

Acoustic pressure and particle motion thresholds in six sciaenid fishes

Andrij Z. Horodysky^{1,*}, Richard W. Brill², Michael L. Fine³, John A. Musick¹ and Robert J. Latour¹

¹Department of Fisheries Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062, USA, ²Cooperative Marine Education and Research Program, Northeast Fisheries Science Center, National Marine Fisheries Service, NOAA, Woods Hole, MA, USA and ³Department of Biology, Virginia Commonwealth University, Richmond, VA, USA

*Author for correspondence (e-mail: andrij@vims.edu)

Accepted 5 March 2008

SUMMARY

Sciaenid fishes are important models of fish sound production, but investigations into their auditory abilities are limited to acoustic pressure measurements on five species. In this study, we used auditory brainstem response (ABR) to assess the pressure and particle acceleration thresholds of six sciaenid fishes commonly found in Chesapeake Bay, eastern USA: weakfish (*Cynoscion regalis*), spotted seatrout (*Cynoscion nebulosus*), Atlantic croaker (*Micropogonias undulatus*), red drum (*Sciaenops ocellatus*), spot (*Leiostomus xanthurus*) and northern kingfish (*Menticirrhus saxatilis*). Experimental subjects were presented with pure 10 ms tone bursts in 100 Hz steps from 100 Hz to 1.2 kHz using an airborne speaker. Sound stimuli, monitored with a hydrophone and geophone, contained both pressure and particle motion components. Sound pressure and particle acceleration thresholds varied significantly among species and between frequencies; audiograms were notably flatter for acceleration than pressure at low frequencies. Thresholds of species with diverticulae projecting anteriorly from their swim bladders (weakfish, spotted seatrout, and Atlantic croaker) were typically but not significantly lower than those of species lacking such projections (red drum, spot, northern kingfish). Sciaenids were most sensitive at low frequencies that overlap the peak frequencies of their vocalizations. Auditory thresholds of these species were used to estimate idealized propagation distances of sciaenid vocalizations in coastal and estuarine environments.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/211/9/1504/DC1>

Key words: animal communication, hearing, particle acceleration, particle velocity, Sciaenidae, soniferous.

INTRODUCTION

Sound in water is composed of two physically linked components, propagating scalar pressure waves and directional particle motion, which differ in the pathways through which they reach the inner ears of fishes (Fay and Popper, 1975). The otoliths of all fishes are biological accelerometers that directly detect the particle motion components of sound as a result of inertial differences between sensory epithelia and otoliths (Lu and Xu, 2002; Popper and Fay, 1999). Additionally, the pressure component of sound may be detected indirectly by some fishes *via* accessory anatomical structures that transform sound pressure waves into particle displacements (Popper and Fay, 1993).

Fishes are categorized as hearing 'specialists' and 'generalists' on the basis of anatomy, the ability to detect the pressure component of sound, and the range of detectable bandwidth. Hearing specialist species have evolved projections of the swim bladder or skeletal connections that enable the indirect re-radiation of the pressure component of sound as particle displacement capable of stimulating the inner ear (Fay and Popper, 1974; Popper and Fay, 1999). Thus hearing-specialist fishes, which include groups such as clupeids, otophysans, mormyrids and osphronemids, may use both direct (particle motion) and indirect (pressure transduction) mechanisms to enhance their hearing sensitivity and extend their detectable auditory bandwidth (Mann et al., 1997; Popper and Fay, 1993; Yan, 1998; Yan and Curtsinger, 2000). By contrast, hearing generalist fishes lack such specialized structures coupling pressure-to-displacement transducers to the otic capsule, resulting in attenuation of the signal and reduced stimulation of the ear *via* sound pressure

(Casper and Mann, 2006). The unaided organs of the inner ear of hearing generalists are thought to be fairly insensitive to the indirect transduction of sound pressure (Sand and Karlsen, 2000; Yan et al., 2000); direct particle motion stimulation of the otoliths is likely more relevant to these fishes (Lu and Xu, 2002; Casper and Mann, 2006). However, few studies have examined the hearing thresholds of fishes with respect to both pressure and particle motion sensitivity (Myrberg and Spires, 1980; van den Berg, 1985; Lovell et al., 2005; Casper and Mann, 2006).

Sciaenid fishes are model organisms of teleost bioacoustics (Ramcharitar et al., 2006a; Roundtree et al., 2006), but comparatively little is known about their auditory abilities. Sciaenid saccular otoliths are enlarged relative to most fishes, and their morphology and proximity to the swim bladder vary widely (Chao, 1978; Ramcharitar et al., 2001). Both hearing specialists and generalists have been identified within the family (Ramcharitar et al., 2004; Ramcharitar et al., 2006b). Unfortunately, the pressure detection abilities of less than two percent of the 270 sciaenid species have been described [Atlantic croaker, spot, weakfish, black drum, silver perch (Ramcharitar, 2003)], and the particle motion sensitivity of these fishes has not been examined. Comparative work on sciaenid fishes has great potential to elucidate form-and-function relationships in the teleost auditory system (Ramcharitar, 2003). We therefore performed auditory brainstem response experiments using a hydrophone and geophone to categorize the pressure and particle acceleration detection thresholds of six sciaenid fishes. The simultaneous recording of the pressure and particle motion

components of sound stimuli allowed us to express audiograms with respect to both. The former allows us to compare our data to previously published results for sciaenid fishes (Ramcharitar and Popper, 2004; Ramcharitar et al., 2006b); the latter allows comparison to recent studies examining particle motion thresholds in other fishes (Casper and Mann, 2006; Mann et al., 2007).

MATERIALS AND METHODS

Experimental animals and design

Weakfish (*Cynoscion regalis* Bloch and Schneider 1801), spotted seatrout (*Cynoscion nebulosus* Cuvier 1830), Atlantic croaker (*Micropogonias undulatus* Linnaeus 1766), red drum (*Sciaenops ocellatus* Linnaeus 1766), spot (*Leiostomus xanthurus* Lacepede 1802) and northern kingfish (*Menticirrhus saxatilis* Bloch and Schneider 1801) were captured in Chesapeake Bay, eastern USA, using hook and line (Table 1). Animals were maintained in recirculating 1855 l aquaria at $20\pm 1^\circ\text{C}$ (winter months) or $25\pm 2^\circ\text{C}$ (summer months) and fed a combination of frozen Atlantic menhaden (*Brevoortia tyrannus*), squid (*Loligo* sp.) and commercially prepared food (AquaTox flakes; Zeigler, Gardners, PA, USA).

Experimental and animal care protocols were approved by the College of William and Mary's Institutional Animal Care and Use Committee, protocol no. 0423, and followed all relevant laws of the United States. Auditory brainstem response (ABR) experiments were conducted on six animals of each species. All subjects were sedated with an intramuscular (i.m.) dose of the steroid anesthetic Saffan (Glaxo Vet, Glaxo Vet Ltd, Uxbridge, UK; 10 mg kg^{-1}) and immobilized with an i.m. injection of the neuromuscular blocking drug gallamine triethiodide (Flaxedil; Sigma, St Louis, MO, USA; 10 mg kg^{-1}). Recording of vertebrate ABR waveforms in anaesthetized and/or immobile subjects is a common practice to minimize the obscuring effect of muscular noise on ABR recordings (Hall, 1992; Kenyon et al., 1998; Casper et al., 2003). Sedated and immobilized animals were suspended within a rectangular $61\times 31\times 16.5\text{ cm}$ Plexiglas tank using foam straps, leaving $<1\text{ mm}$ of the top of the head protruding from the water. Subjects were ventilated (1 l min^{-1}) with filtered, oxygenated, and temperature-controlled seawater ($25\pm 2^\circ\text{C}$). At the conclusion of each experiment, fishes were euthanized with a massive i.m. dose of sodium pentobarbital ($\sim 300\text{ mg kg}^{-1}$).

