



Low-amplitude noise elicits the Lombard effect in plainfin midshipman mating vocalizations in the wild

Nicholas A. W. Brown^{a, b, *} , William D. Halliday^{a, c}, Sigal Balshine^b, Francis Juanes^a 

^a Department of Biology, University of Victoria, Victoria, BC, Canada

^b Department of Psychology, Neuroscience, & Behaviour, McMaster University, Hamilton, ON, Canada

^c Wildlife Conservation Society Canada, Whitehorse, YT, Canada

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Anthropogenic noise pollution is an emerging global threat to fish populations. Among a suite of deleterious effects, noise can potentially impede reproductive success in some fishes by masking their mate advertisement vocalizations. Using the plainfin midshipman fish, *Porichthys notatus*, a marine toadfish that produces a distinctive 'hum' during courtship, we investigated how noise affects male vocalizations and spawning success in the wild. We recorded nesting males for 3 days and measured the frequency (i.e. pitch), amplitude and duration of their vocalizations before, during and after exposure to artificial noise (~118 Hz tone). We also counted eggs in nests exposed to 10 days of artificial noise versus control nests that were not exposed to artificial noise. Males exposed to noise produced fewer vocalizations, reduced the frequency of vocalizations and increased the amplitude of their mating hum (Lombard effect). However, chronic artificial noise exposure did not significantly affect spawning success, suggesting that the Lombard effect allowed males to sustain clear advertisement signals when competing with a relatively weak artificial noise source. Future studies are needed to determine whether such vocal adjustments incur costs for males, and how common anthropogenic noises, such as boat engines, affect spawning and reproductive success.

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Human activities are driving a precipitous increase in ocean noise levels (Frisk, 2012), which is recognized as a global threat to marine life (Duarte et al., 2021; Shannon et al., 2016). Numerous marine animals rely primarily on acoustic over visual signals and information because light penetration attenuates rapidly with depth, whereas sound transmission is enhanced underwater compared to in air (Popper & Hawkins, 2018; Slabbekoorn et al., 2010). Noise pollution can therefore disrupt critical behaviours of marine animals by shrinking communication and listening spaces, damaging sensory organs and inducing physiological stress (André et al., 2011; Carter et al., 2020; Pine et al., 2020; Stanley et al., 2017; Wale et al., 2013).

Recent research has highlighted that noise pollution is an important threat facing fish populations (Cox et al., 2018; Slabbekoorn et al., 2010). Noise affects fish throughout their life span and can impede critical functions such as shoaling, cooperation, parental care and learning (Ferrari et al., 2018; Herbert-Read et al., 2017; Nedelec, Mills, et al., 2017; Nedelec, Radford et al.,

2017). For *soniferous* (sound-producing) fishes, noise can also interfere with mating by masking advertisement vocalizations (de Jong, Heubel et al., 2018; Putland et al., 2018). Some animals compensate for masking by increasing the amplitude of their vocalizations or by altering spectral and temporal characteristics of their vocalizations – a phenomenon known as the Lombard effect (Brumm & Zollinger, 2011; Feng et al., 2006; Lengagne et al., 1999; Slabbekoorn & Peet, 2003). However, these vocal adjustments are often ineffective against high-amplitude and/or broadband noise and can be energetically costly for signallers (Barber et al., 2010; Read et al., 2014). If soniferous fishes cannot effectively and honestly communicate their quality when confronted with noise pollution, they might struggle to attract mates (de Jong, Fox et al., 2018).

Currently, only a limited number of studies have linked noise to acoustic communication and subsequent reproductive success in fish (Blom et al., 2019; de Jong, Fox et al., 2018; Nedelec, Radford et al., 2017; Sierra-Flores et al., 2015). Moreover, we are only aware of one such study that has been carried out in the field (Nedelec, Radford et al., 2017). While laboratory experiments allow researchers to isolate noise effects (e.g. de Jong, Fox et al., 2018; Fakan & McCormick, 2019), replicating natural soundscapes in

* Corresponding author.

E-mail address: brownn20@mcmaster.ca (N. A. W. Brown).

aquaria is a prevailing challenge (Slabbekoorn, 2016). Our objective was to add to this body of literature by studying a marine toadfish, the plainfin midshipman, *Porichthys notatus*, in the wild and investigating how its vocalizations and spawning success are affected by prolonged exposure to artificial noise.

Each year, beginning in the late spring, plainfin midshipman breed in shallow coastal waters, where large guarder males gather and excavate nesting cavities under large rocks (Arora, 1948). Within their nests, guarder males produce vocalizations that attract females and provide sole paternal care to broods of eggs (Arora, 1948; Brantley & Bass, 1994). The male's mating advertisement signal is a protracted, ~95 Hz (fundamental frequency) 'hum' produced by rapid contractions of two specialized sonic muscles attached to the physoclistous swim bladder (Bass & Marchaterre, 1989; McIver et al., 2014). Males nest in large aggregations and broadcast a breeding chorus that dominates the underwater soundscape (Halliday et al., 2018) and is occasionally loud enough to be heard above the water's surface (McCosker, 1986; McIver et al., 2014).

Noises generated by motorized boats and industrial activities are increasingly infiltrating soundscapes around plainfin midshipman breeding sites (Halliday et al., 2018). Such noise pollution could impede mating if it masks male vocalizations or if it interferes with female sound source localization (McKibben & Bass, 1998; Zeddies et al., 2012). However, males might overcome the interfering noise if they can sufficiently adjust the amplitude and/or frequency (i.e. pitch) of their vocalizations. Whether and to what degree males attempt to compete with masking noise is likely mediated by a trade-off between the energetic, metabolic or predation costs of enhanced signalling and the reproductive payoffs for doing so (Harding et al., 2019; Ladich, 2019). If an interfering noise is of a relatively low amplitude or does not broadly overlap with the hum's frequency range, then male vocal adjustments could be an effective adaptation. Conversely, if the interfering noise is of high amplitude and broadband (e.g. motorboat noise), vocal adjustments will likely be ineffective, and males might better reserve their energy for future quiet periods.

To investigate these ideas, we carried out two experiments on plainfin midshipman nesting in the wild. We predicted that when males are exposed to an artificial tonal noise, the amplitude and frequency of their vocalizations would shift to reduce overlap with the noise source (Lombard effect). We then investigated the resulting question: how does spawning success vary between males in nests exposed to the artificial tone over a period of several days versus males in nests exposed to ambient noise conditions?

METHODS

Study Site and Artificial Nest Construction

Between 21 April and 10 June 2019, we carried out artificial noise experiments at a natural plainfin midshipman breeding site on a private beach in Dabob Bay, Washington, U.S.A. (47°76'N, 122°86'W). We used 929 cm² concrete tiles to construct 12 hexagonal groups of six artificial nests; each nest in a group was separated from the next nest/tile by 0.5 m. We constructed a new group every 3 days. All groups were used first in experiment 1, and then reused in experiment 2 (see below). Following the construction of the six grouped nests, snorkellers conducted daily surveys for nest occupancy. When three of a group's six nests were occupied by a territorial guarding male without any eggs, the snorkellers covered these three occupied nests with plastic mesh (1 cm² openings) that was weighted down with rocks to exclude predators, rival males and females. The remaining three tiles and any

natural nests (all 30+ cm diameter rocks) within 5 m of the experimental covered nest trios were removed.

