Reproductive state modulates utricular auditory sensitivity in a vocal fish

Running title: Reproductive state modulates utricular sensitivity

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Conflict of interest: The authors declare no competing financial interests.

Acknowledgments: The authors would like to thank the captain and crew of the R/V Kittiwake for assistance with winter fish collection, and Sujay Balebail and Lorin Gardner for assistance with summer fish collection and fish husbandry. This work was supported by a National Science Foundation grant to JAS (IOS 1933166) and ABC (IOS 1932898). LSR was supported by a National Science Foundation Graduate Research Fellowship (DGE 1762114), National Institutes of Health auditory neuroscience training grant (T32DC005361), and a Howard Hughes Medical Institute Gilliam Fellowship (GT15044).

Abstract

The plainfin midshipman, *Porichthys notatus*, is a seasonally breeding vocal fish that relies on acoustic communication to mediate nocturnal reproductive behaviors. Reproductive females use their auditory sense to detect and localize “singing” males that produce multiharmonic advertisement (mate) calls during the breeding season. Previous work showed that the midshipman saccule, which is considered the primary end organ used for hearing in midshipman and most other fishes, exhibit reproductive state and hormone-dependent changes that enhanced saccular auditory sensitivity. In contrast, the utricle was previously posited to serve primarily a vestibular function, but recent evidence in midshipman and related toadfish suggests that it may also serve an auditory function and aid in the detection of behaviorally-relevant acoustic stimuli. Here, we characterized the auditory evoked potentials recorded from utricular hair cells in reproductive and non-reproductive female midshipman in response to underwater sound to test the hypothesis that variation in reproductive state affects utricular auditory sensitivity. We show that utricular hair cells in reproductive females exhibit up to a 6-fold increase in the utricular potential magnitude and have thresholds based on measures of particle acceleration (re: 1 ms^{-2}) that are 7-10 dB lower than non-reproductive females across a broad range of frequencies, which include the dominant harmonics of male advertisement calls. This enhanced auditory sensitivity of the utricle likely plays an essential role in facilitating midshipman social and reproductive acoustic communication.

Keywords: Utricle, Auditory, Seasonal plasticity, Hair cells

New & Noteworthy

In many animals, vocal-acoustic communication is fundamental for facilitating social behaviors. For the vocal plainfin midshipman fish, the detection and localization of social acoustic signals is critical to the species' reproductive success. Here, we show that the utricle, an inner ear end organ often thought to primarily serve a vestibular function, serves an auditory function that is seasonally plastic and modulated by the animal's reproductive state effectively enhancing auditory sensitivity to courting male advertisement calls.

Introduction

Seasonal changes in sensory processing related to an animal's reproductive cycle occur in many non-mammalian vertebrates including songbirds, amphibians, and fishes [For review see (1–3)]. Furthermore, reproductive-related changes in sensory processing of auditory information occur in a number of seasonally breeding species that rely on acoustic communication to mediate social interactions in a reproductive context [e.g., birds: (4–9); amphibians: (10–12); and fishes: (13, 14)]. However, previous work has primarily focused on reproductive state-dependent changes in sensitivity of the central auditory system (5, 6, 10–12) or primary hearing organs of the peripheral auditory system (4, 13, 14). Here, we consider reproductive state-dependent changes in the frequency sensitivity and auditory gain of the utricle, an end organ not often associated with an auditory function, in a seasonally breeding vertebrate for which the detection and localization of conspecific acoustic signals is critical to its reproductive success.

The plainfin midshipman fish (*Porichthys notatus*) is a seasonally breeding vocal fish that produces social acoustic signals for intraspecific communication during the reproductive season. The social behaviors of this nocturnally-active species are highly dependent upon the production and reception of acoustic signals, which makes the midshipman an excellent model for investigating the neural mechanisms of acoustic communication, especially those related to seasonal changes in vocal-acoustic behavior and auditory reception (15–17). During the late spring and summer, midshipman migrate into the shallow intertidal zone to reproduce and care for their offspring. Courting (type I) males establish nest sites in the rocky substrate where they produce long-duration multiharmonic advertisement calls to attract gravid females for reproduction (18). Previous work has shown that females exhibit reproductive state- and hormone-dependent changes in the auditory sensitivity of the saccule, such that reproductive females are better suited than non-reproductive females to detect conspecific vocalizations (13, 19, 20). This steroid-, reproductive state-dependent modulation of auditory saccular sensitivity is thought to enhance the coupling of sender and receiver in the midshipman acoustic communication system.

In most fishes, the inner ear saccule is often the largest otolithic end organ and most associated with hearing (21, 22), while the smaller utricle has been posited to serve primarily a vestibular function as

a gravistatic organ (23–27). However, recent evidence in toadfish and midshipman (Family Batrachoididae) suggests that the utricle is capable of detecting and encoding behaviorally-relevant acoustic stimuli including conspecific vocalizations (28–30). Yet, the extent to which the utricle may exhibit reproductive-related changes in auditory sensitivity to social acoustic signals remains unknown.

Here, we test the hypothesis that seasonal variation in reproductive state modulates the auditory sensitivity of the utricle in female plainfin midshipman. We compare the auditory evoked utricular potentials of reproductive and non-reproductive females to determine whether there are differences related to reproductive state in the frequency response and auditory threshold of utricular hair cells to behaviorally-relevant auditory stimuli. We show that the utricle serves an auditory function that is seasonally plastic and highly adapted in reproductive females to detect the dominant frequencies of conspecific vocalizations.

Materials and Methods

Animal collection and husbandry

Non-reproductive adult female plainfin midshipman fish, *Porichthys notatus* Girard 1854, were collected via otter trawls (*R/V Kittiwake*, Friday Harbor Laboratories) in January 2021 from the Puget Sound near Edmonds, WA, at depths ranging from 85 to 100 m. Reproductive adult female plainfin midshipman were collected during their breeding season (May – June 2021) by hand at low tide from exposed nest sites in the rocky intertidal area at Seal Rock near Brinnon, WA. Following collection, fish were transported to the University of Washington and housed in a 350 L recirculating artificial saltwater tank maintained at 15 $\pm$ 2 °C and kept on either a winter (9/15-h) or summer (12/12-h) light/dark photoperiod, which corresponds with the non-reproductive and reproductive ambient photoperiods, respectively. Before each physiology experiment, standard length (SL; cm) and body mass (BM; g) were recorded and sex was determined by visual inspection of the gonads. The gonadosomatic index [GSI; defined here as 100 * (gonad mass / (BM – gonad mass))] for each fish was recorded following each experiment. Utricular hair cell potential recordings were performed within 17 days after trawl collection in the winter and 14 days after hand-collection during the summer to minimize any effects of prolonged captivity on midshipman auditory sensitivity while still allowing the animals to recover from capture-related stress.