Auditory brainstem response

Auditory brainstem response (ABR) is a non-invasive recording of the neural activity in the eighth cranial nerve and brainstem in response to synchronized acoustic stimuli (Corwin et al., 1982; Kenyon et al., 1998). The ABR experimental setup and procedure followed that of others (Kenyon et al., 1998). A speaker (model: 40-1034, 27.5 cm in diameter, Radio Shack, Fort Worth, TX, USA), suspended in the air, was mounted 1.5 m directly above the test subject. Two platinum wire needle electrodes (model: F-E7, 10 mm

tip, Grass Technologies, West Warwick, RI, USA) were placed subdermally along the midline of each subject: the active electrode was positioned above the medulla, and the reference electrode in the dorsal musculature above the operculum. The system was grounded to the water of the experimental tank via a $6\text{ cm}\times 26\text{ cm}$ stainless steel plate. An omnidirectional hydrophone (Reson A/S, Slangerup, Denmark; sensitivity: $-211\text{ dB re: }1\text{ V}/\mu\text{Pa}$) was suspended with rubber straps 25 mm below the water surface (i.e. the depth of a subject's otic capsule) and positioned within 2.5 mm of the right opercle-preopercle margin of each subject to measure the sound pressure level of the stimulus and ambient noise.

In the absence of an anechoic chamber, all experiments were conducted in a concrete laboratory. We produced a stochastic differential white noise signal to characterize the echoes resulting from all reflective surfaces at the hydrophone positioned next to the subject. A custom Fourier/inverse Fourier transform algorithm (MATLAB version 6.5, Mathworks, Inc., Natick, MA, USA) was used to analyze these recordings and add to each frequency's pure tone stimulus the appropriate signals needed to destructively interfere with any recorded echoes. Any alteration to the sound field in the laboratory since the last echo-cancellation (i.e. movements, small changes in the tank water level, etc.) required us to re-echo-cancel before proceeding. Visual examination of stimulus waveforms recorded by the hydrophone during ABR experiments (Fig. 1) confirmed that our echo-cancelled stimuli were very similar to pure tone waveforms used in other fish hearing experiments (Kenyon et al., 1998).

A Tucker-Davis Technologies System II (TDT, Inc., Gainesville, FL, USA) and BioSig software were used to produce sound stimuli

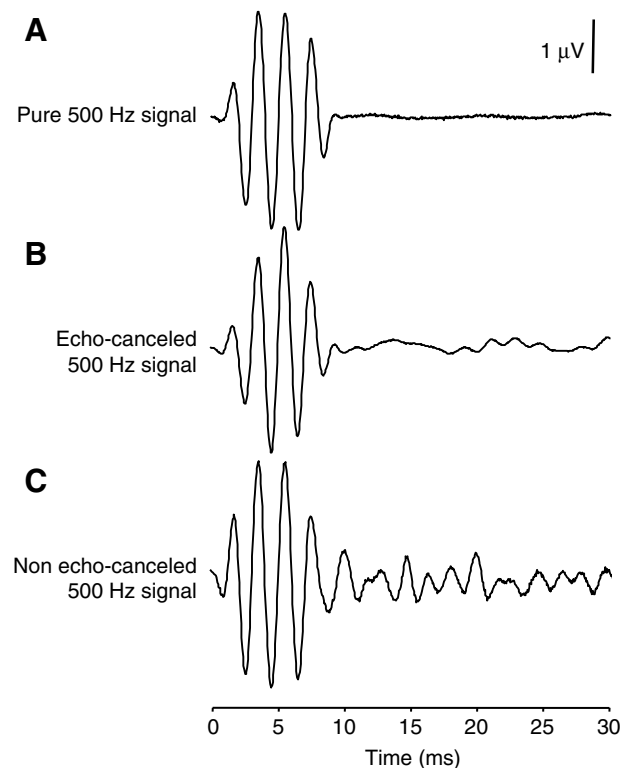


Fig. 1. Sample 500 Hz waveforms: (A) a pure tone 500 Hz stimulus waveform, (B) an echo-canceled 500 Hz stimulus and (C) a 500 Hz signal that was not echo-canceled. B and C were recorded in our experimental chamber by the submersed, omnidirectional hydrophone.

Table 1. Species, sample size, standard length (SL) and mass of the six sciaenid fishes investigated in this study

Species	N	SL (mm)	Mass (g)
<i>Cynoscion regalis</i>	6	230–315	190–460
<i>Cynoscion nebulosus</i>	6	225–515	165–730
<i>Micropogonias undulatus</i>	6	230–485	185–790
<i>Sciaenops ocellatus</i>	6	305–555	585–955
<i>Leiostomus xanthurus</i>	6	115–381	65–405
<i>Menticirrhus saxatilis</i>	6	200–305	140–325

Table 2. Particle accelerations in three orthogonal Cartesian directions and for the magnitude of the three directions combined*

Frequency (Hz)	x-axis acceleration (m s ⁻²)	y-axis acceleration (m s ⁻²)	z-axis acceleration (m s ⁻²)	Magnitude of particle acceleration (m s ⁻²)
100	0.015	0.010	0.182	0.033
200	0.018	0.061	0.578	0.370
300	0.064	0.082	1.17	1.38
400	0.080	0.096	1.01	1.04
500	0.084	0.129	0.428	0.206
600	0.113	0.109	0.670	0.473
700	0.141	0.114	0.482	0.266
800	0.168	0.125	0.510	0.304
900	0.184	0.115	0.305	0.140
1000	0.219	0.124	0.362	0.194
1100	0.218	0.206	0.413	0.260
1200	0.168	0.249	0.339	0.205

*(*sensu* Casper and Mann, 2006).

Sound pressure level (SPL) was measured by hydrophone, and mean SPLs of these recordings (in dB re: 1 μ Pa) were: x axis (116.7 dB), y axis (116.3 dB), z axis (119.7 dB). The x axis was considered to be anterior–posterior along each subject's body whereas the y axis was considered to be lateral (right–left) relative to the subject. Particle acceleration was calculated from the particle velocity measured by the geophone for stimulus acoustic sound pressures. The speaker was mounted in air 1.5 m directly above each test subject. Most of the acoustic energy was along the vertical (z) axis coming from directly above test subjects. The magnitude of particle acceleration (m s⁻²) was calculated as $\sqrt{(x^2+y^2+z^2)}$.

(10 ms stimulus tone bursts in 100 Hz steps from 100 Hz to 1.2 kHz) and record ABR waveforms. Sound bursts were gated using a Blackman window to provide a ramped onset/decay, preventing speaker transients. ABR traces were recorded twice each in two opposing polarities at each frequency and attenuation (250 sweeps each, four total recordings). The polarity of ABR response waveforms is independent of sound stimulus polarity (Kenyon et al., 1998) but the polarity of stimulus artifacts is not. ABR traces of opposite polarity were therefore summed to remove stimulus artifacts. Periodic experiments were also conducted with euthanized fish to ensure that identified ABR responses were not stimulus artifacts.

The two ABR responses at each frequency and sound pressure level were overlaid to assess the response. Sound pressure levels were successively attenuated in roughly 5 dB steps until repeatable ABR waveforms were no longer produced; thresholds were defined as the lowest sound pressure level for which a repeatable ABR trace could be identified visually (Kenyon et al., 1998). Visual threshold assignment provides results similar to quantitative threshold-seeking algorithms (Yan, 1998) and remains the standard method of threshold determination in fish ABRs (Kenyon et al., 1998; Casper et al., 2003). Visually assigned thresholds for each subject of a study species were pooled to produce mean audiograms.

Sound pressure levels of all experimental stimuli were calculated from hydrophone recordings (Burkhard, 1984). Cursors were placed one cycle apart (peak-to-peak) on either side of the largest (i.e. center) cycle of a tone-burst recording of the hydrophone (Kenyon et al., 1998). The Bio-Sig software then calculated the root mean square (RMS) of the waveform between the cursors, and the appropriate gain calibration factors were applied to determine actual sound pressure level (SPL) in dB re: 1 μ Pa.