Experiment 1

After meshing the three nests, snorkellers placed a passive acoustic recorder (SoundTrap ST300; Ocean Instruments NZ, Warkworth, Auckland, New Zealand; 48 kHz sample rate, 5 min per 30 min duty cycle, 16-bit depth, 'high gain' setting active; Halliday et al., 2018) within 0.5 m of the nest group's perimeter (Fig. 1a and b). Snorkellers returned to the group of three nests after 24 h and positioned a 'noise egg' (de Jong et al., 2017) attached to a lead weight on a 5 cm tether in the middle of the nest group (Fig. 1c). These devices produced a continuous tone (mean frequency = 118 Hz, range 113–128 Hz; see Appendix 1 for measured properties of noise eggs) and have been used in previous studies to investigate the effects of artificial noise on gobiid mating (de Jong et al., 2016; de Jong, Heubel et al., 2018; de Jong, Fox et al., 2018). Noise eggs were removed after 24 h. Thus, each group was recorded for 72 h in total: a 24 h baseline period under ambient noise, 24 h under ambient plus artificial noise from the noise eggs and a 24 h follow-up under ambient noise. Following recordings, snorkellers removed the mesh and checked under each tile to confirm the male was still present and that the male had not acquired any eggs (Fig. 1d), then relocated the acoustic recorder to the next group and repeated the protocol. Males were resighted in all nests following the recordings. All recordings were collected between 27 April and 31 May 2019. By staggering start dates for each nest group, trials were dispersed across a range of tidal and weather conditions; thus, it is unlikely that the results were affected by undocumented environmental factors. A reference acoustic recorder (same model and settings as listed above) was placed >10 m away from the nests throughout the entire study to describe ambient conditions in the absence of the noise egg.

Experiment 2

In six of the 12 nest groups, we immediately returned noise eggs to their original positions at the end of the 72 h recording period of experiment 1 (see above). This occurred after removal of mesh from the nests. Nest groups were assigned to either 10 days of ambient noise (control) or 10 days of ambient plus artificial noise exposure. Treatments were assigned to nest groups sequentially, so when a group finished its recording period for experiment 1, it was assigned to the opposite treatment of the nest group that finished before it. This sequential treatment assignment allowed us to control for seasonal effects on spawning success by ensuring control and noise groups were not temporally clustered (Cogliati et al., 2013; Sisneros et al., 2004). Starting dates were offset by 2 days, on average, and temperatures were recorded by local acoustic recorders. The temperatures did not differ significantly between control and noise-exposed nests during experiment 1 (control nests: mean ± SD = 13.1 ± 1.7 °C; noise-exposed nests: mean ± SD = 13.7 ± 2.0 °C; $t_{10} = -0.5$, $P = 0.64$). Following the 10-day noise or control exposure, snorkellers photographed the broods on each tile and used hand-held nets to capture any fish in the nests. Fish were then weighed (± 0.01 g), measured (standard length (SL); ± 1 mm) and gently returned to their nest. Males assigned to the control and artificial noise treatments were of a similar body size (control: mean ± SD SL = 236 ± 20 mm; artificial noise: mean ± SD SL = 247 ± 21 mm). All nests were sampled between 10 May and 10 June 2019.

A rater, blinded to the nest group's noise treatment, counted the number of living and dead eggs present in each nest photograph taken during experiment 2 (see Figure 2 in Bose et al., 2019, for live

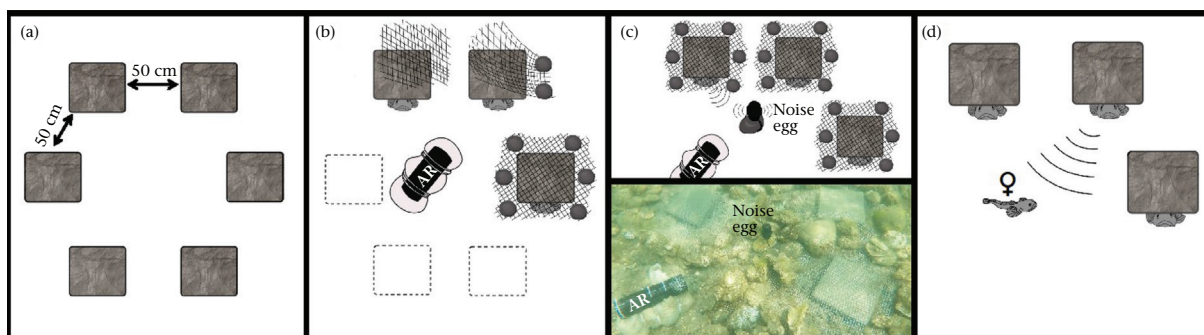


Figure 1. Schematic overview of experiment 1 procedure. (a) Six identical square tiles were deployed in a hexagon pattern. Experimenters checked under each tile daily for the presence of guarder males. After 3–6 days, three of the six tiles were occupied. (b) The three unoccupied tiles were removed, the remaining three were covered over with mesh and an acoustic recorder ('AR'; see details in Methods) was positioned within 50 cm of the nearest nest and no farther than 1 m from either of the other two nests. (c) After 24 h, experimenters placed a noise egg in the centre of the nest group. The inset image in (c) shows the experimental set-up in situ. The noise egg was then removed after 24 h and the acoustic recorder was left for a final 24 h. (d) Following the 72 h recording period of experiment 1, experimenters removed the acoustic recorder and the mesh from the nests, marking the beginning of experiment 2. In 50% of the nest groups, experimenters returned the noise egg to its original position. Males were no longer restricted from spawning with females.

versus dead classification details). The rater also used a staging table (Table 1 in Brown et al., 2021) to record the developmental stages of all eggs and hatched young within each brood. Each developmental stage was assigned a numeric score corresponding to the proportion of total development time it represents, ranging from 0 (newly laid egg) to 1 (nearly free-swimming juvenile with no visible yolk sac; Brown et al., 2021). To calculate the average age of eggs in the brood, we divided the number of eggs at each stage by the total number of eggs, multiplied this proportion by the proportional value for development at the given stage, and then summed these products together. We used these data to infer how quickly a male had attracted a mate/mates by assuming that nests with older eggs (higher average development scores) received spawnings earlier compared to those with younger eggs.