Acoustic stimulus and calibration

The methodology used for acoustic stimulus presentation and calibration was similar to that of previously published work (13, 29, 31–35). Acoustic stimuli were generated by a lock-in amplifier (SR830, Stanford Research Systems, Sunnyvale, CA, USA), which sent pure tone signals to an audio amplifier (BG-1120, TOA Corporation, Hyogo, Japan) and then to an underwater speaker (UW-30, Telex Communications, Burnsville, MN, USA). The midshipman utricle is likely highly sensitive to particle motion along the horizontal plane as both the otolith (i.e., lapillus) and hair cells are oriented in the horizontal plane [Fig. 1b, supplementary fig. 1 https://figshare.com/s/e17c71d36f441dce06e4; but also see fig. 6 in (36)]. Therefore, the underwater speaker was positioned such that the speaker's face resided along the horizontal plane and was fully submerged 2 cm below the water's surface (Figure 1a – 1). Acoustic stimuli consisted of single 500 ms pure tones repeated 8 times at a rate of one every 1.5 s. Acoustic stimuli were randomly presented at the following frequencies 105, 125, 145, 165, 185, 205, 245, 285, 305, 405, 505, 605, 705, 805, 905, and 1005 Hz, which encompasses the dominant bandwidth frequencies contained within the male midshipman advertisement call and avoids frequencies that could potentially cause interference associated with resonance frequencies of the experimental tank [see Rogers and Sisneros, 2020 for tank acoustic properties].

All acoustic stimuli were calibrated relative to the stimuli's sound pressure (dB re: 1 μPa) via a mini-hydrophone (model 8103, Bruel and Kjaer, Naerum, Denmark) connected to a conditioning amplifier (gain = 100 mV/Pa, Nexis 2692-0S1, Bruel and Kjaer, Naerum, Denmark). However, only certain groups of fishes can detect sound pressure via secondary structures that are close in proximity or connect to the inner ear, and function to convert the received sound pressure wave into local particle motion that stimulates the inner ear. Previous midshipman studies showed that both the saccule and lagena are sound pressure sensitive based on their proximity to the swim bladder (35, 37). However, it remains to be determined if the utricle is sensitive to sound pressure; therefore, we also report the equivalent particle acceleration levels (dB re: 1 ms^{-2}) that corresponded to the sound pressure levels (dB re: 1 μPa) used in this study, based on our calibration procedures (detailed below).

Particle acceleration levels (dB re: 1 ms^{-2}) were determined by suspending a neutrally buoyant waterproofed triaxial accelerometer (Model VW3567A12; Sensitivity at 100 Hz: 10.42 mV/ms^{-2} (*x*-axis),

10.03 mV/ms^{-2} (*y*-axis), 10.37 mV/ms^{-2} (*z*-axis); PCB Piezotronics, Depew, NY, USA) that connected to a signal conditioner (gain = ×100/axis; Model: 482A16; PCB Piezotronics, Depew, NY, USA). For both sound pressure (dB re: 1 μPa) and particle acceleration (dB re: 1 ms^{-2}) measurements, the mini-hydrophone and particle accelerometer, respectively, were suspended 10 cm perpendicular to the face of the underwater speaker and 4 cm below the water's surface to coincide with the position of the midshipman inner ear during auditory evoked hair cell potential measurements. Sound pressure level (dB re: 1 μPa) measurements were calibrated by measuring the peak-to-peak (pk-pk) voltage (V_{pk-pk}) amplitude on an oscilloscope (Tektronix, Beaverton, OR, USA) and then equalized in sound pressure level (dB re: 1 μPa) using a custom MATLAB (MathWorks Inc., Natick, MA, USA) script, which measured the power spectral density for all tested frequencies. The signal (V_{pk-pk}) sent to the speaker was scaled until a reference peak-to-peak sound pressure level (SPL_{pk-pk}) output from the speaker of 130 ± 0.5 dB re: 1 μPa was achieved. Particle acceleration level (dB re: 1 ms^{-2}) measurements were acquired by measuring the particle motion amplitude (V_{pk-pk}) of each tested frequency across the entire range of sound levels using a National Instruments data acquisition system (Model: NI USB-6009, National Instruments, Austin, TX, USA) and visualized using LabVIEW software (National Instruments, Austin, TX, USA). Using a custom LabVIEW (National Instruments, Austin, TX, USA) script, particle motion amplitude measurements (V_{pk-pk}) for each axis (*x*-, *y*-, and *z*-axis) were corrected for the gain (sensitivity) of the accelerometer. Particle motion values (dB re: 1 ms^{-2}) for each test frequency at three representative sound levels (130, 142, and 154 dB re: 1 μPa) are displayed in supplementary figure 2 (https://figshare.com/s/e17c71d36f441dce06e4).

Utricular potential measurements

The methodology for recording utricular hair cell potentials follows the techniques used in our previous study, which measured the auditory evoked potentials from the utricular hair cells of adult male plainfin midshipman (29). Midshipman were anesthetized by immersion in a 0.025% ethyl *p*-aminobenzoate (benzocaine) buffered saltwater bath and then given an intramuscular injection of bupivacaine HCL (~1 mg/kg of BM) and cisatracurium besylate (~3 mg/kg of BM) for analgesia and immobilization, respectively. A craniotomy was then performed lateral the sagittal crest of the skull to expose the inner ear saccule and

utricle and the brain (Figure 1b) and a hydrophobic barrier (approximately 2.5 cm dia. x 5 cm height) made of denture adhesive cream (Fixodent, Proctor and Gamble Company, Cincinnati, OH, USA) was constructed around the craniotomy to prevent saltwater contamination during experimental testing (Figure 1a – 2). Fish were then transferred to the experimental tank (40 cm diameter, 20 cm water depth), which was maintained on a vibration-isolation table (TMC Vibration Control, Peabody, MA, USA) inside a sound attenuation chamber (Industrial Acoustics, New York, NY, USA), suspended in the center of the experimental tank using acoustically transparent film (Figure 1a – 3), head-fixed 4 cm below the water's surface via a custom-built acrylic head holder (Figure 1a – 4) and perfused with chilled saltwater (13 – 15°C) throughout experimental testing (Figure 1a – 5).

Auditory evoked utricular hair cell potentials were recorded using borosilicate glass microelectrodes (2 mm outer diameter; 1.16 mm inner diameter; A-M Systems, Sequim, WA) that were pulled using a Narishige puller (Model: PE-21) and filled with 3 M KCl (impedance: 4.0 – 8.0 MΩ). Electrodes were positioned in close proximity (≤ 2 mm) to the medial region of the utricle near the sensory epithelia (Figure 1a – 6). The analog evoked potential signals were pre-amplified (10×; Model 5A, Getting Instruments, San Diego, CA, USA), bandpass filtered (0.07 to 3 kHz), and then amplified (10×) again via a digital filter (model SR650, Stanford Research Systems, Sunnyvale, CA, USA). Using a lock-in amplifier (SR830, Stanford Research Systems, Sunnyvale, CA, USA), the output signal, which was proportional to the utricular hair cell evoked response to the stimulus fundamental frequency, was locked to a reference frequency set to the second harmonic of the pure tone stimulus frequency (i.e., 2 ∗ fundamental frequency), which due to populations of oppositely oriented hair cells in the teleost inner ear corresponds to the greatest evoked potential amplitudes (31, 38–40) (Figure 1c). At the start of each experimental recording session, control trials (i.e., no sound stimulus) were conducted to measure background utricular potential levels (n = 8 measurements) under ambient sound levels (−71 ± 1 dB re: 1 ms^{-2}; 76 ± 1 dB re: 1 µPa). After determining background levels, stimulus trials across the experimental frequency bandwidth were carried out to construct iso-intensity level responses at various sound levels (Figure 1d). All experimental trials were carried out using a custom MATLAB script, which controlled stimulus timing and acquired data, and all data were stored on a desktop computer.

