




Context-dependent effects of anthropogenic noise on nest defence in a singing toadfish

Mackenzie B. Woods ^{a,*} , Nicholas A. W. Brown ^{a,b} , Katrina Nikolich ^a,
William D. Halliday ^{c,d} , 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

^d School of Earth and Ocean Sciences, University of Victoria, Victoria, BC, Canada

ARTICLE INFO

Article history:

Received 20 October 2021

Initial acceptance 26 January 2022

Final acceptance 26 May 2022

MS. number: A21-00612R

Keywords:

acoustics
hydrophones
intertidal
laboratory vs field studies
motorboat noise
nest defence
noise pollution
parental care
plainfin midshipman
soniferous fish

Noise pollution has been steadily increasing in the ocean, negatively affecting aquatic ecosystems by causing both behavioural and physiological deficits for many marine animals, including fishes. To date, most research investigating the effects of noise on fishes has been conducted in controlled laboratory settings; however, these experimental conditions often lack ecological validity, and it is largely unknown whether the effects observed in aquaria are representative of real-world responses for animals experiencing noise in the wild. In this study, we used a combination of laboratory and field playback experiments to assess the effects of noise on nest defence and parental care behaviours in an intertidal-breeding toadfish, the plainfin midshipman, *Porichthys notatus*. Nest-guarding males without eggs in the laboratory exhibited a nearly four-fold increase in defensive behaviours under boat noise playbacks compared to under ambient conditions. However, in the field, no significant changes were detected in the defensive or parental behaviours by guarding males with eggs under exposure to noise. Our study demonstrates that anthropogenic noise can impact nest defence behaviour in plainfin midshipman males, but also that its impact depends on contextual cues such as testing environment, presence of eggs and the noise stimulus used. Our results highlight the urgent need for more field studies and for studies that pair and compare laboratory versus field results to quantify and mitigate the potential harm caused by noise pollution to organisms and populations in coastal environments.

© 2022 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Since the industrial revolution, human activities have drastically changed natural soundscapes (Frisk, 2012; McDonald et al., 2006; Shannon et al., 2016). Activities such as road and air traffic, construction, seismic exploration, commercial shipping and recreational boating generate noises that detrimentally affect numerous species, including birds, mammals, fish and invertebrates (Kight & Swaddle, 2011; Kunc et al., 2016; Morley et al., 2014; Shannon et al., 2016). Accordingly, anthropogenic noise is now recognized as a pollutant of international concern and has been included in national and international legislation, such as the U.S. National Environmental Policy Act (National Environmental Policy Act of 1969, 1970) and the EU's Marine Strategy Framework Directive (European Union, 2008). To craft successful noise mitigation strategies, it is imperative to understand the potential effects this

pervasive pollutant can have on wildlife at the individual, population and ecosystem levels. Noise pollution is of particular concern in aquatic environments because sound travels much faster and further before attenuating underwater than it does in air, and in most aquatic environments, sound also travels much farther than light (Slabbekoorn et al., 2010). Consequently, numerous aquatic animals rely heavily on their hearing, often more so than on vision or other sensory modalities. Because sound travels more efficiently underwater than in air, sound can be effective for long-distance communication and accurate perception and assessment of environmental conditions, but by the same virtue, even distant noise sources can negatively affect aquatic animals (Duarte et al., 2021; Slabbekoorn et al., 2010). Within the ocean, coastal habitats, where the faunal diversity is concentrated (Bowen et al., 2016), are most heavily affected by growing human populations and the corresponding increase in boat noise (Duarte et al., 2021). Noise generated by motorized vessels (including both recreational motorboats and larger vessels and freighters) are predominately low frequency

* Corresponding author.

E-mail address: mbwoods@uvic.ca (M. B. Woods).

(i.e. <1000 Hz), as are the hearing ranges and vocalizations of many coastal marine animals. Thus, vessel noise can be particularly detrimental to species inhabiting coastlines.

Fishes in coastal habitats are prominent contributors to their local soundscapes; many fishes use sound to communicate with members of their own and other species. Even nonvocalizing fish species rely on sound for environmental perception, navigation and detection of predators and prey (Popper, 2003; Simpson et al., 2016; Slabbekoorn et al., 2010). Numerous studies indicate that anthropogenic noise can inflict detrimental physical, physiological and behavioural effects in fishes (Cox et al., 2018; Duarte et al., 2021), including temporary hearing loss (Smith et al., 2004), altered auditory thresholds (Vasconcelos et al., 2007), physical damage to hearing structures (McCauley et al., 2003; Smith et al., 2004) and increased physiological stress responses (Mills et al., 2020; Nichols et al., 2015; Wysocki et al., 2006). Anthropogenic noise can increase vulnerability to predation in fishes by suppressing predator detection and subsequent reaction times (Simpson et al., 2015, 2016; Spiga et al., 2017). It can also alter individual and social behaviours including territoriality, swimming patterns and schooling (Sarà et al., 2007; Sebastianutto et al., 2011), interfere with acoustic communication (Vasconcelos et al., 2007) and negatively affect reproductive success by disrupting courtship and spawning (de Jong, Amorim, Fonseca, Fox, & Heubel, 2018; de Jong, Amorim, Fonseca, & Heubel, 2018; Sierra-Flores et al., 2015) or nest guarding and parental care (Bruintjes & Radford, 2013; Nedelec et al., 2017; Picciulin et al., 2010). Previous research has clearly established that the deleterious effects of noise pollution on fish are widespread and diverse; however, only a few studies have explored how anthropogenic noise affects fish parental care (e.g. Bruintjes & Radford, 2013; McCloskey et al., 2020; Nedelec et al., 2017; Picciulin et al., 2010), and our current understanding of how fish respond to noise exposure over longer time frames is limited.

While many detrimental effects of noise on fishes have been demonstrated in laboratory studies, far fewer studies have examined these effects in situ. Laboratory animals tend to behave differently from those in their natural habitat, and the acoustic properties of glass aquaria present additional challenges for acoustic experiments, such as reverberation and differential propagation (Jones et al., 2019). Nevertheless, laboratory studies present a valuable opportunity to experimentally examine responses to noise in a controlled environment. It is therefore beneficial when studying the impacts of noise on fish to combine laboratory and field experiments (Simpson et al., 2015). It is also important to present a biologically relevant noise stimulus to realistically evaluate in situ impacts of noise. Recreational motorboats are the most prevalent source of anthropogenic noise in coastal waters (Hermannsen et al., 2019). Therefore, recreational motorboat noise is a logical stimulus choice when examining the impacts of noise on coastal fishes.

We addressed these issues and gaps in our knowledge using experiments with the plainfin midshipman, *Porichthys notatus* (Batrachoididae), a vocal marine teleost fish found along the Pacific coast of North America. The species is well known for its powerful mate advertisement 'hum', and males frequently produce agonistic 'grunts' and 'growls' as well (McIver et al., 2014). The plainfin midshipman has been used as a model species for acoustic and neurophysiological research (e.g. Alderks & Sisneros, 2013; Brantley & Bass, 1994; Coffin et al., 2012; Colley et al., 2019; McIver et al., 2014; Sisneros, 2004), and because of its vocal repertoire and reliance on sound for successful reproduction, it is an ideal model for noise pollution studies. After a nest-guarding, or 'guarder', male attracts a fecund female and she lays her eggs in his intertidal nest, he will defend the young against egg predators and defend his nest

against rival males. Guarder males remain in their nests to provide parental care for up to 4 months (Arora, 1948; Cogliati et al., 2013). Plainfin midshipman breeding areas are frequently exposed to noise from coastal boat traffic throughout the spawning and parental care period (Halliday et al., 2018), with recreational boating peaking in the summer breeding months (Knapp et al., 1999; Sisneros, 2004). The plainfin midshipman's reliance on acoustic communication for courtship and competition, their sound sensitivity and their exposure to considerable boat noise in the breeding season all suggest that this species could suffer substantial reproductive consequences from noise pollution (Brown, Halliday, et al., 2021).

In this study, we investigated the potential impacts of motorboat noise on the reproductive success of guarder male plainfin midshipman in two ways. First, we exposed nest-guarding males in aquaria to black-clawed crabs, *Lophopanopeus bellus*, which are frequent nest predators in the wild, and we did so both in the presence and absence of motorboat noise playbacks. Second, we repeated this experiment with brood-guarding males in their natural intertidal habitat using a low-frequency artificial noise stimulus. We measured differences in nest defence behaviours and in parental care, which we define as all egg care and nest-tending behaviours, between noise and control treatments. We predicted that noise would alter nest defence behaviours and decrease parental care. We did not have an a priori prediction about whether defensive behaviour would increase or decrease, as previous studies have shown conflicting results. One study found that noise increased brood defence in a coral reef fish, the spiny chromis, *Acanthochromis polyacanthus* (Nedelec et al., 2017), while another study revealed a decrease in brood defence in the cooperative breeder, *Neolamprologus pulcher*, in relation to noise (Bruintjes & Radford, 2013). More recently, McCloskey et al. (2020) found that boat noise increased vigilance but decreased interaction with a conspecific intruder in nest-guarding Ambon damselfish, *Pomacentrus amboinensis*. Still, relatively few studies have investigated how noise affects nest defence in brooding parents, a knowledge gap our study sought to address.

