

1 **Use of the swim bladder and lateral line in near-field sound source localization by fishes**

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ABSTRACT

We investigated the roles of the swim bladder and the lateral line system in sound localization behavior by the plainfin midshipman fish (*Porichthys notatus*). Reproductive female midshipman underwent either surgical deflation of the swim bladder or cryoablation of the lateral line and were then tested in a monopolar sound source localization task. Fish with nominally “deflated” swim bladders performed similar to sham-deflated controls; however, post-experiment evaluation of swim bladder deflation revealed that a majority of “deflated” fish (88%, 7 of the 8 fish) that exhibited positive phonotaxis had partially inflated swim bladders. In total, 95% (21/22) of fish that localized the source had at least partially inflated swim bladders, indicating that pressure reception is likely required for sound source localization. In lateral line experiments, no difference was observed in the proportion of females exhibiting positive phonotaxis with ablated- (37%) versus sham-ablated (47%) lateral line systems. These data suggest that the lateral line system is likely not required for sound source localization, although this system may be important for fine-tuning the approach to the sound source. We found that midshipman can solve the 180° ambiguity of source direction in the shallow water of our test tank, which is similar to their nesting environment. We also found that the potential directional cues (phase relationship between pressure and particle motion) in shallow water differs from a theoretical free-field. Therefore, the general question of how fish use acoustic pressure cues to solve the 180° ambiguity of source direction from the particle motion vector remains unresolved.

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INTRODUCTION

58 Fish are well equipped to detect underwater acoustic and vibratory stimuli. Most teleost fishes
59 have both auditory and lateral line systems, and each system is responsive to the local fields produced by
60 acoustic/vibratory sources. These systems are used in a variety of behavioral contexts, including
61 behaviors such as prey detection and mate selection, which necessitate sound source orientation and
62 localization (Fay, 2005; Webb et al., 2008). While several studies have established that some fishes can
63 determine sound direction and localize underwater sound sources (Schuijff, 1975; Schuijff and Hawkins,
64 1983; Zeddies et al., 2010; 2012), there is much debate over the mechanism(s) underlying sound source
65 localization ability (see Fay and Simmons, 1999; Rogers and Zeddies, 2008). Sound pressure is a scalar
66 quantity containing no information about direction, and thus is in itself not useful for determining sound
67 source direction. The particle motion component of sound, in contrast, is a vector quantity (containing a
68 directional component) and could thus be useful for determining the direction to a sound source. Fishes
69 possess two systems for particle motion detection, the inner ear and the lateral line (Braun and Coombs,
70 2000; 2010). Based on his work on the lateral line organs of killifish (*Fundulus heteroclitus*), van Bergeijk
71 suggested that pressure reception by the inner ear allowed for detection of a sound source but that the
72 lateral line system was responsible for supplying directional information (Harris and van Bergeijk, 1962;
73 van Bergeijk, 1967). Years later, the relative contributions of the inner ear and lateral line to particle
74 motion detection and source localization are still not firmly understood.

75 Particle motion detection by the inner ear alone seems insufficient for signaling the direction to a
76 sound source because the axis of the particle motion vector points both towards and away from the
77 source, meaning there is a "180° ambiguity" that remains unresolved without further information (Fay
78 2005). In other words, fish sensitive only to particle motion should be able to identify the axis along which
79 the local particle motion lies, but unable to distinguish whether the source is located at one heading or at
80 180° in the opposite direction. Current models for sound source localization by fish depend on the
81 detection and processing of both the pressure and particle motion components of sound for the resolution
82 of this ambiguity (Fay, 2005). A major assumption of several related hypotheses (Chapman and Hawkins,
83 1973; Schuijff, 1975), including a dominant hypothesis of sound source localization known as the "phase
84 model" (Schuijff and Buwalda, 1975), holds that fishes are able to use the phase difference of sound

85 pressure and particle motion components to compute the direction to a sound source (resolving the 180°
86 ambiguity). However, these models assume a far-field pressure-particle velocity relation and require
87 sinusoidal stimuli. An alternative computational model proposed by Rogers et al. (1988) for resolving the
88 180° ambiguity also requires that fish both detect sound pressure and particle motion, but works at all
89 distances from a simple monopole sound source and does not require sinusoidal signals. For sinusoidal
90 signals, the Schuijf and Rogers models make identical predictions, dependent on the phase difference of
91 the pressure and particle velocity components.

92 It is highly likely that all fishes are able to detect the particle motion component of sound via the
93 inner ear otolithic end organs, which function as inertial accelerometers (de Vries, 1950; Dijkgraaf, 1960;
94 Fay, 1984). Recent behavioral studies with the plainfin midshipman fish (*Porichthys notatus*) show that
95 these fish can use local acoustic particle motion to locate a sound source (Zeddies et al., 2010; Zeddies
96 et al., 2012). The extent to which midshipman fish are receptive to sound pressure, or able to resolve the
97 180° ambiguity problem via pressure reception, is not known. More generally, the role that pressure
98 reception or the mechanosensory lateral line, which is sensitive to both particle motion and local pressure
99 gradients (Webb et al., 2008), may play in sound source localization remains empirically untested.

100 Here, we investigate the use of the swim bladder and lateral line in sound source localization by
101 female plainfin midshipman in the near field, wherein the source distance is less than an acoustic
102 wavelength and the ratio of pressure to radial particle velocity is a distance-dependent complex-value
103 smaller in magnitude than the density-sound speed product. Experiments were conducted during the
104 summer of 2010 on wild-caught females in reproductive condition. Across experiments, stimuli were
105 simulated advertisement calls of male midshipman fish, which are approximately sinusoidal (Bass, 1992),
106 and thus well-suited to test the predictions of sound source localization models. Our findings suggest that
107 (1) pressure reception is likely required for near-field source localization, (2) midshipman can resolve the
108 180° ambiguity, though the mechanisms for doing so remain obscure, and (3) the lateral line system may
109 not be necessary for near-field source localization.

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RESULTS

Acoustic field characterization

The spatial characteristics of the acoustic field generated by the J-9 and AQ339 sound projectors were quantified from the tank mapping measurements made with miniature hydrophones (sound pressure) and a tri-axial accelerometer (particle motion). For arcs of constant radius from the source, the azimuthal standard deviation of the pressure at 90 Hz was found to be no more than 10% and 6% for the J-9 and AQ339 projectors, respectively. Standard deviation values at 75 and 80 Hz for the AQ339 were 9% and 6%, respectively. These results confirmed that the sound fields were circumferentially uniform (axisymmetric) within the mapped area of the sound field that contained the fish release points and phonotactic paths of the tested fish.

Biased and unbiased release experiments

Sound playback experiments were conducted at night in an outdoor cylindrical concrete tank, with initial release sites and the speaker position indicated in Figure 1. In this first experiment, we asked if there was a difference in localization behavior for fish that were released so that their initial swimming direction was biased towards the sound source (biased release) vs. fish that were allowed to leave the release site in any direction (unbiased release). The phonotactic responses displayed in the biased versus unbiased release experiments were unambiguous; positive phonotactic responses entailed repeated contact with the source by the fish (see Zeddies et al., 2010 for descriptions of the phonotactic response). Of the 17 reproductive females tested in the “unbiased” (open) release, 65% (n = 11) of the females exhibited a positive phonotactic response. Of the 31 reproductive females tested in the “biased” release experiments, 61% (n = 19) exhibited positive phonotactic responses. A logistic regression showed that there was no difference in the proportion of females that exhibited positive phonotactic responses in the biased (61%) release experiments compared to the unbiased (65%) release experiments ($\beta = 0.14 + 0.67$, $t = 0.23$, $p = 0.82$), suggestive that fish were able to resolve the 180° ambiguity.