190 ***Analyses***

191 Utricular hair cell auditory threshold tuning curves relative to particle acceleration (dB re: 1 ms^{-2}) and
192 sound pressure (dB re: 1 μPa) were determined via input-output measurements of the evoked receptor
193 potentials over the range of tested frequencies (105–1005 Hz) and sound levels (-46.1 – 1.8 dB re: 1 ms^{-2};
194 103 – 154 dB re: 1 μPa). The auditory threshold level was defined as the lowest stimulus level that
195 yielded the lowest mean utricular evoked potential that was at least two standard deviations above the
196 background electrical noise measurement. The frequency that evoked the lowest utricular threshold was
197 defined as the characteristic frequency (CF), while the frequency that elicited the highest evoked utricular
198 hair cell potential response was defined as the best frequency (BF). Particle acceleration level (dB re: 1
199 ms^{-2}) thresholds were calculated as the combined magnitude vector of particle acceleration in dB scale
200 (Eq. 1) (33, 35, 41–44) as follows:

201 $$\text{dB re: } 1\ \text{ms}^{-2} = 20\ \text{Log}_{10}(\sqrt{x^2 + y^2 + z^2}) \quad \text{(Eq. 1)}$$

202 For all statistical tests, the significance level was defined at 0.05. To determine if reproductive
203 state plays a role in modulating utricular hair cell auditory thresholds, the effects of reproductive state and
204 stimulus frequency were analyzed via a repeated-measures analysis of variance (ANOVA, between-
205 subject factor: reproductive state, within-subject factor: frequency * reproductive state). Since we were
206 only interested in how reproductive state modulates frequency sensitivity, *a priori* pairwise t-tests
207 compared the frequency-dependent auditory sensitivity of females from different reproductive states
208 across the stimulus frequency bandwidth (105 – 1005 Hz). Additionally, separate two-sample t-tests were
209 performed to determine significant differences between the SL, BM, and GSI of reproductive and non-
210 reproductive fish. All statistical analyses were performed using MATLAB software (MathWorks Inc.,
211 Natick, MA, USA).

212 **Results**

213 Auditory evoked potentials were recorded from the utricle of 33 adult female plainfin midshipman fish: 16
214 non-reproductive females with standard lengths (SL) that ranged from 12.4 – 19.2 cm (15.0 ± 2.2 cm;
215 mean ± SD), body masses (BM) that ranged from 27.3 – 55.9 g (36.1 ± 9.0 g) and gonadosomatic indices

(GSI) that ranged from 0.4 – 4.0 (1.8 ± 1.1), and 17 reproductive females with SL that ranged from 11.6 – 20.2 cm (16.2 ± 2.4 cm), BM that ranged from 35.5 – 111.0 g (78.3 ± 16.4 g) and GSI that ranged from 15.2 – 40.6 (31.8 ± 5.9). When comparing the morphometrics of non-reproductive and reproductive female plainfin midshipman, there was no difference in SL (two-sample t-test, $t_{1,31}$ = -1.499, p = 0.144); however, both BM (two-sample t-test, $t_{1,31}$ = -9.069, p < 0.001) and GSI (two-sample t-test, $t_{1,31}$ = -19.916, p < 0.001) were larger in the reproductive females, which is reflective of their reproductive status [i.e., gravid (full of eggs) vs. non-gravid females].

Auditory evoked potentials were recorded from utricular hair cells in response to particle acceleration and sound pressure levels that ranged from -46.1 to 1.8 dB re: 1 ms^{-2} and 103 to 154 dB re: 1 µPa, respectively. Iso-level response profiles of the utricular evoked potentials were generated from the presentation of single tone stimuli that ranged from 105 Hz to 1005 Hz for three sound levels: 154 dB re: 1 µPa (-0.8 – 10.8 dB re: 1 ms^{-2}), 142 dB re: 1 µPa (-12.9 – -0.9 dB re: 1 ms^{-2}), and 130 dB re: 1 µPa (-25.9 – -12.5 dB re: 1 ms^{-2}), which corresponded to a broad range of sound levels that have been recorded in the nest of calling type I males (i.e., 154 – 161 dB re: 1 µPa) (29, 34) and recorded at or within 1 m of a calling type I males nest (i.e., 130 – 142 dB re: 1 µPa) (34, 45, 46) [see supplementary figure 2 for details regarding the frequency-specific particle acceleration levels (dB re: 1 ms^{-2}) for the three sound pressure levels: 154, 142, and 130 dB re: 1 µPa; https://figshare.com/s/e17c71d36f441dce06e4]. Figure 2 displays representative utricular evoked iso-level response curves of non-reproductive and reproductive females in response to the bandwidth of tested frequencies (105 – 1005 Hz) at 154, 142, and 130 dB re: 1 µPa. Iso-level response curves consisted of profiles that had best frequencies (BFs, defined as the frequency that evoked the greatest utricular evoked potential magnitude at a given iso-level) ranging from 105 – 205 Hz in non-reproductive and reproductive females. Both non-reproductive and reproductive females had median BFs of 145 Hz at each of the sound levels tested, with no difference in the median BFs observed between non-reproductive and reproductive females at 154 dB re: 1 µPa (0.4 dB re: 1 ms^{-2}) (Friedman test, χ^2 = 0, df = 1, p = 1), 142 dB re: 1µPa (11.6 dB re: 1 ms^{-2}) (Friedman test, χ^2 = 0.2, df = 1, p = 0.6547), and 130 dB re: 1µPa (23.6 dB re: 1 ms^{-2}) (Friedman test, χ^2 = 0, df = 1, p = 1).

The magnitude of the auditory evoked potentials recorded from utricular hair cells in response to pure tone stimuli was greater in reproductive females compared to non-reproductive females. Figure 3 illustrates the mean iso-level response profiles of the evoked utricular potentials from non-reproductive and reproductive females in response to pure tones (105–1005 Hz) at 154, 142, and 130 dB re: 1 μPa. Reproductive females had significantly higher evoked utricular potentials than non-reproductive females within sound levels encompassing a range of biologically-relevant sound levels [One-way repeated measures ANOVA, between-subject factor: reproductive state) at 154 dB re: 1μPa ($F_{1, 912} = 235.4$, $p < 0.001$), 142 dB re: 1 μPa ($F_{1, 912} = 247.0$, $p < 0.001$), and 130 dB re: 1 μPa ($F_{1, 912} = 166.5$, $p < 0.001$)], and exhibited a significant interaction of frequency and reproductive state at 154 dB re: 1 μPa ($F_{1, 15} = 12.0$, $p < 0.001$), 142 dB re: 1 μPa ($F_{1, 15} = 16.7$, $p < 0.001$), and 130 dB re: 1 μPa ($F_{1, 15} = 19.5$, $p < 0.001$). Additionally, frequency-specific differences in the evoked magnitude response of the utricular hair cells were also observed between non-reproductive and reproductive females within each sound level tested (*a prior* t-tests for pair comparisons were used to determine frequency-specific differences in utricular potentials). The magnitudes of evoked utricular potentials were greater in reproductive females compare to non-reproductive females at frequencies ≤ 505 Hz at 154 dB re: 1 μPa ($p < 0.05$; see supplementary table 1; https://figshare.com/s/e17c71d36f441dce06e4), ≤ 805 Hz at 142 dB re: 1 μPa ($p < 0.05$; see supplementary table 2; https://figshare.com/s/e17c71d36f441dce06e4), and ≤ 305 Hz at 130 dB re: 1 μPa ($p < 0.05$; see supplementary table 3; https://figshare.com/s/e17c71d36f441dce06e4). The greatest evoked utricular potential magnitude change with respect to differences in reproductive state occurred at 105 Hz and 125 Hz at a sound pressure level of 130 dB re: 1 μPa (particle acceleration level at 105 Hz = -20.9 dB re: 1 ms^{-2} and 125 HZ = -23.2 dB re: 1 ms^{-2}); at this sound pressure level reproductive females had evoked potentials that were 6.3 and 6.2 times greater than in non-reproductive females, respectively (see supplementary table 3; https://figshare.com/s/e17c71d36f441dce06e4). In sum, reproductive females exhibited greater evoked utricular potentials than non-reproductive females across the frequency bandwidth tested here, with mean magnitudes that were 2.2, 2.7, and 4.1 times greater at sound pressure levels of 154 dB re: 1 μPa (for frequencies ≤ 505 Hz), 142 dB re: 1 μPa (frequencies ≤ 805 Hz) and 130 dB re: 1 μPa (frequencies ≤ 305 Hz), respectively (see supplementary tables 1-3; https://figshare.com/s/e17c71d36f441dce06e4).

