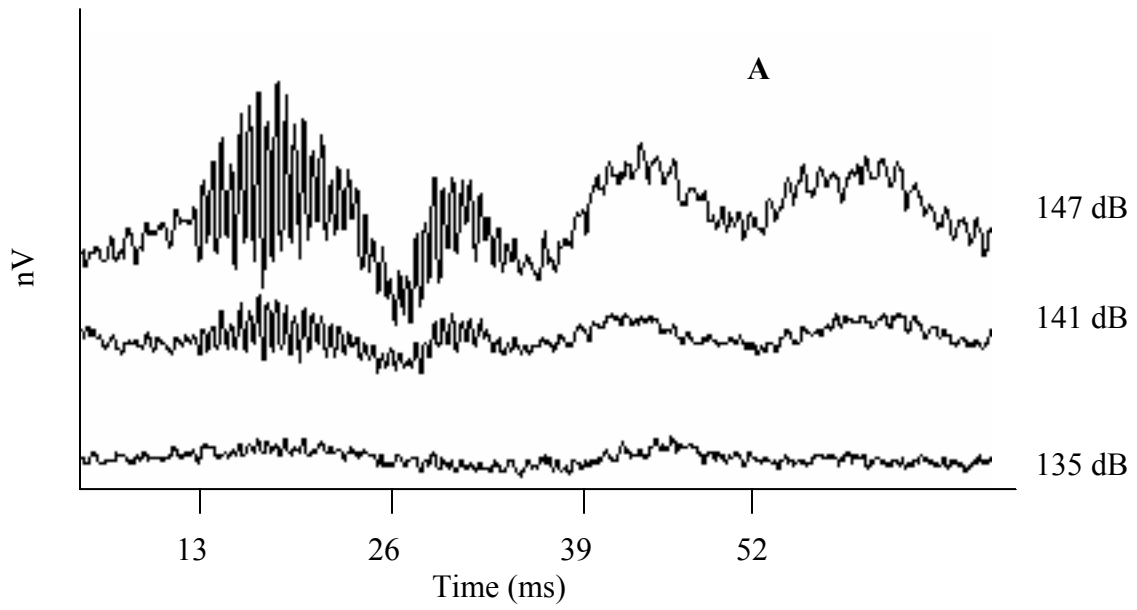


Table 2 Threshold sound levels (dB re 1 μ Pa) for each fish. NR denotes no response from the fish at the loudest sound level played.

GROUP 1 (<30 mm), n=12

fish #	SL (mm)	100	200	400	800	1000	1200	1400	1600
1	11.1	106	107	109	129	142	NR	NR	NR
2	11.7	136	137	121	147	NR	NR	NR	NR
3	12.7	106	113	115	147	NR	NR	NR	NR
4	12.8	112	143	139	147	NR	NR	NR	NR
5	13.2	100	107	109	135	NR	NR	NR	NR
6	19.1	124	125	121	141	NR	NR	NR	NR
7	19.8	112	107	121	141	130	148	NR	NR
8	21.4	106	119	115	141	148	NR	NR	NR
9	21.6	112	107	115	135	148	NR	NR	NR
10	24	106	107	115	141	NR	NR	NR	NR
11	27.3	112	125	127	129	142	148	NR	NR
12	28	112	113	127	147	NR	NR	NR	NR
MEAN		112	117	119	140	142	148	--	--
SD		10	13	9	7	7	0	--	--

GROUP 2 (30-50 mm), n=10

fish #	SL (mm)	100	200	400	800	1000	1200	1400	1600
13	31.7	112	119	121	147	148	NR	NR	NR
14	34.8	112	125	109	129	142	NR	NR	NR
15	37.5	130	149	121	135	NR	NR	NR	NR
16	37.8	118	137	127	135	136	142	NR	NR
17	38.8	130	107	109	129	136	148	NR	NR
18	38.9	142	137	151	NR	NR	NR	NR	NR
19	39.1	112	131	121	141	NR	NR	NR	NR
20	46.1	112	125	127	141	148	NR	NR	NR
21	47.3	124	113	121	117	130	136	142	NR
22	48.7	118	137	133	141	148	NR	NR	NR
MEAN		121	128	124	135	141	142	142	--
SD		10	13	12	9	7	6	--	--

GROUP 3 (>50mm), n=10

fish #	SL (mm)	100	200	400	800	1000	1200	1400	1600
23	51.4	106	125	121	141	148	148	148	NR
24	52.6	118	137	115	147	136	148	136	135
25	57.4	112	125	121	141	124	130	136	129
26	89.6	124	125	121	141	136	148	NR	NR
27	105	118	149	133	141	148	148	148	147
28	108	124	143	133	141	136	142	148	147
29	115	130	131	139	141	NR	NR	NR	NR
30	116	136	137	133	147	148	148	NR	NR
31	120	136	131	121	147	148	148	142	147
32	121	130	131	127	135	148	148	148	147
MEAN		124	133	126	143	141	145	144	142
SD		10	8	8	4	9	6	6	8

Figure 4 Example of ABR response from two different individuals. **A)** fish #8 (21.4 mm, Group 1) **B)** fish #24 (52.6 mm, Group 3)

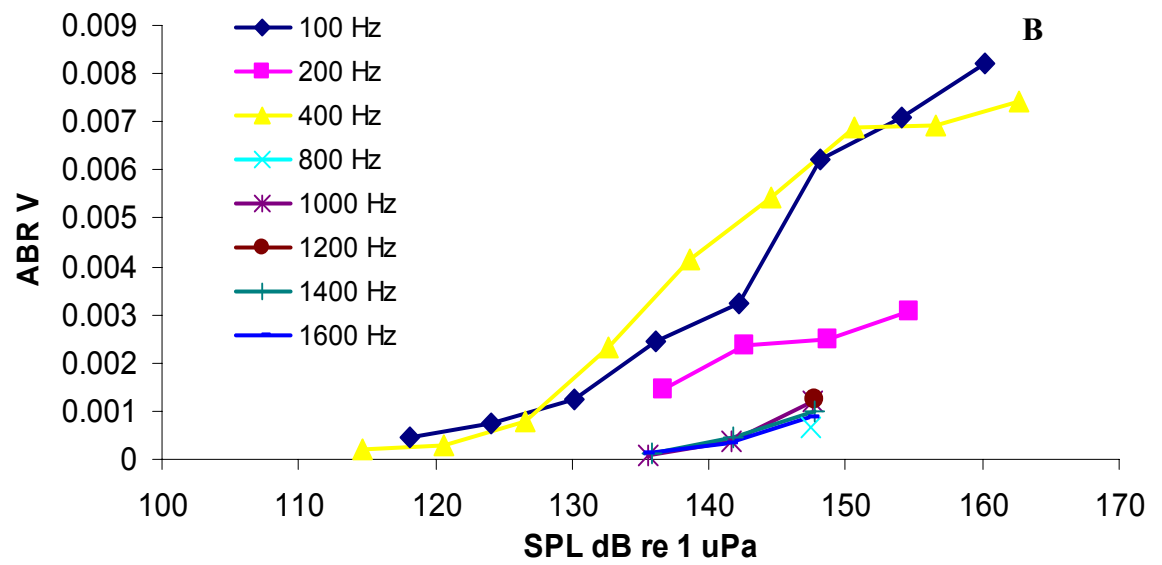
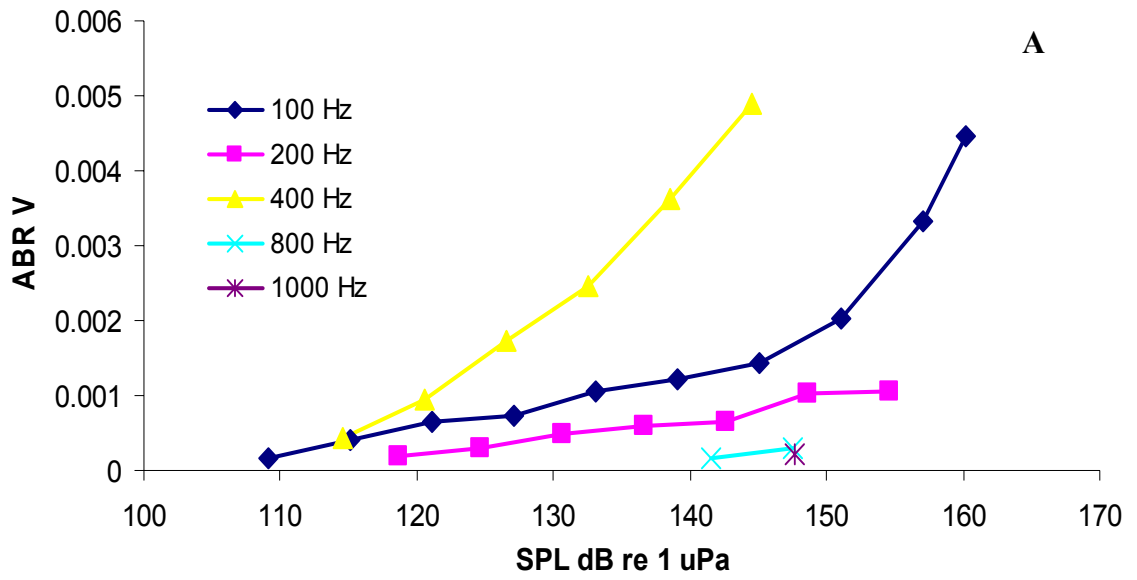