Swim bladder deflation experiments

140 Phonotactic responses were next examined in fish with intact or deflated swim bladders to determine
141 whether the swim bladder was required for near-field sound source localization. Of the 28 reproductive
142 females with sham-deflated swim bladders, 50% (n = 14) exhibited positive phonotactic responses; the
143 trajectories these females took after release from site B are shown in Figure 2A. Of the 21 reproductive
144 females that underwent swim bladder deflation surgery, 38% (n = 8) exhibited positive phonotactic
145 responses (see Figure 2B). An initial logistic regression analysis indicated no difference in the proportion
146 of females with “deflated” (38%) and sham-deflated (50%) swim bladders that exhibited positive
147 phonotaxis ($\beta = 0.49 \pm 0.59$ SE, $t=0.83$, $p = 0.41$). However, upon postmortem examination of the fish that
148 underwent swim bladder deflation surgery, we found that 12 (out of 21) had partially (25 to 50%) to nearly
149 completely (>90%) inflated swim bladders. The observation of partial swim bladder inflation within 24h
150 post deflation surgery (and <12h post-test) suggests rapid re-inflation of the swim bladder in this subset of
151 animals. We thus compared, post-hoc, fish with re-inflated swim bladders (fish with greater than 25%
152 swim bladder re-inflation, n=12) against those with still-deflated swim bladders (fish with 100% swim
153 bladder deflation). Whereas 58% (n=7) of “re-inflated” fish exhibited positive phonotaxis – a proportion not
154 significantly different from that in sham-deflated controls ($\beta = -0.34 \pm 0.67$ SE, $t = 0.48$, $p = 0.68$) - only
155 one of nine “still-deflated” fish exhibited positive phonotaxis – a proportion significantly lower than in the
156 re-inflated sub-group ($\beta = 2.41 \pm 1.21$ SE, $t=1.99$, $p < 0.05$). In sum, we found that 95% (21/22) of fish
157 that localized the source had at least partially inflated swim bladders, indicating that pressure reception is
158 likely required for sound source localization.

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Lateral line ablation experiments

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Morphology

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Phonotaxis responses were next examined in fish with ablated lateral line systems in order to determine
the relative contribution of the lateral line to source localization. Lateral line ablation was achieved with a
liquid N₂-dipped probe. Cryoablation success was qualitatively verified with DASPEI in a subset of fish
immediately after sound source localization experiments were performed (see Figure 3 for an illustration
of lateral line morphology). DASPEI-labelled neuromasts were present in stereotyped patterns on the
head, trunk, and tail of intact and sham-ablated fish, while DASPEI labelling was largely absent from

168 neuromasts in ablated fish (Fig. 4). Only SN could be verified in this way due to the dark skin
169 pigmentation of the skin that obscured CN visualization, and due to the superglue over the canals in
170 ablated animals. SN ablation was quantified in fixed FM 1-43FX-labelled animals as described in the
171 methods (Fig. 5, Table 1). Regions used for quantification are indicated in Fig. 3. There were $16.1 \pm 6.7\%$
172 SN remaining (mean \pm S.D.) in liquid N₂-ablated, FM-labelled fish (n = 9 fish, 177-254 SN quantified per
173 fish), with the highest percentage of intact neuromasts observed on the mandible ($38.9 \pm 15.8\%$) and the
174 lowest percentage seen on the dorsal trunk line ($4.9 \pm 6.3\%$) (Table 1). Two fish with 17.4% and 9.6%
175 intact SN failed to localize the sound source, while a fish with only 6.8% remaining SN localized the
176 sound source, suggesting that the positive phonotaxis seen in many of the ablated fish was not due to
177 excess intact neuromasts.

178 Neuromast structure was visualized in both SN and CN using phalloidin labelling, as shown in
179 Figure 6. The small papillae are clearly visible in intact SN, with a patch of hair bundles lying between the
180 two papillae (Fig. 6 A, B). Intact CN are larger and have many more hair bundles than SN (compare Fig.
181 6B and 6C) and lack peripheral papillae. These papillae are damaged in ablated SN and the hair bundles
182 and surrounding epithelial layer are absent (Fig. 6 D, E). Ablated CN are recognized by the epithelial
183 morphology surrounding the usual hair-cell containing region (Fig. 6F). Intact CN were occasionally
184 detected in ablated fish at a frequency of approximately 1 intact CN per animal, however, CN were not
185 quantified as we could not be certain that some were not damaged during postmortem dissection. It is
186 unlikely that the few remaining CN in ablated fish received any stimulation due to the superglue that
187 obstructed the incurrent and excurrent openings to the canals.

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Localization Behavior

190 Of the 35 reproductive females that had their lateral line system ablated, 37% (n = 13) exhibited positive
191 phonotactic responses. The pathways these females took after release from site A are shown in Figure
192 7A. Of the 15 reproductive females that had sham-ablated lateral lines (photophores only), 47% (n = 7)
193 exhibited positive phonotactic responses. Swimming pathways for these fish after release from site A are
194 shown in Figure 7B. Because some experiments were conducted the day after lateral line ablation, a
195 hierarchical logistic regression was performed to control for the effect of day. There was a significant

196 increase in response on the day after lateral line ablation in both lateral line ablated (28% on Day 1, 55%
197 on Day 2) and sham ablated animals (28% on Day 1, 50% on Day 2) ($\beta = 1.75 + 0.75 \text{ SE}$, $t = 2.33$, $p =$
198 0.02). However, controlling for the effect of day, there was no difference in the proportion of females with
199 ablated- (37%) and sham ablated (47%) lateral lines that exhibited positive phonotaxis ($\beta = 0.67 + 0.69$
200 SE , $t = 0.97$, $p = 0.33$). A mean vector analysis of the swimming pathways at the initial release showed
201 that the direction of movement was biased towards the sound source ($p < 0.001$ for both ablated- and
202 sham-ablated lateral line females).

203 The angles that the positive phonotactic fish took (after released from site A) were compared for
204 females with ablated- and sham-ablated lateral line systems. The mean orientation error relative to the
205 source was significantly different at the initial release for lateral line-ablated females (mean orientation
206 error = 39.0°) compared to lateral line sham-ablated females (mean orientation error = 15.3°) (Watson-
207 Williams test, $F(1, 18) = 6.3$, $p < 0.025$). In addition, the mean orientation relative to the source at the
208 midpoint of the phonotactic pathway taken was also significantly different for lateral line-ablated females
209 (mean orientation error = 33.4°) compared to lateral line sham-ablated females (mean orientation error =
210 12.8°) (Watson-Williams test, $F(1, 18) = 6.39$, $p < 0.025$).

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DISCUSSION

213 The plainfin midshipman fish is a good model to explore how fishes localize underwater sound
214 sources (Zeddies et al., 2010; 2012), in part because reproductive females exhibit robust phonotactic
215 responses to the underwater acoustic playback of natural and synthetic male advertisement calls (Bass et
216 al., 1999; McKibben and Bass, 2001). Previously, we demonstrated that the plainfin midshipman fish can
217 use local acoustic particle motion to guide the animal to a sound source (Zeddies et al., 2010; 2012), but
218 the contribution of swim bladder pressure cues and the mechanosensory lateral line to sound source
219 localization remained unresolved. Thus, the primary objective of this study was to determine whether the
220 swim bladder and/or the lateral line were required for near-field sound source localization. Our findings
221 suggest that pressure reception is likely required for near-field source localization, that fish can solve the
222 180° ambiguity inherent in the particle motion vector, and that the lateral line system is likely not
223 necessary for near-field sound source localization. It is important to note, however, that these

224 experiments were performed in a benthic species that inhabits an extreme shallow-water environment
225 (during the breeding season), and therefore our results may not pertain to all fishes, especially pelagic
226 species that live in the water column.

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228 **Sound pressure reception and resolving the 180° ambiguity**

229 During an acoustic disturbance, an infinitesimal “particle” of fluid undergoes a small linear
230 displacement of oscillating polarity. In the present case, with an axisymmetric sound field, the particle
231 motion vector alternately points towards and away from the acoustic source for equal amounts of time.
232 Therefore, this particle motion vector does not indicate which direction along the line to follow in order to
233 reach the source, giving rise to the so-called 180° ambiguity in particle motion. The behavioral capacity
234 for directional-dependent hearing in fish has been demonstrated (e.g., directional masking in Atlantic cod
235 *Gadus morhua*, Hawkins and Sand, 1977; sound source localization in plainfin midshipman, Zeddies et
236 al., 2010; 2012), but there were no data to demonstrate that fish solve the 180° ambiguity problem during
237 sound source localization. In the present study we found no difference in the positive phonotactic
238 response rate when fish were allowed to swim in any direction upon release (“unbiased” release) versus
239 when they were directed toward the sound source upon release (“biased” release). We also found that
240 the positive response rate was > 60% in both release cases. If fish could not solve the 180° ambiguity,
241 biasing their release toward the source would be expected to increase the positive phonotactic response
242 rate relative to that for the unbiased release, since in the unbiased condition, fish able to detect the
243 source axis but unable to determine “front” from “back” would be expected to swim away from the source
244 on ~half of trials. Moreover, no fish were observed to exit away from the sound source and then correctly
245 turn and move to the sound source (Figure 2). Therefore, because the positive phonotactic response rate
246 was the same in both release conditions, and the positive response rate in the unbiased situation was
247 >60%, we suggest that midshipman effectively resolve the 180° ambiguity problem during sound source
248 localization. While is true that a subset of fish in all experiments failed to localize the sound source, the
249 majority of these fish did not swim 180° in the opposite direction, as would be expected if they were
250 motivated to locate the source but could not solve the 180° ambiguity. Instead, some of the non-localizing

251 fish remained at the release site, while the others swam in various directions towards the edges of the
252 tank, at which point the trial was terminated.

