The effects of anthropogenic noise on the behaviour and vocalizations of plainfin midshipman fish, *Porichthys notatus*

by

Mackenzie B. Woods
BSc Honours, University of Victoria, 2019

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Supervisory Committee

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Abstract

Noise pollution in the ocean has been accelerating at an alarming rate, drastically altering underwater soundscapes and negatively affecting marine life in myriad ways. Many marine animals, including fishes, rely heavily on sound for communication, navigation, and environmental perception, all of which can be negatively affected by anthropogenic noise pollution from activities such as recreational boating, commercial shipping, marine construction, and seismic exploration. While the effects of noise on marine mammals are well documented, far fewer studies have investigated the effects of noise on fishes, and even fewer have been conducted in the field using realistic noise stimuli. In this thesis, I use a combination of field and laboratory studies to investigate how anthropogenic noise affects nesting plainfin midshipman fish (*Porichthys notatus*), which breed in the intertidal zone and rely on acoustic communication for mate attraction and defence. I demonstrate that plainfin midshipman sometimes alter nest defence behaviour in response to boat noise, but that testing context, such as whether they are tested in the laboratory or the field, the presence of eggs, and the noise stimulus used, can greatly affect experimental outcomes. I also found that males in the wild significantly reduced the number of agonistic vocalizations but increased the amplitude (loudness) of these calls when a motorboat was driven continuously near their nests. Males also increased the frequency (pitch) of their mating hums. Such vocal adjustments in response to noisy environments are referred to as the Lombard effect—a widespread phenomenon typically studied in mammals and birds but rarely tested in fishes. This study is amongst the first to report changes in vocalization frequency and amplitude in fishes, and it is the first to demonstrate this effect in wild fish using experimentally introduced noise from a real motorboat. The results in my thesis contribute to our growing understanding of how noise affects fishes and demonstrate the need for additional field studies using realistic noise stimuli and ecologically relevant contexts.

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Chapter 1: Introduction

1.1 Anthropogenic noise in the ocean

Over the last century, rising levels of anthropogenic noise have been introduced into the ocean, greatly altering the underwater soundscape and leading to a multitude of consequences for marine life (Duarte et al., 2021). While anthropogenic noise is a growing concern in most habitats on Earth, the marine environment is of particular concern because under water, sound travels faster than it does in air, and it travels farther before sound levels fall below background noise levels. In most aquatic environments, sound also travels much farther than light (Urick, 1983). Therefore, numerous aquatic animals rely more heavily on their hearing rather than on vision or other sensory modalities (Slabbekoorn et al., 2010). Sound facilitates long-distance communication and accurate perception and assessment of environmental conditions (Putland et al., 2019). Consequently, the properties of underwater sound mean that even distant noise sources can negatively impact aquatic animals (Duarte et al., 2021; Slabbekoorn et al., 2010).

While the focus of research on the effects of noise on aquatic life has historically focused on marine mammals, the field of fish acoustics is rapidly expanding, as additional soniferous (sound-producing) fish continue to be discovered (Looby et al., 2022) and the negative effects of anthropogenic noise become more apparent (Ladich, 2019). Many fishes are reliant on sound for communication, prey and predator detection, and navigation, all of which can be disrupted by noise pollution (Ladich, 2019; Popper, 2003; Slabbekoorn et al., 2010). However, few experiments are conducted in the wild under ecologically valid conditions and fewer still use realistic noise stimuli. My MSc research aimed to fill this gap by examining the effects of anthropogenic noise on a species that relies heavily on acoustic communication for mate attraction and defence.

1.2 Study species: The plainfin midshipman fish

The plainfin midshipman fish (*Porichthys notatus* Girard, 1854), a marine toadfish (Batrachoididae), is an ideal species to study the effects of noise because it is easily accessible in the shallow intertidal zone of many rocky shores in the northeastern Pacific, and it uses a variety of vocalizations for courtship and agonistic encounters (McIver et al., 2014). Plainfin midshipman fish live in deep water (>200 m) most of the year and seasonally migrate to the intertidal zone to breed—an area regularly exposed to recreational boat traffic (Halliday et al., 2018). This boat traffic is especially prevalent during the spring and summer breeding season—typically from May to late July (Arora, 1948). The males dig out nesting cavities under large rocks (Figure 1.1a) and hum to attract females to their nests to spawn (Brantley and Bass, 1994). The males produce this hum, along with agonistic grunts and growls, by rapidly contracting specialized sonic muscles attached to their gas-filled swim bladder, producing a low-frequency tonal hum with several strong harmonics (Bass and Baker, 1990; McIver et al., 2014).

Female plainfin midshipman are extremely sensitive to the fundamental frequency (~100 Hz) and dominant harmonics (~200-400 Hz) of the male mating hum, especially during the breeding season (Sisneros, 2012, 2009a, 2009b), and use it to locate males in their nests (Zeddies et al., 2010). Male plainfin midshipman provide solo parental care; throughout the breeding season, the males continue to attract and spawn with additional females while caring for multiple clutches of eggs, each from a different female (Figure

1.1b; Brantley & Bass, 1994). The plainfin midshipman parental care period is much longer than that of most fishes; eggs take approximately 60 days to reach the free-swimming juvenile stage, and because the males care for multiple clutches laid throughout the breeding season, they may remain in their nests and provide care for up to four months (Cogliati et al., 2013). Males provide care by keeping the young in the nests clean and aerated. They fan the young with their dorsal and caudal fins, and squirt streams of water with their mouths at the young to remove fungus and debris (Arora, 1948; Brown et al., 2021b; Woods et al., 2022). Additionally, the parental males tend their nests by clearing sediment and moving around rocks and shells. Finally, they defend their nests and offspring from rival males and egg predators (Arora, 1948; Woods et al., 2022). Part of this defence against both conspecifics and heterospecifics includes using agonistic vocalizations: short grunts, either alone or in a rhythmic series called a grunt train, and longer, frequency-modulated growls (McIver et al., 2014).

In addition to these Type I, or ‘guarder’, males that court, nest-guard and care for young, there is a second type of male called a Type II, or ‘sneaker’, male (Figure 1.1c). Sneaker males are much smaller, about 1/8th the mass of guarder males (Figure 1.1c), and do not hum, court females, guard nests, or care for young (Brantley and Bass, 1994; Fitzpatrick et al., 2016). Instead, they hide at the edge or inside a guarder male’s nest and wait for him to attract a female. While the guarder and female are spawning, the sneaker male releases his sperm and fertilizes some of the eggs, stealing fertilizations and paternity from the guarder male (Cogliati et al., 2013). In my MSc thesis, I focus on the behaviour of guarder male plainfin midshipman.