Figure 5 Average audiogram for all fish at each tested frequency with standard deviation of thresholds indicated by the bars (n=32).

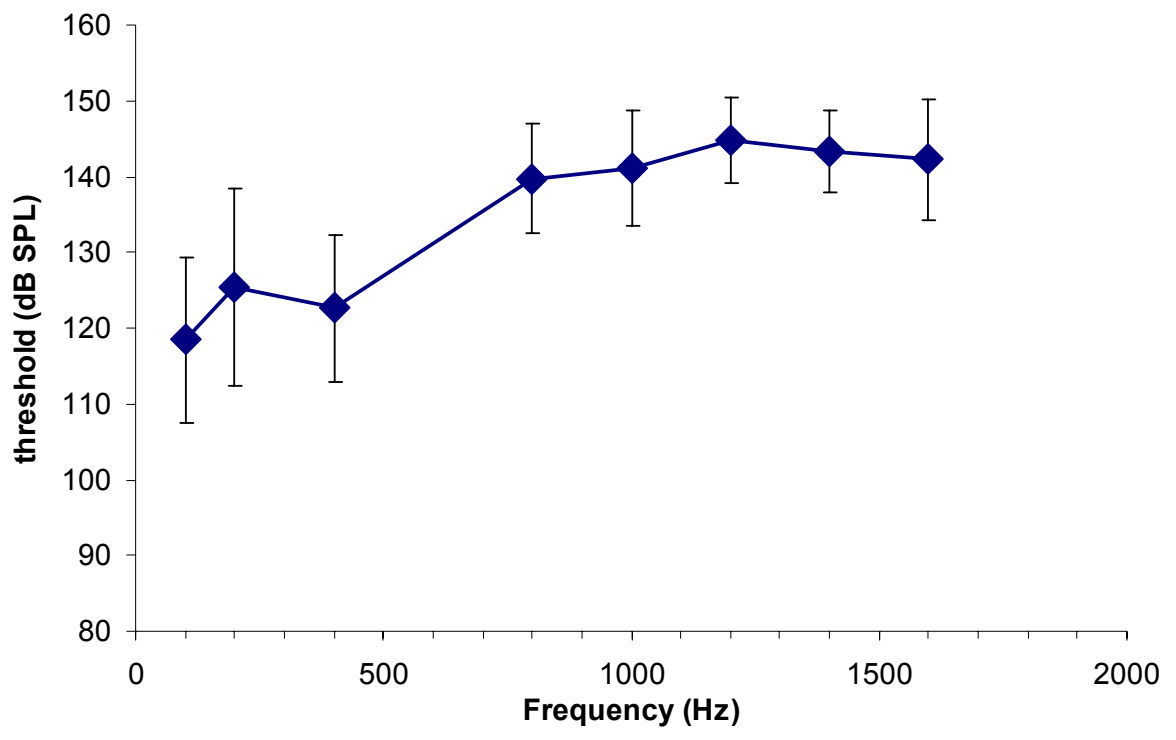


Figure 6 Average audiograms for the three size groups of fish. The threshold sound level for each fish at each frequency for which there was a response was averaged, within size groups, to determine the threshold level at each frequency.