Auditory threshold curves based on particle acceleration (dB re: 1 ms^{-2}) and sound pressure (dB re: 1 μPa) were constructed from the evoked utricular potential recordings. Figure 4 illustrates representative non-reproductive and reproductive female auditory threshold curves based on particle acceleration (dB re: 1 ms^{-2}) and sound pressure (dB re: 1 μPa). In general, the utricular auditory threshold tuning curves of both non-reproductive and reproductive females exhibited the lowest thresholds at frequencies ≤ 205 Hz and steadily increased to the highest thresholds at frequencies ≥ 705 Hz. Characteristic frequencies (CFs, defined as the frequency that evoked the lowest utricular threshold) for non-reproductive females ranged from 105 to 205 Hz (median CF = 105 Hz and 145 Hz based on particle acceleration and sound pressure level tuning profiles, respectively), while for reproductive females CFs ranged from 105 to 185 Hz (median CF = 105 Hz based on both particle acceleration and sound pressure level tuning profiles). The CFs based on particle acceleration did not differ with respect to reproductive state (Friedman test, $\chi^2 = 0.2$, df = 1, $p = 0.6547$); however, the CFs based on sound pressure were lower in reproductive females when compared to non-reproductive females (Friedman test, $\chi^2 = 6$, df = 1, $p = 0.01431$).

The threshold tuning curves of non-reproductive and reproductive females relative to particle acceleration (dB re: 1 ms^{-2}) and sound pressure (dB re: 1 μPa) levels are summarized in Fig. 5. In general, for females of both reproductive states, the lowest utricular thresholds occurred at the lowest frequency tested (i.e., 105 Hz) (non-reproductive females: mean particle acceleration level threshold = -28.9 ± 1.7 dB re: 1 ms^{-2}, mean sound pressure level threshold = 121.5 ± 1.7 dB re: 1 μPa; reproductive females: mean particle acceleration level threshold = -36.5 ± 1.9 dB re: 1 ms^{-2}; mean sound pressure level threshold = 113.4 ± 1.9 dB re: 1 μPa), while the highest auditory threshold levels occurred between 705 Hz to 1005 Hz (non-reproductive females: mean particle acceleration level threshold range = -4 to -1 dB re: 1 ms^{-2}, mean sound pressure level threshold range = 150 to 153 dB re: 1 μPa; reproductive females: mean particle acceleration level threshold range = -8 to -3 dB re: 1 ms^{-2}; mean sound pressure level threshold range = 148 to 151 dB re: 1 μPa). The auditory thresholds were lower (i.e., more sensitive) in reproductive females than in non-reproductive females (One-way repeated measures ANOVA, between-subject factor: reproductive state, particle acceleration level: $F_{1, 893} = 472.6$, $p < 0.001$, sound

pressure level: $F_{1, 893} = 473.6$, $p < 0.001$) and a significant interaction was observed between reproductive state and frequency (One-way repeated measures ANOVA, within-subject factor: frequency * reproductive state, particle acceleration level: $F_{1, 15} = 3.5$, $p < 0.001$, sound pressure level: $F_{1, 15} = 3.7$, $p < 0.001$). Furthermore, frequency-specific differences in auditory thresholds were observed between non-reproductive and reproductive females with reproductive females being more sensitive than non-reproductive females at frequencies from 105 to 805 Hz (*a priori* t-tests for pairwise comparisons of non-reproductive and reproductive females across frequency, $p < 0.001$).

Discussion

The goal of this study was to determine whether seasonal changes in reproductive state modulate the auditory sensitivity of the utricle in female plainfin midshipman. We show that the utricular hair cells of reproductive females exhibit up to a 6-fold magnitude increase in their evoked response to auditory stimuli and have particle acceleration thresholds that are 7-10 dB re: 1 ms^{-2} lower (i.e., more sensitive) than non-reproductive females across a frequency bandwidth that includes the dominant frequencies contained within type I male vocalizations. To our knowledge, this is the first study to demonstrate reproductive state-dependent plasticity of the frequency sensitivity and auditory gain in the teleost utricle, an inner ear end organ not often associated with auditory function. In this discussion, we consider how changes in midshipman utricular auditory sensitivity may facilitate acoustic communication during social and reproductive behaviors.

Auditory sensitivity of the midshipman utricle

In mammals, the utricle primarily serves a vestibular function as it detects linear acceleration, senses horizontal translational movements, and plays an important role in static balance. However, in teleost fishes, the utricle is one of three inner ear otolithic end organs (along with the saccule and lagena) that acts as an inertial accelerometer and responds to direct displacement by acoustic particle motion and linear accelerations primarily in the horizontal plane (21, 47, 48). While the saccule and lagena are most often implicated in sound detection and directional hearing (49–51), the utricle is posited to serve primarily a vestibular role functioning to detect head/body position relative to gravity (i.e., acts as a gravistatic organ) (25–27).

In our current study, we show that the female midshipman utricle, especially in the reproductive state, is sensitive to a broad range of acoustic frequencies with a relatively high gain in particle acceleration sensitivity (dB re: 1 ms^{-2}) from 105 to 1005 Hz (Fig. 5). Our results confirm previous studies, which showed that the utricle in batrachoid fishes (toadfishes and midshipman) serves an auditory function and is capable of detecting behaviorally-relevant acoustic stimuli (28, 29). Further support for the utricle of batrachoids serving an auditory function is the neuroanatomical evidence provided by Highstein et al. (1992) and Sisneros et al. (2002). Highstein et al. (1992) showed that utricular afferents in toadfish project to the rostral "finger" and dorsolateral aspect of the hindbrain descending octaval nucleus (DON), while Sisneros et al. (2002) showed that the midshipman utricle has extensive projections to the intermediate and rostral intermediate auditory zones of the hindbrain DON; note that the rostral "fingerlike" extension described by Highstein et al. (1992) is similar in position and extent to the rostral intermediate zone of the midshipman DON, as described by Bass et al. (2000). Furthermore, Sisneros et al. (2002) showed via transneuronal labeling that the principal cells in the midshipman DON that receive input from utricular afferents subsequently project centrally to terminals in the auditory region of the midbrain torus semicircularis similar to the saccule. Taken together, these physiological and neuroanatomical studies in batrachoid fishes strongly suggest that the utricle serves an auditory function and can detect biologically-relevant acoustic stimuli.