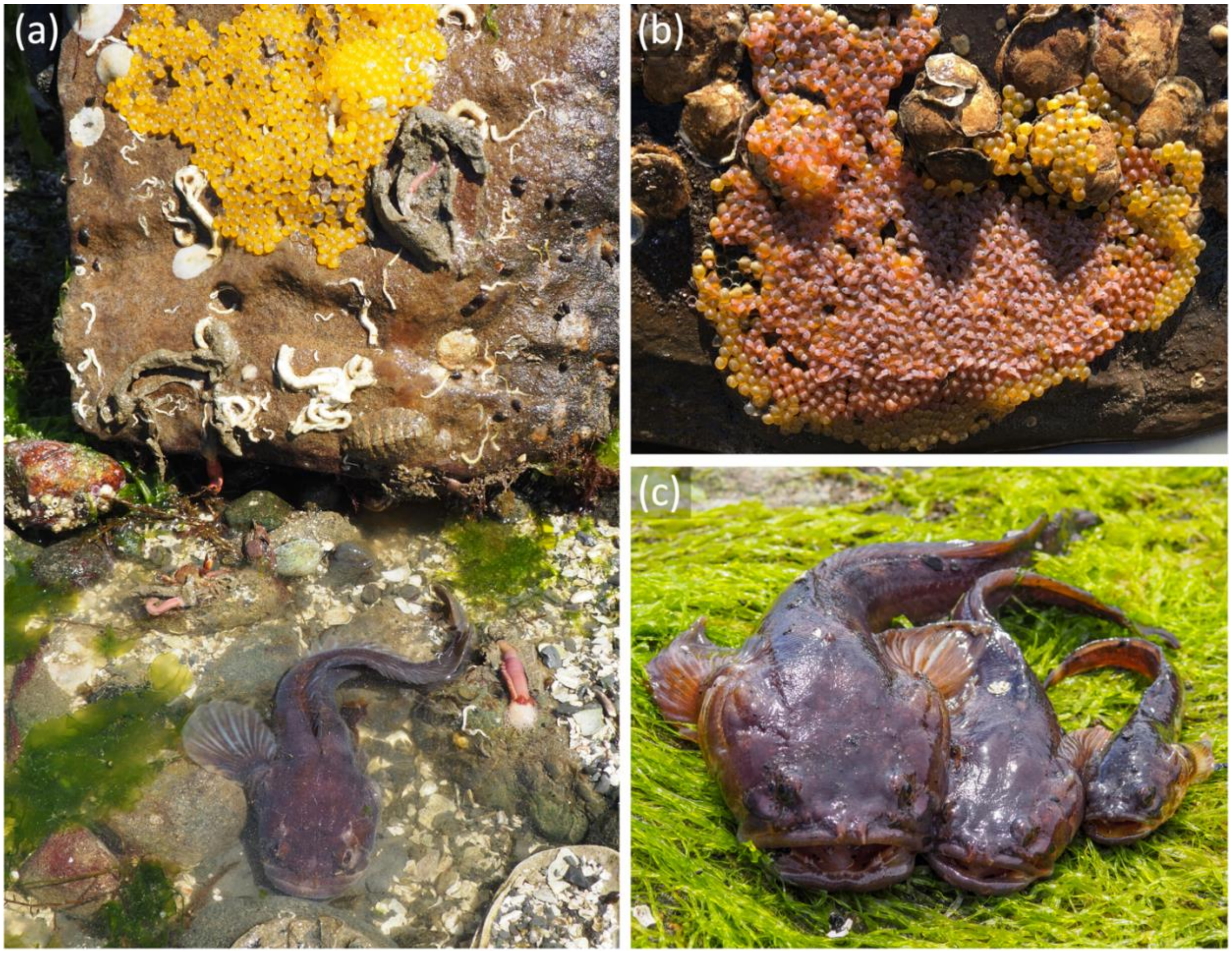

Figure 1.1 Plainfin midshipman in the field. (a) A guarder male in his intertidal nest at low tide (the nesting rock was overturned by a researcher and the yellow eggs on the nest roof are visible). (b) The underside ("roof") of a nest with multiple clutches of young at different and distinct developmental stages, spanning from recently laid (light orange/yellow translucent eggs) to hatched larvae (pink) still attached to their yolk sac. (c) The three reproductive morphs in the plainfin midshipman, from left to right: guarder male (type I male), female, sneaker male (type II male).

1.3 Thesis objectives

The aim of my MSc research was to investigate how anthropogenic noise affects the behaviour and vocalizations of the highly vocal plainfin midshipman fish. My MSc thesis had three primary research objectives:

1) Assess how anthropogenic noise affects plainfin midshipman fish in different contexts (presence/absence of eggs and different noise stimuli) and testing environments (laboratory vs. field) and how this translates to the ways in which noise affects fish in the wild.
2) Determine how noise affects plainfin midshipman parental care and nest defence.
3) Examine if and how boat noise alters plainfin midshipman vocalizations and test whether this species exhibits the Lombard effect in response to boat noise.

1.4 Thesis overview

This thesis is divided into four chapters. Following this first introductory chapter, I use two data chapters to address my research objectives, and then discuss my findings collectively in the fourth and final chapter. Chapter 2 was published in the journal *Animal Behaviour* (Woods et al., 2022), and in this chapter, I investigated how noise affects nest defence and parental care in plainfin midshipman fish under different experimental conditions. In Chapter 2 I also determined whether the effects of noise observed in the laboratory translate to similar impacts in the wild. Building upon the design of the field component of Chapter 2, in Chapter 3 I used a real motorboat to test how wild, nesting plainfin midshipman males alter their vocalizations in response to the type of noise that they are typically exposed to in the shallow intertidal zone. In Chapter 4, I present an overview and general discussion to synthesize the results of my two data chapters and place them in the broader context of the effects of noise on fish behaviour and vocalizations. In this final chapter I re-address each of my thesis research objectives, discuss my conclusions, and outline future directions that would extend this research.

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Chapter 2: Context-dependent effects of anthropogenic noise on nest defence in the plainfin midshipman fish

2.1 Abstract

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 fourfold 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.

2.2 Introduction

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 and 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 US National Environmental Policy Act (United States, 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 (*i.e.* < 1,000 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 non-vocalizing 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., 2016, 2015; 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 et al., 2018a; de Jong 2018b; Sierra-Flores et al., 2015) or nest-guarding and parental care (Bruintjes and 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 timeframes 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.* Brantley and Bass 1994; Sisneros 2004; Coffin et al. 2012; Alderks and Sisneros 2013; McIver et al. 2014; Colleye et al. 2019), and because of its vocal repertoire and reliance on sound for successful reproduction, 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 his nest against rival males. Guarder males remain in their nests to provide parental care for up to four 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 exposure to considerable boat noise in the breeding season all suggest that this species could suffer substantial reproductive consequences from noise pollution (Brown et al., 2021a).

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 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 and 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.

2.3 Methods

2.3.1 Experiment 1: Nest defence during boat noise playback in the laboratory

2.3.1.1 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 and 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-L outdoor holding tanks for a minimum of 24 hours 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^{o}C) seawater 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-L experimental aquarium lined with pebble substrate and supplied with free-flowing, ambient seawater. Each aquarium contained an artificial nest made of a concrete tile supported by five bricks (Figure 2.1). Males were given a minimum 2-day acclimation period to their experimental tank prior to beginning their 6-day trial sequence (Figure S2.1). 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.

2.3.1.2 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 two 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 icListen 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 is a clear example of boat noise that marine fish would be exposed to. A one-minute clip was extracted from the recording and low-pass filtered at 1000 Hz using MATLAB R2015a. This one-minute clip was then continuously looped using Audacity (http://audacityteam.org/) to generate a continuous boat noise playback (Figure 2.2). During noise trials, the boat recording was played through an underwater speaker (University Sound UW30, Lubell Labs, Columbus, OH, USA) continuously for the entire 80-minute 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 to avoid potential boat noise spillover to concurrent ambient trials or to tanks which 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.

2.3.1.2 Behavioural trials

Experimental trials were conducted between July 5–20, 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 (Figure 2.1). For noise trials, a SoundTrap acoustic recorder (Ocean Instruments ST4300 STD, New Zealand) fitted with two HTI hydrophones (High Tech, Inc., Long Beach, MS, USA; flat frequency response 20-24,000 Hz, total sensitivity -164.5 dB re 1V/μPa) was used, with the hydrophones positioned in the tank approximately 40 cm apart and 1.5 cm above the pebble substrate (Figure 2.1). 'Dummy hydrophones' (dark plastic locklines resembling the hydrophones) 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-minute *baseline* period before the threat stimulus was introduced, a 20-minute *threat* period when the crab was placed in the nest, and a 20-minute *follow-up* period after the stimulus was removed. Intertidal crabs commonly prey on plainfin midshipman eggs (NAW Brown, pers. obs.); 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 minutes, the crab was removed from the nest and from the wire and was returned to the housing tank. Recording continued for the 20-minute *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 2.1).

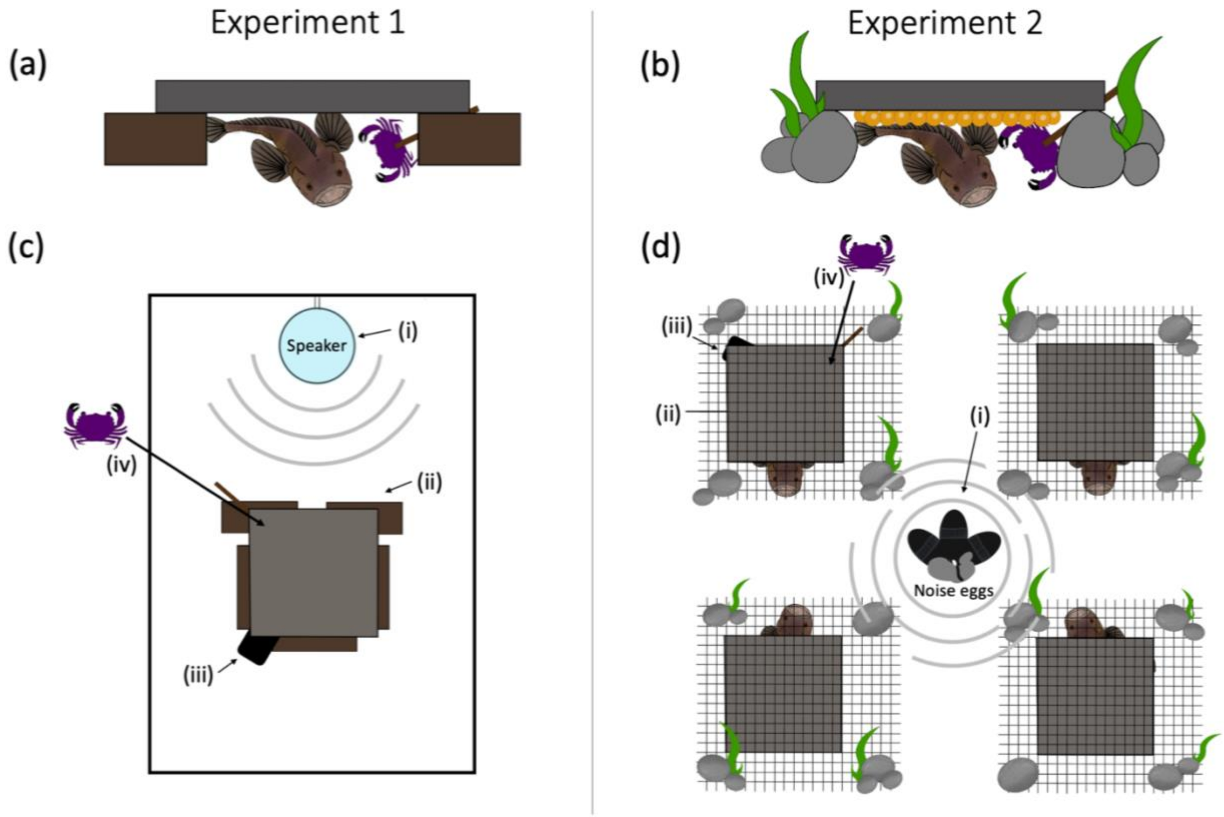

Figure 2.1 Experimental setup for Experiment 1 in laboratory aquaria (a, c) and Experiment 2 in the field (b, d). The illustrations in (a) and (b) show a side view of a guarder male in his nest during the threat period, in the lab for Experiment 1 (a) and in the field for Experiment 2 (b), while (c) and (d) show a top-down view of the experimental setup in the lab (c) and field (d). 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.