Particle velocity was calibrated using an underwater acoustic pressure-velocity probe (Mk. 2, Acoustech Corp, Philadelphia, PA, USA) containing two built-in units: a piezoelectric, omni-directional hydrophone (sensitivity: –200 dB re: 1V/ μ Pa) and a bi-directional

moving-coil geophone (sensitivity: 0.112 V cm⁻¹ s⁻¹). The outer housing of this probe was secured in place of the fish ~25 mm below the water surface with rubberized clamps, and the inner unit of the probe, designed to approximate neutral buoyancy, moved freely in response to our sound stimuli. The omnidirectional hydrophone was suspended by rubber straps to within 2 mm of the pressure–velocity probe. This setup enabled the simultaneous recording of the sound pressure and particle velocity components of the entire range of our experimental stimuli. Subsequently and separately, measurements of particle displacements were recorded in three orthogonal orientations (*sensu* Casper and Mann, 2006). The vertical component (z axis) of particle velocity had substantially greater amplitudes than the x (horizontal: head-to-tail) or y axes (left to right) at each frequency and attenuation (Table 2). This vertical axis was therefore considered most appropriate for expressing thresholds and plotting particle acceleration audiograms.

The otolithic organ systems of fishes are thought to act as accelerometers, and particle motion audiograms have been increasingly expressed in units of acceleration (Kalmijn, 1988; Fay and Edds-Walton, 1997; Casper and Mann, 2006). Therefore, particle velocity (m s⁻¹) was quantified as above for acoustic pressure, and velocity values were converted to particle acceleration using Eqn 1:

$$\mathbf{A} = \mathbf{u}2\pi F, \quad (1)$$

where \mathbf{A} is the particle acceleration (m s⁻²), \mathbf{u} is the particle velocity (m s⁻¹) and F is the frequency (Hz) (see Table S1 in supplementary material).

Table 3. Models of pressure and particle motion data with three candidate covariance structures: first order autoregressive, compound symmetry and unstructured

Analysis	Model	Number of parameters	–ln(likelihood)	AIC _c
Pressure	AR(1)	2	2362	2366
	CS	2	2474	2478
	UN	78	2220	2420
Velocity	AR(1)	2	–6878	–6874
	CS	2	–6758	–6754
	UN	78	n/a	n/a
Acceleration	AR(1)	2	–584	–580
	CS	2	–470	–466
	UN	78	n/a	n/a

AIC_c, Akaike's information criterion; AR(1), first order autoregressive; CS, compound symmetry; UN, unstructured; n/a, not applicable.

The AR(1) model consistently had the lowest values of the small sample adjusted AIC_c. This covariance structure was therefore used in the two-way repeated measures ANOVAs for pressure, velocity and acceleration thresholds. The unstructured covariance model failed to converge for velocity and acceleration analyses.

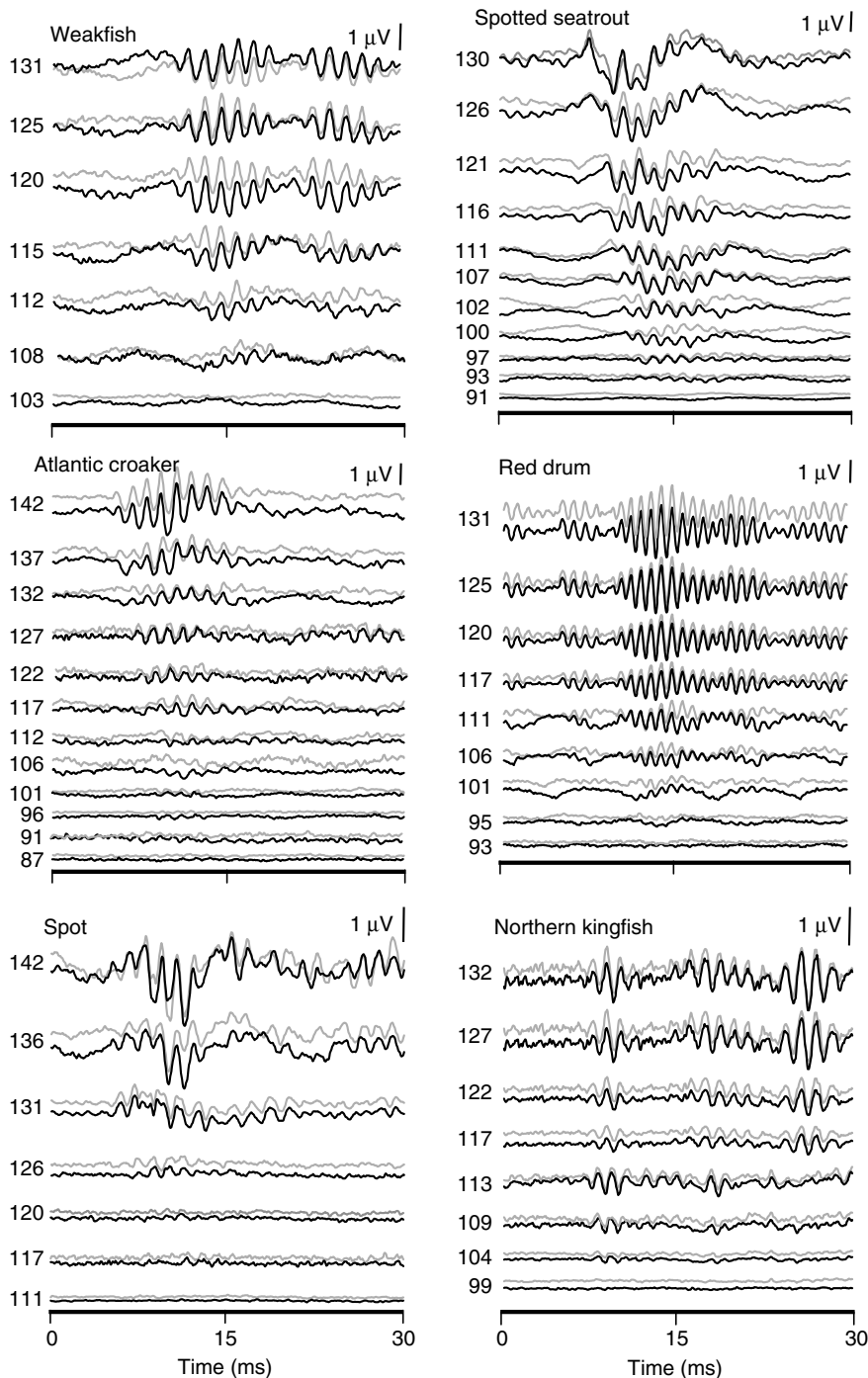


Fig. 2. Sample ABR waveforms from each species, obtained in response to echo-canceled 500 Hz pure tone bursts: weakfish, spotted seatrout, Atlantic croaker, red drum, spot and northern kingfish. Black and grey lines are replicate ABR responses at a given attenuation that each result from the addition of two ABR recordings of opposite polarities. Numbers indicate sound pressure levels (SPL; dB re: 1 μPa).

Statistical analyses

Auditory thresholds are ideally analyzed with repeated measures ANOVA designs because thresholds at different frequencies are non-independent within individual subjects (Underwood, 2002). Considering responses of an individual fish to be independent across frequencies constitutes pseudoreplication (Hurlbert, 1984); valid analyses of such data require that the nature of within-individual autocorrelation is explicitly understood. Inadequate consideration of the variance-covariance structure resulting from repeated measures may result in biased estimates of the variance of fixed effects (Littell et al., 2006). Pressure and particle acceleration thresholds were therefore analyzed separately using two-way

repeated measures ANOVAs with *a priori* contrasts to investigate whether hearing varied between the six sciaenid species and among frequencies. All statistical analyses were conducted using SAS v 9.1 (SAS Institute, Cary, NC, USA). The model for these analyses is given in Eqn 2:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \epsilon_{ijk}, \quad (2)$$

where Y_{ijk} is the value of the response variable (threshold) for the i th species, j th frequency, and the k th level of their interaction; μ is the overall mean of threshold for all species:frequency combinations; α_i is the species (fixed factor); β_j is the frequency (fixed factor); δ_k is the species:frequency interaction; ϵ_{ijk} is the

random error term associated with the observation at each combination of the i th species, the j th frequency, and the k th level of their interaction.