Seasonal auditory plasticity of the utricle

We show that female utricular hair cells exhibit seasonal, reproductive state-dependent changes in evoked responses to auditory stimuli (Figure 3), such that reproductive females exhibit greater evoked utricular potentials compared to non-reproductive females (supplementary tables 1-3; https://figshare.com/s/e17c71d36f441dce06e4). The greatest difference in evoked potential magnitude relative to reproductive state occurred at 105 Hz and a sound pressure level of 130 dB re: 1 μPa (-20.9 dB re: 1 ms^{-2}) such that reproductive females displayed average utricular potentials that were approximately 6.3 times greater than in non-reproductive females. Reproductive state-dependent changes in saccular evoked potential magnitude have previously been examined in reproductive females, which have average evoked potentials approximately 7.4 times greater than in non-reproductive females at 105 Hz and a sound pressure level of 130 dB re: 1 μPa [Sisneros unpublished data; (13)]. One

353 hypothesis for these changes in the magnitude of the hair-cell evoked potentials may, in part, be related
354 to seasonal increases in hair cell density. Coffin et al. (2012) showed that reproductive female
355 midshipman exhibit a 13% increase in saccular hair cell density, which was paralleled by a dramatic
356 increase in the magnitude of evoked saccular potentials. However, reproductive females and type I males
357 do not exhibit reproductive state-dependent changes in the hair cell density of the utricle (36, 40), yet,
358 reproductive females exhibit seasonal changes in the magnitude of evoked utricular potentials. Indeed,
359 seasonal changes in saccular potential magnitude in reproductive females may still be related to the
360 saccular-specific hair cell addition and may explain, in part, some of the evoked potential differences
361 between the saccule and utricle (i.e., the utricle having ~ a 6.3-fold increase vs the saccule having a ~
362 7.4-fold increase). An alternative, but not mutually exclusive, hypothesis for the change in the magnitude
363 of hair cell potentials may be due to reproductive state-dependent changes in ion channel expression and
364 the current kinetics of hair cells in the utricle and saccule [see (59)]. Future studies that characterize the
365 ion channel current kinetics of hair cells in non-reproductive and reproductive females may provide insight
366 into the mechanism responsible for the reproductive state-dependent changes in the magnitude of
367 evoked potentials in the midshipman utricle and saccule.
368 Concurrent with the dramatic increase in utricular potential magnitude, we also observed a
369 remarkable increase in the utricular auditory sensitivity of reproductive females when compared to non-
370 reproductive females. The greatest change in utricular auditory sensitivity occurred from 105–505 Hz (Fig.
371 5), with reproductive females exhibiting particle acceleration thresholds that were 7-10 dB (re: 1 ms^{-2})
372 lower than non-reproductive females (an increase in sensitivity equal to approximately 2-3 times) (Fig.
373 6a). This reproductive state-dependent increase in female auditory sensitivity corresponds with the
374 dominant frequencies contained within type I male vocalizations, which include grunts, growls, and
375 advertisement calls or “hums” (Fig. 6b). Grunts are short duration (50-200 ms), broadband acoustic
376 signals that are produced either individually or in a series of “trains” (Fig.6b, *bottom*), whereas growls are
377 longer duration (> 1s) broadband signals (Fig. 6b, *middle*). In general, these vocalizations are produced in
378 an agonistic context to fend off potential rivals/intruders and during nest defense (52). In contrast, hums
379 are long duration (up 2 hrs in captive conditions) multiharmonic acoustic courtship signals that have
380 fundamental frequencies ranging from 80 to 102 Hz (15, 45, 46). In comparison to broadband grunts and

growls, which have much of their spectral energy at frequencies <600 Hz, hums have prominent harmonics ranging up to ~500 Hz, with additional lower amplitude harmonics ranging up to 1000 Hz (see Fig. 6b, *top*). Together, our results suggest the utricle of reproductive females is better adapted than in non-reproductive females to detect the dominant spectral energy contained within midshipman social acoustic signals (hums, growls, and grunts), which correspond to frequencies < 600 Hz (Fig. 6b). Thus, reproductive state-dependent changes in utricular auditory sensitivity may represent an adaptive auditory plasticity that complements the saccular auditory sensitivity of reproductive female midshipman (supplementary fig. 3; https://figshare.com/s/e17c71d36f441dce06e4) and helps facilitate midshipman social and reproductive acoustic communication.

Potential mechanisms for utricular auditory plasticity

The observed changes in utricular auditory sensitivity are likely due to seasonal changes in circulating gonadal steroids (androgens and estrogens), which are related to seasonal changes in midshipman reproductive state (53). Saccular afferents in non-reproductive females treated with either testosterone or 17β-estradiol exhibit enhanced frequency sensitivity and phase-locking accuracy to higher frequencies within the midshipman hearing range, which effectively enhances acoustic communication (20). Concurrent with reproductive state-dependent changes in gonadal steroid levels are parallel changes in the large-conductance, calcium-activated potassium (BK) channels, which are responsible for the rapid outward currents that contribute to the electrical resonance and low-frequency (<1 kHz) tuning of hair cells in non-mammalian vertebrates (54–56). Rohmann et al. (2013) demonstrated that saccular hair cells of reproductive midshipman exhibit increased expression of calcium-activated BK channels, which is correlated with enhanced higher frequency sensitivity (>145 Hz) and that pharmacological inhibition of BK channels results in decreased saccular sensitivity similar to non-reproductive fish. Together, these studies suggest that gonadal steroids may modulate seasonal changes in frequency sensitivity via the regulation of hair cell BK channel expression to effectively enhance auditory sensitivity for social acoustic communication.

In addition, reproductive state-dependent changes in dopaminergic efferent projections to the inner ear may also be responsible for the observed seasonal, reproductive state-dependent changes in utricular sensitivity. Previous work by Forlano et al. (2015) showed that dopaminergic innervation of the

saccule varied with reproductive state such that reproductive females have a seasonal reduction in dopaminergic input. Furthermore, Perelmuter et al. (2019) showed that dopamine decreases saccular auditory sensitivity via a D2-like receptor and that D2a receptor expression is reduced in the saccule during the midshipman breeding season. Perelmuter et al. (2019) also found that saccular auditory sensitivity is modulated by the dopaminergic efferent system, whereby a release in inhibition effectively mimics the reproductive auditory phenotype and enhances peripheral encoding of social acoustic signals. Furthermore, Perelmuter et al. (2021) recently showed that testosterone treatment mimics the seasonal downregulation of dopamine in the midshipman saccule, which provides evidence that steroid regulation of peripheral auditory sensitivity is mediated, at least in part, by dopamine. Future studies that examine similar reproductive state-dependent, gonadal steroid regulatory mechanisms of hair cell ion channel expression and dopaminergic innervation to the utricle will be instrumental in understanding the neuroendocrine basis of peripheral auditory sensitivity modulation in midshipman fish and other vertebrates, including mammals.

Conclusion

The utricle in mammals primarily serves as a vestibular organ for detecting linear acceleration and sensing translational movements in the horizontal plane. However, in fishes, the utricle is one of three inner ear otolithic end organs (saccule, utricle, and lagena) that act as biological accelerometers and respond to acoustic particle motion and horizontal linear accelerations. While, to some degree, all three otolithic end organs in teleost fishes are posited to serve both an auditory and vestibular function, the teleost utricle is often thought to primarily serve a vestibular function. Here, we show that the utricle in the vocal plainfin midshipman serves an auditory function that is seasonally plastic and modulated by the animal's reproductive state, effectively enhancing the utricle's auditory sensitivity to conspecific acoustic signals. Whether these seasonal-dependent changes extend beyond the auditory system to the vestibular system has yet to be assessed and should be considered in future vestibular research, given the multimodal function of the inner ear end organs.