Bioacoustic Analyses

All recordings collected during experiment 1 were manually analysed in Raven Pro software (version 1.5, Cornell Lab of Ornithology, Ithaca, NY, U.S.A.). The spectrogram was set to use a Hann window with 50% overlap, window size set to 7000 samples, 1400 Hz frequency range, and time to 10 s, although the analyst adjusted the frequency and timescale when necessary. The selection table in Raven Pro was set to measure minimum, maximum, delta (maximum frequency – minimum frequency) and peak frequencies (frequency with peak power); start, stop and delta times (stop time – start time); and peak power (highest decibel level in the vocalization, the peak sound pressure level). In each 5 min file, the fundamental frequency of every clear growl was selected, as well as the first clear grunt from every grunt train (Fig. 2; McIver et al., 2014). Similarly, the fundamental frequency (F0) of the hum at the beginning of the file was selected. Finally, a 10 Hz, 4 s selection was made between 140 Hz and 150 Hz near the beginning of each file to measure the peak power of background ambient sound; this selection was only made in parts of the file without obvious acoustic signals. We chose the 140–150 Hz band for this background sound measurement because this was a consistent frequency range relatively close to the fundamental frequencies of plainfin midshipman vocalizations that we could measure on nearly every file and that almost never contained hum signals. Plainfin midshipman hums are so prolific at this recording site that any wider bandwidth would make it so that we could not calculate background sound in the absence of hums. The peak power for background ambient sound measurements was subtracted from peak power for each selected call within the same

file to measure the signal-to-noise ratio (SNR). All selections were annotated to denote whether they overlapped with other calls or the signal from the noise egg.

Selection data from Raven Pro were then averaged for each file so average peak frequency, delta frequency (frequency range) and SNR were calculated for grunts, growls and hums, and the average duration was calculated for grunts and growls. These averages were only calculated for clear calls that did not overlap with any other calls or with the signal from the noise egg. The total number of grunts and growls, as well as the presence or absence of hums, were also recorded for each file; this count/presence of calls was for all calls, regardless of whether they overlapped with other calls or the signal from the noise egg.

All recordings were also processed in the PAMGuide package (Merchant et al., 2015) in MatLab (version 2017a; MathWorks, Natick, MA, U.S.A.) to calculate sound pressure level (SPL) in third-octave bands for each 1 s of data using a Hanning window with 50% overlap, and then averaged for each 5 min file. All acoustic recorders were factory calibrated when they were purchased in 2018, and we used the end-to-end calibration value in the SPL calculation. These third-octave bands were also summed in linear space into full octave bands. The full octave band between 80 Hz and 160 Hz is important to plainfin midshipman because it includes the F0 for all call types, and therefore represents the sound levels that plainfin midshipman must overcome to effectively communicate (Halliday et al., 2018). Because fish have wider critical bandwidths than other vertebrates, such as marine mammals (Fay, 1988), an octave band is more representative of the critical bandwidths of fish than a third-octave band. Hereafter, all references to decibels (dB) use 1 μ Pa as the reference pressure level. Power spectral densities (PSDs) in 1 Hz by 1 s bins were also calculated for each file in the PAMGuide package between 20 Hz and 1000 Hz using a Hanning window with 50% overlap.

Statistical Analyses

All statistical analyses were conducted in R (version 3.6.0; R Core Team, 2016). All model outputs are presented in Appendix 2. Call characteristics and the number of grunts and growls for each file were analysed using linear mixed effects models (LMMs), with nest group as a random intercept and with call type (growl, grunt, hum), experimental treatment (before, during or after noise egg) and their interaction as fixed effects. The presence of hums was analysed using a binomial generalized linear mixed effects model (GLMM)

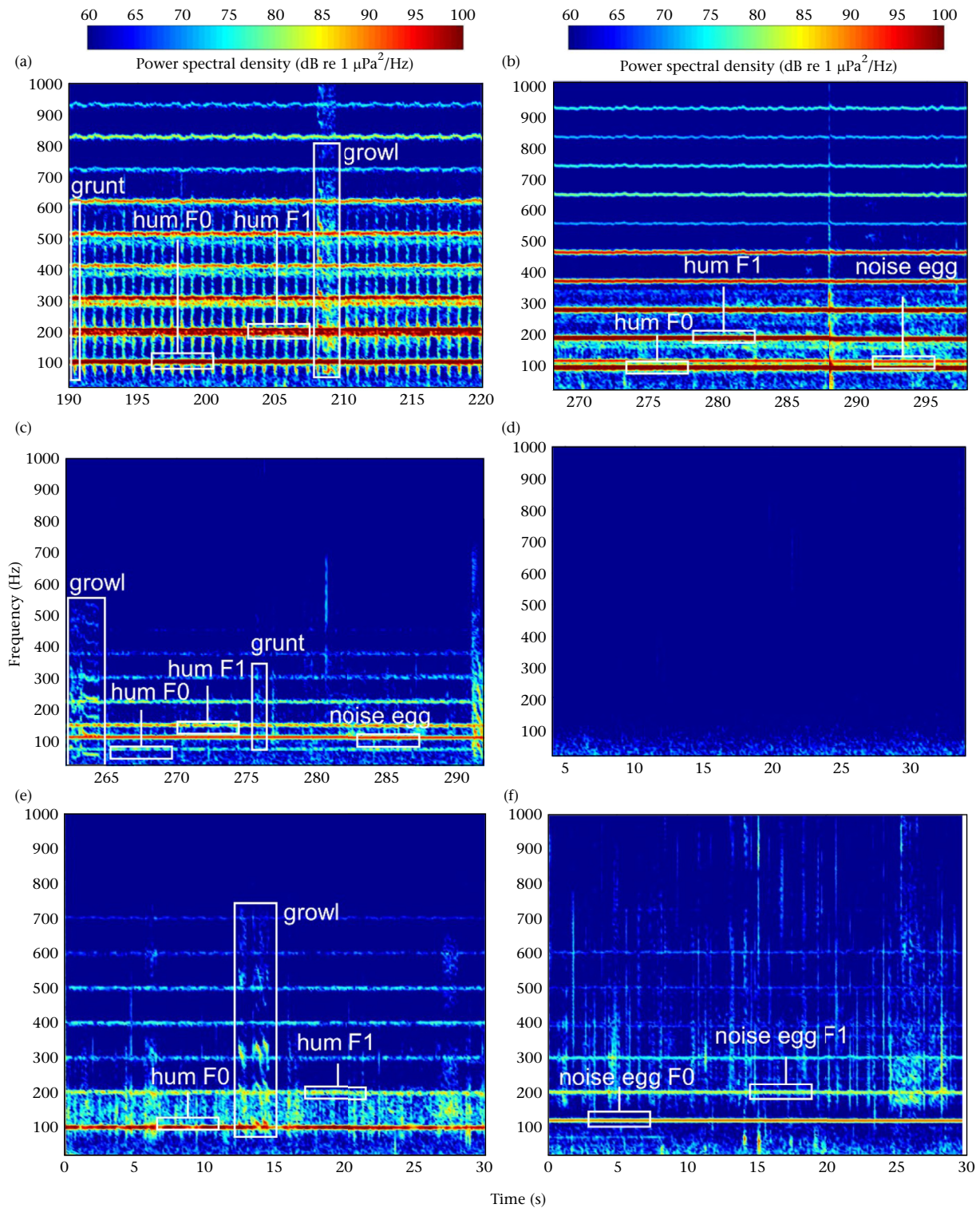


Figure 2. Example spectrograms showing (a) grunts, growls and hum fundamental frequency (F0) and first harmonic (F1) in the absence of the noise egg signal at a local recorder, (b) a close hum signal in the presence of the noise egg signal at a local recorder, (c) all three plainfin midshipman call types in the presence of the noise egg signal at a local recorder, (d) ambient sound levels in the absence of plainfin midshipman calls and the noise egg signal at a local recorder, (e) plainfin midshipman vocalizations (hum and growl) at the reference recorder and (f) the F0 and F1 of the noise egg signal recorder in the field in the absence of plainfin midshipman hums. Note, in (c), we chose an example where all three fish calls occurred when the noise egg was present, and the hum in this example is not from a plainfin midshipman close to the recorder, which is why the noise egg shows a higher power spectral density than the hum F0. All spectrograms were created using 1 s Hanning windows with 50% overlap and fast Fourier transform = 12 000 samples.

with the same fixed and random effects described above. The SPL data in the 80 Hz octave band were analysed using an LMM with treatment, the count of each call type and each of their interactions with treatment as fixed effects and with nest group as a random intercept. Models without the interaction and with either of the main effects were also compared using Akaike's information criterion, corrected for small samples (AICc).