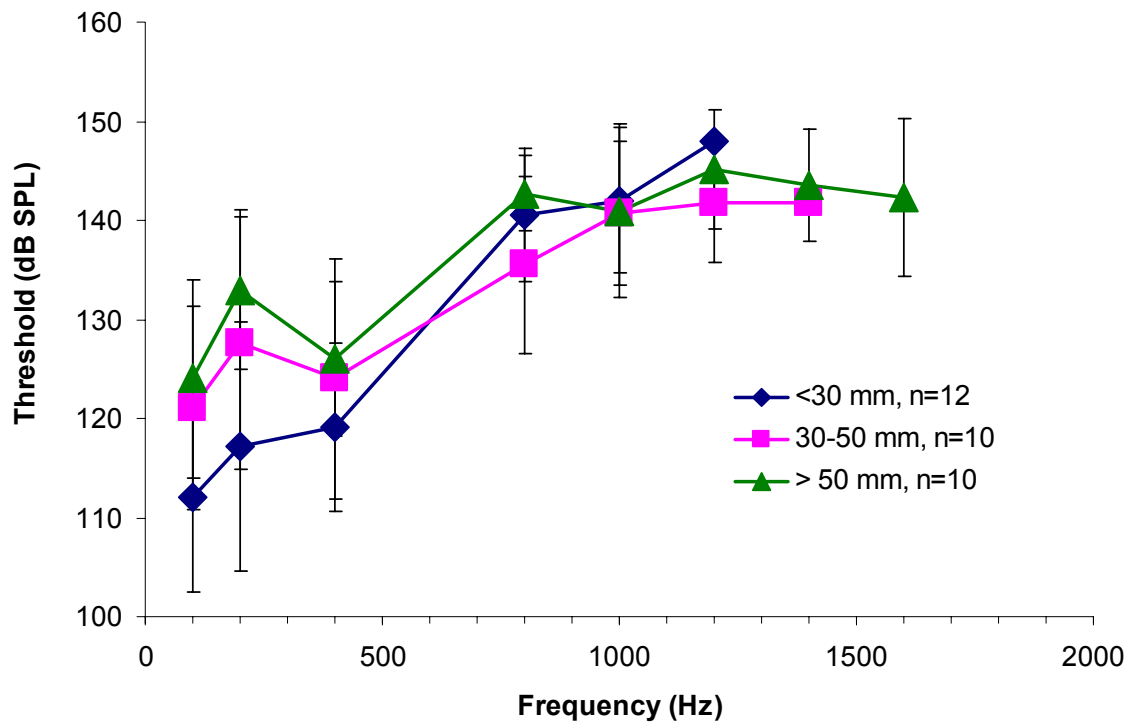
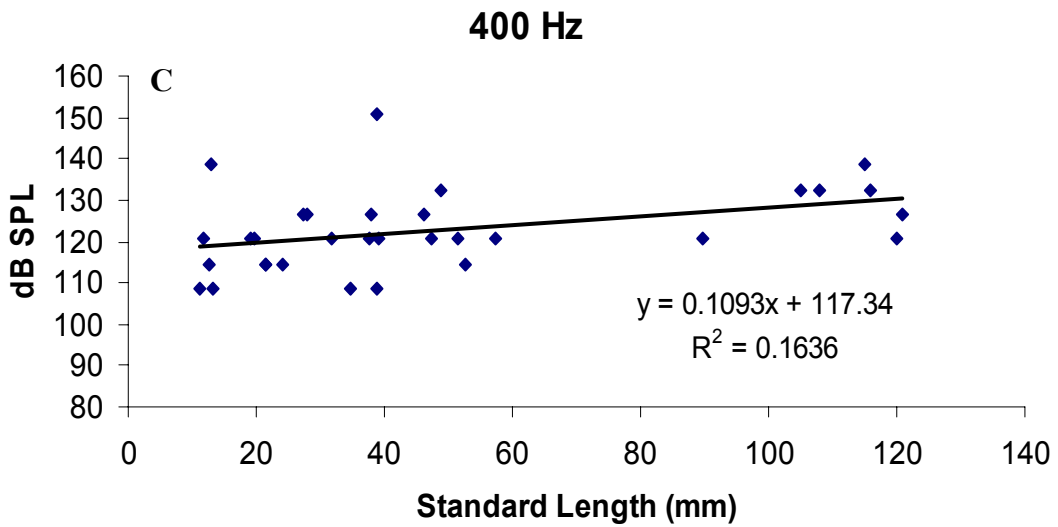
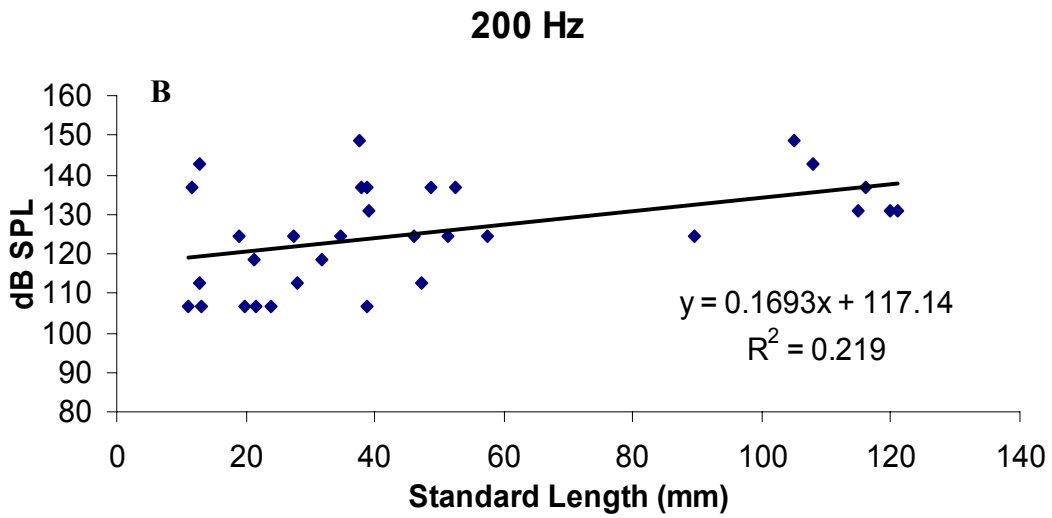
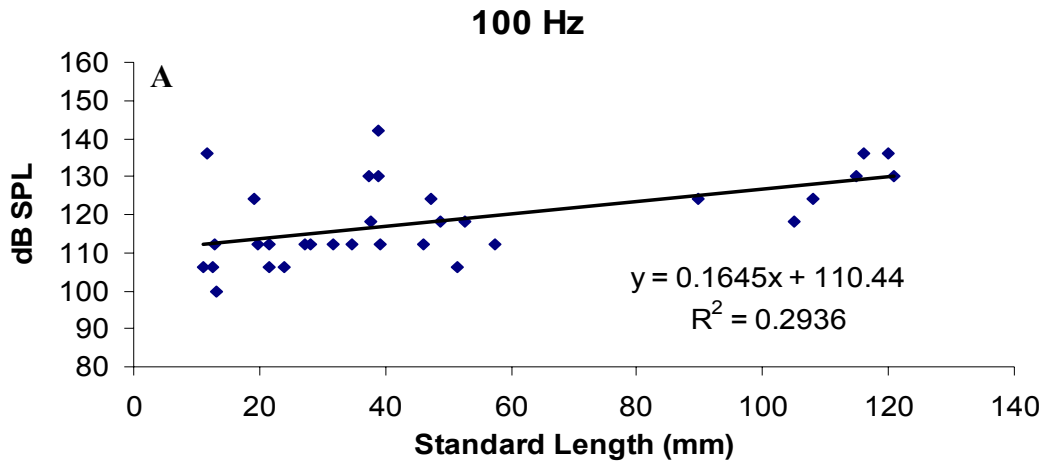


Figure 7 (A-G) Regression lines for frequencies tested with equations and r^2 values indicated. 1600 Hz is not pictured due to the small percentage of fish from which a response was detected.