2.3.1.3 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 guarder male plainfin midshipman; Joseph Sisneros, pers. comm.). 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 fit 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 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 toward the crab were considered defensive behaviours (Table 2.1); 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.

2.3.2 Experiment 2: *In situ* nest defence and parental care in the presence of added noise

2.3.2.1 Field site and nest establishment

We conducted this field experiment in the intertidal zone of Dabob Bay, Washington, USA (47°76′N, 122°86′W) in May and June 2019. We deployed 38 square concrete tiles (929 cm^2) as artificial nests; tiles were grouped into ten 3–4 tile clusters 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-cm^2 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., 2016a; Brown et al., 2020).

2.3.2.2 Acoustic treatments

Each fish underwent one noise treatment trial and one ambient treatment trial, separated by two 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 frequently around 100 Hz and several harmonics (Brown et al., 2021; de Jong et al., 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 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 (Figure 2.2) for the duration of the 60-minute 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.

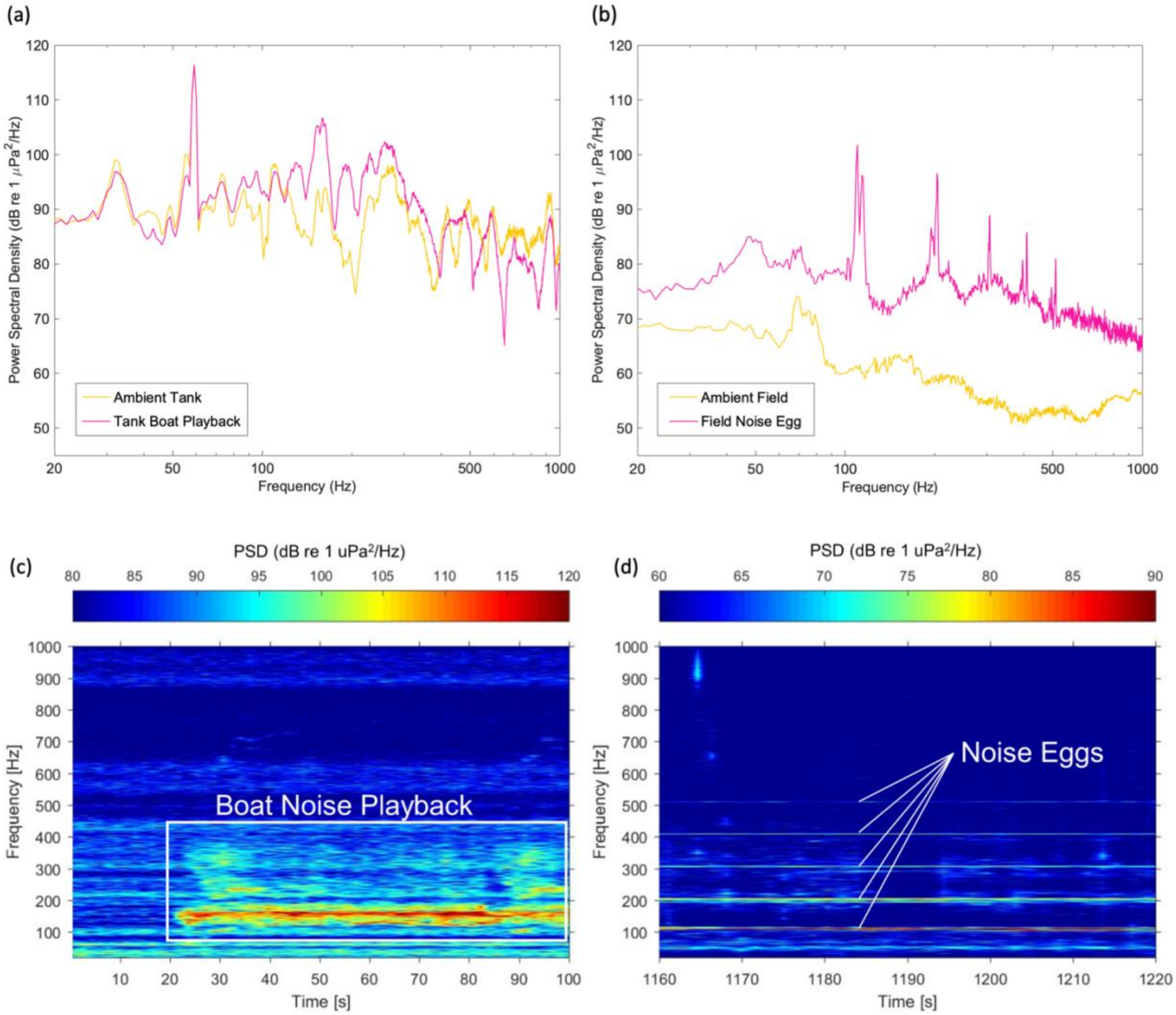

Figure 2.2 Power spectral density (PSD; sound levels; a, b) and spectrograms (c, d) of noise stimuli and ambient sound. (a, c) Sound levels within tanks in Experiment 1, comparing the playback from within the tank during noise trials, and the ambient sound in the tank when no noise was being played through 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 seconds show ambient conditions and after that show 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 is different for (c) and (d) to aid in visualizing the signals.

2.3.2.3 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 & 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-minute 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-minute threat period, which was used to examine defensive behaviour toward the egg predator, in addition to parental care and other natural behaviours. Finally, the snorkeller removed the crab from each nest, initiating the 20-minute follow-up period. In noise trials, the artificial noise continued for the full 60-minute trial (baseline, threat, and 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 et al., 2020 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 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).

2.3.2.4 Statistical analyses

We followed a similar analytical approach to that described for Experiment 1. Continuous covariates in all models were mean-centered and scaled (Schielzeth, 2010). We first fit the number of defensive and parental care behaviours performed in each trial with Poisson GLMMs as in Experiment 1. However, due to overdispersion, we re-fit 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 et al., 2020). We also investigated whether trial order was an important predictor, but model comparisons using 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.

2.3.3 Experimental comparison: Nest defence in the lab (Experiment 1) vs. the field (Experiment 2)

2.3.3.1 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 lab 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.

Table 2.1 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.

2.3.4 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 non-invasive. 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 anesthetic 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.

2.4 Results

2.4.1 Experiment 1: Nest defence in the lab

There was a significant effect of acoustic treatment on the defensive behaviours exhibited by nest-guarding males during the threat period (GLMM: $\chi^2_1 = 22.43$, $P < 0.0001$; Figure 2.3a). Males performed 3.8 times (95% CI: 2.1–6.9) as many defensive behaviours during motorboat noise playback compared to 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. Though the fish guarding eggs was not included in our statistical analyses, it exhibited the same number of defensive behaviours (nine) in both treatments (noise and ambient).

2.4.2 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: $\chi^2_1 = 0.0078$, $P = 0.93$; Figure 2.3b). 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 (Figure 2.4). 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: $\chi^2_1 = 4.79$, $P = 0.03$).