We fitted models with three candidate covariance structures (unstructured, compound symmetry, and first order autoregressive [AR(1)]) to the pressure and particle acceleration threshold data. In the unstructured model (UN), each covariance between measures was estimated individually, allowing the data to dictate the appropriate covariance structure. The second covariance structure, compound symmetry (CS), assumed equal covariances between all pairs of observations. The final covariance structure, first order autoregressive [AR(1)], assumed that the correlation between observations is a function of their lag in space or time; adjacent observations are more likely to be correlated than those taken further apart (Littell et al., 2006). As a simple example involving the relationship between evoked potentials at 200, 300 and 900 Hz, the UN model would calculate the variance–covariance of every pair of observations individually, the AR(1) model would assume that evoked potentials at 200 and 300 Hz are likely more similar than responses at 200 *versus* 900 Hz, whereas the CS model would assume equal covariance.

After models were fitted to data, the appropriate covariance structure was selected using Akaike's information criterion (AIC_c):

$$AIC_c = -2\ln(L) + 2p + 2p(p+1)/(n-p-1), \quad (3)$$

where AIC_c is Akaike's information criterion for small sample size, L is the value of the likelihood function at its maximum, n is sample size (threshold of each fish of each species at each frequency), and p is the number of estimated parameters. AIC_c is a parsimonious measure that strikes a balance between model simplicity and complex overparameterization (Burnham and Anderson, 2002). The small-sample adjustment (AIC_c) is recommended when the ratio of sample size to the number of parameters is less than 40 (Burnham and Anderson, 2002).

RESULTS

The ABR waveforms and audiograms for sound pressure and acceleration were species-specific, but with some commonalities. Auditory evoked potentials of the six sciaenid fishes (Fig. 2) generally began 10–15 ms after stimulus onset and were complete by 30 ms (≥ 400 Hz) or 50 ms (100–300 Hz). Waveform latency varied inversely with frequency and sound pressure level. Sound pressure, particle velocity, and acceleration audiograms of all species (Fig. 3 A–C) exhibited lowest thresholds at low frequencies (100–500 Hz). Velocity and acceleration audiograms were notably flatter at low frequencies. AIC_c values supported the selection of the first order autoregressive [AR(1)] covariance model for both pressure and particle acceleration analyses (Table 3), supporting the assumptions of the AR(1) model. Visual inspection of sciaenid audiograms (Fig. 3) confirms inferences based on AIC_c ; ABR responses at adjacent frequencies were therefore more similar to each other than responses at distant frequencies.

Two-way repeated measures ANOVAs demonstrated significant differences between species for both pressure ($F_{5,48,6}=3.17$, $P<0.02$) and particle motion (velocity: $F_{5,51,4}=3.85$, $P<0.005$; acceleration: $F_{5,52,3}=3.00$, $P<0.02$) thresholds. Sound pressure thresholds of spot were significantly higher ($F_{1,357}=5.05$, $P<0.03$) than those of other sciaenids from 300–700 Hz. Among species with swim bladders, thresholds of those with anteriorly projecting diverticulae (weakfish, spotted seatrout and Atlantic croaker) did not differ from those species without diverticulae (red drum and spot; pressure: $F_{1,357}=2.35$, $P=0.13$). Surprisingly, thresholds of northern kingfish

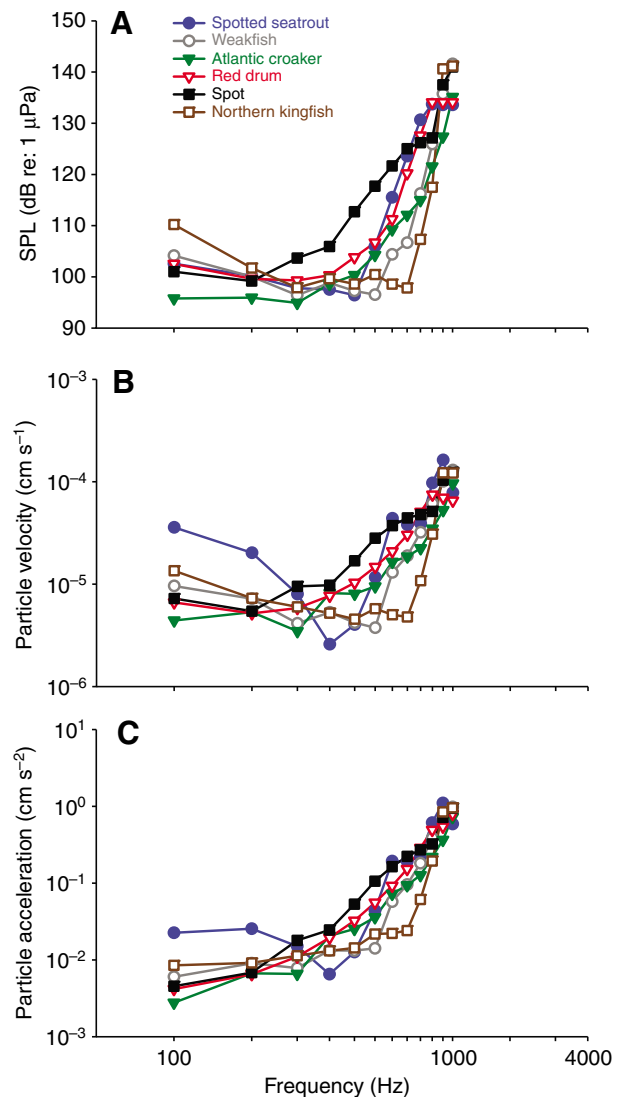


Fig. 3. Audiograms of (A) mean sound pressure (SPL) in dB re: $1 \mu\text{Pa}$, (B) mean velocity in cm s^{-1} and (C) mean acceleration in cm s^{-2} for six sciaenid species: spotted seatrout (filled blue circles), weakfish (open grey circles), Atlantic croaker (filled green triangles), red drum (open red triangles), spot (filled black squares) and northern kingfish (open brown squares). Respective means and standard errors are presented in Table S1 in supplementary material.

were among the lowest at higher frequencies (>600 Hz) even though the swim bladder atrophies in the adults we studied. Detection thresholds varied inversely with frequencies for both pressure ($F_{11,324}=53.01$, $P<0.001$) and particle motion (velocity: $F_{11,317}=78.47$, $P<0.0001$ acceleration; $F_{11,315}=129.24$, $P<0.0001$). Interactions of species and frequencies were significant for both pressure ($F_{55,319}=3.31$, $P<0.0001$) and particle motion (velocity: $F_{55,314}=8.48$, $P<0.0001$; acceleration $F_{55,314}=9.77$, $P<0.0001$) and are visually evident in the crossing of species-specific curves within audiograms (Fig. 3A–C).

DISCUSSION

All fishes are able to directly detect the particle motion components of sound, yet fish auditory thresholds are generally assessed only for sound pressure levels (Popper and Fay, 1993). Few studies

have examined hearing thresholds of fishes with respect to both pressure and particle motion sensitivity (Myrberg and Spires, 1988; van den Berg, 1985; Lovell et al., 2005; Casper and Mann, 2006). Moreover, direct particle motion simulation of the otoliths may be more relevant to hearing generalist fishes than the detection of sound pressure (Fay and Popper, 1975; Popper and Fay, 1993). In this study, we measured thresholds and expressed audiograms of six sciaenid fishes in terms of both sound pressure and acceleration using an omnidirectional hydrophone and a bi-directional geophone. Our experiments are the first to assess particle motion thresholds in sciaenid fishes and include first reports of pressure audiograms for spotted seatrout, red drum, and northern kingfish.

