**SUPPLEMENTARY MATERIALS**

**Reproductive state modulates utricular auditory sensitivity in a vocal fish**

**Running title:** Reproductive state modulatesutricular sensitivity

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**Supplementary table 1: Summary of mean evoked potentials (μV) recorded from utricular hair cells of non-reproductive and reproductive midshipman at 154 dB re. 1 μPa.** Non-reproductive and reproductive female evoked potential (μV) responses are displayed as mean ± SEFold change was calculated as the ratio of the frequency-specific mean evoked response (μV) of reproductive females relative to non-reproductive female midshipman. Non-reproductive and reproductive frequency-specific mean evoked potentials (μV) were compared via *a priori* t-tests, and the *p*-values are shown. \*Significant differences between the two groups (*p*<0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Evoked potential (μV) | |  |  |
| Frequency (Hz) | Non-reproductive | Reproductive | Fold change | *p*-value |
|  |  |  |  |  |
| 105 | 25.3 ± 2.4 | 52.0 ± 4.7 | 2.1 | <0.001\* |
| 125 | 22.6 ± 2.4 | 51.5 ± 3.7 | 2.3 | <0.001\* |
| 145 | 31.8 ± 2.9 | 60.7 ± 4.7 | 1.9 | <0.001\* |
| 165 | 29.0 ± 3.3 | 52.6 ± 5.2 | 1.8 | <0.001\* |
| 185 | 26.1 ± 2.6 | 57.5 ± 4.5 | 2.2 | <0.001\* |
| 205 | 22.5 ± 2.5 | 52.9 ± 3.4 | 2.4 | <0.001\* |
| 245 | 16.5 ± 1.9 | 38.1 ± 3.7 | 2.3 | <0.001\* |
| 285 | 11.1 ± 1.1 | 27.6 ± 2.9 | 2.5 | <0.001\* |
| 305 | 10.0 ± 1.1 | 23.3 ± 2.2 | 2.3 | <0.001\* |
| 405 | 4.7 ± 0.8 | 14.4 ± 1.5 | 3.1 | <0.001\* |
| 505 | 6.0 ± 1.3 | 10.3 ± 1.4 | 1.7 | 0.029\* |
| 605 | 7.1 ± 2.0 | 7.0 ± 1.1 | 1.0 | 0.974 |
| 705 | 9.1 ± 2.5 | 6.9 ± 1.4 | 0.8 | 0.462 |
| 805 | 5.5 ± 1.6 | 4.8 ± 1.3 | 0.9 | 0.721 |
| 905 | 0.9 ± 0.2 | 2.4 ± 0.8 | 2.6 | 0.094 |
| 1005 | 5.4 ± 2.3 | 2.6 ± 1.2 | 0.5 | 0.268 |

**Supplementary table 2: Summary of mean evoked potentials (μV) recorded from utricular hair cells of non-reproductive and reproductive midshipman at 142 dB re. 1 μPa.** Non-reproductive and reproductive female evoked potential (μV) responses are displayed as mean ± SEFold change was calculated as the ratio of the frequency-specific mean evoked response (μV) of reproductive females relative to non-reproductive female midshipman. Non-reproductive and reproductive frequency-specific mean evoked potentials (μV) were compared via *a priori* t-tests and the *p*-values are shown. \*Significant differences between the two groups (*p*<0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Evoked potential (μV) | |  |  |
| Frequency (Hz) | Non-reproductive | Reproductive | Fold change | *p*-value |
|  |  |  |  |  |
| 105 | 11.3 ± 1.2 | 31.1 ± 3.6 | 2.7 | <0.001\* |
| 125 | 7.6 ± 0.8 | 25.9 ± 2.5 | 3.4 | <0.001\* |
| 145 | 13.5 ± 1.6 | 33.5 ± 2.8 | 2.5 | <0.001\* |
| 165 | 13.0 ± 2.1 | 26.3 ± 2.4 | 2.0 | <0.001\* |
| 185 | 9.1 ± 0.9 | 26.0 ± 2.4 | 2.9 | <0.001\* |
| 205 | 6.3 ± 1.1 | 19.3 ± 1.7 | 3.1 | <0.001\* |
| 245 | 3.6 ± 0.9 | 12.2 ± 1.5 | 3.4 | <0.001\* |
| 285 | 2.2 ± 0.6 | 6.8 ± 1.0 | 3.0 | <0.001\* |
| 305 | 1.9 ± 0.6 | 5.8 ± 0.9 | 3.0 | <0.001\* |
| 405 | 0.7 ± 0.2 | 2.7 ± 0.5 | 3.7 | <0.001\* |
| 505 | 0.5 ± 0.1 | 1.4 ± 0.3 | 2.7 | 0.004\* |
| 605 | 0.3 ± 0.1 | 0.6 ± 0.1 | 2.0 | 0.008\* |
| 705 | 0.3 ± 0.03 | 0.4 ± 0.1 | 1.6 | 0.022\* |
| 805 | 0.3 ± 0.03 | 0.5 ± 0.1 | 1.8 | 0.042\* |
| 905 | 0.3 ± 0.03 | 0.3 ± 0.03 | 1.1 | 0.606 |
| 1005 | 0.3 ± 0.03 | 0.3 ± 0.02 | 1.0 | 0.786 |