253 The relationship between particle motion and pressure is central to hypotheses concerning the
254 basis of sound source localization by fish, notably the “phase model” suggested by Schuijf and Buwalda
255 (1975) and the more general model of Rogers et al. (1988). In these models, fish must be receptive to
256 particle motion and pressure in order to solve the 180° ambiguity. To assess the role that pressure
257 reception may play in sound source localization by midshipman, we attempted to deflate the swim bladder
258 in a subset of animals. Collectively, 95% of fish (21/22) that exhibited a positive phonotactic response had
259 at least partially inflated swim bladders (7 re-inflated, 14 sham deflated), while only a single fish with a
260 fully deflated swim bladder (1/9) exhibited a positive phonotactic response. Taken together, these results
261 suggest that sound pressure is likely an important cue used in sound source localization by these fish.
262 The overall reduction in phonotaxis and sound localization in fish with intact or re-inflated swim bladders
263 compared to females in previous studies (Zeddies et al. 2010, 2012) is likely due to the after effects of
264 anesthesia and the observed reduction in swimming behavior in recently anesthetized midshipman that
265 were surgically manipulated, a phenomenon also seen in the lateral line ablation experiments (J.A.S. and
266 A.B.C., data not shown).

267 Although it is apparent that sound pressure is used for source localization by the plainfin
268 midshipman fish, it is not clear how the pressure cue is used to solve the 180° ambiguity problem. The
269 “phase model” (Schuijf and Buwalda, 1975) was developed based on experiments using approximately
270 planar wave conditions, wherein sound pressure and radial particle velocity are in phase, having the
271 same or opposite polarity depending on the direction of propagation. The propagation direction-polarity
272 relationship persists for the in-phase components of the pressure and particle velocity even in the near
273 field for a free-field point source. This is the basis of the Rogers et al. (1988) algorithm for resolving the
274 ambiguity. However, for the propagation conditions in our test tank and for some natural environments,
275 including the midshipman nesting environment, the phase relationship between sound pressure and
276 radial particle velocity is more complicated, and can be significantly different from the field of a free-field
277 point source. We discuss these differences in depth in a modeling study described in the Supplementary
278 Materials accompanying this paper. In short, our results indicate that if a fish responded correctly to a

279 free-field source (at any range), it would actually respond in the *wrong* direction during phonotaxis in the
280 non-free-field Bodega Bay tank. Similarly, our propagation models for conditions similar to the natural
281 nesting environment of the midshipman indicate that if a fish responded in the correct direction for a free-
282 field point source, it would also respond in the *wrong* direction in the nesting environment at distances
283 that ranged from 1 cm to 25-50 m depending on bottom type. Ultimately, since female midshipman
284 consistently exhibited positive phonotaxis in our test tank, we are left to conclude that different source
285 localization strategies are used in shallow-water versus free-field environments, or that midshipman are
286 unable to correctly resolve the 180° ambiguity in deep water. Future studies will be required to determine
287 which of these alternatives is correct and to empirically assess utilization of the directional cues of
288 acoustic pressure and radial particle velocity during sound source localization by midshipman and other
289 fish.

290 All fishes are likely sensitive to particle motion (Fay, 1984) but pressure reception differs greatly
291 among species. Some species, such as flatfish and all elasmobranchs, do not have swim bladders or
292 other gas-filled structures and therefore are not sensitive to pressure, while other species (e.g.,
293 otophysans) have elaborate morphological adaptations for pressure reception (see Popper and Coombs,
294 1982; Popper and Lu, 2000). These adaptations include the development of gas bladders in close
295 proximity to the ear and/or specializations such as Weberian ossicles that form an "otophysic" link
296 between the swim bladder and the inner ear. In these fishes, an incoming sound wave will cause the
297 compression and rarefaction of the air volume in the bladder, which will generate a pressure gradient and
298 a secondary displacement field that is then transmitted to the inner ear, allowing these fish to perceive
299 sound pressure (Popper and Fay, 1999). Sound source localization has not been convincingly
300 demonstrated for fish species with obvious specializations for pressure reception, although few studies
301 have been performed on otophysan fishes (e.g., Schuijf et al., 1977). Pressure sensitivity has also been
302 demonstrated in species without obvious morphological adaptations (Chapman and Hawkins, 1973;
303 Myrberg and Spires, 1980). These species, according to the phase model, could theoretically also resolve
304 the 180° ambiguity. Other species that possess swim bladders but do not detect sound pressure and all
305 species lacking a swim bladder (e.g., flatfishes and elasmobranchs), should theoretically be incapable of
306 resolving the 180° ambiguity (Chapman and Sand, 1974; Hawkins and Johnstone, 1978). Empirical

307 assessment of localization abilities across these different species would further establish the real
308 contributions of pressure sensitivity to source localization/resolution of the 180° ambiguity, toward a test
309 of the phase model and similar models.

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311 **Contribution of the lateral line to sound source localization**

312 Fish possess two mechanosensory systems, the ear and the lateral line, which respond to many
313 of the same stimulus fields (e.g., Coombs et al., 1989; Schellart and Wubbels, 1998; Braun and Coombs,
314 2000). While early theorists suggested that the lateral line was sufficient for source localization in the
315 near field, more recent studies demonstrate that vibrating dipole sources elicit physiological and
316 behavioral responses mediated by both sensory systems, suggesting that either system may be sufficient
317 for source localization (van Bergeijk, 1967; Nauroth and Mogdans, 2009; Braun and Coombs, 2010;
318 Coombs et al., 2010). Single-unit recordings from trunk lateral line afferents in plainfin midshipman
319 demonstrate responses up to 100 Hz, showing that trunk SN are capable of encoding the 75-80 Hz
320 stimulus used in the present experiments (Weeg and Bass, 2002). However, the question of whether the
321 lateral line is required for localization behavior in this species had not been previously explored.

322 In our experiments, animals were released approximately 86 cm from an 80 Hz sound source,
323 i.e., under near-field conditions in which both the inner ear and lateral line may be stimulated (e.g., Braun
324 and Coombs, 2000). We observed no statistical difference in sound source localization by animals with
325 sham-ablated lateral lines as compared to those where the lateral line was ablated with liquid N₂. These
326 data thus suggest that the lateral line is likely not required by this species for source localization in an
327 axisymmetric sound field. Because we never achieved 100% ablation, it is theoretically possible that the
328 remaining neuromasts were sufficient for localization behavior. We consider this hypothesis unlikely
329 because ablation was usually successful over much of the animal, and the propensity for positive
330 phonotaxis did not appear to be related to the proportion of surviving neuromasts (e.g., the animal with
331 the greatest number of surviving neuromasts, 17.6%, failed to localize the source). Nonetheless, we
332 cannot exclude the possibility that a few surviving neuromasts may be sufficient for source localization.
333 Intact neuromasts were most commonly detected along the ventral mandible (see Table 1); thus, if the
334 lateral line is used for localization, the mandibular SN could be involved. Other regions, such as the

335 dorsal trunk line, would not appear to be necessary, given that 100% of dorsal trunk SN were ablated in
336 some fish that demonstrated localization behavior (Table 1).

337 Interestingly, while females with ablated lateral lines demonstrated positive phonotactic
338 responses, there was a significant difference in the mean orientation error (relative to the sound source)
339 of the ablated versus sham-ablated fish. These results suggest a possible role for the lateral line in
340 localization accuracy and imply complementary or synergistic roles for the lateral line and inner ear in
341 source detection (e.g., Braun et al., 2002). Perhaps the lateral line is not indispensable for localization in
342 this context, but is instead used to fine-tune the approach to the target. In the mottled sculpin (*Cottus*
343 *bairdii*), the lateral line mediates orienting behavior to prey, and subtle differences in strike feeding were
344 noted in two predatory fish species (muskellunge *Esox masquinongy* and largemouth bass *Micropterus*
345 *salmoides*) when the lateral line was inactivated with cobalt chloride (New and Kang, 2000; Coombs et
346 al., 2001; Braun and Coombs, 2010). These studies provide behavioral evidence that the lateral line
347 provides information important for orienting behaviors, consistent with the subtle differences in orientation
348 to the sound source we observed in midshipman.