METHODS

Experiment 1: Nest Defence during Boat Noise Playback in the Laboratory

Animal collections and housing

We collected 18 plainfin midshipman guarder males and 14 gravid females from the intertidal zone of Ladysmith Inlet, British Columbia, Canada (49°01'N, 123°83'W) during low spring tides of May and June 2018. Plainfin midshipman nests are cavities excavated by guarder males beneath intertidal rocks. Guarder males are easily identified as the largest and most centrally located fish in a nest, while gravid females can be easily identified by their overall smaller body size and distended, metallic golden bellies (Brantley & Bass, 1994). We transported the fish in aerated plastic bins to the University of Victoria's Outdoor Aquatic Facility, where the males and females were housed separately in 400-litre outdoor holding tanks for a minimum of 24 h before transfer to experimental aquaria. Each fish was weighed (± 0.01 g) and measured (standard length (SL) ± 1 mm) before being placed in an experimental tank (see below) and again after its final experimental trial. Each holding tank was lined with pebble substrate and supplied with free-flowing, ambient temperature (~ 13 °C) sea water and brick shelters. Seven black-clawed crabs (carapace widths: 26–32 mm) were also collected from the intertidal zone of Ladysmith Inlet and held in a separate tank connected to the same water system and supplied with bricks for shelter.

Each guarder male was individually tested in an aerated 175-litre experimental aquarium lined with pebble substrate and supplied with free-flowing, ambient sea water. Each aquarium contained an artificial nest made of a concrete tile supported by five bricks (Fig. 1). Males were given a minimum 2-day acclimation period to their experimental tank prior to beginning their 6-day trial sequence (Appendix, Fig. A1). Females were also introduced into the experimental tanks prior to trials to encourage males to take up residence in the artificial nests and guard them as they would in the wild.

Acoustic treatments

In this experiment, we implemented a paired design; each male underwent one boat noise exposure trial (hereafter ‘noise trial’) and one ambient sound-only control trial (hereafter ‘ambient trial’), separated by 2 days. The audio track used for noise trials was a recording of a recreational boat pass collected in Folger Passage, British Columbia by Ocean Networks Canada’s Folger Deep observatory using an Ocean Sonics iListen HF hydrophone (48°48′50″N, 125°16′29″W, depth: 95 m) in August 2015. This boat noise was from an unknown boat at an unknown distance from the hydrophone, but it is a clear example of boat noise that marine fish would be exposed to. A 1 min clip was extracted from the recording and low-pass filtered at 1000 Hz using MATLAB R2015a. This 1 min clip was then continuously looped using Audacity (<http://audacityteam.org/>) to generate a continuous boat noise playback (Fig. 2). During noise trials, the boat recording was played through an underwater

speaker (University Sound UW30, Lubell Labs, Columbus, OH, U.S.A.) continuously for the entire 80 min trial. During ambient trials, the speaker was present in the tank, but no sound was played from the speaker—fish were exposed only to the ambient conditions in the tank. The order of acoustic treatments was semi-randomly selected for each fish. On each day of trials, a random number generator was used to determine the treatment received by the first in a row of tanks; the order of treatments for adjacent tanks in each row was then alternated between noise and ambient trials to avoid potential boat noise spillover to concurrent ambient trials or to tanks that would be receiving a noise trial later that day. A maximum of two trials were run at a time and concurrent trials were spaced as far apart as possible and were never run in adjacent tanks.

Behavioural trials

Experimental trials were conducted during 5–20 July 2018. At the start of each trial, a video camera (GoPro HERO5 or HERO4) was positioned between two bricks, facing into the nest, and an underwater speaker (Lubell UW30) was set up (Fig. 1). For noise trials, a SoundTrap acoustic recorder (Ocean Instruments ST4300 STD, Auckland, New Zealand) fitted with two HTI hydrophones (High Tech, Inc., Long Beach, MS, U.S.A.; flat frequency response 20–24 000 Hz, total sensitivity -164.5 dB re 1 V/ μ Pa) was used, with the hydrophones positioned in the tank approximately 40 cm apart and 1.5 cm above the pebble substrate (Fig. 1). ‘Dummy hydrophones’ (dark plastic locklines resembling the hydrophones)

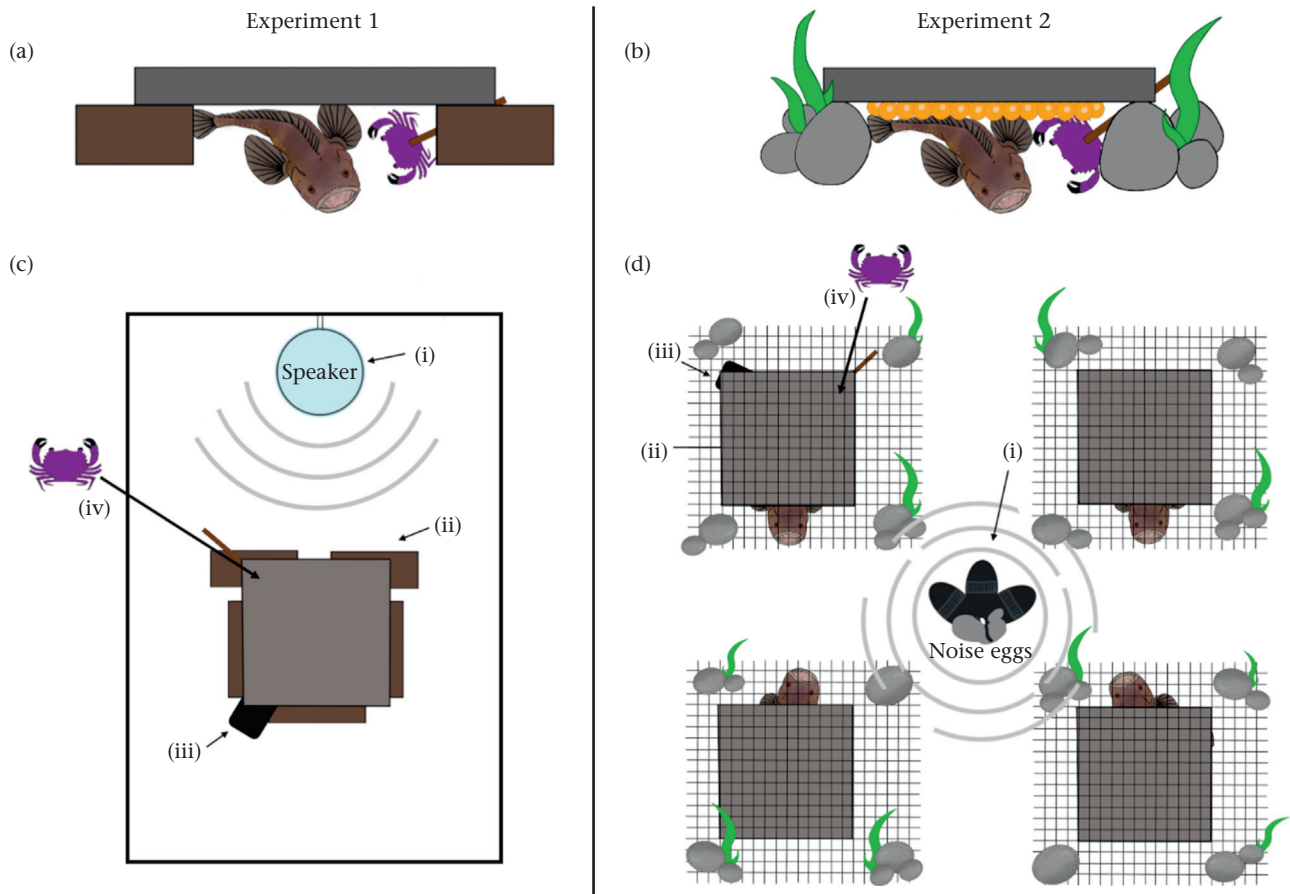


Figure 1. Experimental set-up for experiment 1 in laboratory aquaria and for experiment 2 in the field. (a, b) Side view of a guarder male in his nest during the threat period. (c, d) Top-down view of the experimental set-up. An artificial noise stimulus (i) was used to test the effects of anthropogenic noise on nest defence and parental care in the plainfin midshipman. A guarder male plainfin midshipman resided inside an artificial nest (ii) made of a concrete tile resting on bricks or rocks. A digital video camera (iii) recorded experimental trials from within the nest. A black-clawed crab (iv), a known predator of midshipman eggs, was inserted into the nest during the threat period to act as a threat stimulus.