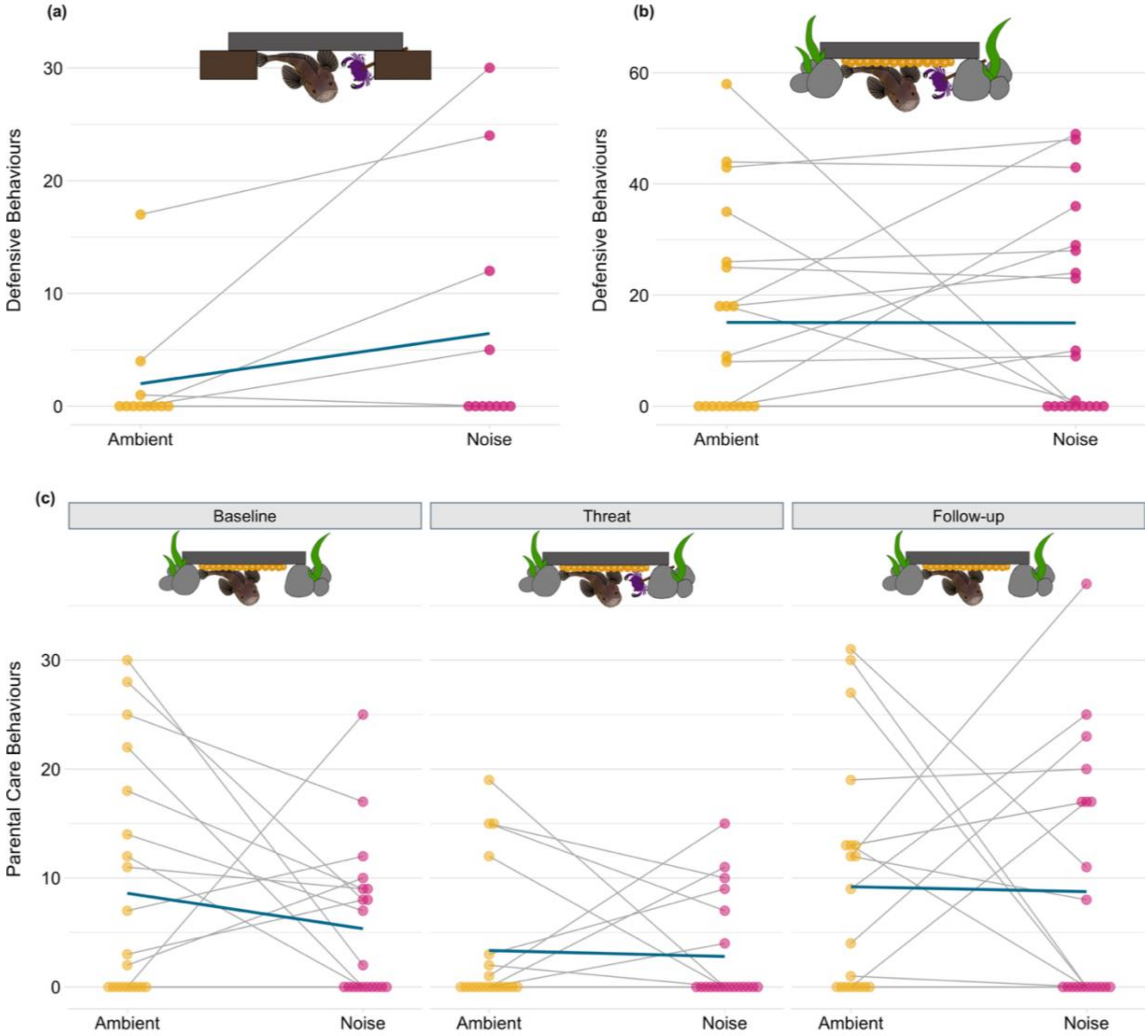

Figure 2.3 Defence and parental care behaviours exhibited in the presence and absence of noise. Number of discrete defence behaviours towards a live crab (egg predator) exhibited by (a) nest-guarding males (N = 11) in laboratory aquaria in Experiment 1, and (b) wild brood-guarding males (N = 20) in the 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 and 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.

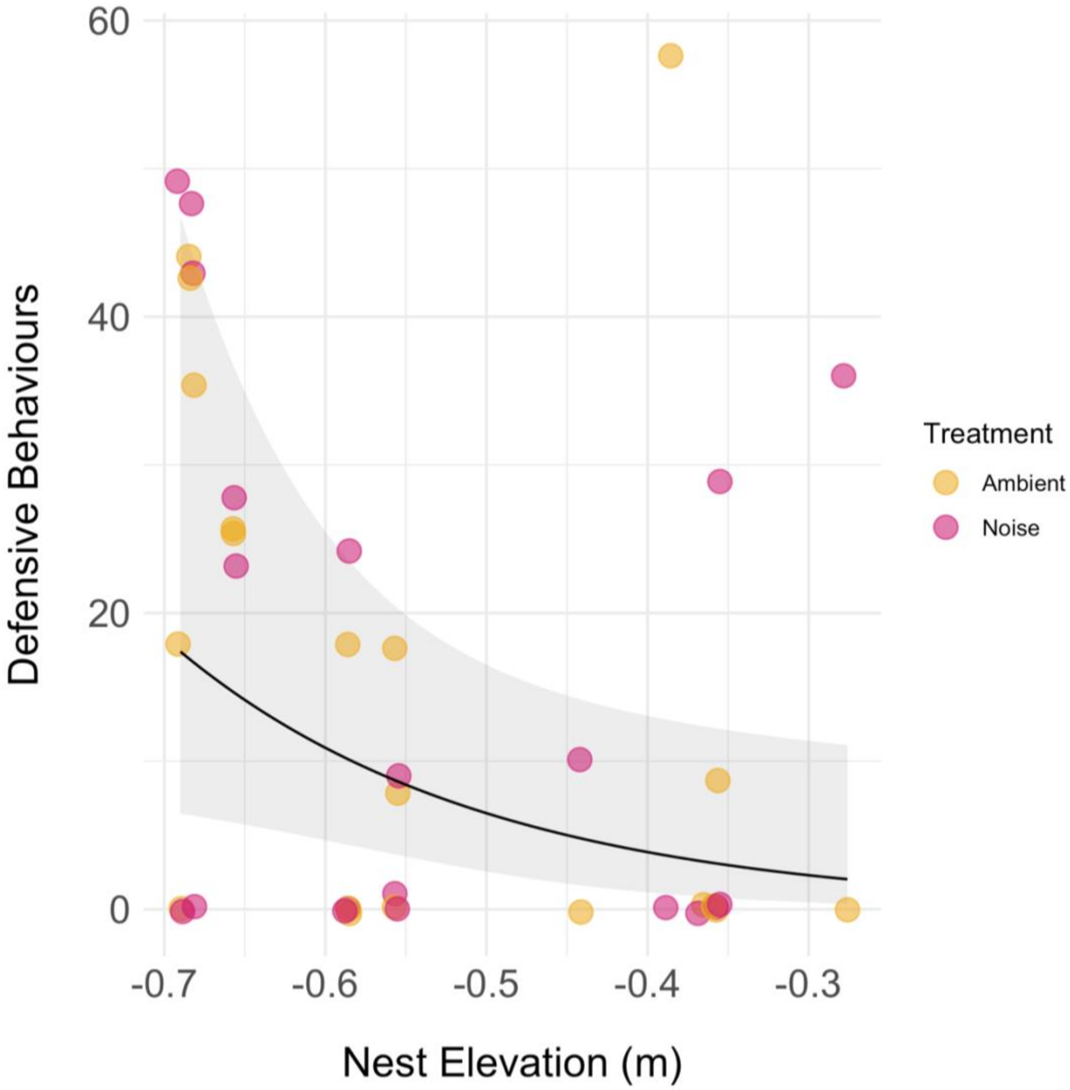

Figure 2.4 Defensive behaviours of parental males nesting at different intertidal elevations. The variation in number of defensive behaviours exhibited by wild brood-guarding males (N = 20) towards a live crab depending on the elevation of their intertidal nests. Pink dots indicate noise trials, while 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.

2.4.3 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: $\chi^2_1 = 0.81$, $P = 0.37$; Figure 2.3c). The extent of parental care differed greatly between trial periods (baseline, threat, and 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 3 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.

2.4.4 Experimental comparison: Defence in the lab (Experiment 1) vs. in the field (Experiment 2a)

The number of defensive behaviours performed by guarder males differed between the lab (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 5 times (95% CI: 1.0–23.5) as many defence behaviours during ambient trials in the field compared to those in the lab.

