SUPPLEMENTARY MATERIALS

Functional plasticity of the swim bladder as an acoustic organ for communication in a vocal fish

Abbreviated title: Functional plasticity of the swim bladder in a vocal fish

Loranzie S. Rogers[1,#a,†,*], Nicholas R. Lozier[1,#b,†] , Yulia P. Sapozhnikova[1,2], Kelly M. Diamond[3,#c], Julian Ly Davis[4,5], and Joseph A. Sisneros[1,5,6]

[1]Department of Psychology, University of Washington, Seattle, WA, USA

[2]Laboratory of Ichthyology, Limnological Institute Siberian Branch of the Russian Academy of Sciences, Irkutsk, Russia

[3]Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, WA, USA

[4]Department of Engineering, University of Southern Indiana, Evansville, IN, USA

[5]Virginia Merrill Bloedel Hearing Research Center, University of Washington, Seattle, WA, USA

[6]Department of Biology, University of Washington, Seattle, WA, USA

[#a]Present address: Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA

[#b]Present address: Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA, USA

[#c]Present address: Department of Biology, Rhodes College, Memphis, TN, USA

[†]These authors contributed equally to this work

*Corresponding author

Email: loranzierogers@fas.harvard.edu (LSR)

Acoustic impedance measurements

The small dimensions (40 cm diameter, 20 cm water depth) and material (Nalgene plastic) of the experimental testing tank directly influences the acoustic environment in which auditory evoked potential recordings were performed. Therefore, as suggested by Popper and Fay (2011) and more recently by Popper et al. (2019), the acoustical impedance (*Z*) of the experimental tank environment should be measured and compared to the acoustic impedance of sea water in a free-field environment, thus allowing for more meaningful comparisons of different experimental tank acoustic environments in other physiology and behavior studies. The *Z* is the complex ratio of sound pressure to particle velocity and is expressed in Rayls [1 Rayl = 1 (Pa s)/m] and was determined in the experimental test tank across all tested frequencies at three sound pressure levels (151, 142 and 133 dB re: 1 μPa). The experimental tank's *Z* was measured and then compared to the *Z* of theoretical "seawater" ($Z_{\text{theoretical seawater}}$ = 1.559 MRayls) in a free-field environment with a salinity of 35 ppt at 15 °C [1,2]. Additionally, the phase (Φ) of the complex *Z* was also determined across all test frequencies at three sound pressure levels (151, 142 and 133 dB re: 1 μPa) by comparing the phase difference between the particle velocity and sound pressure waves. All measurements and analyses for *Z* and phase (Φ) of the complex acoustic impedance were similar to that in previously published studies [3–5].

The *Z* of our experimental tank was determined by simultaneously measuring the sound pressure (dB re: 1 μPa) and particle acceleration (dB re: 1 ms^{-2}) for each tested frequency. Simultaneous measurements were conducted at the position that would be normally occupied by the midshipman inner ear during the physiology experiment using 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) to record sound pressure (dB re: 1 μPa), whereas particle acceleration (dB re: 1 ms^{-2}) was measured using a calibrated 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 was connected to a signal conditioner (Model: 482A16; PCB Piezotronics, Depew, NY, USA) that amplified the particle acceleration signal (gain = ×100/axis). Particle acceleration (dB re: 1 ms^{-2}) and sound pressure (dB re: 1 μPa) measurements were recorded using a data acquisition system (NI myDAQ 16-bit analog to digital conversion at 200 kS s^{-1}, National Instruments, Austin, TX, USA) that was controlled by a custom-written program in LabVIEW software (NI LabVIEW 2016, National Instruments, Austin, TX, USA). Analysis of the complex acoustical impedance followed Colleye et al. (2019), Rogers and Sisneros (2020), and Vetter et al. (2019).

The complex phase of *Z* is equal to the phase difference ($\Delta\Phi p,v$) between the particle velocity (*v*) and the pressure (*p*). The phase (Φ) of the complex *Z* in our experimental test tank was determined by measuring the phase difference (ΔΦ) between the particle acceleration (*a*) and pressure (*p*), where $\Delta\Phi p,a = \Phi p - \Phi a$. All measurements were recorded with a data acquisition system (NI myDAQ 16 bit analog to digital conversion at 200 kS s^{-1}, National Instruments, Austin, TX, USA) that was controlled by a custom-written program in LabVIEW software (NI LabVIEW 2016, National Instruments, Austin, TX, USA). For

sinusoid waves, such as the pure tones examined in our study, the phase of particle acceleration (*a*) will always lead the phase of particle velocity (*v*) by 90 deg. Therefore, the phase difference ($\Delta\Phi p,v$) between the particle velocity and acoustic pressure waves was determined by (eq. 1):

$$\Delta\Phi p, v = \Delta\Phi p, a + 90° \quad \text{(eq. 1)}$$

All measurements were within the near-field approximation; however, we do not expect a simple relationship between velocity and pressure because the complex nature of our experimental tank conditions.