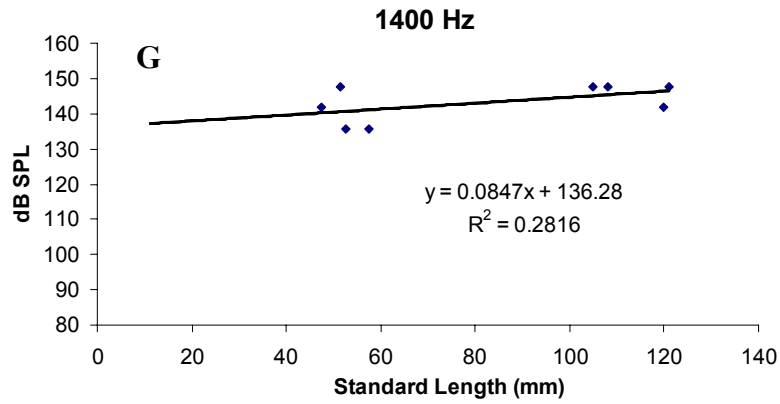
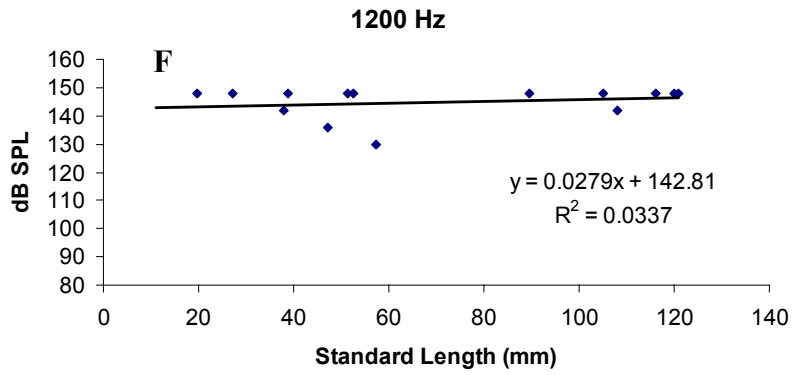
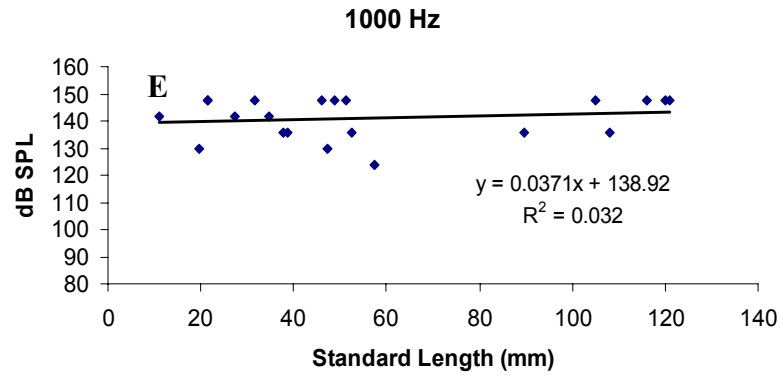
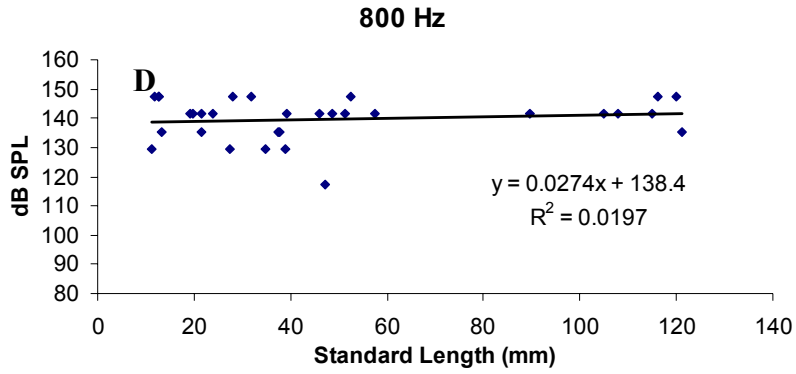


Table 3 Results of ANOVA analyzing the overall goodness of fit on the regression equation at each frequency. Responses at 1600 Hz were too few for statistical analysis (df=4). Bonferroni correction was used to determine significant values (0.05/7). Asterisks denote values considered significantly different ($P < 0.007$).

	Sums of Squares	Degrees of freedom	Mean Squares	P value
100 Hz				
Regression	1083	1	1083	0.001*
Residual	2605	30	86.83	
Total	3688			
200Hz				
Regression	1147	1	1147	0.006*
Residual	4090	30	136.4	
Total	5238			
400 Hz				
Regression	478.4	1	478.4	0.022
Residual	2445	30	81.52	
Total	2924			
800 Hz				
Regression	30.07	1	30.07	0.451
Residual	1496	29	51.58	
Total	1526			
1000 Hz				
Regression	36.65	1	36.65	0.438
Residual	1107	19	58.30	
Total	1144			
1200 Hz				
Regression	13.96	1	13.96	0.530
Residual	400.0	12	33.34	
Total	414.0			
1400 Hz				
Regression	55.75	1	55.75	0.176
Residual	142.25	6	23.71	
Total	198.0			

value at 1400 Hz was lower ($p=0.176$), though not significant. It should be noted that there was a small number of responses detected at this frequency ($df=7$). All of these fish except for one (SL=47.3 mm) are in the largest size group. Effects of size on hearing ability are much more apparent at the lower frequencies tested (100-400 Hz).

In addition, the frequency range detected by the fish appears to be a function of the fish length (Figure 8). The larger fish were more likely to respond to higher frequency sounds. Almost 100% of the tested fish responded to sound presented from 100-800 Hz. As the frequency increased above 800 Hz, the number of smaller fish with a response decreased. A two-sided difference test looking at the difference of proportions of responses detected between the three groups at each frequency at the highest sound level, found significant differences at frequencies 1000-1600 Hz (Table 4).

Figure 8 Percent of individuals in each size group that detected sound at each frequency at the maximum sound level that could be generated.

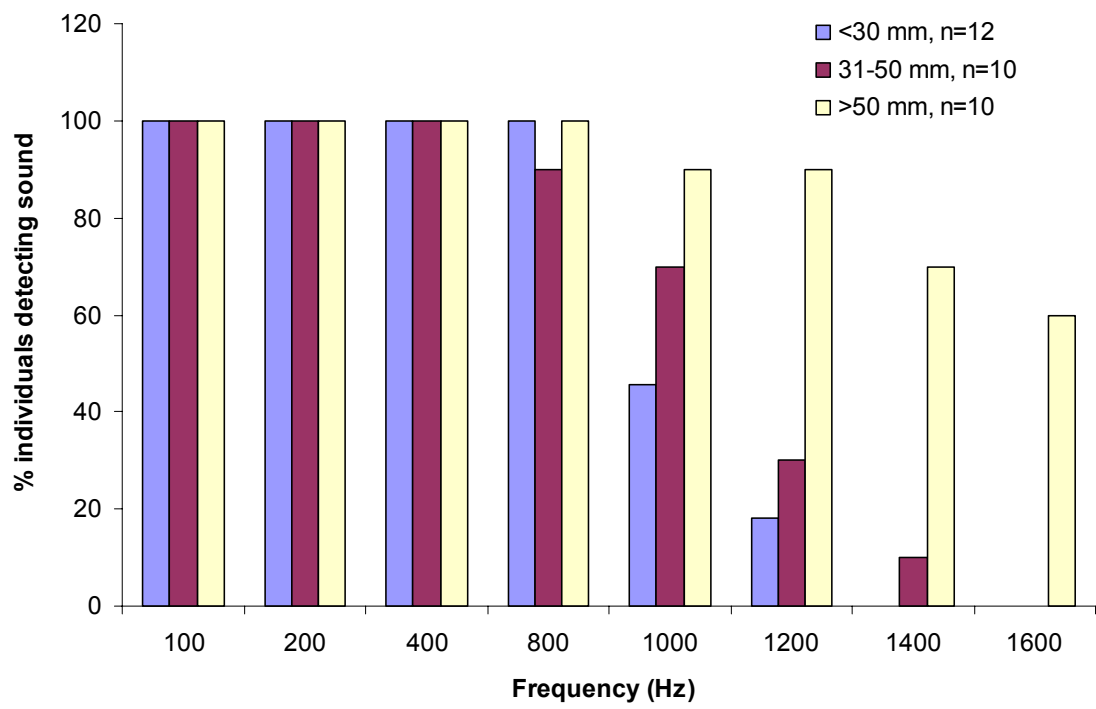


Table 4 Two-sided difference test for significance of differences between proportion of responses at the loudest sound level presented at each frequency. Asterisk denotes values considered significantly different ($p < 0.05$).

	1000 Hz	1200 Hz	1400 Hz	1600 Hz
Groups 1 and 2	0.204	0.478	0.350	
Groups 2 and 3	0.278	0.014*	0.014*	0.010*
Groups 1 and 3	0.030*	0.003*	0.003*	0.006*

DISCUSSION

Auditory sensitivity of sergeant majors

The data from this study suggests that sergeant majors hear lower frequencies (100-400 Hz) at lower thresholds than higher frequencies (800-1600 Hz). Overall, however, they have poor and would be classified as hearing generalists (Figure 9 and 10). This classification is expected because, though 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 1980, Myrberg et al. 1986). Figure 9 shows the sergeant major audiogram with audiograms of two known hearing specialists, the goldfish (Cyprinidae, *Carassius auratus*) and the soldierfish (Holocentridae, *Myripristis kuntee*). The most sensitive frequency for the sergeant major is 100 Hz at 118 dB while the thresholds for the most sensitive frequency for the soldierfish is 50 dB (1000-1600 Hz), and 51 dB for the goldfish (500 Hz) The frequency range detected is greater in the hearing specialists as well.