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Conclusions

351 In this study, the plainfin midshipman fish was used as a general model to investigate the use of swim
352 bladder pressure cues and lateral line mechanosensory information in near-field sound source
353 localization. Our findings suggest that pressure reception via the swim bladder is likely important while the
354 lateral line system may not be required for sound source localization. Our results show that the
355 midshipman can solve the 180° ambiguity in a complex field where the potential directional cues (phase
356 relationship between pressure and particle motion) are the opposite to what they would be in a free field
357 and suggest, therefore, that whatever strategy the female midshipman uses to correctly resolve the 180°
358 ambiguity in our test tank or in the extremely shallow nesting environment would yield an incorrect
359 resolution in a deep water free-field environment. Therefore, the question of how fish use acoustic
360 pressure cues to solve the 180° ambiguity of source direction from the particle motion vector remains
361 unresolved.

362

363

MATERIALS AND METHODS

364

Experimental animals

365 One hundred forty-six adult, reproductive female plainfin midshipman fish (*Porichthys notatus*- Girard
366 1854) were collected during the summer midshipman reproductive season (May and June 2010). Fish
367 were collected from the nests of type I males (Sisneros et al., 2009) during the morning low tides in the
368 intertidal zone of Tomales Bay near Marshall, CA, USA, which is the same geographical location used in
369 previous studies (Zeddies et al., 2010; 2012). Gravid females were visually distinguishable from type I
370 and II males based on the distended appearance of the abdomen due to the presence of eggs (Bass,
371 1996; Bass et al., 1999). Collected fish were transported in coolers with aerated seawater from the field to
372 the Bodega Marine Laboratory (BML) in Bodega Bay, CA, USA. At BML, the fish were maintained in large
373 communal tanks at natural ambient temperatures (12-14°C) until later that night when experiments were
374 conducted. All experimental procedures were approved by the University of California Davis Institutional
375 Animal Care and Use Committee.

376

377

Experimental tank and setup

378 All localization experiments were conducted at BML in an outdoor cylindrical concrete tank (4 m diameter,
379 0.75 m height), the same tank used in previous studies (McKibben and Bass, 1998; 2001; Zeddies et al.,
380 2010; 2012). A sound source (Lubell AQ339, Clark Synthesis, Littleton, CO, USA or a US Navy J-9
381 transducer) was suspended in the center of the tank and positioned 10 cm above the tank floor. Note that
382 two different sound sources were used for these experiments because the J-9 transducer was
383 unavailable for the lateral line ablation experiments. When using the AQ339, it was positioned with its
384 radiating face oriented vertically. When using the J-9, the face of the projector was positioned such that it
385 was oriented away from the tank center. A 2.44 m opaque plastic tarp was placed immediately in front of,
386 but not touching, the sound projector to prevent possible visual cues of the projector that might affect
387 sound source localization behavior.

388

389 The acoustic playback signal consisted of a continuous tone that mimicked the fundamental
390 frequency of the male advertisement call, as this stimulus elicits robust positive phonotaxis in gravid
female plainfin midshipman fish (Bass et al., 1999; Bass and McKibben, 2003). The playback signal used

391 was either 75 or 80 Hz, based on the ambient temperature of the water in the tank; as previous studies
392 report that female midshipman show a temperature-dependent frequency preference in their phonotactic
393 responses (McKibben and Bass, 1998). While temperature may also influence auditory sensitivity in
394 some fish species (Wysocki et al., 2009), in the present experiment both control and experimental
395 animals were tested on a given night using the same temperature parameters, allowing for comparisons
396 between treatment groups. The acoustic playback stimuli were generated by an Agilent 33120A function
397 generator and passed through a Krohn-Hite 3550 filter (30 – 500 Hz bandpass) to a power amplifier
398 (Crown Audio Inc., Elkhart, IN) that drove the sound projector. Acoustic pressure levels were verified
399 nightly prior to behavioral experiments by placing a hydrophone (model 8103, Brüel and Kjaer, Norcross,
400 GA, USA, or model BM8178-7, Sonatech, Santa Barbara, CA, USA) at one of the two sites within the tank
401 where fish would be released (Fig. 1). The tone level at the calibration site was set at 130 dB (re 1 μ Pa
402 peak), consistent with sound pressure levels of the advertisement calls recorded near the nests of type I
403 males (Bass and Clark, 2003).

404 The behavioral responses of female midshipman fish were recorded using a digital video recorder
405 and a CV110 Precision black-and-white camera (0.2 lux minimum light level) mounted approximately 6 m
406 above the outdoor test arena. The video recordings were digitized using a Vixia HV30 camcorder (Canon
407 Inc.) and iMovie 7.0 software (Apple Inc., Cupertino, CA, USA). Windows Movie Maker 5.0 (Microsoft
408 Redmond, WA, USA) and SigmaScan Pro 5.0 (Systat Inc., Chicago, IL, USA) were used for frame-by-
409 frame analysis of the digitized video records. Every fifth frame was analyzed by marking the position of
410 the animal's head (on the midline between the two eyes) relative to the fixed position of the sound
411 projector. The x and y coordinates of the animal's head were then used to track the movement of the
412 animal in relation to the sound source and sound field.

413

414

Acoustic measurements

415 In order to characterize the fields produced by the J-9 and AQ339 sound projectors, acoustic pressure
416 and particle motion measurements were made following procedures described in previous studies
417 (Zeddies et al., 2010; 2012). Pressures were measured using miniature hydrophones (Brüel & Kjaer
418 model 8103; Norcross, GA), and particle motions were measured using a tri-axial accelerometer (PCB

419 model W356A12; PCB Piezotronics, Inc., Depew, New York) that was made neutrally buoyant by
420 embedding it in syntactic foam. Sensor signals were conditioned (Brüel & Kjaer model 2692 and PCB
421 Model 482A amplifier for the hydrophones and accelerometer, respectively), digitized and stored on a
422 Windows-based computer. Using these sensors, the acoustic fields were mapped in a horizontal plane 1
423 cm below the source center. The scan region spanned the “front” half of the test tank, where all
424 midshipman sound exposure experiments were conducted.

425

426

Experimental protocol

427 Sound playback experiments were conducted between 21:00 and 05:00 during May and June in 2010.
428 Three red floodlights were positioned around the tank perimeter that allowed for the observation and
429 videotaping of the female midshipman phonotactic responses. The water temperature in the test tank
430 ranged from 10 to 12° C and was controlled by adjusting the incoming flow rate of seawater to the tank
431 prior to the behavioral tests. Before testing began each night, the water flow to the test tank was shut off
432 and water depth was adjusted to 50 cm, a depth typical of natural nests in the field.

433 Female fish were held individually in 5 gallon buckets filled with water from the test tank for at
434 least 10-15 min prior to testing. Tests began when an individual fish was placed in a 30 cm diameter
435 plastic mesh cylinder at either release site A or B inside the test tank, approximately 86.5 cm from the
436 sound source (Fig. 1). Fish were placed in the cylinder while the acoustic stimulus was continuously
437 playing (no acclimation period). Tests were terminated when the fish swam to the perimeter of the testing
438 arena or after a positive phonotactic response. A positive response was defined as the point when a fish
439 directly touched the speaker face and/or circled in front of or under the sound projector. Although the
440 opaque plastic tarp visually obscured the position of the sound source suspended above the tank
441 substrate, the fish was able to easily swim under the tarp in front of the speaker to reach the source
442 during positive phonotaxis.

443

444

Biased and unbiased release experiments

445 In most experiments the mesh release cylinder was open at the bottom to allow the fish to swim in any
446 direction at onset of the test. In some experiments, three quarters of the bottom circumference was

447 closed off leaving a window for the animals to exit that could be directed toward the sound source. This
448 “biased” release was used to determine whether midshipman fish were able to resolve the 180° ambiguity
449 inherent in the particle motion vectors pointing toward (and away from) the sound source: Fish able to
450 detect the axis of particle motion but unable to resolve the 180° ambiguity would on some trials be
451 expected to exit the cylinder away from the source in the unbiased (open) release experiments. Initial
452 phonotaxis away from the source was physically impossible in the biased release experiments. Therefore,
453 if fish were susceptible to the 180° ambiguity, 180° errors should have occurred at a higher rate given an
454 unbiased release.