Sound stimuli during fish audition experiments contain both pressure and particle motion (Parvulescu, 1967; Lu et al., 1996; Casper and Mann, 2006). Small experimental tanks can have complex particle motion and sound pressure fields, potentially compromising laboratory investigations unless both components of sound stimuli are measured (Kalmijn, 1988; Popper and Fay, 1993). Placing stimulus-generating speakers in air rather than water purportedly reduces the particle motion (Kenyon et al., 1998). Our results, however, demonstrate that speakers in air can produce notable particle motion fields (Table 2). Similar conclusions were reached by others (Casper and Mann, 2006). Particle displacements in small tanks are complex, and for an equal sound pressure level they may be greater in tanks than in an unbounded body of water (Parvulescu, 1967; Rogers and Cox, 1988). General comparisons across studies may be complicated by differences in the location of the sound source in air *versus* water, the proximity of subjects to the sound source and air-water interfaces (Fay and Edds-Walton, 1997). Such concerns demonstrate the utility of routine particle motion assessment of experimental sound stimuli. Submersible units capable of generating and measuring particle motion are available (Casper and Mann, 2007a; Casper and Mann, 2007b). Future fish audition experiments should attempt to measure and report both the pressure and particle motion components of their experimental stimuli if possible (Popper and Fay, 1993; Casper and Mann, 2006).

The frequency range detected by the six sciaenids we studied was similar to those of other hearing generalist fishes (100 to <2000 Hz) (Popper and Fay, 1993; Kenyon et al., 1998; Ramcharitar, 2003; Ramcharitar and Popper, 2004; Ramcharitar et al., 2006b). Pressure detection thresholds of sciaenid fishes were significantly lower at low frequencies from 100–300 Hz. Our mean pressure thresholds for spot, weakfish and Atlantic croaker, obtained with a speaker in air, averaged about 6 dB higher than those obtained by others using a speaker in water (Ramcharitar and Popper, 2004; Ramcharitar et al., 2006b). Whether the different results are a consequence of speaker location/type, different levels of background noise, individual variation due to the use of larger animals in our study, or a combination of these factors, is unclear. Overall, our results generally support the conclusion of Ramcharitar et al. (Ramcharitar et al., 2006b) that enhanced swim bladder–otolith relationships within the Sciaenidae can improve auditory sensitivity. Among sciaenids bearing swim bladders, those possessing diverticulae (weakfish, spotted seatrout and Atlantic croaker) had generally but not significantly lower pressure

Table 4. Approximate propagation distances presuming spherical spreading of sciaenid vocalizations under idealized conditions

Common name	Vocalization frequency (Hz)	Vocalization SPL	Mean auditory pressure threshold	Cylindrical spreading distance (m)
Weakfish	400–500 ¹	127 ²	96.4	32
Spotted seatrout	400–500 ¹	139.6 ³	97.3	128
Atlantic croaker	300 ¹	114 ⁴	94.9	8
Red drum	200 ¹	128 ⁵	99.6	32

¹(Ramcharitar et al., 2006a; Connaughton et al., 1997; Fine et al., 2004); ²(Sprague and Luczkovich, 2004); ³(Baltz, 2002); ⁴(Barimo and Fine, 1998); ⁵J. J. Luczkovich, personal communication. Sound pressure levels (SPL) and auditory thresholds are given in dB re: 1 μ Pa. These calculations assume: spherical spreading (decrease of 6 dB for each distance doubled, in m), uniform water of sufficient depth to not preclude sound propagation, no additional scattering or attenuating objects, and background noise below each species' auditory threshold. Vocalization SPLs are for single individuals except seatrout (an aggregation).

thresholds than species lacking diverticulae (spot and red drum). Swim bladders lacking mechanical coupling to the otic capsule may not enhance sound pressure detection (Yan et al., 2000). Surprisingly, however, we found the lowest sound pressure thresholds at higher frequencies (800–1100 Hz) in northern kingfish, a species with low hair cell densities and swim bladder atrophy in adults (Chao, 1978; Ramcharitar et al., 2001). Since species lacking swim bladders are unlikely to detect sound pressure (Casper and Mann, 2006; Mann et al., 2007), lower 'pressure' thresholds of kingfish at higher frequencies are most likely a response to particle motion during the simultaneous presentation of pressure and particle motion stimuli.

Otoliths are biological accelerometers most sensitive to particle motion on their longitudinal axis (Lu and Xu, 2002), and the larger otoliths of sciaenid fishes may confer higher sensitivity to the particle motion components of low frequency sounds (Lychakov and Rebane, 1993; Ramcharitar et al., 2006b). Our particle acceleration audiograms demonstrate significantly greater sensitivity at low frequencies (Fig. 3C) and are comparable to results obtained with elasmobranchs (Casper and Mann, 2006). Sciaenid species with enhanced connections between the swim bladder and otic capsule (Atlantic croaker, spotted seatrout, weakfish) may be able to obtain different information from the acoustic particle motion and sound pressure fields (van den Berg, 1985; Ramcharitar et al., 2001). By contrast, sciaenid fishes lacking connections between these organ systems (spot, red drum) are more likely to be responsive solely to particle motion fields (Ramcharitar, 2003). Similar conclusions have been reached for elasmobranch and teleost fishes lacking swim bladders (Mann et al., 2007; Casper and Mann, 2006). Adult kingfish (lacking swim bladders) used in our study probably detect acoustic particle motion rather than pressure. The situation is less clear for juvenile kingfish, which do have swim bladders that are distant from the otic capsule (Chao, 1978; Ramcharitar, 2003). Unfortunately, little is known about ontogenetic differences in pressure and particle motion discrimination in most fishes, including sciaenids.

A better understanding of particle motion thresholds in fishes is required, particularly with respect to hearing relative to the direction of stimulus (*sensu* Fay and Edds-Walton, 1997). In our study, maximum particle displacement occurred along the vertical axis (Table 2). But are sciaenids most sensitive to particle motion on this axis? Spawning aggregations, which involve chorusing fish juxtaposed in close proximity (Mok and Gilmore, 1983; Ramcharitar et al., 2006a; Gilmore, 2003), more likely stimulate otoliths in a horizontal direction. Although density and orientation of hair cell bundles in sciaenid fishes differ among species (Ramcharitar,

2003), behavioral sensitivity of oscars (Cichlidae: *Astronotus ocellatus*) to particle motion did not differ among orthogonal axes (Lu et al., 1996). The individual presentation of particle motion stimuli in various orthogonal Cartesian planes to sciaenids would shed light on this question (Lovell et al., 2005; Casper and Mann, 2007a; Casper and Mann, 2007b).

Dominant frequencies of most sciaenid reproductive and disturbance vocalizations [100–500 Hz (Ramcharitar et al., 2006a)] lie well within the frequency bandwidths of the six species we measured. Therefore, if they are within range, sciaenids should be able to detect each others' species-specific vocalizations, which differ in their dominant frequency, pulse duration, repetition rate, number of pulses per call and sound pressure level (Ramcharitar et al., 2006a). The extent to which these sciaenids use auditory cues to discriminate among species or between individuals in generally noisy estuarine environments remains unknown. This ability has, however, been demonstrated in other soniferous fishes (Ladich, 2000; Ripley et al., 2002; Wysocki and Ladich, 2003).

Sound pressure and particle motion detection thresholds in sciaenids were lowest at the lower frequencies at which they communicate, but whether these species primarily detect conspecific and congeneric vocalizations *via* their sound pressure, particle motion, or both components of these sounds remains unknown. Communication in sound-producing fishes occurs over relatively short distances and typically in fairly shallow water, where the acoustic near field is dominated by particle motion (Myrberg, 2001; Bass and Clark, 2002; Weeg et al., 2002). Although the characteristics of sciaenid spawning aggregations differ among species, most occur in waters from 3–50 m depth (Saucier and Baltz, 1993).