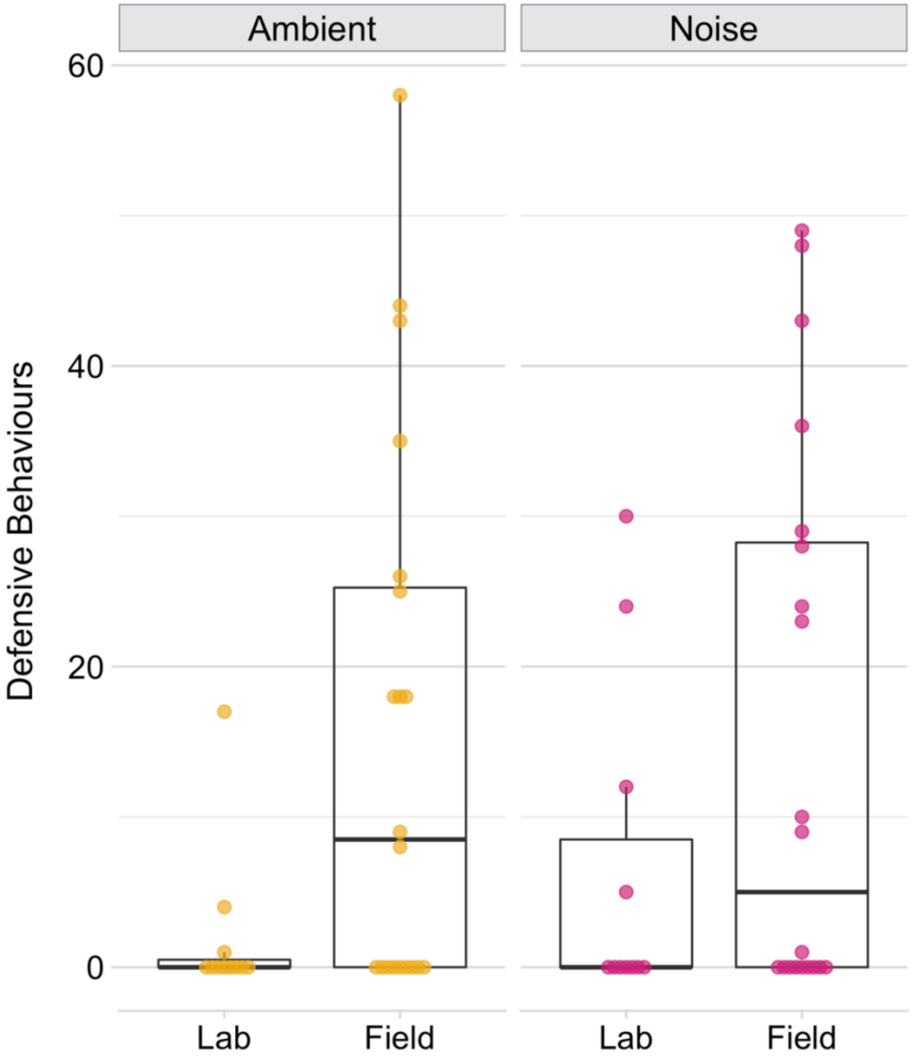

Figure 2.5 Defensive behaviours exhibited in the lab (Experiment 1) and the field (Experiment 2), in the presence and absence of noise. Comparison of the number of defensive behaviours exhibited toward a live crab by nest-guarding males in laboratory aquaria (Experiment 1; N = 11) and wild brood-guarding 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.

2.5 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 lab experiment under ambient conditions. Further, 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 lab and field results may be due to several factors, including: 1) the use of different noise stimuli, 2) presence versus absence of a brood 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 versus 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 $\mu Pa^2/Hz$ at 159 Hz) and consisted of broadband noise, whereas the Noise Egg was quieter (peak field noise egg = 101 dB re 1 $\mu Pa^2/Hz$ at 110 Hz) and contained only a low-frequency tone accompanied by harmonics (Figure 2.2). 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 embryonic damselfish (*Amblyglyphidodon curacao*) heart rates increased in response to both types of engine noise, but the noise from a 2-stroke engine evoked a 2-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. Further, these two engine types differ in the amplitude (~5 dB re 1 μPa higher for the 2-stroke engine) of the noise produced (McCormick et al., 2018). In our study the stronger response was observed in the lab, where the stimulus was 10 dB higher than the noise stimulus used in the field (Figure 2.2). However, the ambient noise was also much greater in the lab than in the field (ambient sound in the tank = 122 dB re 1 μPa in 50-1000 Hz band, peak power = 116 dB re 1 μPa^2/Hz at 59 Hz; ambient sound in the field = 90 dB re 1 μPa in 50–1000 Hz band, peak power = 74 dB re 1 μPa^2/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.

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 and 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 and Bass, 1999; Popper and 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 and Hawkins, 2018). Precisely how fish employ and the degree that they depend on information from the particle motion versus pressure components of sound is not well understood (Nedelec et al., 2016), but the different relative levels of these sound components in our field and lab experiments may have contributed to the differing results. Further, it is possible that sound may have been transmitted as vibrations through the substrate (Popper and Hawkins, 2018), which differed between experiments (pebbles on a glass aquarium bottom vs. 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 and 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 lab 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, Bruintjes and Radford (2013) found that noise had a significant effect on defensive behaviours towards an egg predator in nests without eggs, but 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 lab—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 were more active nest defenders than males nesting in the middle of the intertidal (Brown et al., 2020). Males in the field are likely exposed to egg predators constantly, whereas males in the lab 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 (Zolderdo 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 *Acanthochromis 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. (2017) exhibited increased aggressive acts toward 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 lab study, we suggest that additional field and lab 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 lab and field experiments when studying fish behaviour, especially in response to noise. However, because of the various contextual differences between our lab 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.

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Chapter 3: Boat noise reduces vocalization rate and alters vocal characteristics in wild plainfin midshipman fish

3.1 Abstract

Anthropogenic noise pollution has been accelerating at an alarming rate, greatly altering aquatic soundscapes. Animals use various mechanisms to cope with acoustic masking in noisy environments, including altering calling rates or the frequency (pitch) of their vocalizations or increasing the amplitude (loudness) of their vocalizations (*i.e.* exhibiting the Lombard effect), but few studies have examined this vocal plasticity in fishes. We tested the effects of *in situ* motorboat noise on the agonistic and mating vocalizations of nesting plainfin midshipman fish (*Porichthys notatus*), which breed in the intertidal zone where recreational boat noise is abundant, and found strong evidence that midshipman fish reduce and alter their vocalizations in the presence of boat noise. During boat noise, we observed over four times fewer agonistic vocalizations (grunts and growls) compared to ambient control periods, when the boat engine was off. The fish also increased the frequency of mating hums and increased the amplitude of grunts and growls during boat noise. This study is the first to demonstrate the Lombard effect in fishes by using real motorboat noise. Decreased agonistic vocalizations, as observed in our study, could compromise the effectiveness of nest defence, leading to lower reproductive success. Vocalizing more loudly may be an effective method of avoiding acoustic masking, but it may not be energetically sustainable in chronically noisy environments, especially during an already costly breeding and parental care period. Additional field studies using realistic noise stimuli are needed to establish long-term effects of boat noise on fishes, as well as the effects on mate attraction and reproductive success.

3.2 Introduction

Many aquatic animals rely heavily on sound for communication and to assess their environments. Sound is used to navigate, forage, attract mates and detect predators (Duarte et al., 2021; Myrberg, 1997) and is a highly effective method of underwater communication and sensory perception because it travels more efficiently in water than in air, and underwater sound can propagate much farther without attenuation compared to light (Urick, 1983; Duarte et al., 2021; Slabbekoorn et al., 2010). However, over the last few decades rising levels of anthropogenic noise have greatly altered underwater soundscapes. Human-made noise has been shown to negatively affect aquatic animals in a variety of ways, including reducing the space available for communication, physically damaging hearing organs, altering auditory thresholds, and causing a slew of behavioural and physiological changes (Duarte et al., 2021; Ladich, 2019; Popper and Hawkins, 2019; Shannon et al., 2016). While there is growing evidence of these negative effects, relatively little research exists on how noise affects underwater vocalizations, especially in temperate environments. In this study, we address this research gap and explore how anthropogenic noise influences the quality and quantity of underwater vocalizations.

Several fish species vocalize to attract mates and defend territories and young. One such species is the plainfin midshipman (*Porichthys notatus*), a soniferous (sound-producing) toadfish that produces a loud, multi-harmonic hum to attract mates. The hum has a fundamental frequency of ~100 Hz, with dominant harmonics between 200–400 Hz, and can last for several minutes to upwards of an hour (Halliday et al., 2018; Sisneros, 2012). Plainfin midshipman also produce two agonistic vocalizations—short grunts and longer growls—which are employed during nest defence against rival males and egg predators (Brantley and Bass, 1994; Woods et al., 2022). Grunts (~0.5 s each) can be produced individually or in a rhythmic series called a grunt train, which can last for several minutes and include hundreds of grunts (McIver et al., 2014). Growls are frequency modulated vocalizations that vary in length, lasting up to several seconds (~0.5–10 s; Brantley & Bass, 1994; McIver et al., 2014). Grunts and growls have a fundamental frequency of ~60-100 Hz (McIver et al., 2014). Midshipman produce these three vocalizations by rapidly contracting specialized sonic muscles wrapped around their swim bladder; as the muscles vibrate, they drum on the gas-filled bladder, producing low-frequency, multi-harmonic vocalizations (Balebail and Sisneros, 2022; Cohen and Winn, 1967; Greene, 1924).

Plainfin midshipman fish spend most of the year in deep water (>200 m) before migrating to the shallow intertidal zone in the late spring for their summer breeding season (Arora, 1948). In the intertidal zone, males dig nesting cavities under large rocks and hum to attract incoming females to their nests. Females choose males based on their hum (McKibben and Bass, 2001, 1998; Sisneros, 2012). After spawning, males will begin to care for young but also continue humming to attract additional females (Bose et al., 2016b; Brantley and Bass, 1994). These shallow nearshore midshipman breeding grounds can be bombarded by motorboat noise, during the summer breeding months (May-August), when recreational boat use is at its peak (Halliday et al., 2018). Because midshipman fish are sensitive to and rely heavily on sound, boat noise in their breeding areas could have strong detrimental effects for communication and mate attraction. Furthermore, given that low-frequency boat noise completely overlaps with the dominant frequencies of plainfin midshipman vocalizations, motorboat noise is likely to mask acoustic communication of the plainfin midshipman (Halliday et al., 2018).