455

456 **Swim bladder deflation experiments**

457 To investigate the necessity of the swim bladder for sound source localization, a group of female
458 midshipman underwent surgical swim bladder deflation. Fish were first anesthetized by immersion in a
459 0.025% benzocaine (ethyl p-aminobenzoate) seawater bath until opercular movement ceased. After the
460 animal was anesthetized, a small (5-8 mm) incision in the body wall was made followed by another small
461 (5-6 mm) incision in the swim bladder that allowed the bladder to be deflated. Swim bladder deflation
462 surgeries were performed on the day of animal collection, and completed within 6 to 12 hours before
463 animals were used in behavioral experiments, which included a recovery time of at least 3-4 hours before
464 experiments were performed. Verification of the swim bladder deflation surgeries took place within 12
465 hours (the next morning) after the behavioral experiments were performed. “Sham-deflated” control fish
466 were anesthetized and a similar small (5-8 mm) incision was made in the body wall along the trunk of the
467 fish just dorsal to the swim bladder at a position where the bioluminescent photophores are found on the
468 body. Following surgery, fish were revived by flushing seawater over the gills until opercular movement
469 resumed and the fish showed normal righting behavior.

470

471 **Lateral line ablation experiments**

472 The lateral line was selectively ablated with liquid nitrogen. Fish were anesthetized with a 0.025%
473 concentration of benzocaine until opercular movement ceased. Small stainless steel or copper wire
474 probes were dipped in liquid N₂ and applied to the head and body of the fish to specifically ablate the

475 superficial neuromasts (SN) while the fish was under deep anesthesia. To ablate the canal neuromasts
476 (CN), canals were first opened with microdissection scissors, then the liquid N₂-cooled probe was applied
477 and the canals were re-sealed with super glue (cyanoacrylate). For sham-ablated controls, a subset of
478 ventral photophores were selectively damaged with liquid N₂ using a procedure otherwise comparable to
479 the SN ablation technique. Sham-ablated fish were anesthetized for the same period of time as lateral
480 line-ablated animals. The entire ablation process lasted 30-45 minutes. Fish were then revived by flushing
481 seawater over the gills until opercular movement resumed and the fish showed normal righting behavior.

482 Ablated fish were active upon recovery from anesthesia but did not show normal behavioral
483 escape responses when gently stimulated with a water jet applied to the skin, suggesting that the lateral
484 line was not functioning. We noted that deep anesthesia (fish left in benzocaine for a few minutes after
485 opercular movement ceased) was preferable to shallow anesthesia (fish removed from benzocaine as
486 soon as opercular movement ceased), as fish, after shallow anesthesia, did not swim normally in the
487 hours after ablation, either in the recovery tank or the sound localization test tank. Of the 50 animals that
488 had ablated and sham-ablated lateral line treatment, 39 fish were tested on the same day of surgery while
489 11 fish were tested the day following surgery due to the lethargic swimming response after recovery.
490 Post-hoc assessment confirmed that the lateral line remained ablated in fish tested the day following the
491 ablation procedure, i.e., lateral line hair cells had not regenerated in the intervening <30 hrs. The lethargic
492 response observed in some animals tested shortly after the ablation procedure suggests a potential
493 confounding effect of the benzocaine anesthesia and surgical ablation stress.

494

495 **Lateral line visualization**

496 Ablation success was qualitatively verified in a subset of animals immediately after the behavioral
497 localization experiment. Fish were immersed in 0.005% DASPEI ((2-{4-(dimethylamino-)styryl}-1-
498 ethylpyridinium iodide; Life Technologies) in seawater for 15 minutes in order to visualize remaining
499 neuromasts, rinsed in seawater, and anesthetized in a 0.025% benzocaine bath. Labelling was observed
500 using a Nikon AZ100 stereomicroscope microscope equipped for epifluorescence and images were taken
501 with a Coolsnap HQ2 camera (Photometrix) and NIS Elements software. After imaging, animals were

502 euthanized with an overdose of benzocaine, weighed, measured, and fixed in 4% paraformaldehyde for
503 subsequent lateral line dissection and labelling with fluorescently-tagged phalloidin.

504 Four regions of SN were selected for phalloidin labelling: a 2 cm region from the right dorsal line
505 starting anterior to the dorsal fin, a 1 cm region on the top of the head (left side), 1.5-2 cm from the ventral
506 line (left side) starting at the anterior end of the anal fin, and the entire caudal fin. Given the small number
507 of CN in this species, all canals were dissected out of the head and labelled rather than selecting discrete
508 CN regions for sub-sampling. Sampling regions for SN and CN are shown in Fig. 3. Dissected SN or CN
509 were treated with 20 µg/ml proteinase K for 30 min at 37° C to digest the cupula and increase phalloidin
510 penetration. Samples were then incubated for 30-40 min in Oregon Green phalloidin (Life Technologies)
511 diluted 1:100 in 0.1M phosphate-buffered saline (PBS), rinsed in fresh PBS, and mounted in 50% glycerol
512 on bridged coverslips. Images were taken on an Olympus FV-1000 confocal microscope with associated
513 Fluoview software. Confocal z-series were compressed into brightest-point projections with ImageJ.

514 After behavioral experiments, the majority of animals were labelled with vital dye for post-fixation
515 visualization of the lateral line. These animals were immersed in 3 µM FM 1-43 FX (Life Technologies)
516 for 90 sec, rinsed in salt water, and euthanized with an overdose of benzocaine. Fish were weighed,
517 measured, and then placed in 4% paraformaldehyde (in PBS) and stored at 4°C. Fixed fish were
518 transported back to the University of Washington, rinsed in fresh PBS, and the labelled lateral line was
519 visualized with a Leica MZFLIII stereomicroscope. Images were taken with a Leica DFC350FZ camera
520 and associated Firecam software (v. 3.2).

521 The number of remaining SN in the FM 1-43-labelled animals was quantified in six distinct
522 regions of each animal: the right dorsal lateral line adjacent to the dorsal fin, the SN cluster on the dorsal
523 head surface (right side), the ventral line starting anterior to the pelvic fins and extending to just behind
524 the left pectoral fin, the ventral-most line on the left operculum, the mandibular line, and the entire caudal
525 fin (see Fig. 2). These regions were selected based on initial observations in DASPEI-labelled animals
526 that our ablation procedure was highly effective in some lateral line areas (e.g., the dorsal surface of the
527 head), while other areas were more difficult to access with the frozen probe and therefore contained more
528 surviving neuromasts (e.g., the mandibular line).

529

530

Data Analysis

531 The effects of swim bladder deflation were analyzed using logistic regression. Phonotaxis responses
532 were categorized as 1 (correct localization) or 0 (incorrect) and fit to a logit function using a maximum
533 likelihood method (Menard 2002). Logistic regression was also used to examine the effects of deflated
534 swim bladders as compared to reinflated and sham deflated swim bladders. To analyze the movement of
535 the fish, the difference angles of the bearing of the fish relative to the source and relative to the local
536 sound field were determined. This was done by determining the position of the fish from the video record
537 (at 150 ms intervals) and then calculating the fish's bearing between consecutive positional points. The
538 difference angle relative to the source was the difference between the fish's bearing and the angle from
539 the fish's position to the source and was calculated between each time step (150 ms) for all recorded
540 behavioral tracks. The difference between the fish's bearing and the direction to the source was defined
541 as the orientation error. Statistical analyses were performed to determine whether there were differences
542 among the experimental groups in the difference angles at two specific points in the phonotactic pathway:
543 (1) at the initial movement out of the release point (as defined as the vector between the first observed
544 position outside the release cylinder and the fifth observed position) and (2) at the midpoint between the
545 release point and the speaker.

546 As a measure of performance, the vector strength (r) of the difference angles was computed.
547 Vector strength is a measure of directional tendency or consistency toward the source (Batschelet, 1981);
548 more formally, it is the normalized length of the mean vector of the circular distribution of angles to the
549 source (the vector for each fish is unity length with the angle to the source, or in line with the particle
550 motion vector). If all directions are equally likely, the vector strength is zero, whereas if all fish move in the
551 same direction, the vector strength is 1.0. Difference angles were tested for uniformity using a Hodges-
552 Ajne test (Zar, 1999), which tests the null hypothesis that these angles are randomly distributed. A
553 significant p value indicates a bias in the distribution of difference angles, i.e. the null hypothesis of equal
554 (random) distribution is false. Differences between treatments were analyzed using a Watson-Williams
555 test (the circular equivalent of a two sample t-test) (Watson and Williams, 1956), which analyzes whether
556 two vectors have the same mean direction. The p -values were Bonferroni corrected to $\alpha=0.025$ to reflect
557 the tests conducted at two points in the phonotactic pathway. Though this test assumes that the data have

558 an underlying von Mises unimodal distribution, the test is robust to deviations from this assumption (Zar,
559 1999). All tests were conducted using the circular statistics toolbox (Berens, 2009) for Matlab version
560 2009b (MathWorks, Natick, NJ).