Table S1: Principal component (PC) scores for the first 10 PCs of swim bladder shape analysis.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
PN1	-0.0918	0.0573	-0.1053	-0.0439	-0.0526	0.0067	0.0043	0.0246	-0.0084	0.0131
PN2	-0.0772	0.0720	-0.0347	0.0404	0.0073	-0.0166	-0.0833	0.0668	-0.0380	-0.0089
PN3	-0.1110	0.0137	-0.0087	-0.0884	0.0644	-0.0103	-0.0287	0.0115	-0.0274	-0.0229
PN4	-0.1291	0.0335	0.0281	-0.0362	-0.0176	-0.0496	-0.0798	-0.0142	-0.0305	0.0321
PN5	-0.1322	0.0490	-0.0080	-0.0227	-0.0419	-0.0485	-0.0302	0.0177	-0.0436	-0.0209
PN6	-0.1056	0.0559	0.0594	0.0075	-0.0171	-0.0795	0.0256	0.0267	-0.0377	-0.0002
PN7	-0.0604	0.0577	-0.0142	-0.0508	-0.0696	-0.0346	-0.0514	-0.0035	-0.0588	-0.0048
PN8	-0.1247	0.0447	-0.0296	-0.0228	-0.0457	-0.0260	-0.0598	-0.0156	-0.0209	-0.0263
PN9	0.1220	0.0893	-0.0526	-0.0010	0.0104	-0.0135	-0.0185	-0.0052	-0.0766	0.0083
PN12	0.0987	0.0794	0.0831	-0.0277	-0.0401	0.0533	-0.0338	0.0215	-0.0113	-0.0070
PN14	0.1052	0.0545	-0.0192	-0.0546	-0.0150	-0.0724	-0.0468	0.0409	0.0047	0.0273
PN16	0.0985	0.0975	-0.0262	0.0273	0.0119	-0.0537	-0.0339	-0.0288	0.0107	-0.0158
PN17	0.1154	0.0538	-0.0205	-0.0527	-0.0481	-0.0737	-0.0439	0.0332	-0.0219	-0.0412
PN19	0.0958	0.0513	-0.0028	-0.0489	-0.0251	-0.0450	-0.0417	0.0247	-0.0533	-0.0018
PN20	0.1260	0.05135	-0.0130	-0.0565	0.0220	-0.0397	-0.0383	0.0176	-0.0375	-0.0059
PN22	0.1170	-0.1082	-0.0181	0.0178	-0.0248	-0.0218	-0.0309	0.0111	-0.0275	-0.0037

Table S2: Natural frequencies of representative nonreproductive and reproductive male swim bladders up to 750 Hz.

Nonreproductive	Reproductive
67.9919	35.5784
120.237	39.8727
166.486	43.4527
214.24	61.6258
309.966	89.2682
352.215	150.257
441.484	209.144
490.708	257.877
508.204	302.805
522.299	318.341
613.672	363.981
651.206	371.469
668.491	395.201
697.65	413.426
723.929	436.68
	439.133
	459.204
	521.341
	538.731
	557.978
	599.258
	609.776
	628.013
	643.334
	687.294
	697.3
	736.09