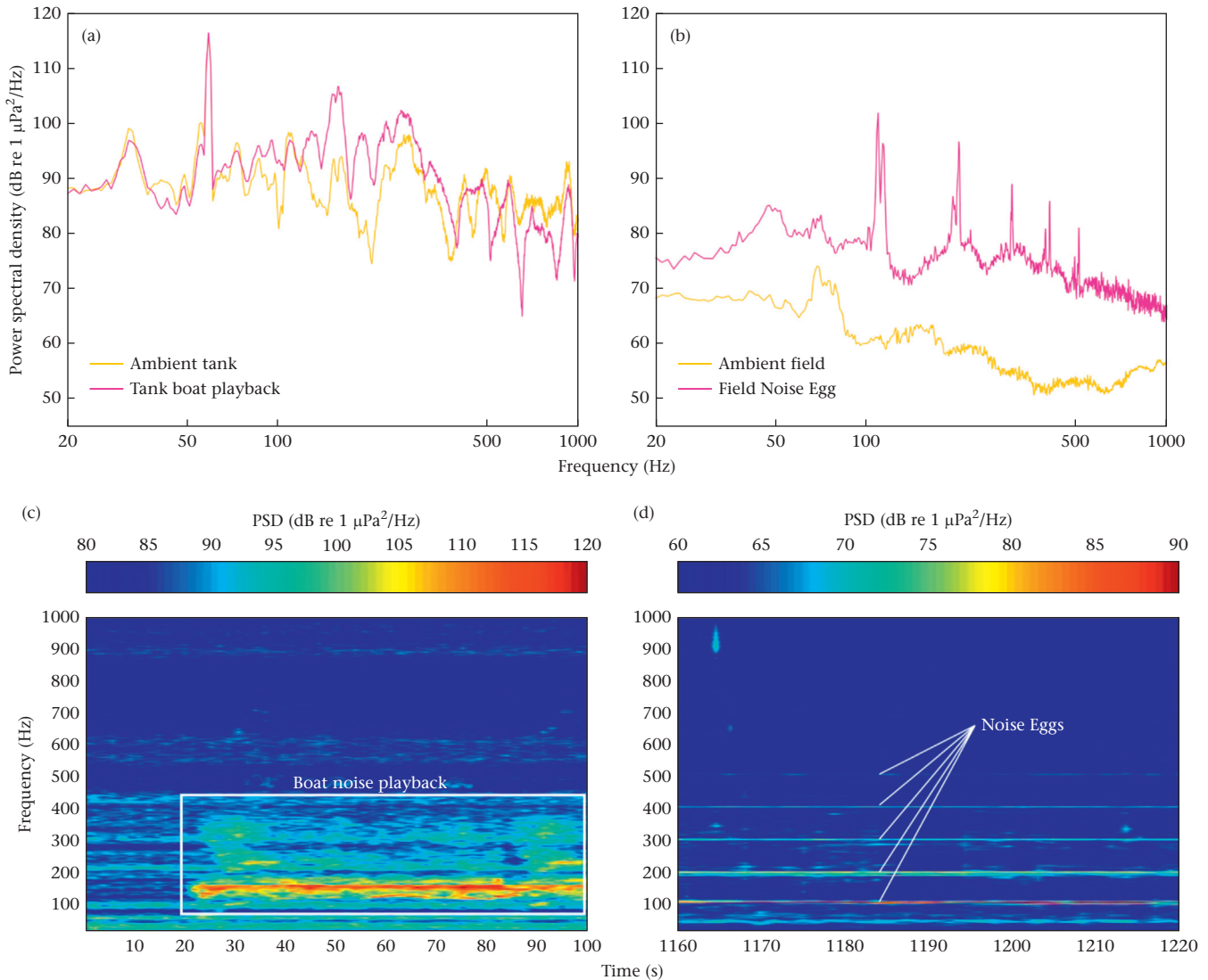


Figure 2. (a, b) Power spectral density (PSD; sound levels) and (c, d) spectrograms of noise stimuli and ambient sound. (a, c) Sound levels within tanks in experiment 1 comparing the playback from within the tank during noise trials and the ambient sound in the tank when no noise was being played through the speaker. (b, d) Sound levels in the field in experiment 2 comparing noise generated by three Noise Eggs tied together and field ambient conditions. The spectrogram in (c) shows an audio clip when the boat noise was turned on in the tank in experiment 1, such that the first ~20 s show ambient conditions, followed by the boat noise signal. The spectrogram in (d) shows the ambient environment in the field in experiment 2, with the tonal signals of the Noise Egg present. Spectrograms were built using a Hanning window with 50% overlap and 6000 samples; note that the colour scale differs for (c) and (d) to aid in visualizing the signals.

were set up within the tanks during ambient trials in the same position as the hydrophones in the noise trials to provide similar types of objects in the tank during noise and ambient treatments.

Each trial consisted of three phases: an initial 40 min baseline period before the threat stimulus was introduced, a 20 min threat period when the crab was placed in the nest and a 20 min follow-up period after the stimulus was removed. Intertidal crabs commonly prey on plainfin midshipman eggs (N. A. W. Brown, personal observation); therefore, we simulated a nest invasion by a crab to evoke a defensive response from the nest-guarding males. The individual crab used in each trial was randomly selected, secured to a flexible plastic rod via a wire wrapped around its carapace, and placed in the nest at the end of this rod during the threat period. The crab had full range of movement of its claws and limbs but could not move away because it was tethered to the rod. After 20 min, the crab was removed from the nest and from the wire and was returned to the housing tank. Recording continued for the 20 min follow-up period after the crab was removed. Behavioural displays and acts by the guarder male were scored as counts from

the video recordings by a trained observer. Defensive behaviours included biting, lunging, frontal fin display and grunt and growl agonistic vocalizations (Table 1).

Statistical analyses

Because the objective of this experiment was to assess how noise affects defensive behaviour during nest guarding, we needed to ensure that the males used in the study treated the artificial nests as their territory. To determine whether males had taken up residence in the artificial nests, we developed a classification system based on observable signs of territoriality in plainfin midshipman guarder males, including (1) whether males were in or out of their nests at the beginning of each trial (territorial males do not usually leave their nests), (2) the presence of a midden at the nest entrance (territory owners will modify their nest entrances with their fins and mouths by building up a small barrier at the nest entry from the substrate found inside the nest) and (3) whether or not males had a distended ventral surface (a morphological change indicating an inflated swim bladder, a sign of vocal courtship activity in

Table 1
Ethogram of behaviours exhibited by plainfin midshipman guarder males that was used to score behaviour in both experiment 1 and 2

Behaviour	Description
Defence	
Bite	Mouth opens and closes rapidly, directed towards the crab
Frontal fin display	While facing the crab, males would spread out their pectoral and dorsal fins; a threat display
Lunge	A quick burst of forward movement towards the crab
Vocalizations	A short-duration 'grunt' (~0.5 s) or a medium-duration 'growl' (up to several seconds)
Parental care	
Egg fanning	Moving fins rapidly under the eggs and providing aeration
Egg squirting	Squirting a stream of water with their mouths at individual eggs to keep them clean and remove debris
Sediment spitting	Using mouth to pick up stones, shells and other large pieces of sediment and spit them outside or towards the perimeter of nest
Sediment clearing	Using fins to clear fine sediment from bottom of nest

Note that the parental care behaviours were only scored in experiment 2 because males did not have young in experiment 1.

guarder male plainfin midshipman; J. Sisneros, personal communication). The territoriality scale ranged from 0 (no signs) to 3 (all signs present); signs were recorded before each trial, and we summed the territoriality scores for each male across its two trials for a maximum score of 6. Six of the 18 males scored 0, showing no signs of territoriality; these males were removed from all our statistical analyses. One spawning event occurred prior to the first day of trials, and this male was also removed from all analyses, as guarding eggs represents a different reproductive stage (Knapp et al., 1999). Therefore, from the 18 males originally collected for this experiment, we used the trials from a final sample of 11 males (SL: 177–243 mm).

We conducted all statistical analyses in R (v.3.5.1; R Core Team, 2018). We defined nest defence as the sum of all observed defensive behaviours (Table 1) and fitted a Poisson generalized linear mixed effects model (GLMM) ('glmmTMB' package; Brooks et al., 2017) to these counts of nest defence behaviours performed by each focal male in its two trials (noise and ambient). We included male identity (ID) as a random intercept to reflect the within-subjects study design, acoustic treatment as a fixed effect, and an additional fixed effect of trial order (i.e. whether the ambient or noise trial was received first). Only behaviours exhibited towards the crab were considered defensive behaviours (Table 1); therefore, only counts from the threat period (when the crab was present) were modelled. We used likelihood ratio tests to assess the statistical significance of the acoustic treatment effect. We used an additional Poisson GLMM with the same random and fixed effects to test the effect of acoustic treatment on the most escalated defence behaviours: bites and lunges.

Experiment 2: In Situ Nest Defence and Parental Care in the Presence of Added Noise

Field site and nest establishment

We conducted this field experiment in the intertidal zone of Dabob Bay, Washington, U.S.A. (47°76'N, 122°86'W) in May and June 2019. We deployed 38 square concrete tiles (929 cm²) as artificial nests; tiles were grouped into 10 clusters of three to four tiles that were spaced at least 9 m apart. Nests were then checked daily by snorkellers for fish occupancy. Once a male had excavated a cavity and was observed by snorkellers to be guarding a brood of eggs, the snorkellers covered the nest with plastic mesh (1 cm² openings) weighed down by rocks to exclude rivals, further matings and predators and to ensure that the same individual remained for the duration of the experiment (Bose et al., 2016; Brown, Houpt et al., 2021).

Acoustic treatments

Each fish underwent one noise treatment trial and one ambient treatment trial, separated by 2 days without manipulation, as in

experiment 1. The acoustic treatment received first was determined randomly for each group of nests. To simulate continuous anthropogenic noise underwater, we employed 'Noise Eggs' (de Jong et al., 2017), which generate a tone with a fundamental frequency around 100 Hz and several harmonics (Brown, Halliday, et al., 2021; de Jong, Amorim, Fonseca, Fox, & Heubel, 2018). In the field, a single Noise Egg added <10 dB re 1 μPa to ambient noise (in the dominant 80 Hz octave band; Brown, Halliday, et al., 2021), so we combined three Noise Eggs together to increase the signal strength. We positioned a group of three Noise Eggs secured to a lead weight in the middle of each nest cluster. Noise Eggs emitted continuous noise (Fig. 2) for the duration of the 60 min trial. No Noise Eggs (and no dummy Noise Eggs) were present during ambient trials; the males were exposed only to natural ambient conditions. The noise emitted by the Noise Eggs attenuated quickly in the shallow intertidal water, and nest groupings were spaced far apart (9–22 m), so any noise spillover to the ambient trials was very unlikely.