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ACKNOWLEDGEMENTS

564 We would like to thank Andrew Bass and Margaret Marchaterre for field assistance and logistical support,
565 Edwin Rubel for microscopy support, and the University of California Bodega Marine Laboratory where
566 these experiments were performed. We also thank two reviewers for feedback that greatly improved the
567 manuscript.

568

AUTHOR CONTRIBUTIONS

569 A.B.C., D.G.Z., R.R.F., M.D.G., P.H.R. and J.A.S. conceived and designed the experiments. P.H.R. and
570 M. D. G. are responsible for the analysis contained in the Supplementary Material. All authors collected
571 and analyzed the data. All authors contributed to the draft and/or revision of this article.

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573

FUNDING

574 This collaborative research was supported by the National Science Foundation (IOS-0642214 to J.A.S.
575 and R.R.F).

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694 **Table 1.** Quantification of FM-labelled SN from 6 regions of liquid N₂-ablated fish (n = 9). Data are
695 represented as the number and percentage of intact SN (as visualized with FM) relative to the total
696 number of SN. Neuromast position was determined by epithelial morphology (e.g., fleshy papillae) and
697 pigmentation differences as compared to the surrounding non-sensory areas, allowing for unambiguous
698 identification of SN even in the absence of FM labelling. Data for sham-ablated animals are not shown but
699 missing neuromasts were rarely seen in these animals (0-2 unlabelled SN per fish).

700 *ablated fish that did not localize the sound source.

701 ** phonotactic response, FM labelling, and sacrifice occurred one day post-ablation.

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Fish	Dorsal line		Top of head		Ventral line		Mandible		Caudal fin		Operculum		Total	
	Intact (%)	Total	Intact (%)	Total	Intact (%)	Total	Intact (%)	Total	Intact (%)	Total	Intact (%)	Total	Intact (%)	Total
376	3 (3.9%)	76	0 (0%)	13	0 (0%)	43	9 (22.5%)	40	23 (33.8%)	68	3 (21.4%)	14	38 (15.0%)	254
377	16 (20.8%)	77	0 (0%)	9	7 (14%)	50	28 (65%)	43	10 (40%)	25	1 (6.7%)	15	62 (28.3%)	219
391*	0 (0%)	88	0 (0%)	10	5 (15.6%)	32	16 (57.1%)	28	14 (29.8%)	47	3 (23.1%)	13	38 (17.4%)	218
392*	0 (0%)	73	4 (40%)	10	3 (7.1%)	42	11 (33.3%)	33	2 (5.3%)	38	0 (0%)	12	20 (9.6%)	208
432	4 (5.2%)	77	1 (7.1%)	14	0 (0%)	49	23 (52.3%)	44	0 (0%)	32	1 (5.5%)	18	29 (12.4%)	234
433**	5 (5.5%)	90	0 (0%)	13	1 (2.1%)	47	16 (40%)	40	13 (31.7%)	41	0 (0%)	15	35 (14.2%)	246
441	4 (4.5%)	89	0 (0%)	8	0 (0%)	50	9 (31.0%)	29	28 (70%)	40	1 (5.9%)	17	42 (18.0%)	233
447	2 (2.8%)	70	0 (0%)	10	10 (20.0%)	50	8 (22.2%)	36	33 (78.6%)	42	0 (0%)	18	53 (23.4%)	226
449	1 (1.6%)	62	0 (0%)	11	0 (0%)	24	10 (26.3%)	38	0 (0%)	27	1 (6.7%)	15	12 (6.7%)	177

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743 **Figure Legends**

744

745 Fig. 1. Schematic of the experimental setup (tank diameter = 4 meters; water depth = 50 cm) showing the
746 monopole sound source (Lubell AQ339, Clark Synthesis, Littleton, CO, USA or a US Navy J-9
747 transducer), the opaque screen and the animal release sites (A and B).

748

749 Fig. 2. Phonotactic response pathways of reproductive female midshipman fish that underwent swim
750 bladder deflation surgery. (A) Response pathways of females with sham-deflated swim bladders that
751 exhibited positive (green circle) phonotaxis and negative (red circle) responses to the simulated
752 advertisement call stimulus. (B) Response pathways of females with deflated and re-inflated swim
753 bladders (see Results) that exhibited positive phonotaxis with either deflated (green circle) or re-inflated
754 (blue triangle) swim bladders and negative responses with either deflated (red square) or re-inflated
755 (orange diamond) swim bladders. The axes are the distance from the center of the tank in cm where the
756 monopole J-9 speaker (black square) was located and the dotted line represents the position of the
757 opaque screen.

758

759 Fig. 3: Schematic illustration of the plainfin midshipman fish lateral line mechanosensory system (adopted
760 from Greene, 1899). All illustrated neuromasts and several stitches not illustrated (SN primarily on the
761 head and underbelly) were targeted for ablation. Four regions of SN were selected for phalloidin labelling
762 (neuromasts filled in black): a 2 cm region from the right dorsal line starting at the anterior edge of the
763 dorsal fin (SN 1), a 1 cm region on the top of the head (left side; SN 2), 1.5-2 cm from the ventral line (left
764 side) starting at the anterior edge of the anal fin (SN 3), and the entire caudal fin (SN 4). Given the small

765 number of CN in this species, all canals were dissected from the head and labelled rather than selecting
766 discrete CN regions for sub-sampling (CN, arrows; neuromasts within canals not illustrated).

767

768

769 Fig. 4. DASPEI labelling of neuromasts in live, anesthetized female midshipman. Images are from the
770 dorsal lateral line that runs along the base of the dorsal fin. (A) Intact animal, (B) sham-operated animal
771 (ventral photophores ablated), (C) lateral line ablation performed same day, (D) lateral line ablation
772 performed one day prior to behavioral testing and imaging. Arrows in C and D delineate examples of
773 neuromast locations. Arrowheads in B and D indicate photophores, which are autofluorescent. Scale bar
774 in A is 500 μm and applies to all panels.

775

776 Fig. 5. FM 1-43FX-labelled neuromasts in fixed animals. Images are from the ventromedial surface
777 between the pectoral fins. (A) Intact animal, (B) sham-operated animal, (C-D) lateral line ablation
778 performed same day, (E) lateral line ablation performed one day prior to behavioral testing and imaging.
779 White arrows in C-E show examples of neuromast locations where all hair cells were successfully
780 ablated. Black arrows in D point to remaining neuromasts in a fish subjected to liquid N_2 ablation. Scale
781 bars = 500 μm .

782

783 Fig. 6. Phalloidin-labelled neuromasts from intact (A-C) and ablated (D-F) animals. (A) Low-magnification
784 image of a ventral SN showing intact papillae. The neuromast between these structures is indicated with
785 an arrow and is shown at higher magnification in (B). (C) Intact CN with a full complement of hair bundles.
786 (D) Low magnification image of a damaged ventral SN. The papillae are still present but appear
787 damaged. The arrow indicates the position of the ablated neuromast, which is shown in higher
788 magnification in (E). (F) Liquid N_2 -ablated CN. The arrowhead marks scattered hair bundle remnants.
789 Scale bar = 100 μm in A, D; = 10 μm in B, E; = 30 μm in C, F.

790

791 Fig. 7. Phonotactic response pathways of reproductive female midshipman fish with ablated- and sham
792 ablated lateral line systems. Positive response pathways of females with ablated lateral line systems (A)

793 and sham ablated lateral line systems (B) are colored green and negative responses are colored red. The
 794 axes are the distance from the center of the tank in cm where the monopole AQ 339 speaker (black
 795 circle) was located and the dotted line represents the position of the opaque screen.

796

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798

799 Supplementary Materials: Resolving the 180° ambiguity.

800

801 The field of a point monopole in a free-field is an archetype for the directionalization problem. It is the
 802 simplest possible case, yet one which occurs often in nature. It would be expected that any method that
 803 a fish might use to resolve the ambiguity would have to work for this case to be of general use. The
 804 mathematics can be simplified further by assuming that the signal is sinusoidal. This is a good
 805 approximation for the advertisement call of the midshipman and was exactly the case in our experiment.
 806 We focus here on the directionalization cues present in the stimulus, not on the algorithm employed by
 807 fish such as those proposed by Rogers et al (1988) or Schuijf and Buwalda (1975). Any proposed
 808 algorithm must exploit these cues. We assume therefore that the fish can detect both the acoustic
 809 particle velocity vector (relative to its own body axis) as well as the acoustic pressure. We also assume
 810 that the discrimination is to be made by simultaneous sensing both quantities at the same point in the
 811 water. For a sinusoidal signal the only parameters that can be measured are the amplitude and phase of
 812 the two field quantities.