We assessed the efficacy of both SPL in the 80 Hz band and SNR at reflecting the amplitude generated by plainfin midshipman hums. We selected a subset of acoustic files for this analysis that had clear hum signals that would have been generated by males within the nest group between 0.5 m and 1 m away from the acoustic recorder. The frequency range of the hum was defined by the minimum and maximum frequency of a hum in each file. Three of the 12 nest groups (representing 9 males) were randomly selected for this comparison. We calculated the hum's received level in 30 of the 5 min files (10 from each period of the experiment – baseline, noise treatment and follow-up) that were randomly selected from each of the three nest groups, for a total of 90 files used in this analysis. Received level for each hum was calculated by extracting the PSDs for the frequency range of the hum in each file, summing these PSDs in linear space for each 1 s time bin, then calculating the median across all time bins within a 5 min file. However, a hum was detected in only one recording of one group's noise treatment period. Therefore, we compared received levels to SPL in the 80 Hz octave band of 81 files. The SNR of hums and SPL in the 80 Hz octave band were compared with SPL in the frequency range of the hum (effectively the received level of the hum) using simple linear regression. Received levels and SPL in the 80 Hz octave band were highly correlated ($R^2 = 0.93$; Appendix 1, Fig. A1a) and were moderately correlated with SNR ($R^2 = 0.64$; Appendix 1, Fig. A1b).

Acoustic recorders positioned close to nest groups in experiment 1 recorded vocal activity from all three males indiscriminately, as well as vocalizations from any transient individuals and from males at more distant nests, which could have obscured responses of individuals across all three trial periods. To strengthen our inferences about individual responses, we carried out two additional analyses on the recordings from experiment 1. First, we filtered the data to include only hums with SPL ≥ 120 dB to theoretically isolate calls produced by the male whose nest was closest to the recorder in each group. This threshold was based on the clear bimodal distribution of the SPL data when hums were present (Appendix 1, Fig. A2), which seemed to be indicative of hums produced by males close to the acoustic recorder versus those who were farther away. We then fitted the filtered hums with a linear mixed effects model and included trial period as a fixed effect and nest group as a random intercept. We compared these results to those obtained using all recorded hums to assess consistency in the responses.

To determine how spawning success varied between the control and artificial noise exposure groups in experiment 2, we used a negative binomial GLMM to model the total number of eggs (alive and dead) photographed in a nest. To determine how brood age differed between control and artificial noise exposure groups, we used a beta GLMM to model the average development of broods. In both models, treatment (control or artificial noise exposure) and male size (SL; DeMartini, 1988) were included as fixed effects and nest group was included as a random intercept.

Ethical Note

All work complied with guidelines set forth by the ASAB/ABS (2012) and the Canadian Council on Animal Care (Olfert et al., 1993) and was approved by the University of Victoria Animal Care Committee (Animal Use Protocol: Juanes-2017-003). No animals were sacrificed for this study.

RESULTS

Experiment 1

All plainfin midshipman vocalizations occurred less often during the noise playback than before or after the noise treatment. During the noise treatment, there was, on average, one fewer grunt or growl during each 5 min period than before or after the treatment (estimated difference (est.) \pm SE = -0.6 ± 0.1 calls; $t_{3433} = 4.50$, $P < 0.001$; Appendix 2, Table A3). Hums were similarly less frequent during the noise treatment (est. \pm SE = -0.3 ± 0.1 calls; $z_{1720} = 2.00$, $P = 0.045$; Appendix 2, Table A4). Despite the difference in the number of hums, grunts and growls, the durations of grunts and growls were not significantly affected by the treatment ($F_{2,1571} = 0.53$, $P = 0.59$; Appendix 2, Table A5).

Sound pressure levels in the 80 Hz octave band recorded by the reference acoustic recorder ranged from 79.1 dB to 133.0 dB (median = 92.2 dB) and were strongly driven by the presence of hums. SPLs in the 24 h baseline, noise playback and follow-up periods for both the reference recorder and experimental recorders are shown in Table 1. We compared the local acoustic recorder at the nest group to the reference recorder and focused only on files where hums were present. In doing so, we found that SPLs at the reference recorder did not differ significantly between the baseline, noise playback and follow-up periods ($P > 0.27$; Appendix 2, Table A6). We also found that SPLs recorded during the baseline period at the local acoustic recorder (the recorder at the experimental nest group) did not differ significantly from those recorded across all periods by the reference recorder ($P = 0.26$). However, SPLs at the local recorder were approximately 7.9 ± 1.8 dB (est. \pm SE) higher during the noise playback period compared to the baseline period ($t_{1215} = 4.46$, $P < 0.0001$) and were approximately 5.7 ± 1.7 dB (est. \pm SE) higher during the follow-up period compared to the baseline period ($t_{1215} = 3.31$, $P < 0.001$; Fig. 3, Appendix 2, Table A6). This increase in SPL was mostly driven by increased amplitude of plainfin midshipman hums rather than additional pressure from the noise egg (Table 1), which had a comparatively low amplitude that was overpowered by the plainfin midshipman hum (see Appendix 1 for acoustic characteristics of the noise egg).

Noise eggs added approximately 8.8 ± 0.8 dB (est. \pm SE) to SPL in the 80 Hz octave band ($t_{1708} = 11.42$, $P < 0.001$), and the average SPL in the absence of any plainfin midshipman calls or the noise egg was approximately 87.6 ± 0.8 dB (est. \pm SE; Appendix 2, Table A7). Hums added an additional 16.2 ± 0.9 dB (est. \pm SE) to SPL before the noise egg was deployed ($t_{1711} = 17.5$, $P < 0.001$) and added a similar amount even while the noise egg was deployed ($t_{1709} = 0.23$, $P = 0.82$), but added approximately 6.1 ± 1.3 dB (est. \pm SE) more to SPL after the noise egg was removed ($t_{1708} = 4.80$, $P < 0.001$; Appendix 2, Table A7). The number of growls had no significant effect on SPL during any treatment ($t_{1601} = 1.24$, $P = 0.21$), whereas grunts consistently added 0.2 ± 0.1 dB per grunt train ($t_{1709} = 2.34$, $P = 0.02$; Appendix 2, Table A7).