Behavioural trials

Immediately prior to each field trial, snorkellers confirmed the presence of a guarder male with a brood of eggs under the meshed nest before deploying the Noise Eggs and cameras. Once snorkellers had positioned the Noise Eggs in the centre of a group of nests (if that group was receiving the noise treatment), the snorkellers then placed a digital camera (models: GoPro Hero5 Black and Sony AS300 and AS50 Action Cameras) in the corner of each nest in that group. The procedure then unfolded similarly to experiment 1. The trial began with a 20 min baseline period, during which the male's natural behaviour—which largely consists of parental care—was recorded. Next, snorkellers placed a locally collected black-clawed crab (carapace width: 20–29 mm) secured to a thin bamboo rod by a wire wrapped around its carapace, into each nest. This initiated a 20 min threat period, which was used to examine defensive behaviour towards the egg predator, in addition to parental care and other natural behaviours. Finally, the snorkeller removed the crab from each nest, initiating the 20 min follow-up period. In noise trials, the artificial noise continued for the full 60 min trial (baseline, threat, follow-up) and behaviours were recorded throughout. At the end of each trial, all cameras and the Noise Eggs were removed by a snorkeller.

After a male's final trial (ambient or noise), we captured, weighed (± 0.01 g) and measured (SL ± 1 mm) each male and photographed the eggs in his brood. We then removed the mesh and gently returned the male to his nest. Eggs in the photographs were later counted and their developmental stages were determined by an individual blind to experimental conditions (see Brown, Houpt et al., 2021 for details). We used these data to quantify brood size and the health of the brood from each nest.

We tested a total of 23 males in this experiment, but two were removed from analysis because the fish abandoned their nest

during a trial (one abandoned during a noise trial and one abandoned during an ambient trial), and a third fish was removed because one of its videos was obstructed by a rock in front of the camera and could not be scored. This left us with a final sample size of 20 guarder males (SL: 181–261 mm).

Statistical analyses

We followed a similar analytical approach to that described for experiment 1. Continuous covariates in all models were mean-centred and scaled (Schielzeth, 2010). We first fitted the number of defensive and parental care behaviours performed in each trial with Poisson GLMMs as in experiment 1. However, due to overdispersion, we refitted the models using negative binomial GLMMs (Bolker et al., 2009). Both models included male ID as a random intercept to reflect the within-subjects experimental design, and noise treatment was included as a fixed effect. Trial date was added as an additional random effect to account for unmeasured environmental variations (e.g. weather, tides) in the field – this variable was not included in experiment 1 analyses, as the fish were in a controlled laboratory environment. An additional fixed effect was added for nest elevation because males nesting at higher tidal elevations exhibit more frequent parental care behaviours (Brown, Houpt et al., 2021). We also investigated whether trial order was an important predictor, but model comparisons using Akaike's information criterion corrected for small samples (AICc) revealed no significant effect, so trial order was not included in the final model. As in experiment 1, our defence model consisted of counts only from the threat period (when the crab was present). Trial period was included as an additional fixed effect in the parental care model. We used likelihood ratio tests to test for statistical significance of terms in both models. We used an additional negative binomial GLMM with the same random and fixed effects to test the effect of acoustic treatment on the most escalated defence behaviours: bites and lunges.

Experimental Comparison: Nest Defence in the Laboratory (Experiment 1) versus the Field (Experiment 2)

Statistical analysis

Following a similar analytical protocol as those described for experiment 1 and experiment 2, we used GLMMs to compare the number of defensive behaviours exhibited in experiment 1 in the laboratory versus experiment 2 in the field. We used one negative binomial GLMM to test for differences between behaviours exhibited in experiments 1 and 2 during noise treatment trials, and a second model for differences during the ambient treatment trials. In both models, we included test order (whether fish experienced the noise or ambient treatment first) as an additional fixed effect because it was a significant term in our model for defensive behaviours in experiment 1.

Ethical Note

All procedures in this study complied with guidelines set by the ASAB/ABS (2012) and the Canadian Council on Animal Care (Olfert et al., 1993) and were approved by the University of Victoria Animal Care Committee (AUP: Juanes-2017-003). All procedures implemented were noninvasive. Fish tested in the field were released back into the wild following experimentation; all equipment was removed, and fish were allowed to continue guarding their brood in their artificial nests at the study site for the remainder of the breeding season. Fish tested in the laboratory were humanely euthanized by overdose in an anaesthetic bath (TMS-222) and cervical dislocation, as release of fish back into the wild is not permitted by the Canadian Council on Animal Care (2005; Guideline 115) once the animal has been held in a captive environment.

RESULTS

Experiment 1: Nest Defence in the Laboratory

There was a significant effect of acoustic treatment on the defensive behaviours exhibited by nest-guarding males during the threat period (GLMM: $\chi^2_1 = 22.43$, $P < 0.0001$; Fig. 3a). Males performed 3.8 times (95% confidence interval (CI): 2.1–6.9) as many defensive behaviours during motorboat noise playback than during ambient control conditions, during which no sound was played. When we performed the same analysis examining only the most escalated defence behaviours—bites and lunges—we found the same pattern (GLMM: $\chi^2_1 = 15.39$, $P < 0.0001$), with 9.5 times (95% CI: 2.9–31.8) as many of these escalated behaviours exhibited in the noise trials compared to ambient trials. Six of the 11 laboratory fish (54.5%) did not perform any defensive behaviours in either of their trials. Although it was not included in our statistical analyses, the one egg-guarding male exhibited the same number of defensive behaviours (nine) in both treatments (noise and ambient).

Experiment 2a: Nest Defence in the Field

In contrast to experiment 1, the number of defensive behaviours performed by wild brood-guarding males did not significantly differ between the noise and ambient treatments (GLMM: $\chi^2_1 = 0.0078$, $P = 0.93$; Fig. 3b). However, unlike in the laboratory experiment, the majority of fish exhibited defensive behaviour, with only seven of the 20 field fish (35%) not performing any defensive behaviours. Regardless of noise treatment, defensive behaviours were most frequently exhibited by males in the lowest-elevation nests (GLMM: elevation: $\chi^2_1 = 4.49$, $P = 0.03$); for every 10 cm increase in elevation, males performed approximately half as many (95% CI: 0.3–0.9) defensive behaviours (Fig. 4). As in experiment 1, we found the same result when the analysis was repeated with only the most energetically costly behaviours: bites and lunges. We did not observe an effect of acoustic treatment (GLMM: $\chi^2_1 = 0.0004$, $P = 0.98$), but bites and lunges were observed more frequently at lower tidal elevations (GLMM; elevation: $\chi^2_1 = 4.79$, $P = 0.03$).

Experiment 2b: Parental Care in the Field

The number of parental care behaviours performed by wild brood-guarding males did not significantly differ between sound treatments (GLMM: $\chi^2_1 = 0.81$, $P = 0.37$; Fig. 3c). The extent of parental care differed greatly between trial periods (baseline, threat, follow-up; GLMM: $\chi^2_2 = 14.38$, $P = 0.0008$); males exhibited the fewest parental care behaviours during the threat period, i.e. when the egg predator crab was in the nest. Approximately 2.5 times as many parental behaviours were performed in the baseline period (95% CI: 1.3–5.2) and three times as many performed in the follow-up period (95% CI: 1.4–6.3), relative to the threat period. This indicated that the males reduced their parental care activities temporarily while interacting with the crab.

Experimental Comparison: Defence in the Laboratory (Experiment 1) versus in the Field (Experiment 2a)

The number of defensive behaviours performed by guarder males differed between the laboratory (experiment 1) and the field (experiment 2) during ambient trials ($\chi^2_1 = 4.37$, $P = 0.04$), but not during noise trials ($\chi^2_1 = 0.21$, $P = 0.17$). Males exhibited approximately five times (95% CI: 1.0–23.5) as many defence behaviours during ambient trials in the field compared to those in the laboratory (Fig. 5).