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Figures

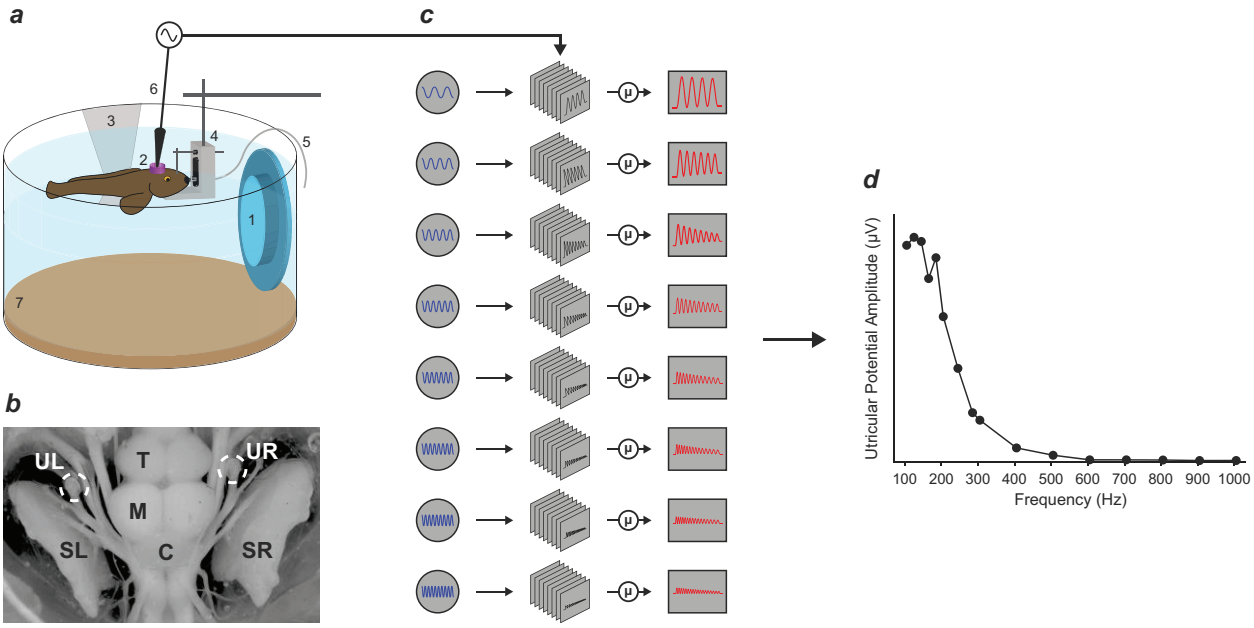

Figure 1: Experimental design. (*a*) Schematic of experimental physiology tank. For all physiology experiments, fish were suspended 4 cm below the water's surface and positioned such that the face of the underwater speaker was 10 cm rostral of the otic capsule. Labels are as follows: *1* underwater speaker, *2* hydrophobic water dam, *3* parafilm suspension, *4* head holder, *5* respiration tube, *6* glass microelectrode, and *7* rocky sediment. Physiology tank dimensions: 40 cm diameter and 20 cm water depth. (*b*) Dorsal view of midshipman cranial cavity. The dashed circles indicate the position of the left (UL) and right (UR) inner ear utricle. Abbreviations are as follows: *SL* and *SR* left, and right saccule, respectively, *T* telencephalon, *M* midbrain, *C* cerebellum. (*c*) For all experiments, pure tone acoustic stimuli (right; 500 ms duration; 8 repetitions) were delivered via an underwater speaker and evoked hair cell responses were recorded to each acoustic stimulus presentation. Consecutive utricular hair cell evoked responses (middle) were averaged across stimulus frequency, and frequency-dependent averaged output signals (right) were used to construct (*d*) iso-intensity level response curves.

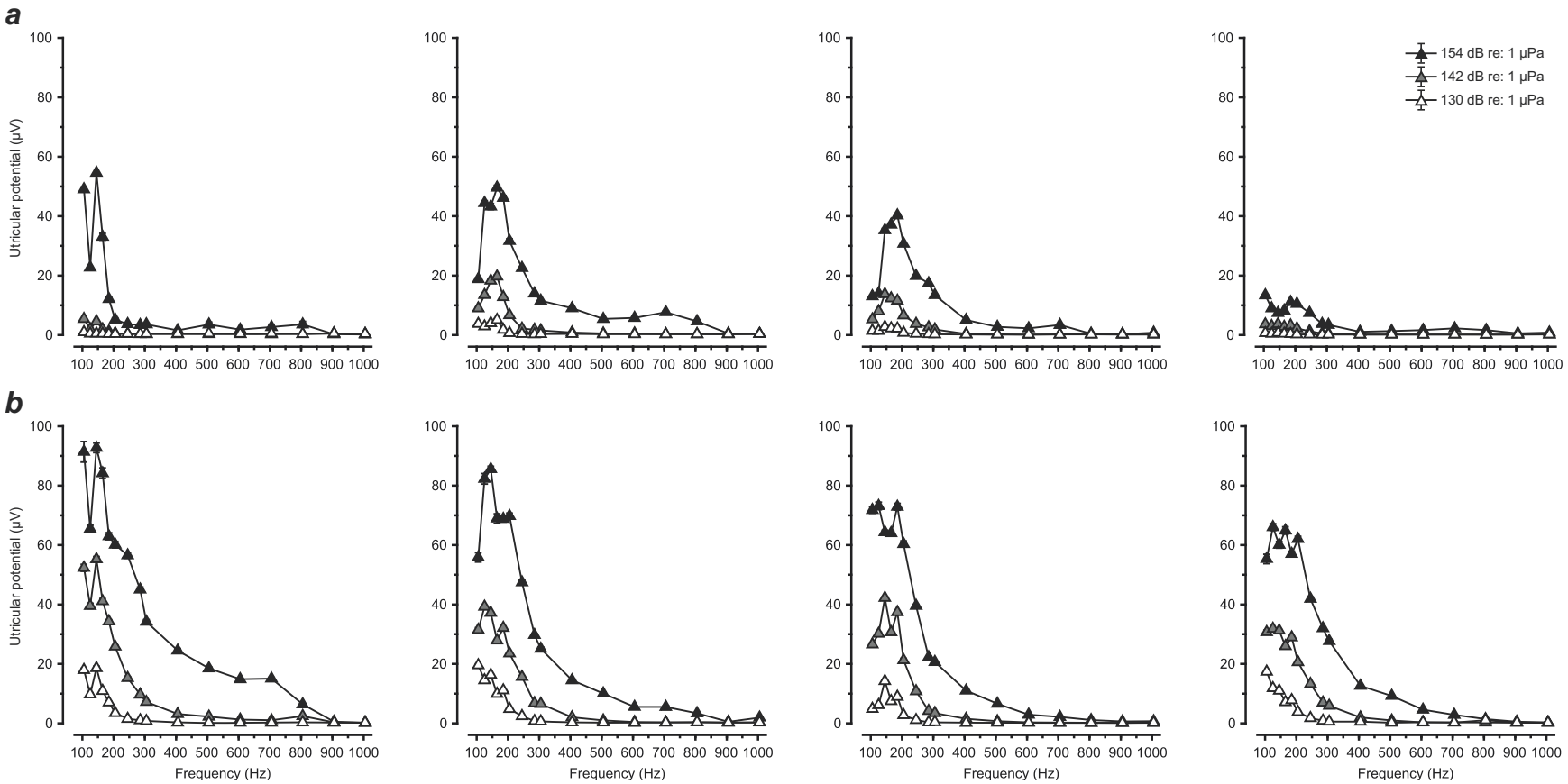

Figure 2: Representative examples of iso-intensity level curves recorded from utricular hair cells of (*a*) non-reproductive and (*b*) reproductive female plainfin midshipman. Iso-intensity responses were recorded in response to single tone playbacks at sound pressure levels of 154 (black), 142 (gray), and 130 (white) dB re: 1 μPa. Data are represented as mean ± 1 SD; note that some error bars are minimal, and the symbols may obscure the bars.