In a subset of the data that included only hums with SPL ≥ 120 dB (to focus on individuals closest to the recorder), SPLs were approximately 2.4 ± 0.9 dB (est. \pm SE) higher during the noise playback compared to the baseline period ($t_{222} = 2.8$, $P = 0.006$) and approximately 4.7 ± 0.8 dB higher during the follow-up compared to the baseline period ($t_{224} = 5.6$, $P < 0.001$; Appendix 2, Table A8).

The peak frequencies of all call types were lower in the presence of a noise egg than either before or after the noise egg treatment (est. \pm SE difference = -4.6 ± 0.9 Hz; $t_{2054} = 5.14$, $P < 0.001$; Appendix 2, Table A9). Hums also had the highest peak frequency (mean = 98 Hz), followed by growls (mean = 87 Hz) and grunts (mean = 85 Hz; Appendix 2, Table A9). The range of hum F0 was

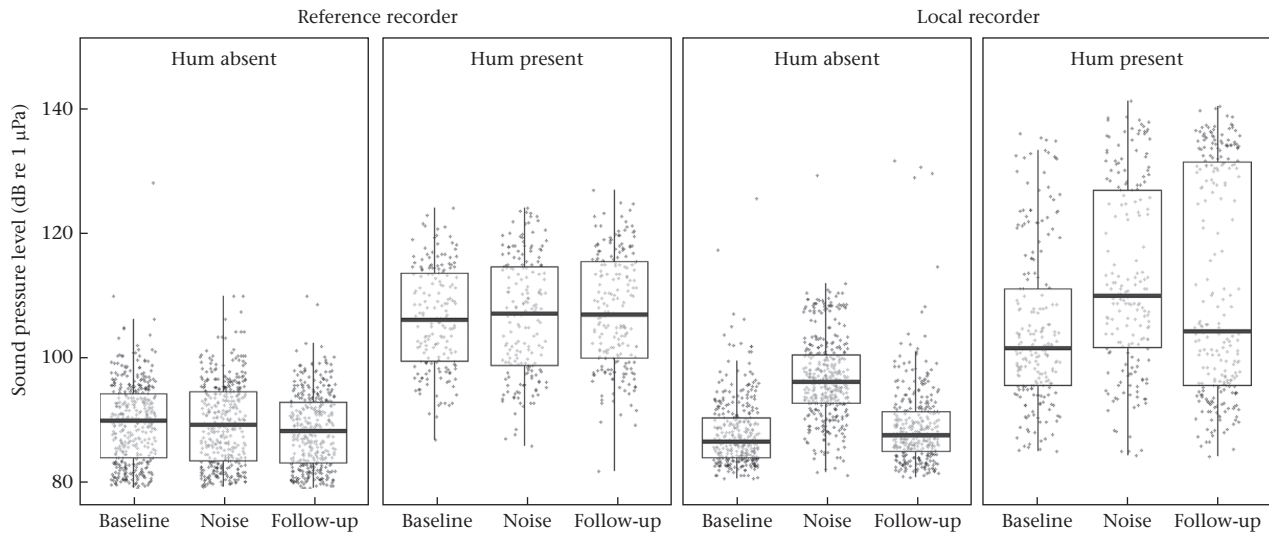


Figure 3. Received sound pressure level (SPL) at acoustic recorders positioned near nest groups in experiment 1 ('Local') and at the reference recorder. Box plots show the median (thick line), interquartile range (box) and 95% quantile range (whiskers).

greater in the presence of noise eggs (est. \pm SE difference = 6.7 ± 2.1 Hz; $t_{2050} = 3.21$, $P = 0.001$) and after the noise egg treatment (est. \pm SE difference = 5.2 ± 1.8 Hz; $t_{2049} = 2.86$, $P < 0.001$) compared to before the noise egg treatment, whereas growls had a narrower frequency range in the presence of noise eggs (est. \pm SE difference = -5.6 ± 1.2 Hz; $t_{2050} = 4.55$, $P < 0.001$); the frequency range of grunts was not significantly affected by the noise eggs ($t_{2049} = 1.32$, $P = 0.19$; Appendix 2, Table A10). Overall, hums had the lowest frequency range (27 Hz), followed by growls (52 Hz) and grunts (55 Hz; Appendix 2, Table A10).

Experiment 2

For every 1 cm increase in body size, males received 135 ± 39 (est. \pm SE) more eggs ($z_{32} = 3.97$, $P < 0.001$; Appendix 2, Table A11); however, there was no significant difference in egg number between control nests and noise treatment nests after 10 days ($z_{32} = 0.08$, $P = 0.94$; Appendix 2, Table A11, Table 2). We found older broods in the noise treatment compared to control nests after 10 days (noise treatment – control: est. \pm SE = 0.06 ± 0.02 ; $z_{21} = 2.29$, $P = 0.02$), but male size did not significantly affect brood age ($z_{21} = -1.09$, $P = 0.27$; Appendix 2, Table A12, Table 2).

DISCUSSION

Plainfin midshipman males lowered the frequency of their vocalizations in the presence of artificial noise and increased the amplitude of their mating hum, consistent with our first hypothesis. The Lombard effect occurs when individuals adjust the amplitude or pitch of their vocalizations. As our recordings were

taken from groups of three fish, it is possible that the vocal shifts we observed resulted from individual adjustments (Lombard effect) or changes that occurred at the level of the tri-male chorus. We argue that our results reflect the former because, when we filtered the data by SPL to include primarily hums produced by the closest male in each group, the results were equivalent.

The noise eggs produced tonal noises comparable to other tonal anthropogenic noise sources, including aspects of boat noise (de Jong et al., 2017; Halliday et al., 2018). However, compared to other artificial noise sources in the plainfin midshipman's soundscape, the noise eggs were relatively low amplitude. And yet, noise eggs effected measurable vocal adjustments in nearby males. The tones produced by the noise eggs were similar in frequency to male hums, so they might have mimicked the addition of a fourth male to the experimental groups. If so, the Lombard effect could be a strategy that allows males to compete vocally with their neighbours to attract more females. Female plainfin midshipman preferentially approach higher-amplitude hums (McKibben & Bass, 1998). Additionally, females can resolve frequency differences of as little as 10–20 Hz, and in water temperatures similar to those recorded during experiment 1 (~ 13.5 °C), females preferentially approach 90 Hz hums over 110 Hz hums (McKibben & Bass, 1998). Thus, when the FO of male hums decreased from ~ 98 Hz to ~ 93 Hz during exposure to the noise egg, their hums were more closely aligned with female preference. The frequency and amplitude adjustments that we observed in response to the noise egg in experiment 1 are thereby consistent with the Lombard effect and aligned the male advertisement signals with female preferences.