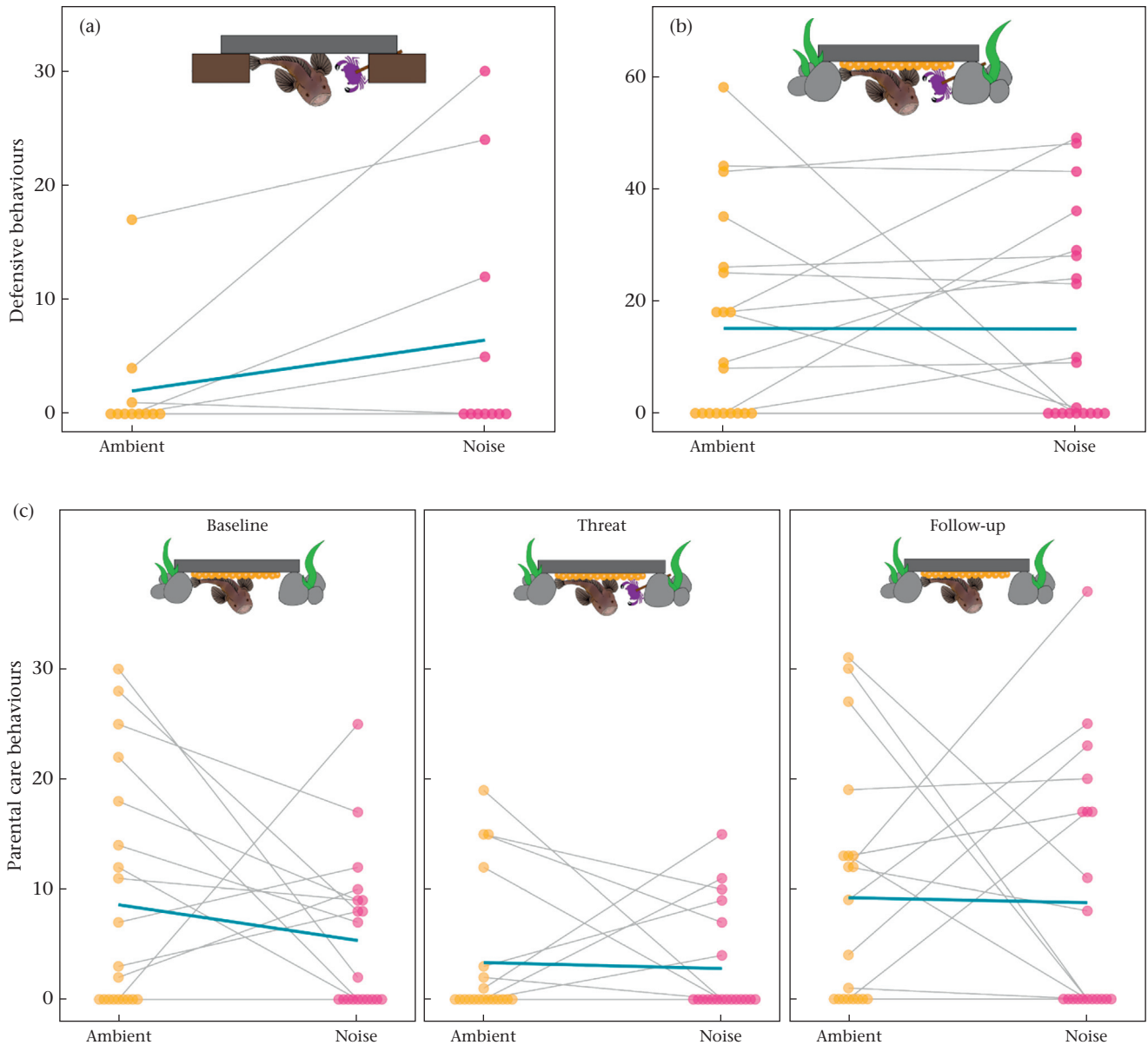


Figure 3. Defence and parental care behaviours exhibited in the presence and absence of noise. Number of discrete defence behaviours towards a live crab (egg predator) exhibited by (a) nest-guarding males ($N = 11$) in laboratory aquaria in experiment 1 and (b) wild brood-guarding males ($N = 20$) in the field in experiment 2. (c) Number of parental care behaviours exhibited by wild brood-guarding males ($N = 20$) in experiment 2 in the presence and absence of noise during each of the three trial periods. The live crab was placed in the nest during the threat period only. Noise was generated by boat noise playback through a speaker in experiment 1 (a) and by vibrating Noise Eggs in experiment 2 (b, c). The thin grey lines connect each fish's two trials, and the thick blue line connects the mean numbers of behaviours performed under each acoustic treatment.

DISCUSSION

We found evidence that boat noise can cause plainfin midshipman guarder males to increase their defensive behaviour. However, the responses to noise were context specific. In laboratory aquaria, males guarding an empty nest exhibited more defensive behaviours towards crabs during boat noise playback compared to ambient conditions. In contrast, we did not observe an effect of a tonal artificial noise stimulus on the nest defence of brood-guarding males in our complementary field experiment. Overall, the males guarding eggs in the field experiment exhibited more than three times as many defence behaviours on average compared to those guarding empty nests in the laboratory experiment. When examined within each acoustic treatment (noise and ambient), males in the field experiment exhibited nearly five times as many defence behaviours in ambient conditions compared to

those in the laboratory experiment under ambient conditions. Furthermore, 55% of the laboratory fish did not exhibit any defence behaviours, while in the field, only 35% of the fish did not exhibit defensive behaviours. Our results demonstrate that responses to noise depend on context and are not uniform across testing environments.

The differences observed between laboratory and field results may be due to several factors, including (1) the use of different noise stimuli, (2) presence versus absence of eggs and (3) the degree of environmental complexity. The difference in noise stimuli likely played a key role in the different outcomes between our laboratory and field experiments. While both stimuli produced low-frequency noise, the playback in our laboratory study had a higher amplitude (peak laboratory boat noise playback = 111 dB re $1 \mu\text{Pa}^2/\text{Hz}$ at 159 Hz) and consisted of broadband noise, whereas the Noise Egg was quieter (peak field Noise Egg = 101 dB re $1 \mu\text{Pa}^2/$

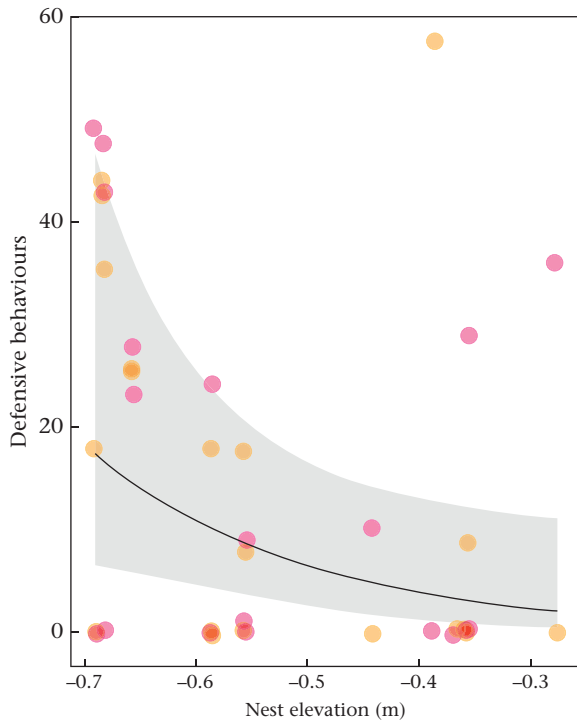


Figure 4. Defensive behaviours of parental males nesting at different intertidal elevations. The variation in number of defensive behaviours exhibited by wild brood-guarding males ($N = 20$) towards a live crab depending on the elevation of their intertidal nests. Pink dots indicate noise trials, while gold dots indicate ambient trials (no added noise). Darker colours represent overlap of points. Points are also slightly 'jittered' to show multiple points with the same value. The trendline shows the model predictions with a 95% confidence interval.

Hz at 110 Hz) and contained only a low-frequency tone accompanied by harmonics (Fig. 2). Differences in the amplitude and frequency spectra of noise stimuli are known to elicit different responses. For example, McCormick et al. (2018) found that boat noise reduces boldness and activity of juvenile damselfish (*Pomacentrus wardi*) when generated by a 2-stroke engine, but not by a 4-stroke engine, demonstrating that even small differences in noise stimuli can lead to different behavioural outcomes. Similarly, Jain-Schlaepfer et al. (2018) found that heart rates of embryonic damselfish (*Amblyglyphidodon curacao*) increased in response to both types of engine noise, but the noise from a 2-stroke engine evoked a two-fold increase in heart rates compared to a 4-stroke engine. Differences in frequency distribution between these two motor types may be subtle to our ears, but are potentially more salient to fish with high acoustic sensitivity in this frequency range. Furthermore, these two engine types differ in the amplitude (~5 dB re $1 \mu\text{Pa}$ higher for the 2-stroke engine) of the noise produced (McCormick et al., 2018). In our study, the stronger response was observed in the laboratory, where the stimulus was 10 dB higher than the noise stimulus used in the field (Fig. 2). However, the ambient noise was also much greater in the laboratory than in the field (ambient sound in the tank = 122 dB re $1 \mu\text{Pa}$ in 50–1000 Hz band, peak power = 116 dB re $1 \mu\text{Pa}^2/\text{Hz}$ at 59 Hz; ambient sound in the field = 90 dB re $1 \mu\text{Pa}$ in 50–1000 Hz band, peak power = 74 dB re $1 \mu\text{Pa}^2/\text{Hz}$ at 69 Hz), resulting in a more pronounced difference in amplitude between ambient and noise conditions in the field, where behavioural differences were not detected. Further study is warranted to determine how differences in frequency distribution, amplitude or amplitude shift affect behavioural changes in nest- or brood-guarding plainfin midshipman.

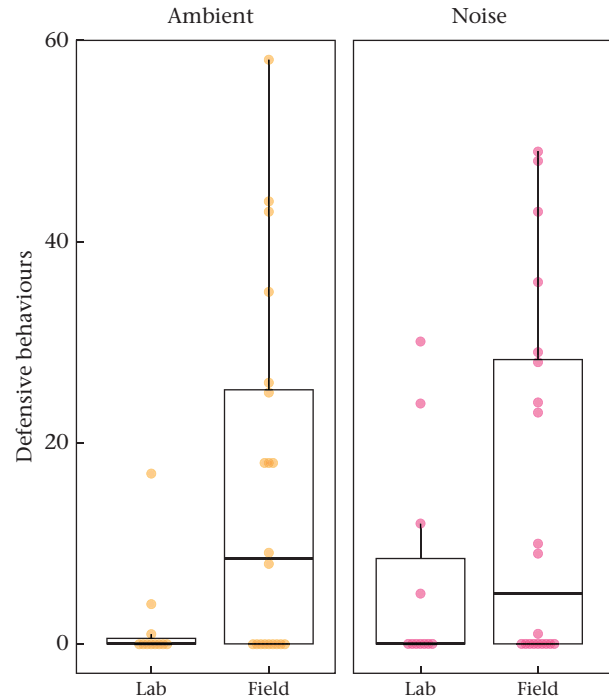


Figure 5. Defensive behaviours exhibited in the laboratory (experiment 1) and the field (experiment 2) in the presence and absence of noise. Comparison of the number of defensive behaviours exhibited towards a live crab by nest-guarding males in laboratory aquaria (experiment 1; $N = 11$) and by wild brood-guarding males in the field (experiment 2; $N = 20$), compared within each of the two acoustic treatments: noise and ambient. Noise was generated by boat noise playback through a speaker in experiment 1 (lab) and by vibrating Noise Eggs in experiment 2 (field), and no noise was introduced in the ambient treatment in either experiment.