Only two reef fish families, Chaetodontidae and Holocentridae, have been classified as hearing specialists. Chaetodontids have a laterophysic connection, a unique connection between the lateral line and the ear, which is believed to enhance hearing sensitivity (Webb 1998; Webb and Smith 2000). The Holocentridae have a range of morphologies from close apposition of the anterior end of the swim bladder to the inner ear to essentially no apparent specialization (Coombs and Popper 1979). Perhaps in a loud environment such as a reef, hearing specializations are not as necessary, or hearing specializations in untested families are yet to be found.

Figure 10 shows the audiogram produced for the sergeant majors along with other teleost species of the coral reef classified as hearing generalists. ABR is considered more conservative in judging threshold levels than the classical behavioral approaches that

Figure 9 Sergeant major audiogram with audiograms of two hearing specialists, the goldfish (*Carassius auratus*) (Popper, 1971) and soldierfish (*Myripristis kuntee*) (Coombs and Popper 1971).

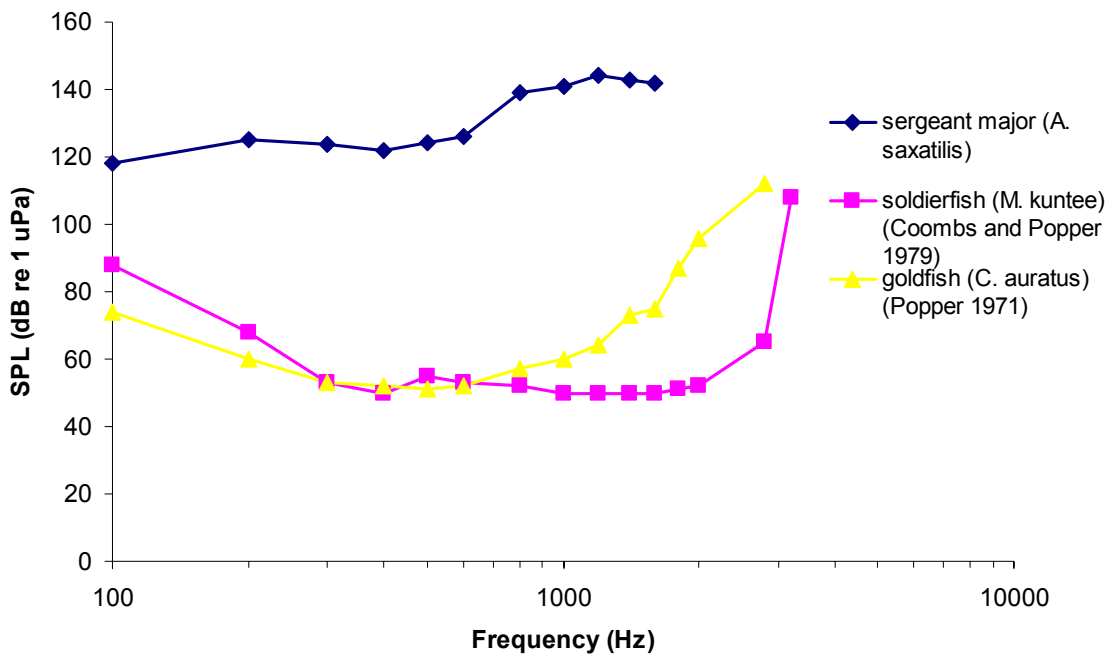
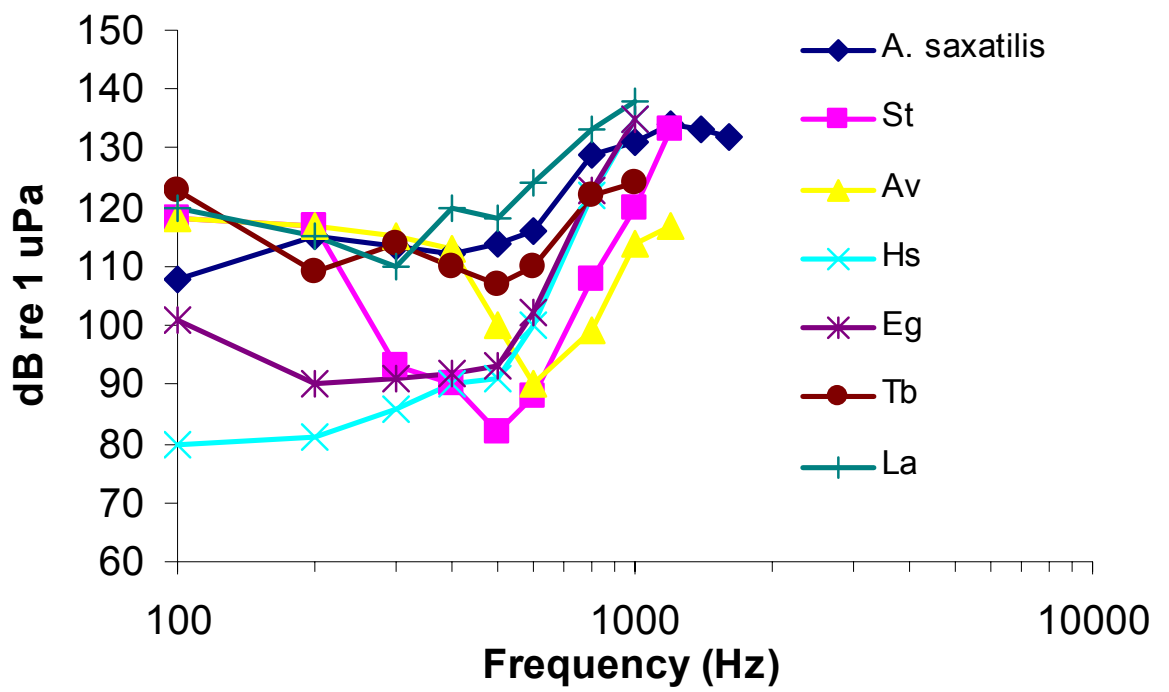


Figure 10 Audiogram of sergeant majors (thresholds adjusted for comparisons by subtracting 10 dB) and other hearing generalists. St: average for 6 species of *Stegastes* (Myrberg and Spires 1980); Av: *Adioryx vexillarius*, Hs: *Haemulon sciurus*, Eg: *Epinephalus guttatus*, Tb: *Thalassoma bifasciatum*, La: *Lutjanus apodus* (Tavolga and Wodinsky 1963).



were used to create the other audiograms (Kenyon et al. 1998). ABR is a relatively new technique for determining thresholds for fish, however, work with humans has demonstrated that auditory thresholds determined using tone-burst ABR are generally higher, by 10-20 dB, than those obtained using behavioral methods (Kenyon et al. 1998, Gorga et al. 1988). Kenyon et al. (1998) found no significant difference in thresholds determined using ABR and those found with behavioral methods but they did see that ABR thresholds were generally higher than behavioral values below 1500 Hz. This was taken into account for comparisons in this study by subtracting 10 dB from the threshold levels found for the sergeant major.