Ours is the third study to report evidence of the Lombard effect in a fish (Ladich, 2019), which further suggests the phenomenon

Table 1
Mean \pm SE (median; interquartile range [IQR]; N) sound pressure levels (SPL; dB re 1 μ Pa; bandwidth = 80–160 Hz) recorded throughout experiment 1 by acoustic recorders positioned close to nest groups ('Local') and by a reference acoustic recorder positioned >10 m from the nearest nest group

Acoustic recorder	Hum presence	Baseline	Noise playback	Follow-up
Local	No Hum	101.3 \pm 0.3 (86.7; [84.3–90.6]; 359)	105.7 \pm 0.3 (96.4; [93.0–101.0]; 389)	111.3 \pm 0.4 (87.8; [85.3–91.7]; 333)
	Hum	122.4 \pm 0.8 (101.6; [95.9–111.0]; 217)	129.0 \pm 1.1 (110.1; [102.0–127.0]; 187)	130.4 \pm 1.2 (104.4; [95.8–132.0]; 239)
Reference	No Hum	102.9 \pm 0.3 (90.1; [84.2–94.5]; 403)	94.9 \pm 0.3 (89.5; [83.8–94.8]; 392)	93.2 \pm 0.3 (88.4; [83.4–93.2]; 370)
	Hum	112.8 \pm 0.6 (106.3; [99.6–114.0]; 180)	114.3 \pm 0.7 (107.3; [99.1–115.0]; 187)	114.9 \pm 0.6 (107.0; [100.0–116.0]; 205)

SPLs are given in the presence and absence of plainfin midshipman hums during the 24 h periods before, during, and after the noise egg was deployed in the center of each group.

Table 2

Summary of nests sampled in experiment 2 after 10 days of exposure to either ambient noise ('control') or ambient noise plus artificial noise ('noise'). Spawning success was defined as the total number of eggs (living or dead) counted in a male's nest. The brood age index ranged from 0–1, indicating the average ago of embryos in the nest (see details in Methods). Higher age index values indicate older broods and thereby earlier spawnings. Sample sizes (*N*) refer to the number of nests sampled

Nest group manipulation	Male size (SL)	Number of eggs in nest	Brood age index
	Mean \pm SD (<i>N</i>)	Median [IQR] (<i>N</i>)	Median [IQR] (<i>N</i>)
Control	236 \pm 20 mm (16)	466 [166–816] (18)	0.05 [0.02–0.08] (14)
Noise	247 \pm 21 mm (17)	512 [319–882] (18)	0.09 [0.05–0.15] (16)

could be prevalent among soniferous fishes, as it is in birds and mammals (Brumm & Zollinger, 2011). An additional effect commonly observed in birds and mammals confronted with interfering noise is increased vocalization rate, which increases signal redundancy (Shannon et al., 2016). We observed the opposite pattern; the rates of plainfin midshipman male vocalizations decreased in the presence of artificial noise, which has also been observed in the Lusitanian toadfish, *Halobatrachus didactylus* (Vieira et al., 2019) and in two goby species, *Gobiusculus flavescens* and *Pomatoschistus pictus* (de Jong, Fox et al., 2018). In blacktail shiner, *Cyprinella venusta*, growl durations decreased in the presence of noise; and while the rate of 'bursts' – periods of amplitude spikes ('pulses') within a single growl – increased, pulses also decreased, resulting in lower overall signal redundancy (Holt & Johnston, 2014). In contrast to these results, in brown meagre, *Sciaena umbra*, repeated exposures to boat noise were associated with an approximately two-fold increase in vocalization rates, although it was not determined whether individuals vocalized more frequently or whether more individuals joined the chorus (Picciulin et al., 2012). Overall, the strategy of increasing signal redundancy by increasing vocalization rates appears less common among fishes than among birds and mammals and is likely dependent on acoustic properties of the interfering noise (e.g. duration, uniformity) and the behavioural patterns of individual species (Ladich, 2019).

Exposure to 10 days of artificial noise during experiment 2 did not significantly affect brood sizes compared to control nests. In fact, males in noise-exposed nests received spawnings earlier than males in control nests. Experiment 1 showed that males increased the amplitude of their mating advertisement hum when confronted with live noise eggs. Our spawning success data suggest that this response allowed males to successfully drown out the noise eggs and attract females with similar success to males in control nests. By producing louder hums, males in noise-exposed nests might have attracted females more quickly than those in control nests if their vocalizations propagated further and were received by approaching females earlier. Female plainfin midshipman possess sophisticated neural architecture in the saccula that allows them to localize individual males amid competition from several overlapping hums (McKibben & Bass, 1998). Accordingly, the tonal noise eggs employed in our study did not likely interfere with female sound source localization. Noise eggs are useful in a field setting because they are compact, battery-powered devices that can produce artificial noise continuously for several days (de Jong et al., 2017). While anthropogenic noises confronting plainfin midshipman (e.g. motorboats) often have clear tones similar to the noise egg (de Jong et al., 2017; Halliday et al., 2018), they are typically more transient (lasting minutes, not hours) and more broadband in nature. Therefore, the noise egg signal – a pure tone with weak harmonics – likely has a less potent masking effect on plainfin midshipman hums compared to boat noise, which occupies a broad swath of the frequency spectrum containing the F0 and all harmonics of the hum (Halliday et al., 2018). Other types of anthropogenic noise, such as underwater drilling sounds, create long-lasting tonal signals that are more like the noise egg signal. However, the majority of plainfin

midshipman in shallow breeding grounds are likely not exposed to such noise sources. Future studies that employ realistic stimuli, such as motorboat noise or playbacks thereof (e.g. Nedelec, Radford et al., 2017), are still needed to determine how noise pollution specifically affects spawning success.

Although the noise exposure in our study did not directly impede male spawning success, it might have inflicted unobserved physiological consequences. Our recordings suggest that males responded to the noise egg with the Lombard effect, which could incur energetic or survival costs if sustained over a longer period (Read et al., 2014). To our knowledge, energetic costs of the Lombard effect in fish have not been investigated, but there is mixed evidence for metabolic costs in other animals (Gillooly & Ophir, 2010; Oberweger & Goller, 2001; Ryan, 1988; Zollinger et al., 2011). Although the artificial noise in our study was a pure tone, playbacks of inboard and outboard engine noise elicited the Lombard effect in the confamilial oyster toadfish, *Opsanus tau* (Luczkovich et al., 2016). Sound production does not produce measurable metabolic costs in oyster toadfish (Amorim et al., 2002), but it does lead to sonic muscle fatigue (Mitchell et al., 2008). As anthropogenic noise can elicit the Lombard effect in at least some soniferous fishes (this study; Ladich, 2019), it is important to understand how it could affect their physiology and/or fitness. Additionally, amplitude increases in male hums persisted after the artificial noise source in our study was removed, an effect also observed in the oyster toadfish (Luczkovich et al., 2016). Plainfin midshipman predators, such as harbour seals, *Phoca vitulina* (Luxa & Acevedo-Gutiérrez, 2013), likely localize prey by their vocalizations (Bodson et al., 2006), so an unnecessarily high amplitude might increase a male's susceptibility to predation (Patricelli & Blickley, 2006). Further research is needed to determine whether the noise-induced vocal responses that we are discovering in fishes can inflict physiological or predation costs on the signaller.

Author Contributions

Nicholas Brown and William Halliday contributed to conceptualization, methodology, investigation, formal analysis, writing – original draft preparation and funding acquisition. Sigal Balshine and Francis Juanes contributed to conceptualization, writing – review and editing and funding acquisition.

Competing Interests

We declare no competing interests.