A related issue is that sound can be measured in terms of particle motion or pressure. In our study, we reported only sound pressure levels. Pressure differences are typically detected by fish using their gas-filled swim bladder and/or by the inner ear otolithic organs, while particle motion is primarily detected in the lateral line and otolithic organs (Popper & Fay, 2011). While all fish perceive particle motion, plainfin midshipman detect both pressure and particle motion (Coffin et al., 2014; Colleye et al., 2019; McKibben & Bass, 1999; Popper & Hawkins, 2018; Simpson et al., 2016). Plainfin midshipman females use particle motion as their primary mechanism of nearfield sound source localization when moving into the intertidal to locate humming males in their nests (Zeddies et al., 2012). The relative levels of sound pressure and particle velocity would have varied greatly between tank and natural environments (Campbell et al., 2019; Popper & Hawkins, 2018). Precisely how fish use particle motion and pressure components of sound and the degree to which they depend on these sources of information is not well understood (Nedelec et al., 2016), but the different relative levels of these sound components in our field and laboratory experiments may have contributed to the differing results. Furthermore, it is possible that sound may have been transmitted as vibrations through the substrate (Popper & Hawkins, 2018), which differed between experiments (pebbles on a glass aquarium bottom versus sand and rocks in the field), potentially leading to a difference in signal detection between experiments. This may be particularly important to plainfin midshipman fish since they are in direct contact with the substrate in their nests. However, no studies have tested whether plainfin midshipman fish can detect sound through the substrate, nor is there empirical evidence that any fishes can detect substrate signals (Popper & Hawkins, 2018). Future studies considering particle motion and

perception of anthropogenic noise by the plainfin midshipman and other acoustically sensitive species are still needed to better understand the impacts of these effects.

The second major factor that likely contributed to the different behavioural responses to noise between the field and the laboratory were the differences in the reproductive states of the males. Males are expected to exhibit higher overall defence against an egg predator when they have eggs and territories to defend, compared to when they are simply defending their territory (Sowersby et al., 2017). It is possible that males with young in the nest are defending at maximal rates, and that extra predation cues matter little. Similar to our results, Brintjes and Radford (2013) found that noise had a significant effect on defensive behaviours towards an egg predator in nests without eggs, but they did not observe a significant change in defence when eggs were present in nests. Anecdotally, the single egg-guarding male in our laboratory study, who was excluded from analyses, exhibited the same number of defensive behaviours in each of the acoustic treatments. We also did not see a change in parental care behaviours performed between the two acoustic treatments in the field; however, we did see a significant decrease in the amount of parental care exhibited in the threat period—when the crab was in the nest—compared to the baseline and follow-up periods. This was likely because the male was spending more time on defensive behaviours when there was an active threat in the nest, and therefore had less time to spend on parental care.

A third factor that likely contributed to the difference in experimental outcomes between the laboratory and field is the difference in environmental cues or enrichment. Males nesting in the intertidal zone are privy to a full range of natural biotic sounds (in particular, the loud chorus of plainfin midshipman hums) and abiotic sounds (e.g. wave action/water movement). Males in the field would have had numerous biological interactions with neighbours, rivals, passing females and predators, whereas ambient noise in the laboratory was far more homogenous and less naturalistic. Sounds in the laboratory consisted of mechanical noise from pumps, bubbles from air lines and water running over tables and through pipes, and our experimental males were housed apart from other males. The abundance of biological stimuli in the field may have resulted in our noise stimulus not being as significant a disruption to the defending males as the noise playback in the laboratory—an artificial habitat lacking those natural interactions and cues. Our finding that nest defence behaviours were increased at lower tidal elevations aligns with previous work showing that subtidal males are more active nest defenders than males nesting in the middle of the intertidal (Brown, Houpt et al., 2021). Males in the field are likely exposed to egg predators constantly, whereas males in the laboratory were only exposed to egg predators during the experimental trials. The risk of egg predation by aquatic animals presumably increases at lower tidal elevations (MacDonald et al., 1995), and the corresponding increase in nest defence behaviours we observed among lower-nesting males suggests nest defence is tuned to egg predation risk, which may have overshadowed a subtler effect of the artificial noise we employed in the field.

The results of our laboratory study contribute to growing evidence that vessel noise can negatively affect fish by altering their behaviour. Increasing defensive behaviour and vigilance is likely energetically costly (Zoldero et al., 2016), and because passing boats do not represent a predation threat, expending additional energy on unneeded defence during their passage could be detrimental. Furthermore, additional time spent on unnecessary defence and vigilance would detract from time and energy normally spent on parental care or mate attraction, as we saw with the decrease in parental care while defending against the crab, which in turn could have population level consequences. For example, Nedelec et al. (2017) observed total brood mortality in six of 19 nests exposed to

in situ motorboat playbacks in the reef fish *A. polyacanthus*; in contrast, none of the 19 nests exposed to ambient playbacks suffered complete mortality. Similar to our laboratory study results, the parental males in Nedelec et al.'s (2017) study exhibited increased aggressive acts towards potential predators while exposed to boat noise. Exposed parental males also decreased feeding and parent–offspring interactions (Nedelec et al., 2017).

While our field study did not show the same increase in defensive behaviours in the presence of noise, this disparity could be the result of several factors, such as differing received amplitude of ambient and noise conditions, different environmental stimuli and different reproductive states between males in the field and in the laboratory. Because our study was not designed to investigate the individual effects of each of these factors on plainfin midshipman behaviour, and due to the limited sample size in our laboratory study, we suggest that additional field and laboratory studies should be performed on the plainfin midshipman and other species with similar breeding ecology.

By pairing experiments in the field and in the laboratory, we were able to identify patterns that might have been obscured in one context or the other. Our results reveal the utility of combining laboratory and field experiments when studying fish behaviour, especially in response to noise. However, because of the various contextual differences between our laboratory and field experiments, our results are not conclusive, and this topic warrants additional research. Nevertheless, this study contributes to our growing understanding of how noise affects fish. As anthropogenic noise levels in the ocean continue to rise, it is vital that we understand how this changing soundscape is affecting some of the most ecologically and economically important animals in the ocean.

Author Contributions

Mackenzie Woods: conceptualization, methodology, investigation, formal analysis, visualization, writing – original draft and funding acquisition. **Nicholas Brown:** conceptualization, methodology, investigation, formal analysis, writing – review and editing and funding acquisition. **Katrina Nikolich:** conceptualization, methodology, investigation, formal analysis, and writing – review and editing. **William Halliday:** formal analysis, visualization, writing – review and editing. **Sigal Balshine** and **Francis Juanes:** conceptualization, supervision, writing – review and editing and funding acquisition.

Acknowledgments

We sincerely thank Eileen Carr and family for supporting our field team and granting access to the field site. We thank Jennifer Curtis, Dylan Paterson, Noah Houpt and Breanna Nelson for their assistance with fieldwork, laboratory work and analysis. We also thank Kieran Cox, Nicholas Planidin and Kelsie Murchy for their thoughtful guidance and analytical assistance. This study was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants to S.B. and F.J., an NSERC Michael Smith Foreign Study Supplement to N.B., a Liber Ero Foundation grant to F.J. and a University of Victoria Jamie Cassels Undergraduate Research Award to M.B.W.

References

- Alderks, P. W., & Sisneros, J. A. (2013). Development of the acoustically evoked behavioral response in larval plainfin midshipman fish, *Porichthys notatus*. *PLoS One*, 8(12), Article e82182. <https://doi.org/10.1371/journal.pone.0082182>
- Arora, H. L. (1948). Observations on the habits and early life history of the batrachoid fish, *Porichthys notatus* Girard. *Copeia*, 1948(2), 89–93. <https://doi.org/10.2307/1438409>

- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24(3), 127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Bose, A. P. H., Kou, H. H., & Balshine, S. (2016). Impacts of direct and indirect paternity cues on paternal care in a singing toothfish. *Behavioral Ecology*, 27(5), 1507–1514. <https://doi.org/10.1093/beheco/arw075>
- Bowen, B. W., Gaither, M. R., DiBattista, J. D., Iacchi, M., Andrews, K. R., Grant, W. S., Toonen, R. J., & Briggs, J. C. (2016). Comparative phylogeography of the ocean planet. *Proceedings of the National Academy of Sciences of the United States of America*, 113(29), 7962–7969. <https://doi.org/10.1073/pnas.1602404113>
- Brantley, R. K., & Bass, A. H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). *Ethology*, 96(3), 213–232. <https://doi.org/10.1111/j.1439-0310.1994.tb01011.x>
- Brown, N. A. W., Halliday, W. D., Balshine, S., & Juanes, F. (2021). Low-amplitude noise elicits the Lombard effect in plainfin midshipman mating vocalizations in the wild. *Animal Behaviour*, 181, 29–39. <https://doi.org/10.1016/j.anbehav.2021.08.025>
- Brown, N. A. W., Houpt, N. S. B., Yee, N. L., Curtis, J. E. M., Bolker, B. M., Juanes, F., & Balshine, S. (2021). Consequences of nest site selection vary along a tidal gradient. *Journal of Animal Ecology*, 90(2), 528–541. <https://doi.org/10.1111/1365-2656.13385>
- Bruinijes, R., & Radford, A. N. (2013). Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. *Animal Behaviour*, 85(6), 1343–1349. <https://doi.org/10.1016/j.anbehav.2013.03.025>
- Campbell, J., Shafiei Sabet, S., & Slabbekoorn, H. (2019). Particle motion and sound pressure in fish tanks: A behavioural exploration of acoustic sensitivity in the zebrafish. *Behavioural Processes*, 164, 38–47. <https://doi.org/10.1016/j.beproc.2019.04.001>
- Coffin, A. B., Mohr, R. A., & Sisneros, J. A. (2012). Sacculus-specific hair cell addition correlates with reproductive state-dependent changes in the auditory sacculus sensitivity of a vocal fish. *Journal of Neuroscience*, 32(4), 1366–1376. <https://doi.org/10.1523/JNEUROSCI.4928-11.2012>
- Coffin, A. B., Zeddes, D. G., Fay, R. R., Brown, A. D., Alderks, P. W., Bhandiwad, A. A., Mohr, R. A., Gray, M. D., Rogers, P. H., & Sisneros, J. A. (2014). Use of the swim bladder and lateral line in near-field sound source localization by fish. *Journal of Experimental Biology*, 217(12), 2078–2088. <https://doi.org/10.1242/jeb.093831>
- Cogliati, K. M., Neff, B. D., & Balshine, S. (2013). High degree of paternity loss in a species with alternative reproductive tactics. *Behavioral Ecology and Sociobiology*, 67(3), 399–408. <https://doi.org/10.1007/s00265-012-1460-y>
- Colley, O., Vetter, B. J., Mohr, R. A., Seeley, L. H., & Sisneros, J. A. (2019). Sexually dimorphic swim bladder extensions enhance the auditory sensitivity of female plainfin midshipman fish, *Porichthys notatus*. *Journal of Experimental Biology*, 222(14), jeb.204552. <https://doi.org/10.1242/jeb.204552>
- Cox, K., Brennan, L. P., Gerwing, T. G., Dudas, S. E., & Juanes, F. (2018). Sound the alarm: A meta-analysis on the effect of aquatic noise on fish behavior and physiology. *Global Change Biology*, 24(7), 3105–3116. <https://doi.org/10.1111/gcb.14106>
- Duarte, C. M., Chapuis, L., Collin, S. P., Costa, D. P., Devassy, R. P., Eguiluz, V. M., Erbe, C., Gordon, T. A. C., Halpern, B. S., Harding, H. R., Havlik, M. N., Meekan, M., Merchant, N. D., Miksis-Olds, J. L., Parsons, M., Predragovic, M., Radford, A. N., Radford, C. A., Simpson, S. D., Slabbekoorn, H., Staaterman, E., et al. (2021). The soundscape of the Anthropocene ocean. *Science*, 371(6529). <https://doi.org/10.1126/science.aba4658>
- European Union. (2008). Marine Strategy Framework Directive: Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy. *Official Journal of the European Union*, 164, 19–40. <http://data.europa.eu/eli/dir/2008/56/oj>
- Frisk, G. V. (2012). Noiseconomics: The relationship between ambient noise levels in the sea and global economic trends. *Scientific Reports*, 2, 437. <https://doi.org/10.1038/srep00437>
- Halliday, W., Pine, M., Bose, A., Balshine, S., & Juanes, F. (2018). The plainfin midshipman's soundscape at two sites around Vancouver Island, British Columbia. *Marine Ecology Progress Series*, 603, 189–200. <https://doi.org/10.3354/meps12730>
- Hermanssen, L., Mikkelsen, L., Tougaard, J., Beedholm, K., Johnson, M., & Madsen, P. T. (2019). Recreational vessels without Automatic Identification System (AIS) dominate anthropogenic noise contributions to a shallow water soundscape. *Scientific Reports*, 9(1), Article 15477. <https://doi.org/10.1038/s41598-019-51222-9>
- Jain-Schlaepfer, S., Fakan, E., Rummer, J. L., Simpson, S. D., & McCormick, M. I. (2018). Impact of motorboats on fish embryos depends on engine type. *Conservation Physiology*, 6(1), coy014. <https://doi.org/10.1093/conphys/coy014>
- Jones, I. T., Stanley, J. A., Bonnel, J., & Mooney, T. A. (2019). Complexities of tank acoustics warrant direct, careful measurement of particle motion and pressure for bioacoustic studies. *Proceedings of Meetings on Acoustics*, 37(1), Article 010005. <https://doi.org/10.1121/2.0001073>
- de Jong, K., Amorim, M. C. P., Fonseca, P. J., Fox, C. J., & Heubel, K. U. (2018). Noise can affect acoustic communication and subsequent spawning success in fish. *Environmental Pollution*, 237, 814–823. <https://doi.org/10.1016/j.envpol.2017.11.003>
- de Jong, K., Amorim, M. C. P., Fonseca, P. J., & Heubel, K. U. (2018). Noise affects multimodal communication during courtship in a marine fish. *Frontiers in Ecology and Evolution*, 6, 113. <https://doi.org/10.3389/fevo.2018.00113>
- de Jong, K., Schulte, G., & Heubel, K. U. (2017). The noise egg: A cheap and simple device to produce low-frequency underwater noise for laboratory and field experiments. *Methods in Ecology and Evolution*, 8(2), 268–274. <https://doi.org/10.1111/2041-210X.12653>
- Kight, C. R., & Swaddle, J. P. (2011). How and why environmental noise impacts animals: An integrative, mechanistic review: Environmental noise and animals. *Ecology Letters*, 14(10), 1052–1061. <https://doi.org/10.1111/j.1461-0248.2011.01664.x>
- Knapp, R., Wingfield, J. C., & Bass, A. H. (1999). Steroid hormones and paternal care in the plainfin midshipman fish (*Porichthys notatus*). *Hormones and Behavior*, 35(1), 81–89. <https://doi.org/10.1006/hbeh.1998.1499>
- Kunc, H. P., McLaughlin, K. E., & Schmidt, R. (2016). Aquatic noise pollution: Implications for individuals, populations, and ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 283(1836), Article 20160839. <https://doi.org/10.1098/rspb.2016.0839>
- MacDonald, J. F., Bekkers, J., Maclsaac, S. M., & Blouw, D. M. (1995). Intertidal breeding and aerial development of embryos of a stickleback fish (*Gasterosteus*). *Behaviour*, 132(15–16), 1183–1206. <https://doi.org/10.1163/156853995X00522>
- McCaughey, R. D., Fewtrell, J., & Popper, A. N. (2003). High intensity anthropogenic sound damages fish ears. *Journal of the Acoustical Society of America*, 113(1), 638–642.
- McCloskey, K. P., Chapman, K. E., Chapuis, L., McCormick, M. I., Radford, A. N., & Simpson, S. D. (2020). Assessing and mitigating impacts of motorboat noise on nesting damselfish. *Environmental Pollution*, 266, Article 115376. <https://doi.org/10.1016/j.envpol.2020.115376>
- McCormick, M. I., Allan, B. J. M., Harding, H., & Simpson, S. D. (2018). Boat noise impacts risk assessment in a coral reef fish but effects depend on engine type. *Scientific Reports*, 8(1), 3847. <https://doi.org/10.1038/s41598-018-22104-3>
- McDonald, M. A., Hildebrand, J. A., & Wiggins, S. M. (2006). Increases in deep ocean ambient noise in the Northeast Pacific west of San Nicolas Island, California. *Journal of the Acoustical Society of America*, 120(2), 711–718. <https://doi.org/10.1121/1.2216565>
- McIver, E. L., Marchaterre, M. A., Rice, A. N., & Bass, A. H. (2014). Novel underwater soundscape: Acoustic repertoire of plainfin midshipman fish. *Journal of Experimental Biology*, 217(13), 2377–2389. <https://doi.org/10.1242/jeb.102772>
- McKibben, J. R., & Bass, A. H. (1999). Peripheral encoding of behaviorally relevant acoustic signals in a vocal fish: Single tones. *Journal of Comparative Physiology*, 184, 563–576.
- Mills, S. C., Beldade, R., Henry, L., Laverty, D., Nedelec, S. L., Simpson, S. D., & Radford, A. N. (2020). Hormonal and behavioural effects of motorboat noise on wild coral reef fish. *Environmental Pollution*, 262, Article 114250. <https://doi.org/10.1016/j.envpol.2020.114250>
- Morley, E. L., Jones, G., & Radford, A. N. (2014). The importance of invertebrates when considering the impacts of anthropogenic noise. *Proceedings of the Royal Society B: Biological Sciences*, 281(1776), Article 20132683. <https://doi.org/10.1098/rspb.2013.2683>
- National Environmental Policy Act of 1969, 42 U.S.C. § 4331 et seq.(1970). <https://ceq.doe.gov/laws-regulations/laws.html>
- Nedelec, S. L., Campbell, J., Radford, A. N., Simpson, S. D., & Merchant, N. D. (2016). Particle motion: The missing link in underwater acoustic ecology. *Methods in Ecology and Evolution*, 7(7), 836–842. <https://doi.org/10.1111/2041-210X.12544>
- Nedelec, S. L., Radford, A. N., Pearl, L., Nedelec, B., McCormick, M. I., Meekan, M. G., & Simpson, S. D. (2017). Motorboat noise impacts parental behaviour and offspring survival in a reef fish. *Proceedings of the Royal Society B: Biological Sciences*, 284(1856), Article 20170143. <https://doi.org/10.1098/rspb.2017.0143>
- Nichols, T. A., Anderson, T. W., & Širović, A. (2015). Intermittent noise induces physiological stress in a coastal marine fish. *PLoS One*, 10(9), Article e0139157. <https://doi.org/10.1371/journal.pone.0139157>
- Picciulin, M., Sebastianutto, L., Codarin, A., Farina, A., & Ferrero, E. A. (2010). *In situ* behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a Marine Protected Area. *Journal of Experimental Marine Biology and Ecology*, 386(1–2), 125–132. <https://doi.org/10.1016/j.jembe.2010.02.012>
- Popper, A. N. (2003). Effects of anthropogenic sounds on fishes. *Fisheries*, 28, 24–31. [https://doi.org/10.1577/1548-8446\(2003\)28\[24:EOASOF\]2.0.CO;2](https://doi.org/10.1577/1548-8446(2003)28[24:EOASOF]2.0.CO;2)
- Popper, A. N., & Fay, R. R. (2011). Rethinking sound detection by fishes. *Hearing Research*, 273(1), 25–36. <https://doi.org/10.1016/j.heares.2009.12.023>
- Popper, A. N., & Hawkins, A. D. (2018). The importance of particle motion to fishes and invertebrates. *Journal of the Acoustical Society of America*, 143(1), 470–488. <https://doi.org/10.1121/1.5021594>
- Sarà, G., Dean, J., D'Amato, D., Buscaino, G., Oliveri, A., Genovese, S., Ferro, S., Buffa, G., Martire, M., & Mazzola, S. (2007). Effect of boat noise on the behaviour of bluefin tuna *Thunnus thynnus* in the Mediterranean Sea. *Marine Ecology Progress Series*, 331, 243–253. <https://doi.org/10.3354/meps331243>
- Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, 1(2), 103–113. <https://doi.org/10.1111/j.2041-210X.2010.00012.x>
- Sebastianutto, L., Picciulin, M., Costantini, M., & Ferrero, E. A. (2011). How boat noise affects an ecologically crucial behaviour: The case of territoriality in *Gobius cruentatus* (Gobiidae). *Environmental Biology of Fishes*, 92(2), 207–215. <https://doi.org/10.1007/s10641-011-9834-y>
- Shannon, G., McKenna, M. F., Angeloni, L. M., Crooks, K. R., Frstrup, K. M., Brown, E., Warner, K. A., Nelson, M. D., White, C., Briggs, J., McFarland, S., & Wittermyer, G. (2016). A synthesis of two decades of research documenting the effects of noise on wildlife: Effects of anthropogenic noise on wildlife. *Biological Reviews*, 91(4), 982–1005. <https://doi.org/10.1111/brv.12207>
- Sierra-Flores, R., Atack, T., Migaud, H., & Davie, A. (2015). Stress response to anthropogenic noise in Atlantic cod *Gadus morhua* L. *Aquacultural Engineering*, 67, 67–76. <https://doi.org/10.1016/j.aquaeng.2015.06.003>