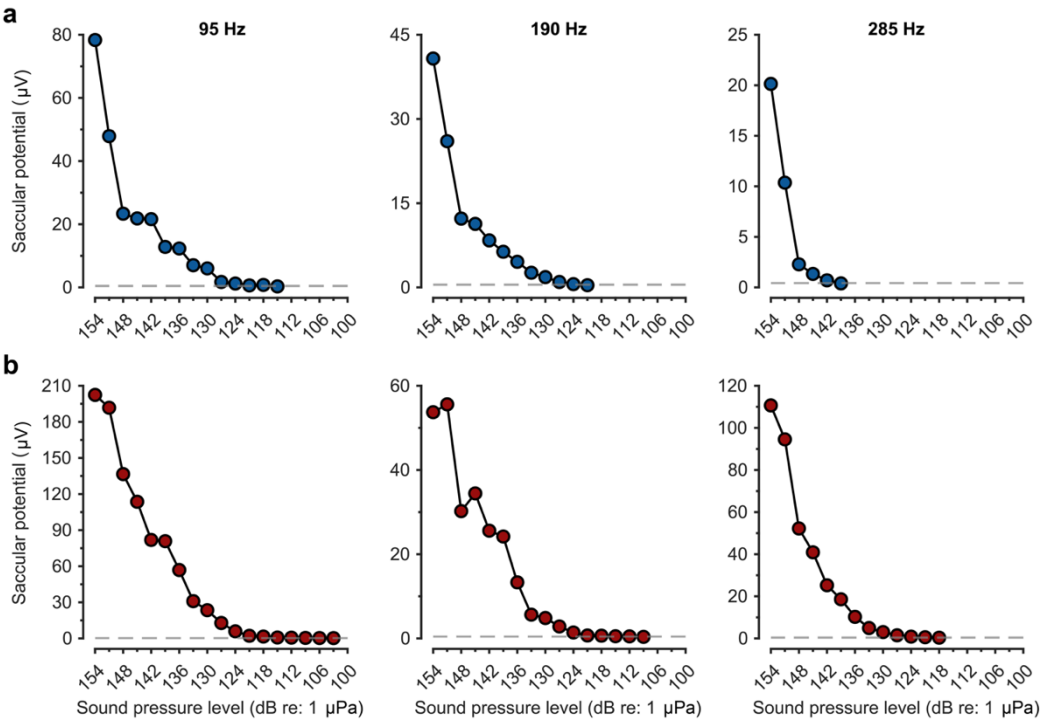

Figure S1: Representative evoked saccular hair cell potential input-output response curves. Input-output measurements were recorded from representative **a,** non-reproductive and **b,** reproductive male midshipman made at varying sound pressure levels (dB re: 1 μPa). Input-output response curves are plotted at three representative frequencies: 95 (left), 190 (middle), and 285 (right) Hz. Auditory threshold levels were defined as the lowest stimulus level (dB re: 1 μPa) that yielded the lowest mean saccular evoked potential at least two standard deviations above background levels (dashed line).