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Appendix 1

Acoustic Properties of the Noise Eggs

We measured the acoustic properties and propagation of nine individual noise eggs in the submerged intertidal zone. Noise eggs were always tethered to a lead dive weight such that they floated ≈ 0.3 m above the sea bed. An acoustic recorder (SoundTrap ST300; Ocean Instruments NZ), housed in a 4-inch (10.2 cm) diameter PVC pipe and strapped to a sandbag, was then positioned at fixed distances from the noise egg for 1 min and set to record continuously at a 48 kHz sample rate, 16-bit depth and with the 'high gain' setting active. The recorder was placed at 0.5 m, 1.0 m, 2.0 m, 5.0 m and 10.0 m from the noise egg. We measured distances starting at 0.5 m because this was the minimum distance between a noise egg and a nest during the noise egg exposure experiment. Recordings were processed in PAMGuide for both sound pressure level (SPL; in third-octave bands), as well as power spectral density (PSD) between 20 and 1000 Hz. We used the median PSD and SPL values for the central 30 s of each recording at each distance from the noise egg to characterize the acoustic properties and propagation of the noise eggs.

The nine noise eggs showed some variability around their fundamental frequency (FO) and received level (Table A1); FO ranged from 108 to 128 Hz (mean = 118 Hz). Measured from 0.5 m away, PSD at FO ranged from 83 to 96 dB re $1 \mu\text{Pa}^2/\text{Hz}$ (mean = 94 dB re $1 \mu\text{Pa}^2/\text{Hz}$). Also measured from 0.5 m away, SPL in the 80 Hz octave ranged from 87 to 99 dB re $1 \mu\text{Pa}$ (mean = 97 dB re $1 \mu\text{Pa}$ at 0.5 m). Most of the signal of the noise egg was masked into the background ambient noise once the recorder was ≥ 2.0 m from the noise egg. The signal lost >10 dB between 0.5 and 1.0 m (Table A2).

Table A1

Acoustic properties of the noise eggs measured from 0.5 m – the distance noise eggs were positioned away from the focal nests

Noise egg	FO (Hz)	PSD at FO (dB re $1 \mu\text{Pa}^2/\text{Hz}$ at 0.5 m)	SPL in 80 Hz octave (dB re $1 \mu\text{Pa}$ at 0.5 m)
1	116	93	97
2	122	96	98
3	108	94	99
4	128	95	97
5	119	83	87
6	113	89	93
7	121	97	99
8	120	96	98
9	116	95	98
Mean	118	94	97

Measurements include fundamental frequency (FO), power spectral density (PSD) at the fundamental frequency, and sound pressure level (SPL) in the 80 Hz octave band.

Table A2

Acoustic properties of the noise egg signal at different distances, as tested on three separate noise eggs, where F0 is the fundamental frequency of the noise egg signal

Noise egg	F0	0.5 m	1.0 m	2.0 m	3.0 m	4.0 m	5.0 m	10.0 m
1	117	93/98	80/90	74/90	74/91	65/82	71/86	63/83
2	122	96/99	81/87	69/82	75/86	70/83	85/90	79/87
3	109	94/97	75/61	58/61	58/60	58/59	59/59	58/58

Under the distance column headers (0.5 m, 1.0 m, 2.0 m, etc.), data are displayed in each cell as power spectral density (PSD; dB re 1 $\mu\text{Pa}^2/\text{Hz}$) at the fundamental frequency and sound pressure level (SPL; dB re 1 μPa) in the 80 Hz full octave band, shown as PSD/SPL.

Appendix 2. Outputs from Statistical Models

Table A3

Final model output for a linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) on the number of plainfin midshipman grunts and growls counted within 5 min audio files

Parameter	Estimate \pm SE	<i>t</i>	<i>P</i>
Intercept	2.16 \pm 0.44	4.89	<0.001
Call type (Grunt)	0.25 \pm 0.11	2.19	0.03
Noise exposure	-0.62 \pm 0.14	4.50	<0.001
Follow-up	-0.01 \pm 0.14	0.09	0.93

N = 3448 files. Final model structure: dependent variable = call count; fixed effects = call type, treatment; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

Table A4

Final model output for a binomial general linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) on the presence of plainfin midshipman hums within 5 min audio files

Parameter	Estimate \pm SE	<i>z</i>	<i>P</i>
Intercept	-0.69 \pm 0.33	2.08	0.04
Noise exposure	-0.27 \pm 0.13	2.00	0.045
Follow-up	0.22 \pm 0.13	1.64	0.10

N = 1724 files. Final model structure: dependent variable = hum presence; fixed effect = treatment; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

Table A5

Final model output for a linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) on the duration (s) of plainfin midshipman grunts and growls

Parameter	Estimate \pm SE	<i>t</i>	<i>P</i>
Intercept	2.51 \pm 0.21	12.04	<0.001
Call type (Grunt)	-2.18 \pm 0.19	11.33	<0.001
Noise exposure	-0.07 \pm 0.24	0.31	0.76
Follow-up	0.16 \pm 0.23	0.73	0.47

N = 1577 files. Final model structure: dependent variable = call duration; fixed effects = call type, treatment; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

Table A6

Final model output for a linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) and whether the acoustic recorder was a local or reference recorder on sound pressure level (SPL; dB re 1 μPa) in the 80 Hz octave band when plainfin midshipman hums were present

Parameter	Estimate \pm SE	<i>t</i>	<i>P</i>
Intercept	104.79 \pm 1.71	61.26	<0.001
Noise exposure	0.27 \pm 1.29	0.21	0.83
Follow-up	1.37 \pm 1.26	1.09	0.28
Recorder (Local)	-1.40 \pm 1.25	1.13	0.26
Noise exposure : Recorder (Local)	7.92 \pm 1.78	4.46	<0.001
Follow-up : Recorder (Local)	5.66 \pm 1.71	3.31	0.002

N = 1215 files. Final model structure: dependent variable = SPL; fixed effects = - treatment, recorder location, treatment \times recorder location; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

Table A7

Final model output for a linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) plainfin midshipman vocalizations on sound pressure level (SPL; dB re 1 μPa) in the 80 Hz octave band when plainfin midshipman hums were present

Parameter	Estimate \pm SE	<i>t</i>	<i>P</i>
Intercept	87.64 \pm 0.80	109.59	<0.001
Noise exposure	8.84 \pm 0.77	11.42	<0.001
Follow-up	1.05 \pm 0.80	1.30	0.19
Hum present	16.24 \pm 0.93	17.55	<0.001
Number of growls	0.10 \pm 0.08	1.24	0.21
Number of grunts	0.18 \pm 0.08	2.34	0.02
Noise exposure : Hum present	-0.30 \pm 1.31	0.23	0.82
Follow-up : Hum present	6.12 \pm 1.28	4.80	<0.001

N = 1724 files. Final model structure: dependent variable = SPL; fixed effects = - treatment, hum presence, number of grunts, number of growls, treatment \times hum presence; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

Table A8

Final model output for a linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) and plainfin midshipman vocalizations on sound pressure level (SPL; dB re 1 μPa) in the 80 Hz octave band when plainfin midshipman hums were present and filtered to \geq 120 dB re 1 μPa