Simpson, S. D., Purser, J., & Radford, A. N. (2015). Anthropogenic noise compromises antipredator behaviour in European eels. *Global Change Biology*, 21(2), 586–593. <https://doi.org/10.1111/gcb.12685>

Simpson, S. D., Radford, A. N., Nedelec, S. L., Ferrari, M. C. O., Chivers, D. P., McCormick, M. I., & Meekan, M. G. (2016). Anthropogenic noise increases fish mortality by predation. *Nature Communications*, 7, Article 10544. <https://doi.org/10.1038/ncomms10544>

Sisneros, J. A. (2004). Steroid-dependent auditory plasticity leads to adaptive coupling of sender and receiver. *Science*, 305(5682), 404–407. <https://doi.org/10.1126/science.1097218>

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., & Popper, A. N. (2010). A noisy spring: The impact of globally rising underwater sound levels on fish. *Trends in Ecology & Evolution*, 25(7), 419–427. <https://doi.org/10.1016/j.tree.2010.04.005>

Smith, M. E., Kane, A. S., & Popper, A. N. (2004). Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). *Journal of Experimental Biology*, 207(Pt 3), 427–435.

Sowersby, W., Lehtonen, T. K., & Wong, B. B. M. (2017). Temporal and sex-specific patterns of breeding territory defense in a color-polymorphic cichlid fish. *Hydrobiologia*, 791(1), 237–245. <https://doi.org/10.1007/s10750-016-2889-1>

Spiga, I., Aldred, N., & Caldwell, G. S. (2017). Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.). *Marine Pollution Bulletin*, 122(1–2), 297–305. <https://doi.org/10.1016/j.marpolbul.2017.06.067>

Vasconcelos, R. O., Amorim, M. C. P., & Ladich, F. (2007). Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. *Journal of Experimental Biology*, 210(12), 2104–2112. <https://doi.org/10.1242/jeb.004317>

Wysocki, L. E., Dittami, J. P., & Ladich, F. (2006). Ship noise and cortisol secretion in European freshwater fishes. *Biological Conservation*, 128(4), 501–508. <https://doi.org/10.1016/j.biocon.2005.10.020>

Zeddies, D. G., Fay, R. R., Gray, M. D., Alderks, P. W., Acob, A., & Sisneros, J. A. (2012). Local acoustic particle motion guides sound-source localization behavior in the plainfin midshipman fish, *Porichthys notatus*. *Journal of Experimental Biology*, 215(1), 152–160. <https://doi.org/10.1242/jeb.064998>

Zolderdo, A. J., Algera, D. A., Lawrence, M. J., Gilmour, K. M., Fast, M. D., Thuswaldner, J., Willmore, W., & Cooke, S. J. (2016). Stress, nutrition and parental care in a teleost fish: Exploring mechanisms with supplemental feeding and cortisol manipulation. *Journal of Experimental Biology*, 219(8), 1237–1248. <https://doi.org/10.1242/jeb.135798>

Appendix

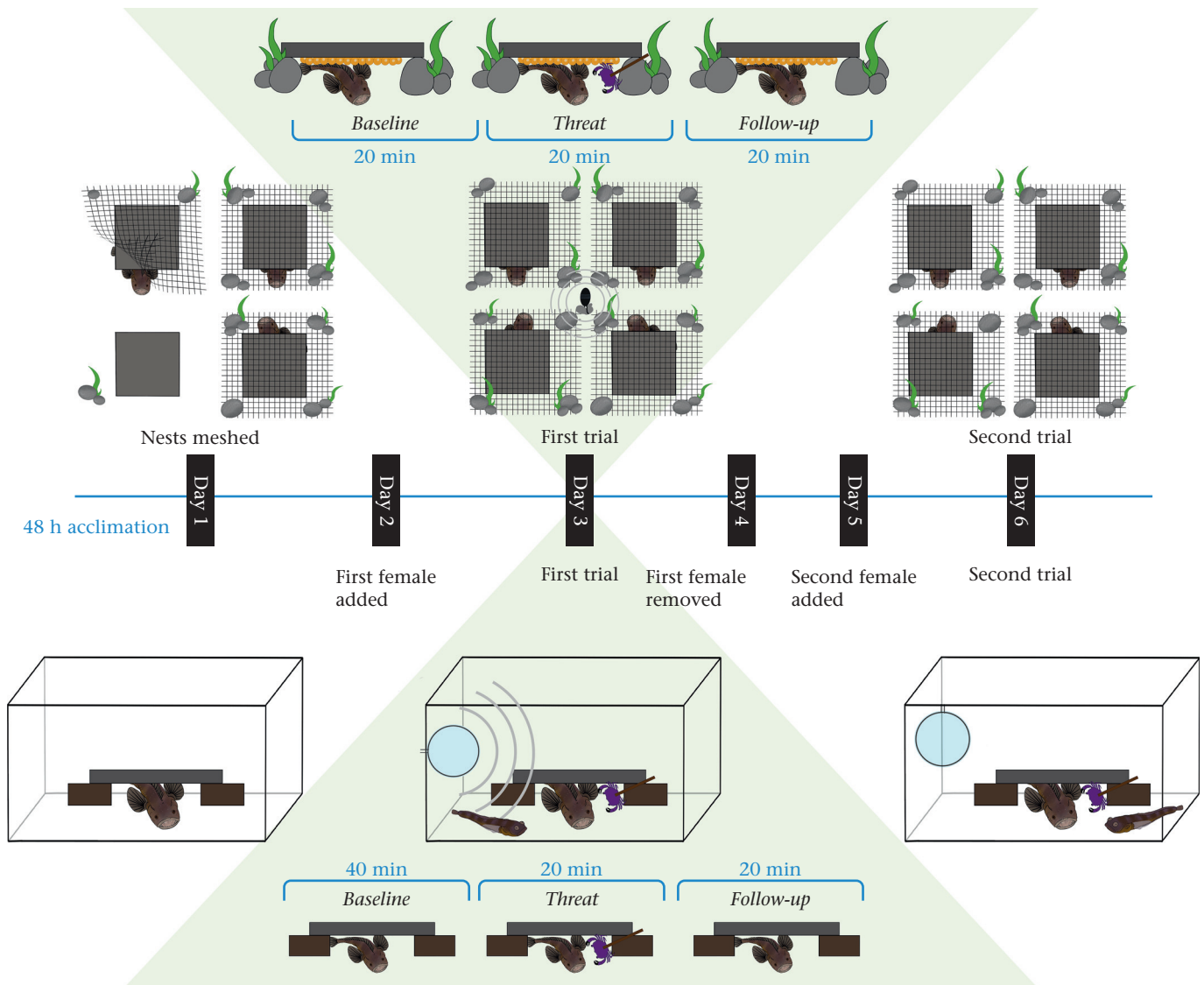


Figure A1. Schematic methods diagram showing the 6-day trial sequence and three-period experimental trials in the field (top) and laboratory (bottom) experiments. The order of noise and ambient treatments was determined randomly for each fish; in this figure, the first trial (day 3) is shown as a noise trial and the second trial (day 6) is shown as an ambient trial.