Sciaenids and other soniferous fishes communicate in shallow coastal and estuarine waters despite high levels of background noise and the theoretical short-distance propagation of low frequency sounds in shallow water (Lugli et al., 2003; Ramcharitar et al., 2006a). Under idealized conditions, we estimate that sciaenid calls may propagate 8–128 m from the source, based on their amplitudes, simple spherical spreading (a loss of 6 dB for every distance doubled) and auditory thresholds (Table 4). Further, our calculations assumed that background noise was below the auditory thresholds, which is unlikely. For example, background ambient noise levels measured in a North Carolina estuary ranged from 110 to 125 dB re: 1 μ Pa (Sprague and Luczkovich, 2004). There is evidence for frequency selectivity amidst background masking within the Sciaenidae, suggesting that some species may still detect certain sounds amidst the masking din of background noise in coastal environments (Ramcharitar and Popper, 2004). Therefore, the distances at which these vocalizations can be heard depend on the source's sound pressure level, the pressure sensitivity and masked hearing ability of the listener, and environmental variables such as background noise, depth, bottom type and habitat complexity (Mann, 2006). Unfortunately, masked auditory thresholds are known for only two sciaenids [Atlantic croaker and black drum (Ramcharitar and Popper, 2004)]. Additionally, the propagation of pressure and particle motion fields and actual attraction distances of sound sources in shallow, complex, high-scattering, high-background estuarine habitats, are not well understood at present (Mann, 2006; Casper and Mann, 2006; Lugli and Fine, 2007).

In this study, we presented the pressure and particle motion thresholds of six sciaenid fishes, including the first reports of particle acceleration thresholds in this teleost family and first reports of pressure thresholds for three species. Together, emerging data on sciaenid auditory abilities and sonifery support growing efforts to

identify and manage their spawning habitats in environments with ever-increasing anthropogenic noise (Wahlberg and Westerberg, 2005; Ramcharitar et al., 2006a; Vasconcelos et al., 2007). Sciaenid bioacoustics therefore remains a fruitful research avenue and critical link between sensory physiology and behavioral ecology (Popper et al., 2005; Ramcharitar et al., 2006a; Roundtree et al., 2006). Such research promotes multidisciplinary syntheses that can mechanistically link processes from the cellular to the individual to the population level in support of fisheries management.

We thank B. Deffenbaugh for designing the echo-cancellation software used for these experiments, and M. Luckenbach, R. Bonniwell, and S. Fate for their logistical assistance, patience and extreme flexibility in support of these experiments. We also thank M. Fabrizio for advice regarding statistical methods and J. Luczkovich for providing red drum vocalization sound pressure levels. J. Lucy, J. Smith, Capt. S. Wray, and the vessels *Bada Bing* and *Sea Beaver* helped collect study animals. Assistance with animal husbandry was graciously provided by C. Magel, P. Lynch, A. Buchheister, P. Bushnell, J. Woodward, and K. McNamee. This research was partially funded by the National Marine Fisheries Service and by a small grant to A.Z.H. from the International Women's Fishing Association. This is VIMS contribution number 2907.

REFERENCES

- Baltz, D.** (2002). Spotted seatrout spawning requirements and essential fish habitat: a microhabitat approach using hydrophones. In *Listening to Fish: Passive Acoustic Applications in Marine Fisheries* (ed. R. Rountree, C. Goudey and T. Hawkins), pp. 90–93. Cambridge, MA: Massachusetts Institute of Technology.
- Barimo, J. F. and Fine, M. L.** (1998). Relationship of swim-bladder shape to the directionality pattern of underwater sound in the oyster toadfish *Can. J. Zool.* **76**, 134–143.
- Bass, A. H. and Clark, C. W.** (2002). The physical acoustics of underwater sound communication. In *Acoustic Communication* (ed. A. M. Simmons, A. N. Popper and R. R. Fay), pp. 15–64. New York, Berlin, Heidelberg: Springer.
- Burkhard, R.** (1984). Sound pressure level measurement and spectral analysis of brief acoustic transients. *Electroencephalogr. Clin. Neurophysiol.* **57**, 83–91.
- Burnham, K. P. and Anderson, D. R.** (2002). *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach* (2nd edn). New York: Springer Science and Business Media.
- Casper, B. M. and Mann, D. A.** (2006). Evoked potential audiograms of the nurse shark (*Ginglymostoma cirratum*) and the yellow stingray (*Urobatis jamaicensis*). *Environ. Biol. Fishes* **76**, 101–108.
- Casper, B. M. and Mann, D. A.** (2007a). Dipole measurements in elasmobranch fishes. *J. Exp. Biol.* **210**, 75–81.
- Casper, B. M. and Mann, D. A.** (2007b). The directional hearing abilities of two species of bamboo sharks. *J. Exp. Biol.* **210**, 505–511.
- Casper, B. M., Lobel, P. S. and Yan, H. Y.** (2003). The hearing sensitivity of the little skate, *Raja erinacea*: a comparison of two methods. *Environ. Biol. Fishes* **68**, 371–379.
- Chao, L. N.** (1978). A basis for classifying western Atlantic Sciaenidae (Teleostei: Perciformes). *NOAA Tech. Rep. NMFS Tech. Circ.* **415**, 1–64.
- Connaughton, M. A., Fine, M. L. and Taylor, M. H.** (1997). The effects of seasonal hypertrophy and atrophy on fiber morphology, metabolic substrate concentration and sound characteristics of the weakfish sonic muscle. *J. Exp. Biol.* **200**, 2449–2457.
- Corwin, J. T., Bullock, T. H. and Schweitzer, J.** (1982). The auditory brainstem response in five vertebrate classes. *Electroencephalogr. Clin. Neurophysiol.* **54**, 629–641.
- Fay, R. R. and Edds-Walton, P. L.** (1997). Directional response properties of saccular efferents of the toadfish, *Opsanus tau*. *Hear. Res.* **111**, 1–21.
- Fay, R. R. and Popper, A. N.** (1974). Acoustic stimulation of the ear of goldfish. *J. Exp. Biol.* **61**, 243–260.
- Fay, R. R. and Popper, A. N.** (1975). Modes of stimulation of the teleost ear. *J. Exp. Biol.* **62**, 379–387.
- Fine, M. L., Shrinel, J. and Cameron, T. M.** (2004). The effect of loading on disturbance sounds of the Atlantic croaker *Micropogonias undulatus*: air versus water. *J. Acoust. Soc. Am.* **116**, 1271–1275.
- Gilmore, R. G.** (2003). Sound production and communication in the spotted seatrout. In *Biology of the Spotted Seatrout* (ed. S. A. Bortone), pp. 177–195. Boca Raton, FL: CRC Press.
- Hall, J. W.** (1992). *Handbook of Auditory Evoked Responses*. Boston MA: Allyn and Bacon.
- Hurlbert, S. H.** (1984). Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **54**, 187–211.
- Kalmijn, A. J.** (1988). Hydrodynamic and acoustic field detection. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 83–130. New York: Springer-Verlag.
- Kenyon, T. N., Ladich, F. and Yan, H. Y.** (1998). A comparative study of the hearing ability in fishes: the auditory brainstem response approach. *J. Comp. Physiol. A* **182**, 307–318.
- Ladich, F.** (2000). Acoustic communication and the evolution of hearing in fish. *Proc. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1285–1288.
- Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D. and Schabenberger, O.** (2006). *SAS for Mixed Models* (2nd edn). Cary, NC: SAS Institute.