813 Consider a point monopole source, oscillating at angular frequency ω , located at the origin of a spherical
 814 coordinate system. Using complex notation, the acoustic pressure at a distance r from the source is
 815 given by

$$816 \quad p(r, t) = \text{Re} \left[\hat{p}(r) e^{-i\omega t} \right] = \text{Re} \left[\hat{p}_0 \frac{r_0 e^{ikr}}{r} e^{-i\omega t} \right] \quad (1)$$

817 where $\hat{p}(r)$ is the complex amplitude of the received acoustic pressure, \hat{p}_0 is the complex source
 818 level of the source, $k (= \omega / c)$ is the wavenumber, r_0 is some reference distance and Re denotes the
 819 real part of the complex quantity in brackets.

820 From Euler's equation, the acoustic particle velocity in the radial direction is given by

821
$$v_r(r, t) = \text{Re} \left[\hat{v}_r(r) e^{-i\omega t} \right] = \text{Re} \left[\frac{\hat{p}_0}{\rho c} \left(1 + \frac{i}{kr} \right) \frac{r_0 e^{ikr}}{r} e^{-i\omega t} \right] \quad (2)$$

822 where $\hat{v}_r(r)$ is the complex amplitude of the radial particle velocity and ρ and c are the density and
823 speed of sound in water.

824

825 From Eqs 1 and 2, it is evident that the complex radial velocity is proportional to the complex acoustic
826 pressure:

827
$$\hat{v}_r(r) = \left[\frac{1}{\rho c} \left(1 + \frac{i}{kr} \right) \right] \hat{p}(r) \quad (3)$$

828 The quantity in brackets is the acoustic admittance, the inverse of the acoustic impedance. The real part
829 of the term in brackets is proportional to the component of the radial velocity that is in phase with the
830 pressure (the far-field component) and the second term is proportional to the component in quadrature
831 with the pressure (the near-field component). The sign of the real part is certain but the sign of the
832 imaginary part is actually unknown since it depends on the true phase of the source, which is unknown.

833 This can be seen by noting that the sign of the imaginary term in Eq. 3 would be negative if an $e^{i\omega t}$
834 time dependence was used rather than the $e^{-i\omega t}$ time dependence that was used in Eq. 1.

835 The acoustic power density (intensity) which given by $\vec{I} = p\vec{v}$ consists of a radiated term, which has a
836 nonzero time average and a reactive part with zero time average. The general expression time averaged
837 radial intensity is given by

838
$$\langle I_r \rangle = \frac{1}{2} \text{Re} \left[\hat{p}(r) \hat{v}_r(r)^* \right] \quad (4)$$

839 Substituting Eq 3 in Eq 4, the radiated power for the free-field point monopole is given by

840
$$\langle I_r \rangle = \frac{r_0^2}{2\rho c r^2} |\hat{p}_0|^2 \quad (5)$$

842

843 Note that $\langle I_r \rangle$ is always positive, indicating power being radiated away from the source. Normalizing this
844 quantity by the magnitude of I_r , we get a localization metric which we will designate by $\Gamma(r)$ which is
845 given by

846
$$\Gamma(r) = \frac{(kr)^2}{\sqrt{1+(kr)^2}} \quad (6)$$

847

848 for the free-field point source, which always falls between 0 and +1, while for an

849 arbitrary field

850

851
$$\Gamma(r) = \frac{\text{Re}(\hat{p}(r)\hat{v}_r(r)^*)}{|\hat{p}(r)\hat{v}_r(r)|} \quad (7)$$

852

853 which must fall somewhere between -1 and +1, with positive values corresponding to a sound
854 propagating in the positive r direction and negative values corresponding to sound propagating in the
855 negative r direction. The discrimination between plus and minus becomes increasingly difficult as the
856 value of Γ approaches 0.

857 The fish measures particle velocity in its own body-centered coordinate system. For simplicity, assume
858 that the fish is oriented so that its rostral-caudal acoustic axis is aligned with a radial to the source. The
859 measured particle velocity is

860
$$\hat{v}_m(r) = \pm \hat{v}_r(r) \quad (8)$$

861 The sign ambiguity in Eq 4, which reflects the 180° ambiguity, occurs because the positive rostral-caudal
862 axis may be pointing towards or away from the source. If the positive direction for the measured particle
863 velocity is toward the tail of the fish then positive sign in Eq 4 corresponds to the fish being oriented with
864 its head towards the source and the negative sign corresponds to the fish being oriented with its tail
865 towards the source. Using this choice of sign we get

866
$$(9) \quad \Gamma_m(r) = \frac{\text{Re}(\hat{p}_m(r)\hat{v}_m(r)^*)}{|\hat{p}_m(r)\hat{v}_m(r)|}$$

867

868 where $\hat{p}_m(r)$ and $\hat{v}_m(r)$ are measured (or modeled) values of pressure and particle velocity at a point r .

869 There are many possible ways in which $\Gamma_m(r)$ could be evaluated in practice.

870 In figures S1 and S2 we plot the metric, $\Gamma_m(r)$, at 80 Hz, for five cases, (1) a point source located at $r=0$,
871 (2) the field as measured in the Bodega Bay tank, and cases based on the results from a “method of
872 images” propagation model (Jenson et al, 2011) in .5 m deep water with source and receiver located
873 .05m from the bottom with three acoustically fast bottom types, (3) gravel, (4) fine sand and (5) sandy silt,
874 with sediment parameters derived from Hamilton, 1972. Figure S1 gives the results out to 5m from the
875 source and Fig. S2 gives the results out to 100 m from the source. It is clear from Figure S1 that if a fish

876 responded correctly to a free-field source, it would respond in the wrong direction in the Bodega Bay tank.
877 Propagation models for the nesting environment indicate that close to the source a fish which would
878 respond the correctly to a free-field source, would respond in the wrong direction for ranges from a
879 centimeter out to 25-50 m from the source depending on bottom type. At large ranges the theory
880 indicates the fish would respond in the correct direction. This latter conclusion would be tempered by the
881 fact that the pressure 100m from the source is 60dB lower than it is at 1 meter and that the depth of the
882 water is unlikely to remain at 0.5 m at that distance.

883 Since the female midshipman in fact responds in the correct direction in the tank and likely does so in the
884 nesting environment as well, we conclude that she must either alter her localization method when seeking
885 males or is unable to correctly resolve the 180° ambiguity in deep water.

886

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892 *morhua*). *J. Comp. Physiol. A* 98, 333-344.