**Supplementary table 3: Summary of mean evoked potentials (μV) recorded from utricular hair cells of non-reproductive and reproductive midshipman at 130 dB re. 1 μPa.** Non-reproductive and reproductive female evoked potential (μV) responses are displayed as mean ± SEFold change was calculated as the ratio of the frequency-specific mean evoked response (μV) of reproductive females relative to non-reproductive female midshipman. Non-reproductive and reproductive frequency-specific mean evoked potentials (μV) were compared via *a priori* t-tests and the *p*-values are shown. \*Significant differences between the two groups (*p*<0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Evoked potential (μV) | |  |  |
| Frequency (Hz) | Non-reproductive | Reproductive | Fold change | *p*-value |
|  |  |  |  |  |
| 105 | 2.1 ± 0.3 | 13.2 ± 2.1 | 6.3 | <0.001\* |
| 125 | 1.2 ± 0.2 | 7.2 ± 0.9 | 6.2 | <0.001\* |
| 145 | 2.7 ± 0.5 | 11.1 ± 1.2 | 4.1 | <0.001\* |
| 165 | 2.5 ± 0.6 | 8.7 ± 1.3 | 3.5 | <0.001\* |
| 185 | 1.6 ± 0.3 | 6.4 ± 0.8 | 3.9 | <0.001\* |
| 205 | 1.0 ± 0.4 | 3.4 ± 0.5 | 3.3 | <0.001\* |
| 245 | 0.5 ± 0.1 | 2.0 ± 0.4 | 4.1 | 0.001\* |
| 285 | 0.3 ± 0.1 | 1.0 ± 0.2 | 2.8 | 0.011\* |
| 305 | 0.3 ± 0.04 | 0.8 ± 0.2 | 2.7 | 0.008\* |
| 405 | 0.3 ± 0.03 | 0.4 ± 0.1 | 1.6 | 0.068 |
| 505 | 0.3 ± 0.02 | 0.3 ± 0.03 | 1.1 | 0.426 |
| 605 | 0.2 ± 0.02 | 0.3 ± 0.02 | 1.2 | 0.258 |
| 705 | 0.3 ± 0.02 | 0.2 ± 0.02 | 1.0 | 0.916 |
| 805 | 0.2 ± 0.02 | 0.3 ± 0.03 | 1.2 | 0.232 |
| 905 | 0.3 ± 0.02 | 0.3 ± 0.01 | 1.0 | 0.968 |
| 1005 | 0.3 ± 0.03 | 0.3 ± 0.02 | 1.0 | 0.697 |



**Supplementary figure 1: Midshipman inner ear morphology.** μCT reconstruction of midshipman otolithic end organs, which illustrates variation in otolithic end organ orientation. S, L, and U represent saccule, lagena, and utricle, respectively. μCT data was obtained and adapted from the Virtual Natural History Museum (http://131.220.133.140/VNHM/).

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**Supplementary figure 2: Frequency-specific particle acceleration levels (dB re: 1 ms-2) for three representative sound levels [130 (white), 142 (gray), and 154 dB re: 1 μPa (black)].** Particle acceleration level (dB re: 1 ms-2) measurements were measured using 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). The accelerometer was positioned 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. Particle motion at each tested frequency was acquired 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 (Vpk-pk) for each axis (*x-*, *y-*,and *z-*axis) were corrected for the gain (sensitivity) of the accelerometer and then calculated as the combined magnitude vector of particle acceleration in dB scale (Eq. 1).



**Supplementary figure 3: Comparison of reproductive female utricular and saccular auditory sensitivity.** Particle acceleration level (dB re: 1 ms-2) threshold curve of utricular (gray) and saccular (white) hair cells from summer reproductive female plainfin midshipman. 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. Saccular hair cell data has been adapted from Colleye et al., 2019.