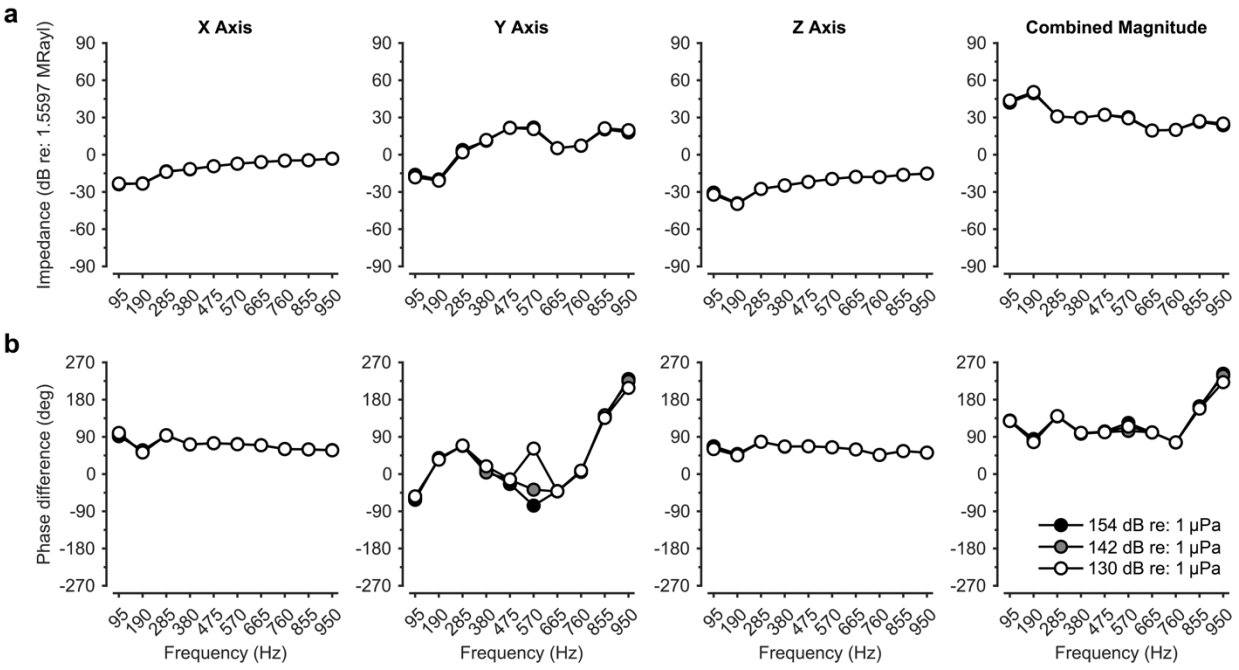

Figure S2: Acoustic characteristics of the experimental speaker and tank. a, Acoustic impedance (dB re: 1.5597 MRayl), which is the complex ratio of sound pressure to particle velocity and is expressed in Rayls [1 Rayl = 1 (Pa s)/m]. **b,** Phase difference (deg) between the pressure and particle velocity wave. All measurements were made using a triaxial accelerometer placed in the center of the tank at the position of the fish head during testing, with measurements made at three sound pressure levels (154, 142, and 130 dB re: 1 μPa) for all tested frequencies (95, 190, 285, 380, 475, 570, 665, 760, 855, and 950 Hz). Data are represented along the x-, y-, and z-axes and the combined magnitude.

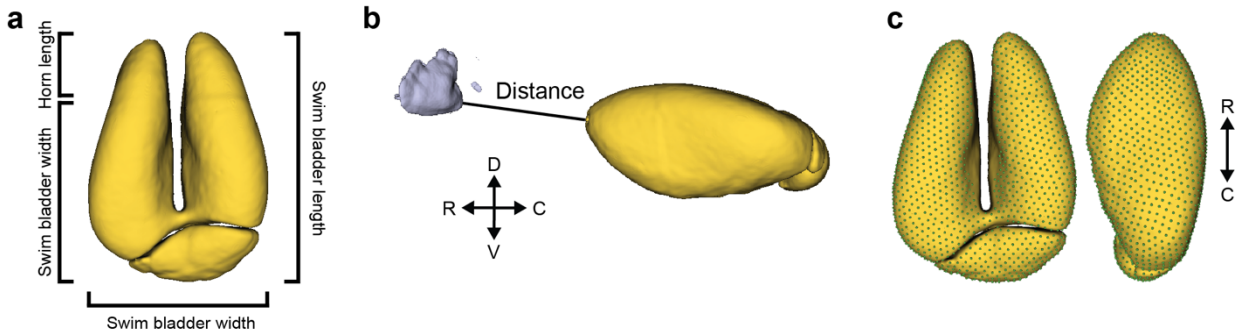

Figure S3: Representative image illustrating swim bladder microCT morphometric measurements. **a,** Characterization of swim bladder length, width, and horn length. Horn length was defined as the difference between swim bladder length and width. **b,** Swim bladder-to-otolith distance, which was characterized as the distance between the rostral most point of the swim bladder and caudal most point of the saccular otolith. Note that for calculations, distance was measured bilaterally and averaged within each subject to account for differences in laterality. **c,** Representative pseudo-landmarked (n = 2,483) swim bladder in the dorsal and lateral view, respectively.

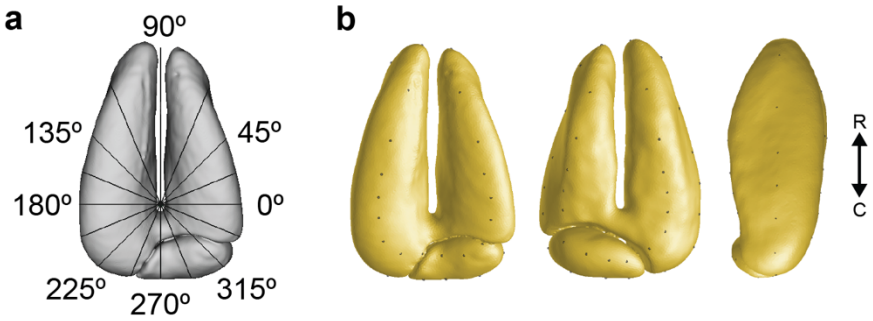

Figure S4: Methods for finite element modeling. a, Loading angles ranging from 0° – 315° in 45° increments. **b,** Representative points where displacement and power analyses were quantified. Points were positioned at the rostral horns, and the dorsal, midline, and ventral surface of the swim bladder at each of the 16 sections.