- Lovell, J. M., Findlay, M. M., Moate, R. M., Nedwell, J. R. and Pegg, M. A. (2005). The inner ear morphology and hearing abilities of the paddlefish (*Polyodon spathula*) and the lake sturgeon (*Acipenser fulvescens*). *Comp. Biochem. Physiol.* **142A**, 286-296.
- Lu, Z. and Xu, Z. (2002). Effects of saccular otolith removal on hearing sensitivity of the sleeper goby (*Dormitator latifrons*). *J. Comp. Physiol. A* **188**, 595-602.
- Lu, Z., Popper, A. N. and Fay, R. R. (1996). Behavioral detection of acoustic particle motion by a teleost fish (*Astronotus ocellatus*): sensitivity and directionality. *J. Comp. Physiol. A* **179**, 227-233.
- Lugli, M. and Fine, M. L. (2007). Stream ambient noise, spectrum and propagation of sounds in the goby *Padogobius martensii*: sound pressure and particle velocity. *J. Acoust. Soc. Am.* **122**, 2881-2892.
- Lugli, M., Yan, H. Y. and Fine, M. L. (2003). Acoustic communication in two freshwater gobies: the relationship between ambient noise, hearing thresholds and sound spectrum. *J. Exp. Biol.* **189**, 309-320.
- Lychakov, D. V. and Rebane, Y. T. (1993). Effect of otolith shape on directional sound perception in fish. *J. Evol. Biochem. Physiol.* **28**, 531-536.
- Mann, D. A. (2006). Propagation of fish sounds. In *Communication in Fishes* (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 107-120. Enfield, NH: Science Publishers.
- Mann, D. A., Lu, Z. and Popper, A. N. (1997). Ultrasound detection by a teleost fish. *Nature* **389**, 341.
- Mann, D. A., Cott, P. A., Hanna, B. W. and Popper, A. N. (2007). Hearing in eight species of northern Canadian freshwater fishes. *J. Fish Biol.* **70**, 109-120.
- Mok, H. K. and Gilmore, R. G. (1983). Analysis of sound production in estuarine aggregations of *Pogonias cromis*, *Bairdiella chrysoura*, and *Cynoscion nebulosus* (Sciaenidae). *Bull. Inst. Zool. Acad. Sinica* **22**, 157-186.
- Myrberg, A. A. (2001). The acoustical biology of elasmobranchs. *Environ. Biol. Fishes* **60**, 31-45.
- Myrberg, A. A. and Spires, J. Y. (1980). Hearing in damselfishes: an analysis of signal detection among closely related species. *J. Comp. Physiol. A* **140**, 135-144.
- Parvulescu, A. (1967). The acoustics of small tanks. In *Marine Bioacoustics*. Vol. 2 (ed. W. N. Tavolga), pp. 7-13. Oxford: Pergamon Press.
- Popper, A. N. and Fay, R. R. (1993). Sound detection and processing by fish: critical review and major research questions. *Brain Behav. Evol.* **41**, 14-38.
- Popper, A. N. and Fay, R. R. (1999). The auditory periphery in fishes. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 43-100. New York, Berlin, Heidelberg: Springer.
- Popper, A. N., Ramcharitar, J. and Campana, S. E. (2005). Why otoliths? Insights from inner ear physiology and fisheries biology. *Mar. Freshw. Res.* **56**, 497-504.
- Ramcharitar, J. (2003). *Sciaenids: model species for investigating structure-function relations in the teleost inner ear*. PhD thesis, University of Maryland, College Park, USA.
- Ramcharitar, J. U. and Popper, A. N. (2004). Masked auditory thresholds in sciaenid fishes: a comparative study. *J. Acoust. Soc. Am.* **166**, 1687-1694.
- Ramcharitar, J. U., Higgs, D. M. and Popper, A. N. (2001). Sciaenid inner ears: a study in diversity. *Brain Behav. Evol.* **58**, 152-162.
- Ramcharitar, J. U., Deng, X., Ketten, D. and Popper, A. N. (2004). Form and function in the unique inner ear of a teleost: the silver perch (*Bairdiella chrysoura*). *J. Comp. Neurol.* **475**, 531-539.
- Ramcharitar, J., Gannon, D. P. and Popper, A. N. (2006a). Bioacoustics of the fishes of the Family Sciaenidae (croakers and drums). *Trans. Am. Fish. Soc.* **135**, 1409-1431.
- Ramcharitar, J. U., Higgs, D. M. and Popper, A. N. (2006b). Audition in sciaenid fishes with different swim bladder-inner ear configurations. *J. Acoust. Soc. Am.* **119**, 439-443.
- Ripley, J. L., Lobel, P. S. and Yan, H. Y. (2002). Correlation of sound production with hearing sensitivity in the Lake Malawi cichlid *Tramitichromis intermedius*. *Bioacoustics* **12**, 238-240.
- Rogers, P. and Cox, M. (1988). Underwater sound as a biological stimulus. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 131-149. New York: Springer-Verlag.
- Roundtree, R. A., Gilmore, R. G., Goudey, C. A., Hawkins, A. D., Luczkovitch, J. J. and Mann, D. A. (2006). Listening to fish: applications of passive acoustics to fisheries science. *Fisheries* **31**, 433-446.
- Sand, O. and Karlsen, H. E. (2000). Detection of infrasound and linear acceleration in fishes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1295-1298.
- Saucier, M. H. and Baltz, D. M. (1993). Spawning site selection by spotted seatrout, *Cynoscion nebulosus*, and black drum, *Pogonias cromis*, in Louisiana. *Environ. Biol. Fishes* **36**, 257-272.
- Sprague, M. W. and Luczkovitch, J. J. (2004). Measurement of an individual silver perch *Bairdiella chrysoura* sound pressure level in a field recording. *J. Acoust. Soc. Am.* **116**, 3186-3191.
- Underwood, A. J. (2002). Some common and some particular experimental designs. In *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*, pp. 385-418. New York: Cambridge University Press.
- van den Berg, A. V. (1985). Analysis of the phase difference between particle motion components of sound by teleosts. *J. Exp. Biol.* **119**, 183-197.
- Vasconcelos, R. O., Amorim, M. C. P. and Ladich, F. (2007). Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. *J. Exp. Biol.* **210**, 2104-2112.
- Wahlberg, M. and Westerberg, H. (2005). Hearing in fish and their reactions to sounds from offshore wind farms. *Mar. Ecol. Prog. Ser.* **288**, 295-309.
- Weeg, M. S., Fay, R. R. and Bass, A. H. (2002). Directionality and frequency tuning of primary saccular afferents of a vocal fish, the plainfin midshipman (*Porichthys notatus*). *J. Comp. Physiol. A* **188**, 631-641.
- Wysocki, L. E. and Ladich, F. (2003). The representation of conspecific sounds in the auditory brainstem of teleost fishes. *J. Exp. Biol.* **206**, 2229-2240.
- Yan, H. Y. (1998). Auditory role of the suprabranchial chamber in gourami fish. *J. Comp. Physiol. A* **183**, 325-333.
- Yan, H. Y. and Curtsinger, W. S. (2000). The otic gasbladder as an ancillary auditory structure in a mormyrid fish. *J. Comp. Physiol. A* **186**, 595-602.
- Yan, H. Y., Fine, M. L., Horn, N. S. and Colon, W. E. (2000). Variability in the role of the gasbladder in fish audition. *J. Comp. Physiol. A* **186**, 435-445.