893

894 Supplemental Figures

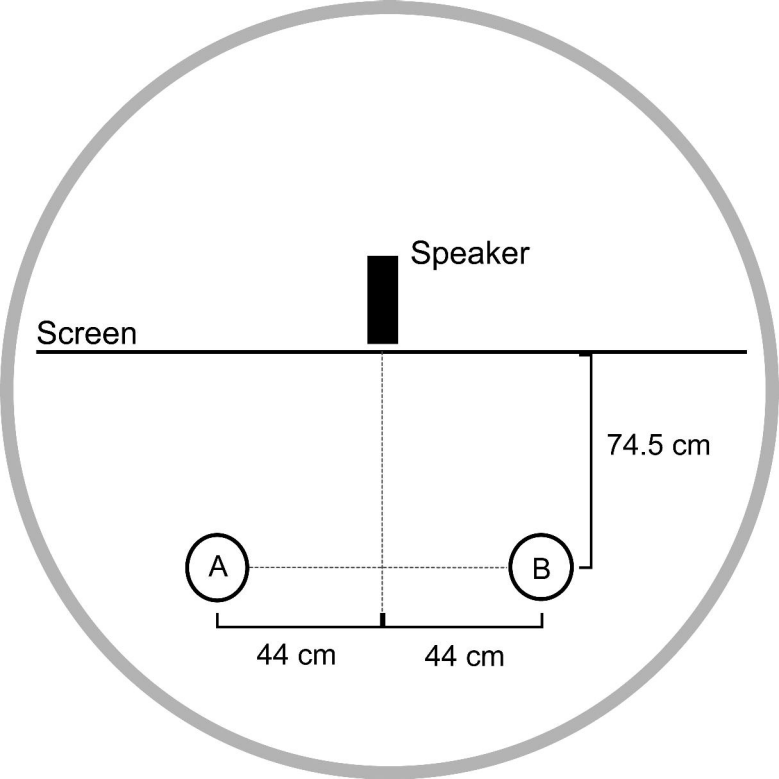
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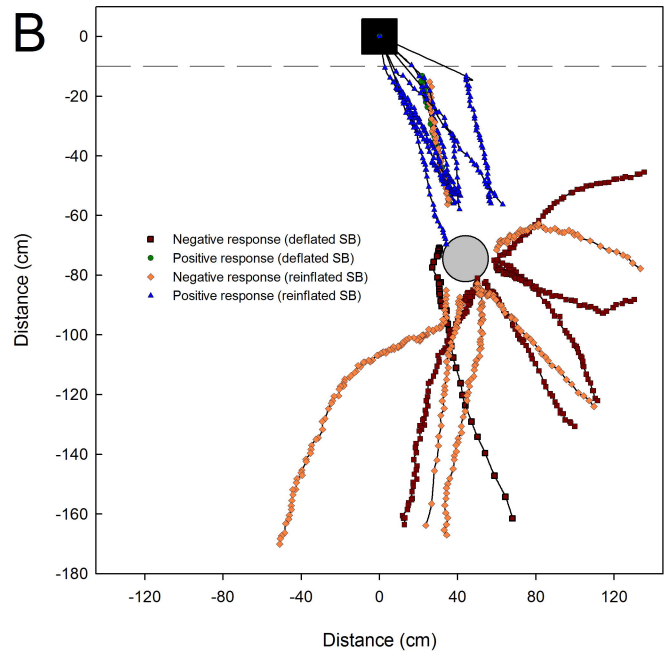
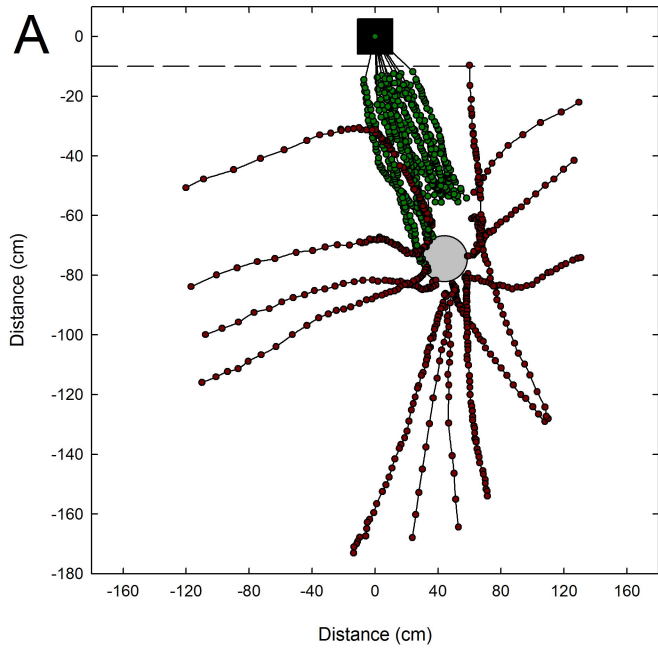
896 Fig. S1. Comparison of the localization metric Γ_m , at 80 Hz, as given in Eq. 9 (see discussion), as a
897 function of range out to 5 m from the source for a free-field point source (black), for the measured field in
898 the Bodega Bay tank (cyan), and for modeled results for 0.5 m deep water, with source and receiver 0.05
899 m from the bottom, and with three different sediment types: gravel (blue), fine sand (green) and sandy silt
900 (red). It would be expected that positive values for Γ_m would indicate the source to be located at $r = 0$ and
901 negative values would indicate that the source is located in the opposite direction.

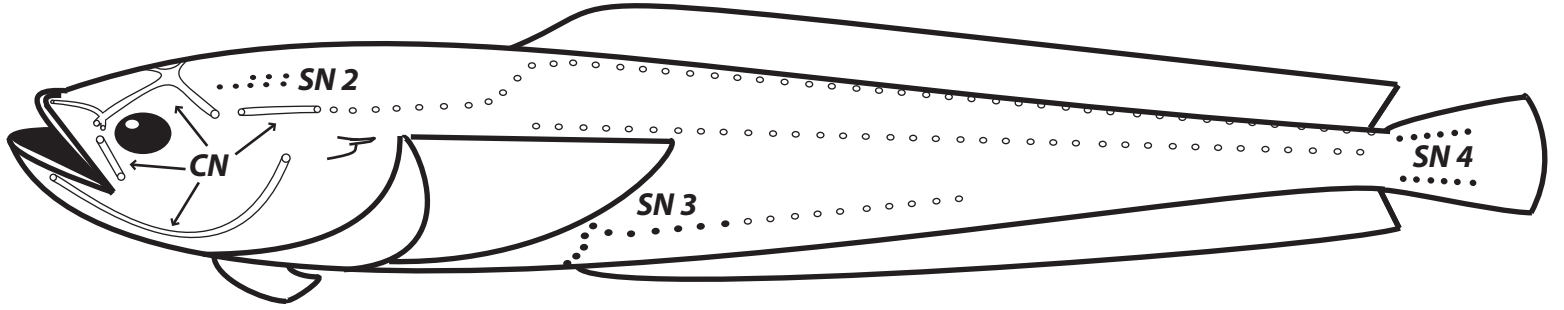
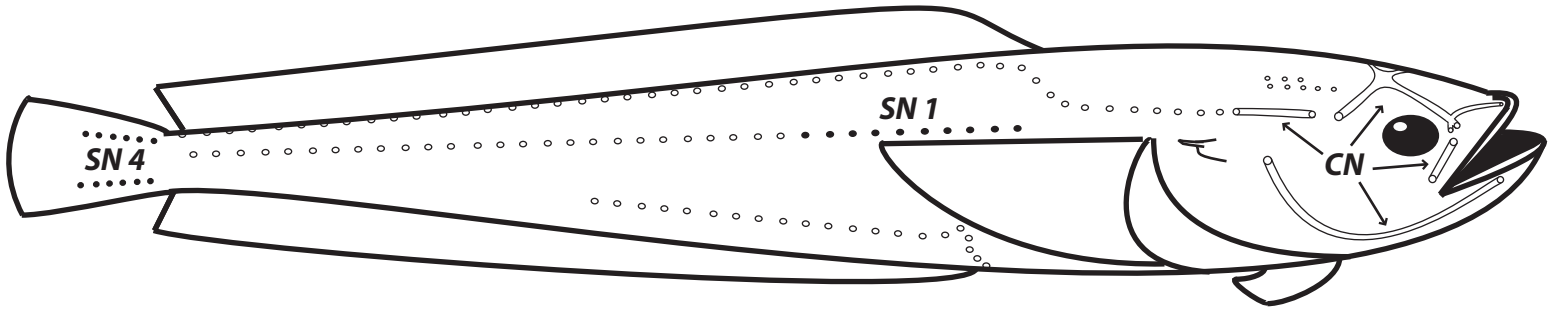
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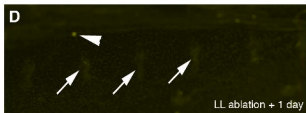
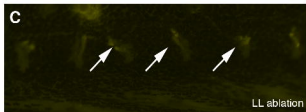
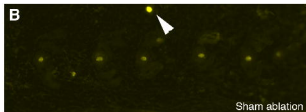
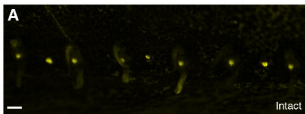
903 Fig. S2. Comparison of the localization metric Γ_m , at 80 Hz, as given in Eq. 9 (see discussion), as a
904 function of range out to 100 m from the source for a free-field point source (black), for the measured field
905 in the Bodega Bay tank (cyan), and for modeled results for 0.5 m deep water, with source and receiver 0

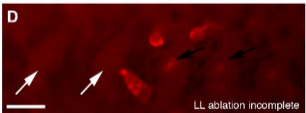
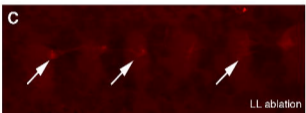
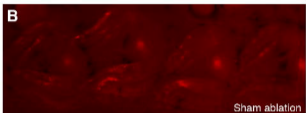
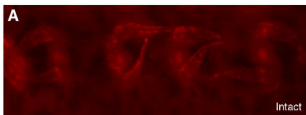
906 .05 m from the bottom, and with three different sediment types: gravel (blue), fine sand (green) and sandy
907 silt (red). It would be expected that positive values for Γ would indicate the source to be located at $r = 0$
908 and negative values would indicate that the source is located in the opposite direction
909











Intact

Damaged