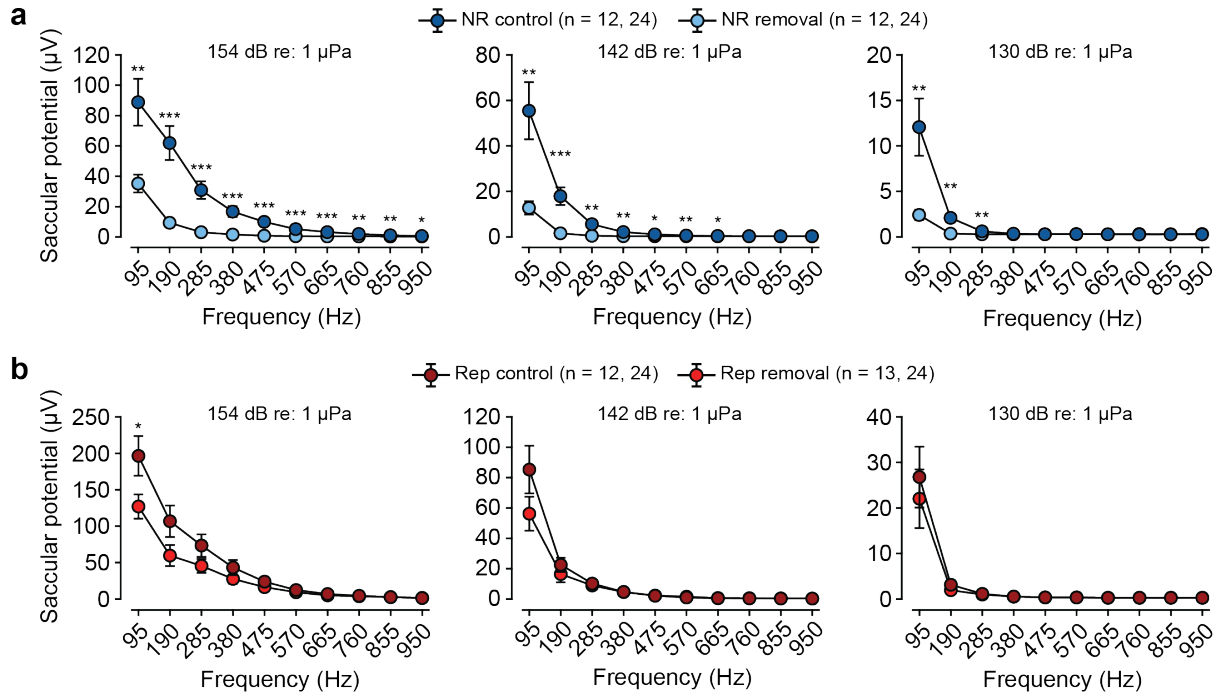

Figure S5: Saccular hair cell iso-level responses when stimulated by biologically relevant pure tone playbacks at sound pressure levels of 154, 142, and 130 dB re: 1 µPa, respectively. a, Response curves of non-reproductive male plainfin midshipman with intact (control; dark blue) and removed (removal; light blue) swim bladders. **b,** Response curves of reproductive male plainfin midshipman with intact (control; dark red) and removed (removal; light red) swim bladders. Data are represented as mean ± 1 SE; note that some error bars are minimal, and the symbols may obscure the bars. Asterisks indicate significant differences across frequencies: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. The number of animals and records for each group is indicated in parentheses.

References

1. Bradley, D. & Wilson, W. Acoustic impedance of sea water as a function of temperature, pressure and salinity. (1966).
2. Erbe, C. *Underwater acoustics: noise and the effects on marine mammals*. (JASCO Applied Sciences, Brisbane, 2011).
3. Colleye, O., Vetter, B. J., Mohr, R. A., Seeley, L. H. & Sisneros, J. A. Sexually dimorphic swim bladder extensions enhance the auditory sensitivity of female plainfin midshipman fish, Porichthys notatus. *Journal of Experimental Biology* **222**, jeb204552 (2019).
4. Vetter, B. J., Seeley, L. H. & Sisneros, J. A. Lagenar potentials of the vocal plainfin midshipman fish, Porichthys notatus. *J Comp Physiol A* bas163-175 (2019) doi:10.1007/s00359-018-01314-0.
5. Rogers, L. S. & Sisneros, J. A. Auditory evoked potentials of utricular hair cells in the plainfin midshipman, Porichthys notatus. *Journal of Experimental Biology* **223**, (2020).