Table S1. Auditory brainstem response thresholds for six sciaenid fishes expressed as the relevant sound pressure (dB re: 1 mPa) recorded by the hydrophone and particle velocity (m s^{-1}) recorded by the geophone

Species	Sound pressure (dB re: 1 μPa) \pm s.e.m.	Recorded particle velocity (m s^{-1})	Calculated particle acceleration (m s^{-2}) \pm s.e.m.
<i>C. nebulosus</i>			
100 Hz	102.6 \pm 3.2	3.59310 ⁻⁵	0.023 \pm 0.008
200 Hz	100.2 \pm 2.7	2.03310 ⁻⁵	0.026 \pm 0.006
300 Hz	97.8 \pm 1.9	8.01310 ⁻⁶	0.015 \pm 0.003
400 Hz	97.5 \pm 2.1	2.59310 ⁻⁶	0.007 \pm 0.002
500 Hz	96.4 \pm 1.6	4.03310 ⁻⁶	0.013 \pm 0.002
600 Hz	105.9 \pm 2.7	1.17310 ⁻⁵	0.044 \pm 0.009
700 Hz	115.5 \pm 2.8	4.38310 ⁻⁵	0.193 \pm 0.043
800 Hz	123.6 \pm 1.0	3.80310 ⁻⁵	0.191 \pm 0.017
900 Hz	130.7 \pm 1.8	3.90310 ⁻⁵	0.220 \pm 0.028
1000 Hz	133.7 \pm 1.6	9.73310 ⁻⁵	0.611 \pm 0.093
1100 Hz	133.9 \pm 1.5	1.60310 ⁻⁴	1.10 \pm 0.168
1200 Hz	133.7 \pm 1.6	7.80310 ⁻⁵	0.587 \pm 0.078
<i>C. regalis</i>			
100 Hz	104.2 \pm 4.0	9.60310 ⁻⁶	0.006 \pm 0.002
200 Hz	100.0 \pm 4.3	7.20310 ⁻⁶	0.009 \pm 0.004
300 Hz	96.4 \pm 2.7	4.15310 ⁻⁶	0.008 \pm 0.002
400 Hz	98.8 \pm 2.9	5.30310 ⁻⁶	0.013 \pm 0.004
500 Hz	97.3 \pm 2.0	4.21310 ⁻⁶	0.013 \pm 0.002
600 Hz	96.5 \pm 1.4	3.75310 ⁻⁶	0.014 \pm 0.002
700 Hz	104.4 \pm 5.4	1.30310 ⁻⁵	0.057 \pm 0.030
800 Hz	106.7 \pm 6.8	1.90310 ⁻⁵	0.095 \pm 0.052
900 Hz	116.3 \pm 6.2	3.21310 ⁻⁵	0.018 \pm 0.067
1000 Hz	125.9 \pm 5.9	6.15310 ⁻⁵	0.387 \pm 0.124
1100 Hz	135.7 \pm 4.1	1.10310 ⁻⁴	0.697 \pm 0.133
1200 Hz	141.6 \pm 0.7	1.30310 ⁻⁴	0.979 \pm 0.010
<i>M. undulatus</i>			
100 Hz	95.8 \pm 3.3	4.41310 ⁻⁶	0.003 \pm 0.001
200 Hz	95.9 \pm 4.4	5.33310 ⁻⁶	0.007 \pm 0.003
300 Hz	94.9 \pm 2.3	3.47310 ⁻⁶	0.007 \pm 0.002
400 Hz	98.6 \pm 5.2	8.18310 ⁻⁶	0.021 \pm 0.013
500 Hz	100.3 \pm 5.2	8.04310 ⁻⁶	0.025 \pm 0.010
600 Hz	104.3 \pm 4.0	9.5310 ⁻⁶	0.036 \pm 0.011
700 Hz	109.3 \pm 4.8	1.65310 ⁻⁵	0.073 \pm 0.029
800 Hz	112.1 \pm 3.9	1.86310 ⁻⁵	0.094 \pm 0.032
900 Hz	115.0 \pm 3.6	2.24310 ⁻⁵	0.127 \pm 0.038
1000 Hz	121.5 \pm 3.2	3.48310 ⁻⁵	0.022 \pm 0.040
1100 Hz	127.4 \pm 2.7	5.27310 ⁻⁵	0.364 \pm 0.070
1200 Hz	135.1 \pm 4.0	9.62310 ⁻⁵	0.725 \pm 0.140
<i>S. ocellatus</i>			
100 Hz	102.6 \pm 1.6	6.63310 ⁻⁶	0.004 \pm 0.001
200 Hz	99.6 \pm 1.9	5.18310 ⁻⁶	0.007 \pm 0.001
300 Hz	99.3 \pm 3.3	5.83310 ⁻⁶	0.011 \pm 0.003
400 Hz	100.3 \pm 4.4	7.69310 ⁻⁶	0.019 \pm 0.009
500 Hz	103.8 \pm 4.5	1.03310 ⁻⁵	0.032 \pm 0.013
600 Hz	106.7 \pm 5.2	1.47310 ⁻⁵	0.056 \pm 0.025
700 Hz	111.2 \pm 5.2	2.09310 ⁻⁵	0.092 \pm 0.040
800 Hz	120.2 \pm 2.4	3.03310 ⁻⁵	0.152 \pm 0.027
900 Hz	127.6 \pm 1.6	5.08310 ⁻⁵	0.287 \pm 0.039
1000 Hz	133.4 \pm 0.7	7.47310 ⁻⁵	0.470 \pm 0.023
1100 Hz	132.3 \pm 1.0	6.98310 ⁻⁵	0.482 \pm 0.035
1200 Hz	131.4 \pm 1.1	6.54310 ⁻⁵	0.493 \pm 0.038
<i>L. xanthurus</i>			
100 Hz	101.0 \pm 3.6	7.23310 ⁻⁶	0.005 \pm 0.002
200 Hz	99.2 \pm 2.7	5.44310 ⁻⁶	0.007 \pm 0.002
300 Hz	103.7 \pm 4.2	9.52310 ⁻⁶	0.018 \pm 0.006
400 Hz	105.9 \pm 2.8	9.77310 ⁻⁶	0.025 \pm 0.005
500 Hz	112.7 \pm 2.4	1.69310 ⁻⁵	0.053 \pm 0.011
600 Hz	117.7 \pm 3.8	2.81310 ⁻⁵	0.106 \pm 0.030
700 Hz	121.7 \pm 3.8	3.72310 ⁻⁵	0.164 \pm 0.041
800 Hz	125.0 \pm 2.8	4.44310 ⁻⁵	0.223 \pm 0.039
900 Hz	126.2 \pm 2.7	4.78310 ⁻⁵	0.270 \pm 0.043
1000 Hz	127.2 \pm 2.8	5.14310 ⁻⁵	0.323 \pm 0.049
1100 Hz	137.5 \pm 2.3	1.04310 ⁻⁴	0.717 \pm 0.094
1200 Hz	141.0 \pm 0.2	1.25310 ⁻⁴	0.943 \pm 0.010
<i>M. saxatilis</i>			
100 Hz	110.23 \pm 2.2	1.35310 ⁻⁵	0.0085 \pm 0.002
200 Hz	101.72 \pm 3.3	7.27310 ⁻⁶	0.0091 \pm 0.003
300 Hz	97.88 \pm 4.0	6.01310 ⁻⁶	0.0113 \pm 0.006
400 Hz	99.63 \pm 1.9	5.21310 ⁻⁶	0.0131 \pm 0.002
500 Hz	98.61 \pm 1.3	4.56310 ⁻⁶	0.0143 \pm 0.002
600 Hz	100.44 \pm 2.3	5.75310 ⁻⁶	0.0217 \pm 0.004
700 Hz	98.58 \pm 2.8	5.03310 ⁻⁶	0.0221 \pm 0.005
800 Hz	97.84 \pm 2.9	4.79310 ⁻⁶	0.0241 \pm 0.006
900 Hz	107.31 \pm 2.4	1.08310 ⁻⁵	0.0611 \pm 0.015
1000 Hz	117.49 \pm 5.1	3.08310 ⁻⁵	0.1937 \pm 0.061
1100 Hz	140.63 \pm 0.5	1.22310 ⁻⁴	0.8453 \pm 0.027
1200 Hz	141.18 \pm 0.5	1.26310 ⁻⁴	0.9536 \pm 0.031

Particle acceleration (m s^{-2}) was calculated from the recorded particle velocity using Eqn 1.