One strategy to overcome masking by loud background noise, such as boat noise, is to alter the rate, duration, frequency (pitch), or loudness of vocalizations (Brumm and Zollinger, 2011). By altering the temporal characteristics (rate or duration) of vocalizations, calling more often or for longer, animals can increase the redundancy of their vocal signals, leading to a greater chance of the receiver hearing them in a noisy environment (Shannon et al., 2016). Increasing the amplitude (sound-level, or loudness) of calls in the presence of loud background noise is another strategy, allowing the sender to be heard above the noise source; this phenomenon specifically is often referred to as the Lombard effect (Brumm and Zollinger, 2011). Individuals may also shift the frequency of vocalizations out of the range of the noise source that is masking them, which again can allow the signal to be received against loud background noise (Brumm and Zollinger, 2011). Changes in vocalization frequency and amplitude in response to noise sources have been observed in many terrestrial and aquatic mammals and birds (Kunc et al., 2022), but have only been investigated in three fish species to date, two of which were toadfishes (Brown et al., 2021a; Holt and Johnston, 2014; Luczkovich et al., 2016).

Shifting the frequency of ones’ vocalizations may not always be a viable strategy, and increasing the amplitude of energetically costly vocalizations may not be sustainable, especially over the long term. Instead of calling more often or more loudly, signallers may instead reduce their calling rates and wait for the background noise to decrease before resuming normal vocalizations (Ladich, 2019). A reduction in calling rate when confronted by noisy environments has been observed in several fish species (Ladich, 2019), including the plainfin midshipman (Brown et al., 2021a), Lusitanian toadfish

(Vieira et al., 2019), oyster toadfish (Mackiewicz et al., 2021), and two goby species (de Jong et al., 2018a).

In a previous study using wild plainfin midshipman fish, Brown et al. (2021a) found that nesting males increased their call amplitude, decreased their call frequency, and produced calls less often in response to an artificial low-amplitude tonal stimulus. However, this artificial noise stimulus did not have the broadband frequency typical of boat noise but instead strongly resembled a midshipman hum in fundamental frequency and accompanying harmonics. It is possible that the males in that previous study may have changed their calls because the artificial noise mimicked having additional competing, vocalizing males in the area, and may not tell us how these fish respond to anthropogenic noise, such as noise from boat engines. Few studies have used boat noise from a real motorboat to experimentally test the Lombard effect; most have used playbacks or noise already occurring in the area (*e.g.* near a ferry terminal or marina), the latter of which is not a controlled stimulus.

In this study, we experimentally manipulated the exposure to anthropogenic noise by systematically introducing motorboat noise. We did this by driving a motorboat near experimental nests to assess if and how nesting plainfin midshipman males alter their vocalizations. We predicted that in the presence of motorboat noise, plainfin midshipman fish would 1) decrease their calling rate, in particular their agonistic grunts and growls, as these shorter vocalizations may be easier to temporarily forego during short-term boat noise, 2) shift the frequency of their calls away from the dominant boat harmonics to avoid masking, and 3) increase the amplitude (loudness) of their vocalizations (*i.e.* exhibit the Lombard effect).

3.3 Methods

3.3.1 Field site and nest construction

We conducted this study in the intertidal zone of Hood Canal in Brinnon, Washington, USA on a private beach during May-June 2022. We constructed 55 artificial nests out of 30 cm x 30 cm concrete tiles (Figure 3.1c), which plainfin midshipman guarder males readily inhabit (Brown et al., 2021b; DeMartini, 1988; Woods et al., 2022), and placed them in five groups, each consisting of either eight (single row) or 16 (double row) tiles, along the middle and lower intertidal (Figure 3.1b), with 1 m between tiles and 5 m between groups. Nests were checked for occupancy every few days at low tide, or by snorkelers when the nests were submerged. Once at least six nests in a group were occupied by males guarding eggs, we considered that group ready for testing.

3.3.2 Noise stimulus

We used a 4-m flat-bottom aluminum hull motorboat equipped with a 9.9 hp Yamaha outboard engine driven near the nests as our noise stimulus. During boat noise periods, the boat was driven in continuous loops at full-throttle for the entirety of each 10-min noise period. The closest point of the loop was approximately 20 m from the nest group being tested (Figure 3.1a). At the furthest point, the boat was approximately 60 m from the nest group. We chose this distance because plainfin midshipman nests are typically in shallow water, so the noise they usually experience in the intertidal would not normally come from boats driven directly over the nests. For consistency, the boat was always driven by the same individual (MBW).

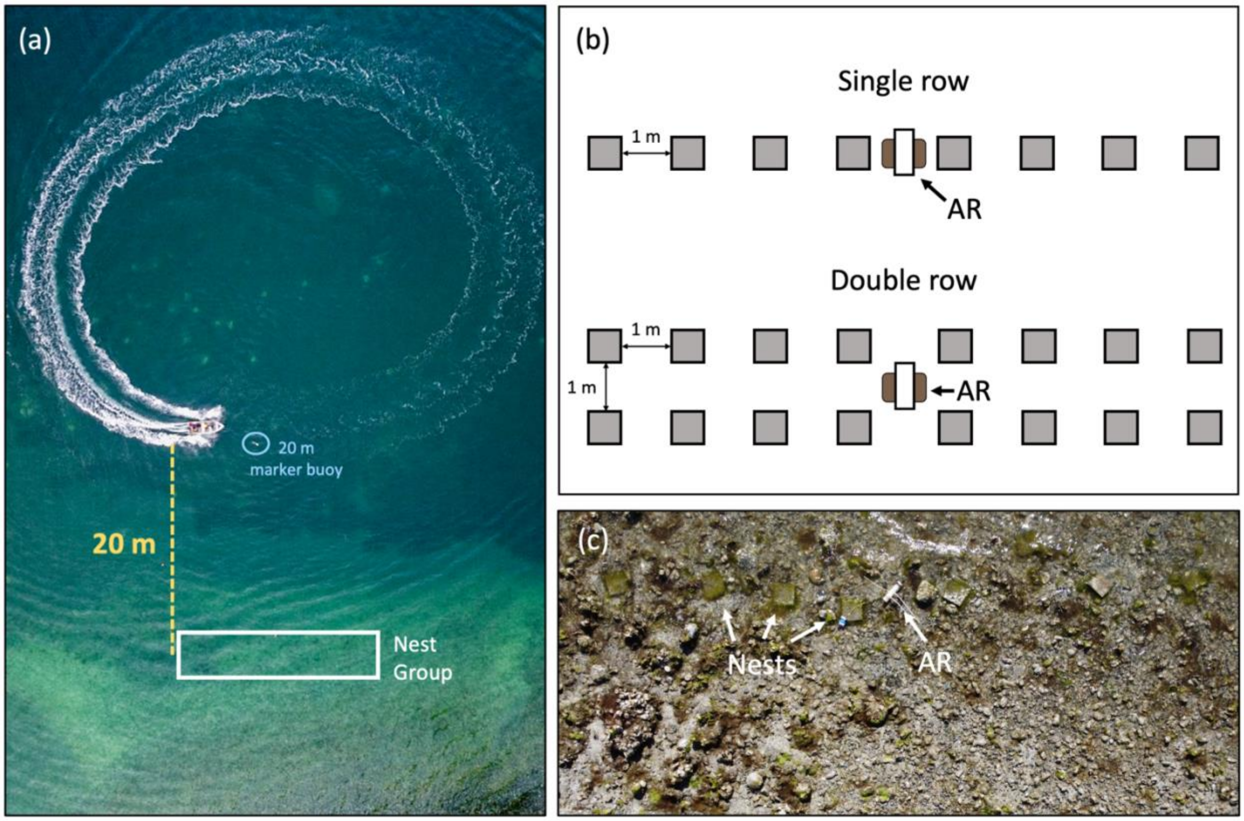

Figure 3.1 Experimental setup. (a) Aerial photograph taken during the boat noise period of an experimental trial, showing the path of the boat loop, location of the submerged intertidal nests in the nest group being tested, and the 20 m marker buoy (20 m from the center of the nest group) which was used to steer the boat in consistent loops at the correct distance from the nests. (b) schematic diagram of the two nest group arrangements for the five groups tested: 8 nests in a single row or 16 nests in a double row, with the acoustic recorder (AR) positioned in the middle of the nest groups. (c) An aerial photograph of a single-row nest group (consisting of 8 nests).

3.3.3 Experimental trials

Each of the five nest groups received one trial, each on a different night, with at least two nights without noise between groups. At least 24 hours before each trial began, a SoundTrap acoustic recorder (ST300 STD or ST300 HF; Ocean Instruments New Zealand) was placed in the centre of the nest group to be tested (Figure 3.1) to record the vocalizations of the fish in the focal group, as well as the boat noise we were exposing them to.