Parameter	Estimate \pm SE	<i>t</i>	<i>P</i>
Intercept	126.33 \pm 1.28	98.65	<0.001
Noise exposure	2.44 \pm 0.88	2.78	0.006
Follow-up	4.67 \pm 0.84	5.55	<0.001

N = 232 files. Final model structure: dependent variable = SPL; fixed effect = - treatment; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

Table A9

Final model output for a linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) and the type of plainfin midshipman vocalization on the peak frequency (Hz) of plainfin midshipman hums, grunts and growls

Parameter	Estimate \pm SE	<i>t</i>	<i>P</i>
Intercept	86.88 \pm 2.27	38.19	<0.001
Call type (Grunt)	-2.23 \pm 0.81	2.75	0.01
Call type (Hum)	10.80 \pm 0.96	11.28	<0.001
Noise exposure	-4.61 \pm 0.90	5.14	<0.001
Follow-up	-0.84 \pm 0.82	1.03	0.30

N = 2068 files. Final model structure: dependent variable = peak frequency; fixed effects = call type, treatment; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

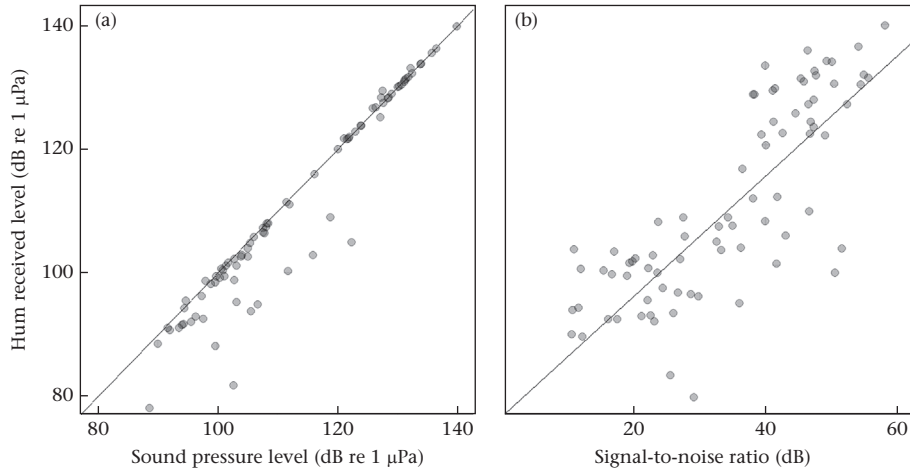


Figure A1. Received levels of the plainfin midshipman’s hum, measured only within the frequency range of an individual hum, from fish clearly within the nesting group (0.5–1 m from the acoustic recorder) compared to two other acoustic measurements from the same 5 min file: (a) sound pressure level (SPL) in the 80–160 Hz octave band and (b) signal-to-noise ratio (SNR) of the individual hum. The diagonal black line in both panels shows a 1:1 slope, for visual reference.

Table A10

Final model output for a linear mixed effects model examining the influence of the treatment (baseline [intercept], noise exposure, follow-up) and the type of plainfin midshipman vocalization on the frequency range (Hz) of plainfin midshipman hums, grunts and growls

Parameter	Estimate ± SE	<i>t</i>	<i>P</i>
Intercept	51.63 ± 1.24	41.55	<0.001
Call type (Grunt)	3.70 ± 1.15	3.22	0.001
Call type (Hum)	-25.19 ± 1.32	19.05	<0.001
Noise exposure	-5.58 ± 1.23	4.55	<0.001
Follow-up	-0.94 ± 1.18	0.80	0.42
Call type (Grunt) : Noise exposure	-2.30 ± 1.74	1.32	0.19
Call type (Hum) : Noise exposure	6.66 ± 2.08	3.21	<0.001
Call type (Grunt) : Follow-up	1.38 ± 1.64	0.84	0.40
Call type (Hum) : Follow-up	5.25 ± 1.84	2.86	0.01

N = 2068 files. Final model structure: dependent variable = frequency range; fixed effects = call type, treatment, call type × treatment; random intercept = nest group. Significant effects (*P* < 0.05) are shown in bold.

Table A11

Final model output for a negative-binomial generalized linear mixed effects model with a log link function examining the influence of the treatment (noise egg presence or absence [control] for 10 days) on the spawning success (brood size) of plainfin midshipman nests

Parameter	Estimate ± SE	<i>z</i>	<i>P</i>
Intercept	-1.24 ± 1.89	-0.66	0.51
Noise egg present	0.03 ± 0.39	0.08	0.94
Male SL	0.30 ± 0.08	3.97	<0.001

N = 33 nests. Final model structure: dependent variable = brood size; fixed effects = treatment, male standard length (SL); random intercept = nest group. Estimates and standard errors reported in the main text have been backtransformed via the inverse link function whereas those presented here have not. Significant effects (*P* < 0.05) are shown in bold.

Table A12

Final model output for a beta generalized linear mixed effects model with a logit link function examining the influence of the treatment (noise egg presence or absence [control] for 10 days) on the average age of broods in plainfin midshipman nests

Parameter	Estimate ± SE	<i>z</i>	<i>P</i>
Intercept	-0.32 ± 2.23	-0.14	0.89
Noise egg present	0.73 ± 0.32	2.29	0.02
Male SL	-0.10 ± 0.09	-1.09	0.27

N = 27 nests (6 nests had no eggs when inspected). Final model structure: dependent variable = brood age; fixed effects = treatment, male standard length (SL); random intercept = nest group. Estimates and standard errors reported in the main text have been backtransformed via the inverse link function whereas those presented here have not. Significant effects (*P* < 0.05) are shown in bold.

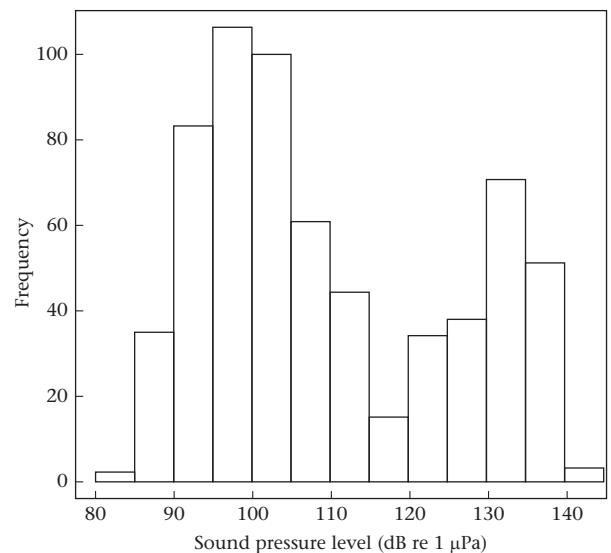


Figure A2. Histogram of SPL in the 80 Hz octave band from the local recorder when plainfin midshipman hums were detected in experiment 1. This bimodal distribution shows one group of values between 80 and 110 dB, and a second between 120 and 140 dB, which suggests a distinction between plainfin midshipman calling in nests closest to the acoustic recorder (latter) and those calling from nests further away from the acoustic recorder (former).