Thresholds for the sergeant majors are still comparatively high. The adjusted threshold at the most sensitivity frequency of the sergeant major (108 dB, 100 Hz) is above the majority of the other species' most sensitive frequency (*Stegastes* [average of six species]: 82 dB, 500 Hz; *Adioryx vexillarius*: 90 dB, 600 Hz; *Haemulon sciurus*: 80 dB, 100 Hz; *Epinephalus guttatus*: 90 dB, 200Hz; *Thalassoma bifasciatum*: 107 dB, 500 Hz; *Lutjanus apodus*: 110 dB, 300 Hz). It should be noted that the frequency range detected by the sergeant major is greater than for most of the other species represented, however, this is most likely a limitation of the maximum sound levels used in the other studies.

In comparison to other Pomacentrids, sergeant major thresholds are especially high. Despite no known auditory specializations, many Pomacentrid species have been found to have low hearing thresholds. Sergeant majors are not as vocal as many other members of the Pomacentrid family. This species is also not as territorial as other damselfish such as the bicolor damselfish (*Stegastes partitus*) (Myrberg 1997). 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). Sergeant majors are likely to be soniferous during the spawning season, not only due to the territorial behavior that occurs, but even more because they have been observed performing the "signal jump" during courtship (Prappas et al. 1991). The male courtship display in damselfishes is known as the signal jump, where the male rises in the water column and swims down rapidly while producing a

pulsed sound anywhere in the frequency range of 100-1000 Hz, depending on the species (Fishelson 1964, Myrberg 1972, Spanier 1979, Lobel and Mann 1995).

It is thought that *Abudefduf sordidus* do not rely on sound during courtship as much as other Pomacentrids such as *Dascyllus* and *Stegastes* spp. (Lobel and Kerr 1999). The courtship associated sounds produced by the *A. sordidus* were highly variable in pulse and number length, making courtship sounds appear rudimentary in comparison to the repetitious acoustic display exhibited by other Pomacentrids.

Studies on the propagation of fish sounds in shallow water suggest that they attenuate greatly over short distances (Mann and Lobel 1997, Rogers and Cox 1988, Forrest et al. 1993). Due to attenuation and background noise levels, damselfish sounds are thought to be used over distance less than 11-12 m (Mann and Lobel 1997). The study on *A. sordidus* found that courtship associated behavior of this species could be detected only within two meters (Lobel and Kerr 1999). The threshold levels of this species are probably low enough to detect sounds produced by neighboring fish.

The most sensitive frequency for *Stegastes* is 500 Hz (Figure 10). This has adaptive value because the peak frequency of *Stegastes* sound falls within a frequency band centered around 500 Hz (Kenyon 1996). The most sensitive frequency for the sergeant major was determined to be 100 Hz. Further study on sergeant major sound production could determine whether the most sensitive frequency of 100 Hz has adaptive significance as well. A study on *Abudefduf luridus* found the most common frequencies produced to be in the frequency range of 50-500 Hz, with most of the energy concentrated on the lower frequencies (Santiago and Castro 1997). This data correlates well with what was found in this study.

Effect of size on the auditory sensitivity of the sergeant major

Size of fish significantly affected auditory sensitivity and the frequency range the fish was able to detect. At the lower frequencies (100-200 Hz), thresholds increased with an increase in length. In addition, the larger fish more readily responded to the higher frequencies (800-1600 Hz) at a significant level (Table 4). However, the most sensitive frequency was 100 Hz for each size group.

These results are in contrast to a study on the bicour damselfish (*Stegastes partitus*), which found an exponential decrease in threshold with an increase in size (Kenyon 1996) (Figure 11). All size groups responded to sounds in the frequency range of 300-1500 Hz. The most sensitive frequency at 500 Hz remained the same in each size group.

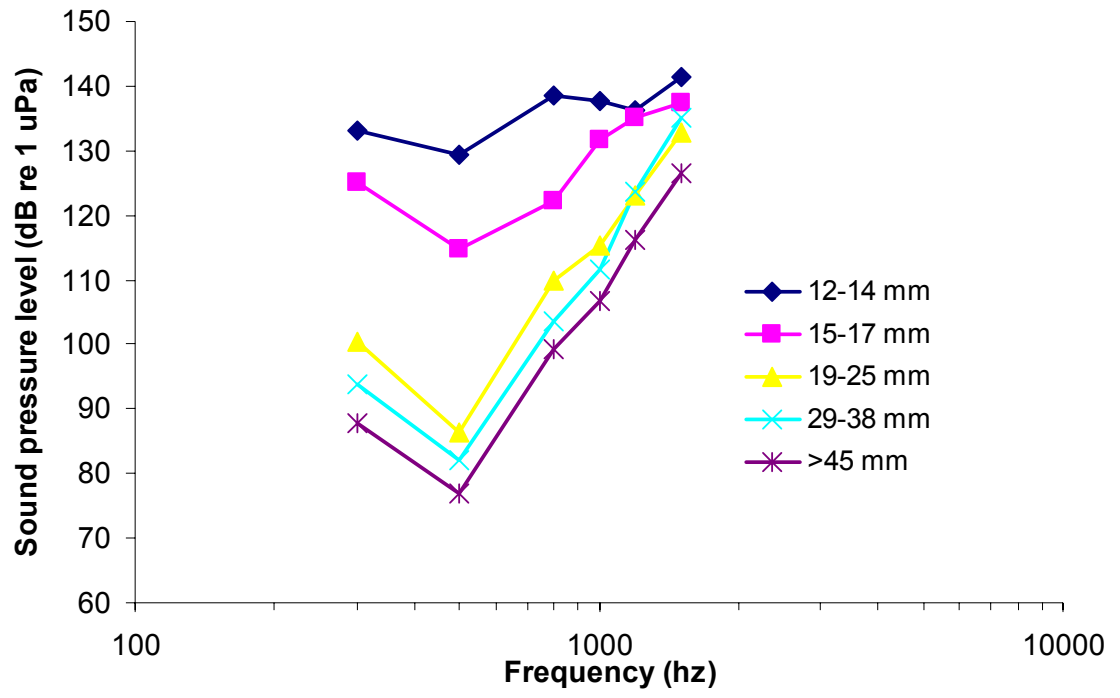
Popper (1971) found that there was no difference in audition between two size groups of goldfish (*Carassius auratus*). Kenyon (1996) notes that the difference in results may be due to the physiological differences between the hearing specialist goldfish and the hearing generalist bicour damselfish (*S. partitus*). Another explanation may be that only two size groups of goldfish were tested (Kenyon 1996, Wysochi and Ladich 2001).

It is important to note that the methods used by Kenyon, Popper and in this study are different. Rather than the auditory brainstem response technique, Kenyon (1996) conducted psychophysical experiments where the fishes were trained to associate sound with an electric shock. The fish eventually showed an avoidance response with the onset of detectable sound. Popper (1971) used a similar behavioral shock conditioning technique where the fish were shocked if they did not swim across a barrier in response to a sound. As mentioned previously, ABR is thought to be more conservative in determining thresholds (Kenyon 1998).

It is interesting that at the lower frequencies, where length appeared to be a factor in the sound level the fish was able to detect, threshold levels actually increased with an increase in size. 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 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 likely closer to the brain which may have caused a larger ABR response in some of the smaller fish compared to larger fish.

Figure 11 Average audiograms of four size groups of juveniles (12-14 mm: n=5, 15-17mm: n=6, 19-25 mm: n=7, 29-38 mm: n=6) and adult *Stegastes partitus* (n=8). (Redrawn from Kenyon 1996).



Though Kenyon (1996) and Popper (1971) 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 and 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. The role of the swim bladder in hearing generalists is not well understood, but due to the fact that there is no connection between the swim bladder and the otolith in the sergeant major, it is unlikely that any increase in size of the swimbladder would affect audition (Yan et al. 2000).