As plainfin midshipman fish are nocturnal and primarily hum at night, we conducted trials at dusk, between 20:20 and 21:20. We did not conduct trials later at night, as recreational boat noise is rare after dusk, and safety protocols favoured driving boats while there was still some daylight. Directly before trials began, the boat was quietly rowed away from the nests (>150 m) and into deep water, where the engine was then started and allowed to warm up. The first boat noise treatment began approximately 45 minutes before sunset.

Each trial night consisted of three 10-minute boat noise treatments and four 10-minute ambient control treatments, including the ambient baseline and follow-up periods. During boat noise periods, the boat was driven continuously at full throttle. During the ambient control periods, the boat engine was turned off, the boat was away from the nests, and no noise was introduced.

Trials began with a 10-minute baseline period before the boat engine was started. There were then three 10-minute intervals of continuous boat noise, interspersed by 10-minute quiet periods where the boat engine was off. Finally, a 10-minute follow-up period was conducted once the boat engine was turned off.

3.3.4 Acoustic Analysis

All bioacoustic analyses were performed using Raven Pro acoustic software (version 1.6.5; K. Lisa Yang Center for Conservation Bioacoustics, Cornell Lab of Ornithology, Ithaca, New York, USA). Spectrograms were built in Raven Pro with 7000 samples, a

Hanning window with 50% overlap, and time and frequency axes set to 10 seconds and 1000 Hz, respectively, and vocalizations were annotated manually. We selected three 1–3 second measurements of hum vocalizations present during each trial period: one near the beginning, one in the middle, and one at the end of the trial period, taking care to pick the loudest and clearest hums present. Both the fundamental frequency (F_0) and the first harmonic (F_1) were measured for each hum when these signals were both audible and visible in the spectrograms. Every individual clear grunt and growl during each of the trial periods was enumerated and analyzed and the presence/absence of hums in each trial period was also recorded. In the case of grunt trains, the clearest grunt in the train was selected for amplitude and frequency analyses and the rest of the grunts in that train were simply counted. For the peak power density (amplitude) analysis, we used the first harmonic (F_1) of each vocalization, as F_1 was often the most prominent harmonic. No vocalizations were scored or analyzed if they occurred during the minutes between trial periods when the boat was being started or driven away from the nests.

Metrics of underwater sound levels were processed in two ways using the PAMGuide package (Merchant et al. 2015) in Matlab (version 2016b; Mathworks, Natick, Massachusetts, US). First, power spectral densities (PSD), a high-resolution metric of amplitude which quantifies sound levels for every Hz in a frequency range, were computed using a Hanning window with 50% overlap and 1 second bins in the 20 Hz to 10 kHz range. Second, sound pressure levels (SPL), which measure the sum of power across a broad frequency range, were calculated in third octave bands using a Hanning window with 50% overlap and 1 second bins. We then summed the power of the three bands centered on 80, 100, and 125 Hz and converted back to decibels, thus calculating SPL in the octave band centered on the midshipman hum fundamental frequency.

3.3.5 Statistical Analysis

We conducted all statistical analyses in R (v.4.0.4; R Core Team, 2021). Acoustic masking from the boat noise could have made it harder to detect quieter calls during noise trial periods compared to ambient control periods. Thus, to account for this potential masking effect, we set a threshold for the ambient periods such that the lowest amplitude vocalization in the ambient periods could not be lower than the lowest amplitude vocalization in the boat noise periods. Therefore, we used the peak power density of the lowest-amplitude vocalizations during the boat noise treatment (82 dB re 1 µPa) as a cutoff threshold for the vocalizations in the ambient periods. Julian date was not included as a term in any of the models because each group was tested on a different day, which would lead to collinearity if both group and date were included in the models.

For all models, we used ANOVA Wald chi-squared tests (*car* package), with contrasts summed to zero in any models with an interaction term to properly account for the interaction, and we used contrast ratios (*emmeans* package) to calculate the magnitude of any observed effects. The presence/absence of hums was not modelled, as hums were present in every trial period.

3.3.5.1 Agonistic vocalization rates

We fit a negative binomial generalized linear mixed-effect model (GLMM; *glmmTMB* package) to the counts of agonistic vocalizations (grunts and growls) recorded for each nest group in each trial period to test the effects of noise treatment (ambient control vs. boat noise). We included trial period and nest group as random intercepts to account for the repeated-measures design, and noise treatment as the fixed effect. We used ANOVA type II Wald chi-squared tests (*car* package) to test the effect of boat noise on agonistic vocalizations and we used contrast ratios (*emmeans* package) to calculate the magnitude of the observed effect. We fit additional negative binomial GLMMs with the same fixed and random effects to test the effect of boat noise on growls and grunts individually.

We also modelled the number of grunts in each grunt train during boat noise vs. ambient periods, using an additional negative binomial GLMM with the same fixed and random effects as above.

3.3.5.2 Duration of agonistic vocalizations

We built LMMs to examine the effect of noise treatment on the duration of agonistic vocalizations: grunts and growls. We examined each separately because grunts are typically much shorter than growls. The growl data were log-transformed to achieve normality. Trial period and nest group were included as random intercepts in both models, with noise treatment as the fixed effect. We could not analyze the duration of hums because it is difficult to distinguish between when hums of different fish are starting and stopping, and we also only measured a uniform duration of hums within a file for consistency.

3.3.5.3 Peak frequency of vocalizations

We used linear mixed-effects models (LMMs; *lme4* package) to test the effect of noise treatment on the peak frequency (in Hz) of all three vocalization types. We fit a single LMM for the fundamental frequency (F_0) of all vocalizations. Noise treatment (ambient control or boat noise), vocalization type (grunt, growl, or hum), and their interaction were included as fixed effects in the model. Temperature was included as an additional fixed effect (mean-centered), as previous studies have shown a correlation between water temperature and frequency of plainfin midshipman vocalizations (Halliday et al., 2018; McIver et al., 2014). Nest group and trial period were both included as random intercepts to account for the repeated-measures design.

To further examine the effect of noise on hum peak frequency, we fit an additional LMM for the first harmonic (F_1) of hums. Due to the acoustic properties of shallow water, the propagation of the various harmonics differed, and F_1 often propagated more strongly than F_0, especially for hums, giving us a larger sample size for F_1 hums. The F_1 hum model included noise treatment and temperature (mean-centered) as fixed effects and trial period and nest group as random intercepts.

3.3.5.4 Amplitude of vocalizations

Peak power density (PPD), measured in decibels (dB re 1 µPa), was used as the primary measurement of amplitude to examine the effects of boat noise on amplitude of all three plainfin midshipman vocalizations. Peak power density is defined as the amplitude of the grid cell (grid cell dimensions are defined by the Fast Fourier Transform used to calculate the spectrogram) with the greatest amplitude within an annotation.

We fit a single LMM to examine the effect of noise treatment on the PPD of the first harmonic (F_1) of all vocalizations. Noise treatment (ambient control or boat noise), vocalization type (grunt, growl, or hum), and their interaction were included as fixed effects in the model, and nest group and trial period were included as random intercepts.

Sound pressure levels (SPL), again measured in dB re 1 µPa, were also modelled using an LMM with treatment as the fixed effect and nest group and trial period as random intercepts. This examined the overall sound levels during ambient control and boat noise periods rather than the amplitude of individual vocalizations.

3.3.6 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-2021-012). All procedures implemented were non-invasive. After experiments concluded, all equipment was removed, and the experimental fish were allowed to continue guarding their brood in their artificial nests at the study site for the remainder of the breeding season.

3.4 Results

3.4.1 Rates of agonistic vocalizations

We observed a significant decrease in midshipman agonistic vocalizations during boat noise ($\chi^2_1 = 26.58$, $p < 0.0001$), with an average of 4.4 times (95% CI: 2.4–7.8) fewer vocalizations in boat noise periods compared to ambient control periods when the boat engine was off (Figure 3.2a). There were 4.4 times (95% CI: 2.2–8.9) fewer grunts during boat noise (Figure 3.2b) and 3.6 times (95% CI: 2.4–5.6) fewer growls during boat noise compared to ambient control periods (Figure 3.2c).

Additionally, grunt trains were shorter during boat noise compared to ambient periods ($\chi^2_1 = 8.76$, $p = 0.0031$). On average, grunt trains during boat noise periods contained 2.8 times fewer grunts (95% CI: 1.4–5.4).