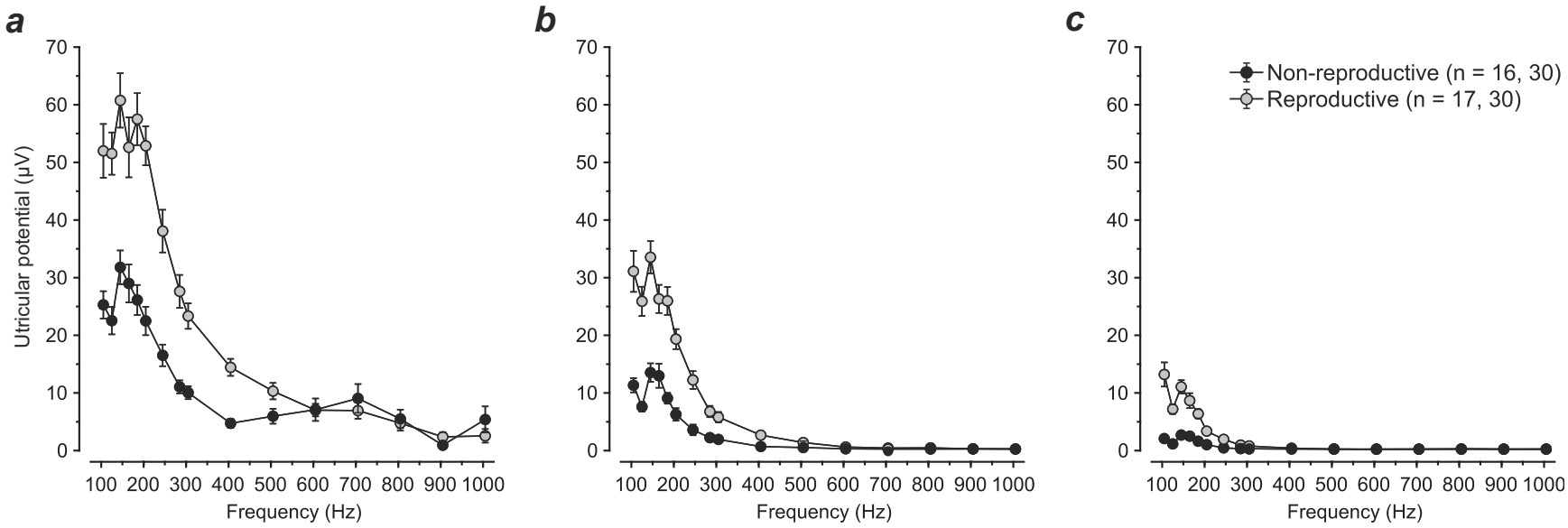

Figure 3: Iso-level response curves recorded from utricular hair cells of non-reproductive (black) and reproductive (gray) female plainfin midshipman in response to single tone playbacks at sound pressure levels of (*a*) 154, (*b*) 142, and (*c*) 130 dB re: 1 μPa. Data are represented as mean ± 1 SE; note that some error bars are minimal, and the symbols may obscure the bars. The number of animals and records for each group is indicated in parentheses.

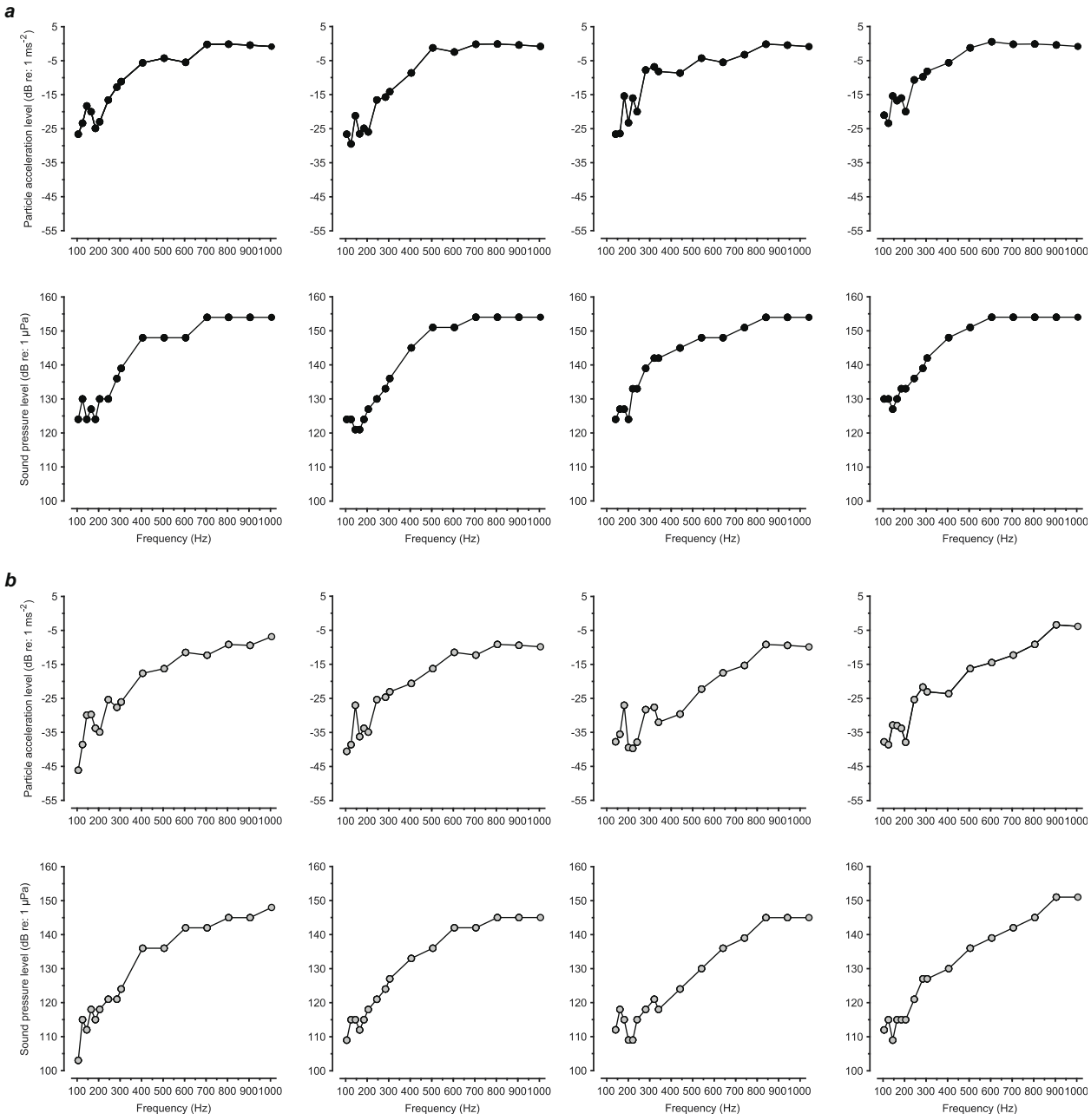

Figure 4: Representative (top) particle acceleration (dB re: 1 ms^{-2}) and (bottom) sound pressure (dB re: 1 µPa) level auditory threshold tuning curves recorded from (a) non-reproductive (black) and (b) reproductive (gray) female plainfin midshipman. Tuning curves were constructed using utricular hair cell evoked responses, with thresholds defined as the lowest sound pressure level (dB re: 1 µPa) needed to evoke a utricular potential at least 2 SD above the background electrical noise level.