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 and 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. Perhaps the ongoing addition of the sensory hair cells on the sensory epithelium of the otolithic organs and the exact placement of the new sensory hair bundles plays a role in the frequencies the fish can detect.

It should be noted that the differences in hearing range could partly be a reflection of the maximum sound level that could be generated by the system. Perhaps if the speaker were capable of broadcasting the higher frequencies at a higher sound level, a response could have been detected from the smaller fish at the higher frequencies.

Use of reef sound as a navigational cue for pelagic larvae

In order for larvae to detect a reef from a few kilometers away they need to hear reef sound levels below approximately 80 dB re: $1 \mu\text{Pa}/\sqrt{\text{Hz}}$, and to recognize these sounds against broadband background noise (Montgomery et al. 2001). The majority of sound from the reef is the result of snapping shrimp (Leis et al. 2002) and fish choruses (Cato 1992). The data in this study suggest that it is highly unlikely that larval sergeant majors use coral reef noise as a navigational cue (Figure 12).

To determine the possibility of the use of sound as a navigational cue, it is important to consider the distance a fish must be able to detect the sound. McCauley (1997) measured the chorus spectra from inshore fish calling that were recorded at 4.3 km from Feather Reef (Queensland, Australia). This spectra is redrawn in figure 12 in comparison to the audiogram of smallest size group of sergeant majors. Recordings done off of Lizard Island, Great Barrier Reef show that at 75 meters from the reef some of the clicks of the broadcast sounds are evident above the background noise (Leis et al. 2002). At 160 meters, only an occasional click is evident above the background noise. Pelagic larvae are tens to hundreds of kilometers from the reef (Cowen 2002). Judging from the data collected from this study in comparison to recorded reef sounds from other sources, it is more likely the fish use sound and their auditory sense in close-range orientation.

Though Kenyon's (1996) study on the hearing of damselfish juveniles supports the idea that the larvae cannot use sound as a navigational cue, studies have been conducted which suggest a role for audition in settlement (Tolimieri et al. 2000, Leis et al. 2002, 2003) Tolimieri and Leis both found that significantly more larvae of specific taxa (Tolimieri: Tripterygiidae, Leis: Apogonidae, Mullidae, Pomacentridae, Serranidae and Sphyrinae) were caught in light traps broadcasting recorded reef sounds than in "silent" traps (Tolimieri et al. 2000, Leis et al. 2003). It is important to consider, however, that the distance the larvae were coming from was unknown. All of the trapped larvae could have arrived from within a few meters, where particle velocities would be higher than at greater distances.

McCauley's (1997) data on a reef chorus measured 4.3 km from the Great Barrier Reef was used to determine the farthest distance from which juvenile sergeant majors can detect reef sounds (Figure 13). The equation $\text{Transmission Lost} = 20 \log r$ (r =distance), which assumes spherical spreading, was used to determine that 6 dB was lost per distance doubled. The farthest distance from which sergeant major juveniles can detect a reef chorus was approximated to be about .54 km from the reef. The farthest distance from which a juvenile fish in this study would be able to detect this reef chorus is 2.15 km.

Another consideration when studying sound detection as a navigational cue is the ability of the fish to localize the sound. This study looked only at the ability of the fish to detect certain frequencies and sound levels. Even if the fish do have the ability to detect the sound from the pelagic environment, they must be able to determine the direction the sound is coming from.

A study on the black axil chromis (*Chromis atripectoralis*) was conducted in which reefs sounds were broadcasted to larvae released 50-100m from the speaker (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 the fish had the ability to localize the sound.

Due to the high speed of sound in water and the small distance between the ears of fish, fish are presumably unable to use interaural time, phase and intensity differences of sound pressure to localize a sound source like vertebrates in air (Fay and Feng 1987). However, localization has been demonstrated in some adult fishes including Pomacentrids (Myrberg and Spires 1980, Myrberg et al. 1986). Different ciliary bundle types are found in different regions of the maculae (Myrberg and Fuiman 2002). There is evidence for the ability of a fish to determine the direction of a sound source depending on which part of the macula is most stimulated (Platt and Popper 1984, Enger 1976, Popper 1977, Lu and Popper 1998). The theoretical problem with this is that a 180° ambiguity results from the oscillations of the hair cells. In order to use directional information from reef sound, larvae need to be able to hear directionally and resolve the 180° ambiguity.

Although sound may be unlikely as the sole navigational cue for the reef fish larvae, it is probable that the return to the reef from the pelagic environment is not by chance (Leis and McCormick, 2002). Most pelagic larvae do not settle on the first reef they come upon. If reefs were difficult to locate from open water, most larvae would not leave a reef once it was found (Leis and Carson-Ewart 1998, 1999). In addition, it has been observed that settlement stage larvae in open water tend to have roughly linear

Figure 12 Audiogram of smallest group size group of sergeant majors (11-30mm), with threshold range at each of the tested frequencies indicated by the bars, in comparison to spectrum level ambient reef noise; morning and night choruses (Cato 1980), snapping shrimp (Tavolga 1974), chorus spectra from inshore reef calling recorded 4.3 km from Feather Reef (Queensland, Australia) (redrawn from McCauley 1997). Sergeant major audiogram was altered for comparison purposes, taking into account a frequency bandwidth of 10% of test frequency.