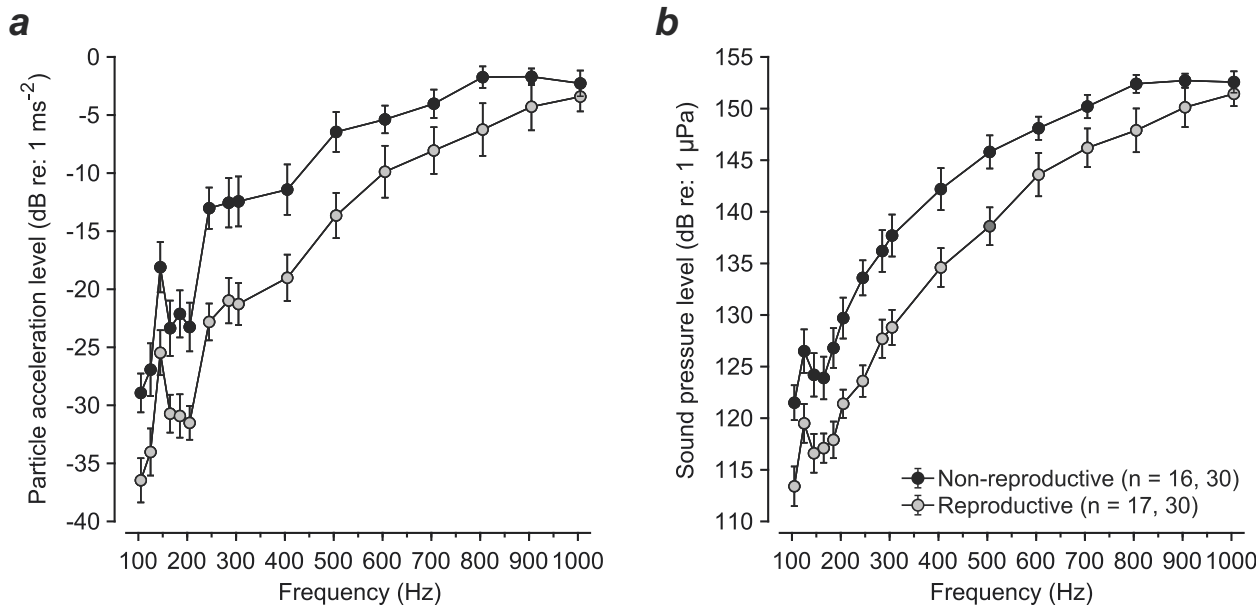

Figure 5: (*a*) Particle acceleration (dB re: 1 ms^{-2}) and (*b*) sound pressure (dB re: 1 µPa) level auditory threshold tuning curves recorded from non-reproductive (black) and reproductive (gray) female midshipman utricular hair cells. The auditory thresholds were defined as the lowest auditory stimulus level needed to evoke utricular potentials at least 2 SD above the background electrical noise level. All data are plotted as mean ± 95% confidence interval. The number of animals and records for each group is indicated in parentheses.

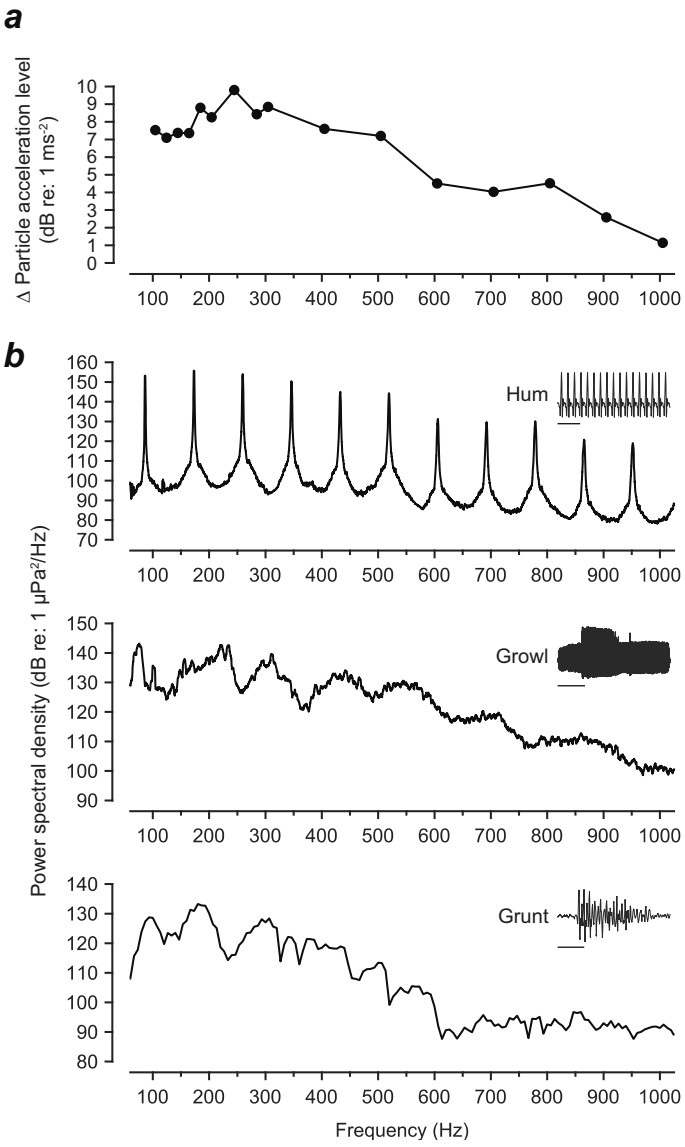

Figure 6: Comparison of female midshipman particle motion (dB re: 1 ms^{-2}) sensitivity and male midshipman vocalizations. *a)* Utricular hair cell particle acceleration threshold difference (Δ dB re: 1 ms^{-2}) between reproductive and non-reproductive female midshipman. *(b)* Power spectral density (dB re: 1 μPa^2/Hz) curves of a male midshipman hum (top), growl (middle), and grunt (bottom). *Inset:* Waveform of male midshipman hum, growl, and grunt, respectively. Scale bars represent 5, 1000, and 50 ms, respectively. All vocalizations were recorded from a reproductive type I male midshipman housed in a large, indoor concrete tank (3 m diameter; 14.1 °C) at the University of Washington Friday Harbor Laboratories. Source level recordings were made using a mini-hydrophone placed directly in front of the entrance of an artificial nest.