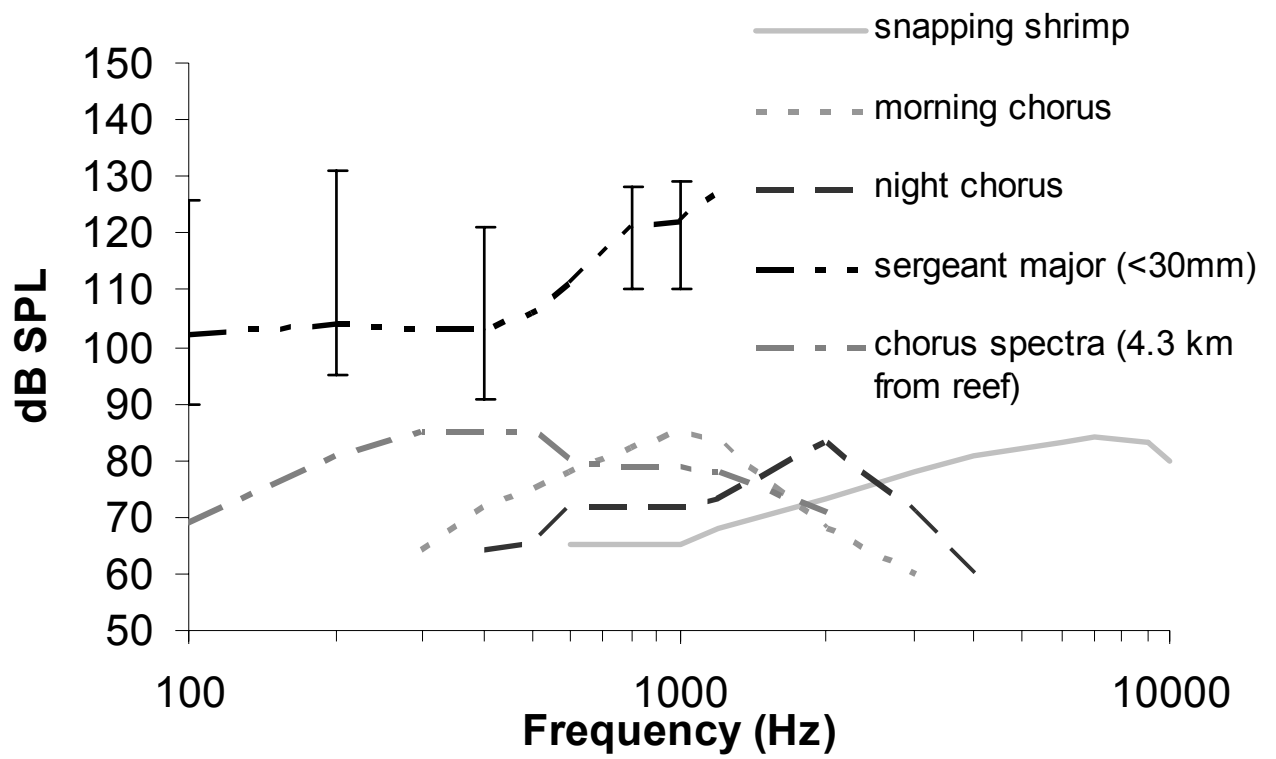
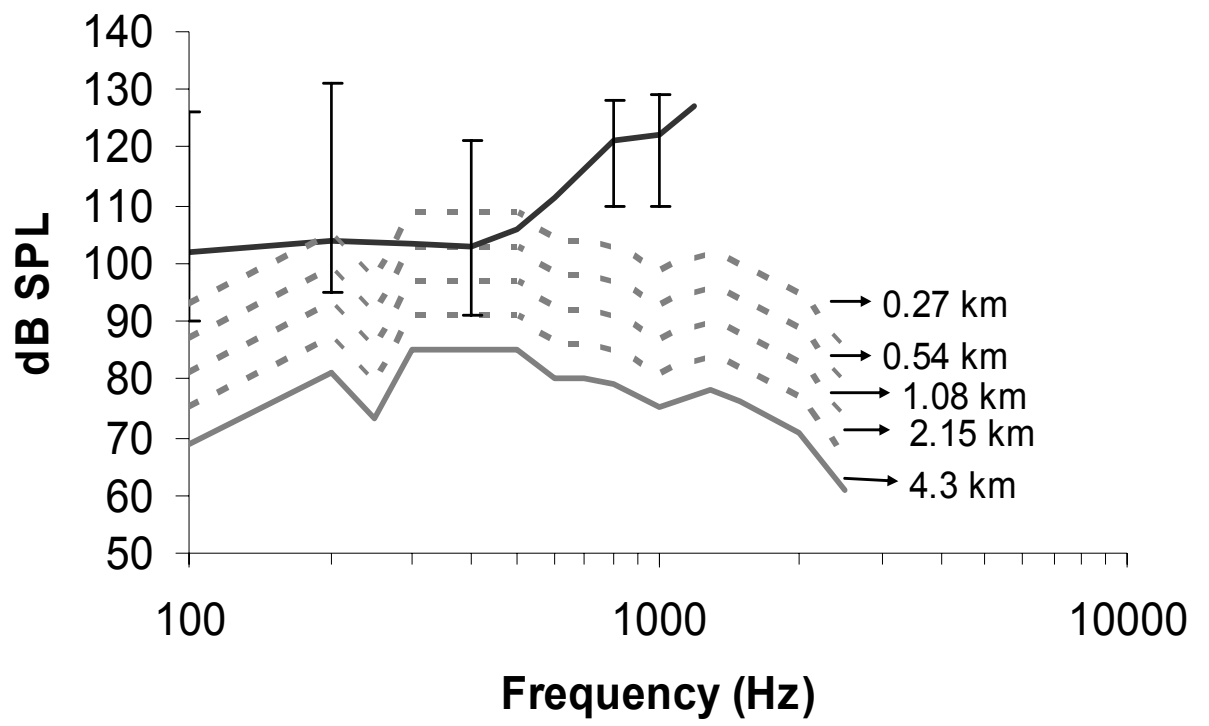


Figure 13 Sounds of a reef chorus spectra measured 4.3 km from the reef (McCauley 1997) and the audiogram of Group 1 (<30mm) were used to approximate the distance the juvenile fish in the study would be able to detect the sounds. Sergeant major audiogram was altered for comparison purposes, taking into account a frequency bandwidth of 10% of test frequency. The bars indicate threshold range for the 12 fish in the smallest size group of sergeant majors. The equation $\text{Transmission Lost} = 20 \log r$ (r =distance), which assumes spherical spreading, was used to determine that 6 dB is lost per distance doubled.



horizontal trajectories, or at least trajectories that are significantly different from random (Leis et al 1996, Leis and Carson-Ewart 1999, 2001).

Armsworth (2000) modeled the swimming responses of weak and strong swimming larvae reacting to current dependent and current independent cues emanating from a reef. Current dependent cues include odor, while current independent would include sound. He concluded that larval swimming and sensory abilities are far more important than circulation in determining enhancement of settlement. Rather than audition and sound from the reef being the sole cue for pelagic larval fish to follow, it is more likely that the larvae use a combination of their sensory organs to navigate their return to the reef. Vision is obviously important over small scales (5-15 m) (Leis and 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 tens of meters (Sweetman 1988, Elliot et al. 1995, Arvedlund et al. 1999, Atema et al. 2002). Other possible cues include differences in wind or wave induced turbulence, gradients in abundance of fish, plankton or reef detritus and differences in temperature of lagoonal or reef flat water flowing from a reef (Leis and McCormick 2002). Cues may vary with ontogeny and with distance from the reef. The use of cues is most likely different among species depending on their sensory abilities.

Though most likely quite complex, understanding the navigational process of larval reef fish on their return to the reef is important for the many groups exploiting reef fish populations and for managing reef fisheries. The coral reef is a highly exploited ecosystem and an understanding of the supply rate of larvae to coral reefs is critical in determining the structure of coral reef populations (Doherty and Williams 1988, Doherty and Fowler 1994, Armsworth 2000). Studies such as this one which provide information on the sensory capabilities of larval reef fish offer insight on population connectivity and are important to management decisions such as the design of marine protected areas.

Future directions

This study provided important hearing data on one of the less vocal and less studied, though very abundant, damselfishes. The differences between the hearing

abilities of this particular Pomacentrid species in comparison to other species in the same family are interesting due to the large variation. More audiograms should be produced for other Pomacentrids and more *Abudefduf* species to see if a similar pattern of higher thresholds with a greater frequency range exists for large versus small fish. The higher sensitivity to low frequency sounds of juveniles in comparison to the adults is different than what has been found in previous ontogenetic studies. The production of more audiograms for coral reef fish in general is important because the reef is such a noisy habitat and a highly exploited environment.

Determining the frequency range and sound levels a newly settled juvenile fish can detect is the first step in understanding the use of audition as a guide for larval fish. The auditory brainstem response technique is a rapid method for determining the hearing abilities of a fish. A better understanding of how ABR thresholds are related to behavioral thresholds would be beneficial. In addition, other factors must be considered such as the distance from which the fish must be able to detect the sounds and the ability of a larval fish to localize the sound. More measurements of reef sounds as a function of distance from the reef, particularly in the Florida Keys where the fish in this study were collected, would be beneficial for comparisons with fish hearing ability. In addition, more recordings need to focus on lower frequency sounds from the reef such as breaking waves.

According to the data from this study it is not likely that reef noise is used by pelagic larval sergeant majors as the only source for navigating their way to a reef for settlement. Further study on auditory capabilities of newly settled juveniles, or possibly larval fish, of many more species is important in fully understanding ontogenetic changes in audition and the possibility of using sound as a guide.

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