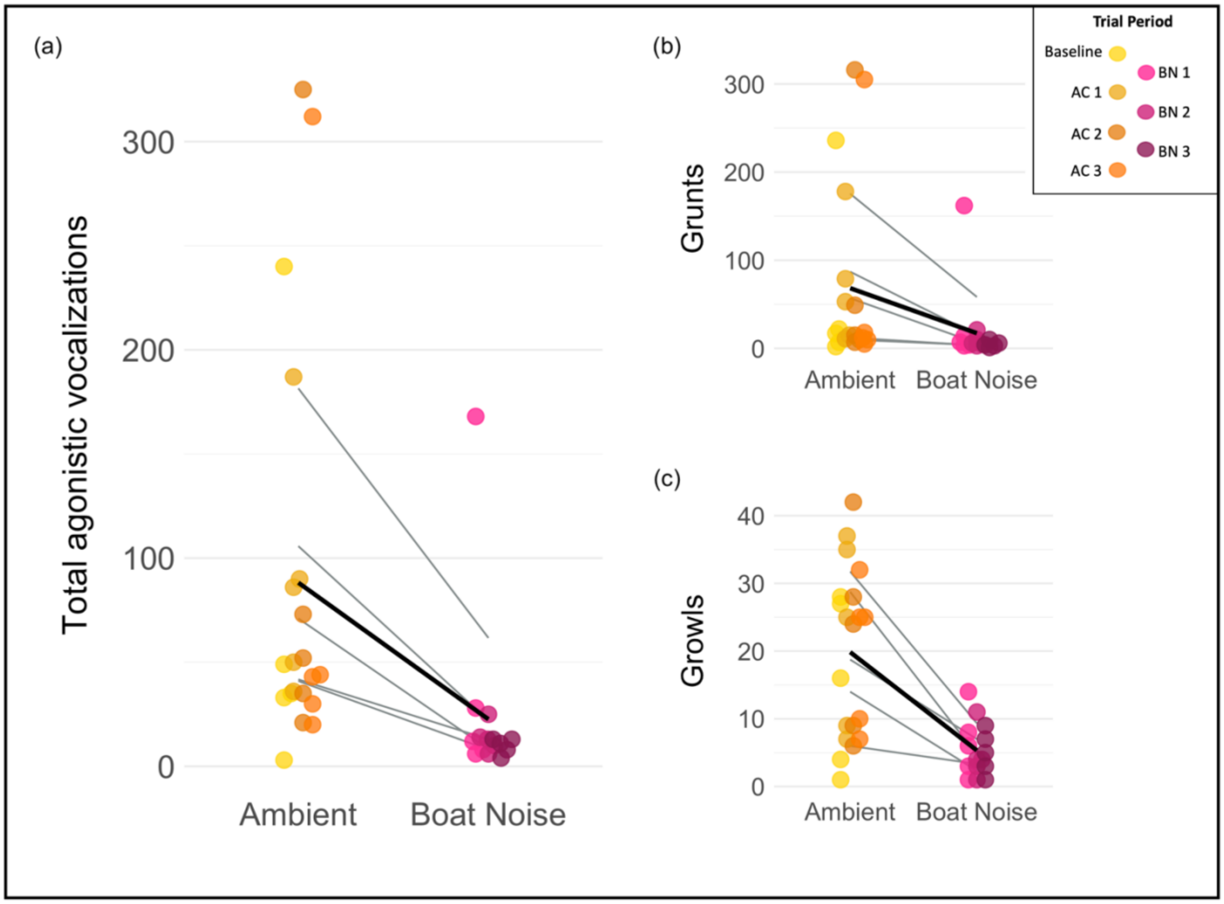

Figure 3.2 Sum of agonistic vocalizations produced by groups of guarder male plainfin midshipman during 10-min trial periods of continuous boat noise or ambient control periods when the boat was turned off and away from the nests. (a) Total number of combined grunts and growls (agonistic vocalizations), (b) number of grunts, and (c) number of growls recorded from each of the five nest groups in each trial period. Colours represent trial periods, with colours darkening with the time of night.

3.4.2 Duration of agonistic vocalizations

The duration of both grunts and growls were affected by noise treatment (Figure 3.3). Grunts were longer on average during boat noise periods compared to ambient control periods (χ^2_1 = 3.90, p = 0.048), while growls were shorter during boat noise periods (χ^2_1 = 5.67, p = 0.02). On average, grunts lasted for 0.164 seconds and were 0.013 ± 0.007 seconds longer during boat noise periods compared to ambient periods, where they lasted for an average of 0.149 seconds. Growls were approximately 1.3 times (95% CI: 1.0–1.7) shorter during boat noise periods, where they lasted an average of 1.64 s, compared to growls in the ambient periods, which had an average duration of 2.31 s.

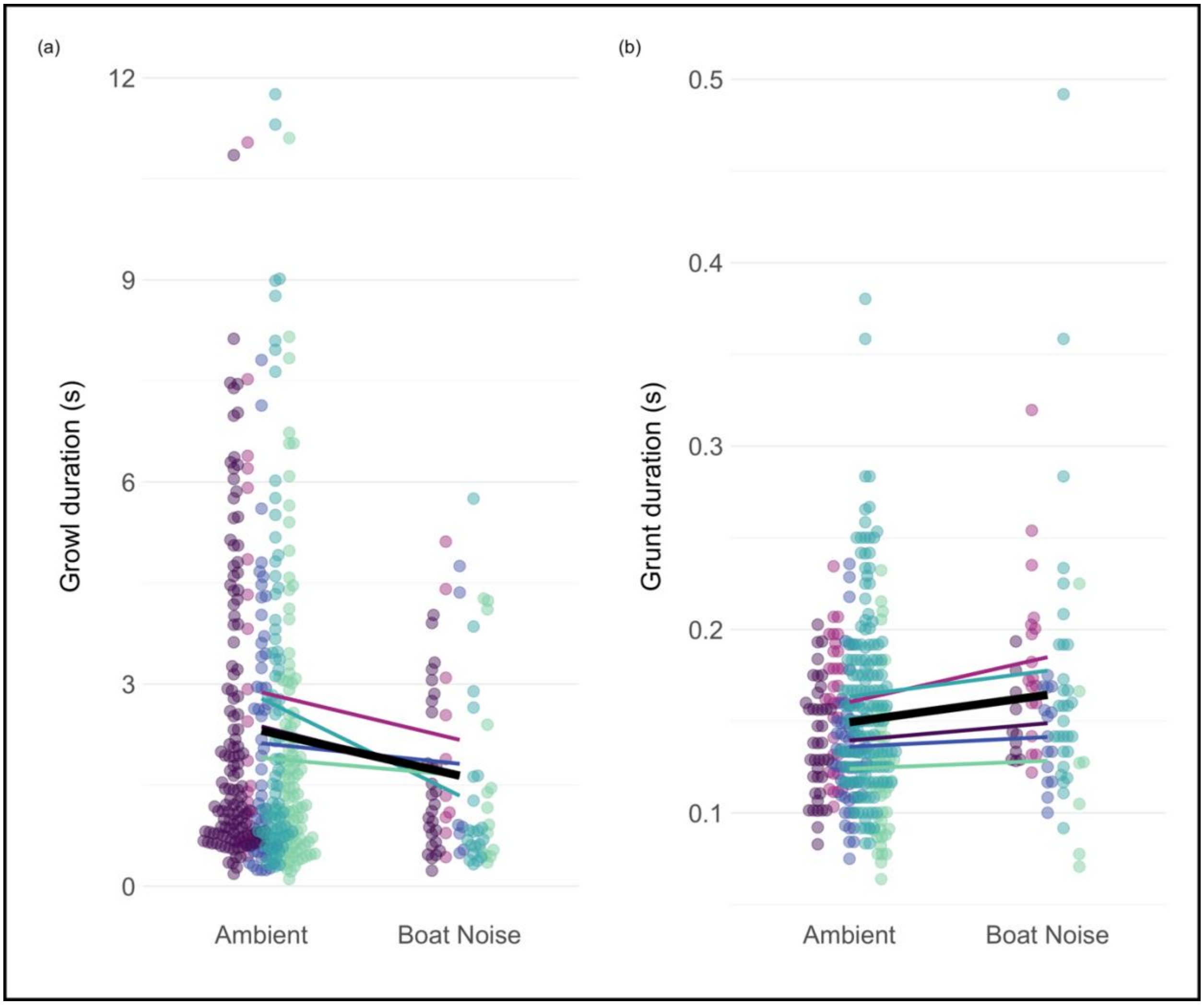

Figure 3.3 Duration (in seconds) of agonistic growl (a) and grunt (b) vocalizations produced by guarder male plainfin midshipman fish in each of five nest groups (denoted by the different colours) during boat noise and ambient control periods. Coloured lines show the mean peak frequency for each group within ambient and boat noise periods, and the thick black lines show the mean across all five groups.

3.4.3 Peak frequency of vocalizations

Boat noise had a significant effect on the peak frequency of the hum first harmonic (F_1), which was on average approximately 4.18 ± 1.44 Hz (est. ± SE) higher in the boat noise treatment compared to the ambient control treatment ($\chi^2_1 = 8.48$, p = 0.004; Figure 3.4).

Water temperature also had a significant effect on hum F_1, with an average increase of approximately 10.33 ± 2.88 Hz for every 1ºC increase in water temperature (Figure S3.1).

We did not observe an effect of boat noise on the fundamental frequency (F_0) peak frequency of any of the vocalization types ($\chi^2_1 = 0.12$, $p = 0.73$). However, temperature had a significant effect on F_0 peak frequency ($\chi^2_1 = 6.60$, $p = 0.01$), and the different vocalization types differed significantly in their peak frequencies ($\chi^2_2 = 23.68$, $p < 0.0001$). For every 1ºC increase in water temperature, there was an increase of approximately 11.28 ± 4.39 Hz (Figure S3.2; Figure S3.3).

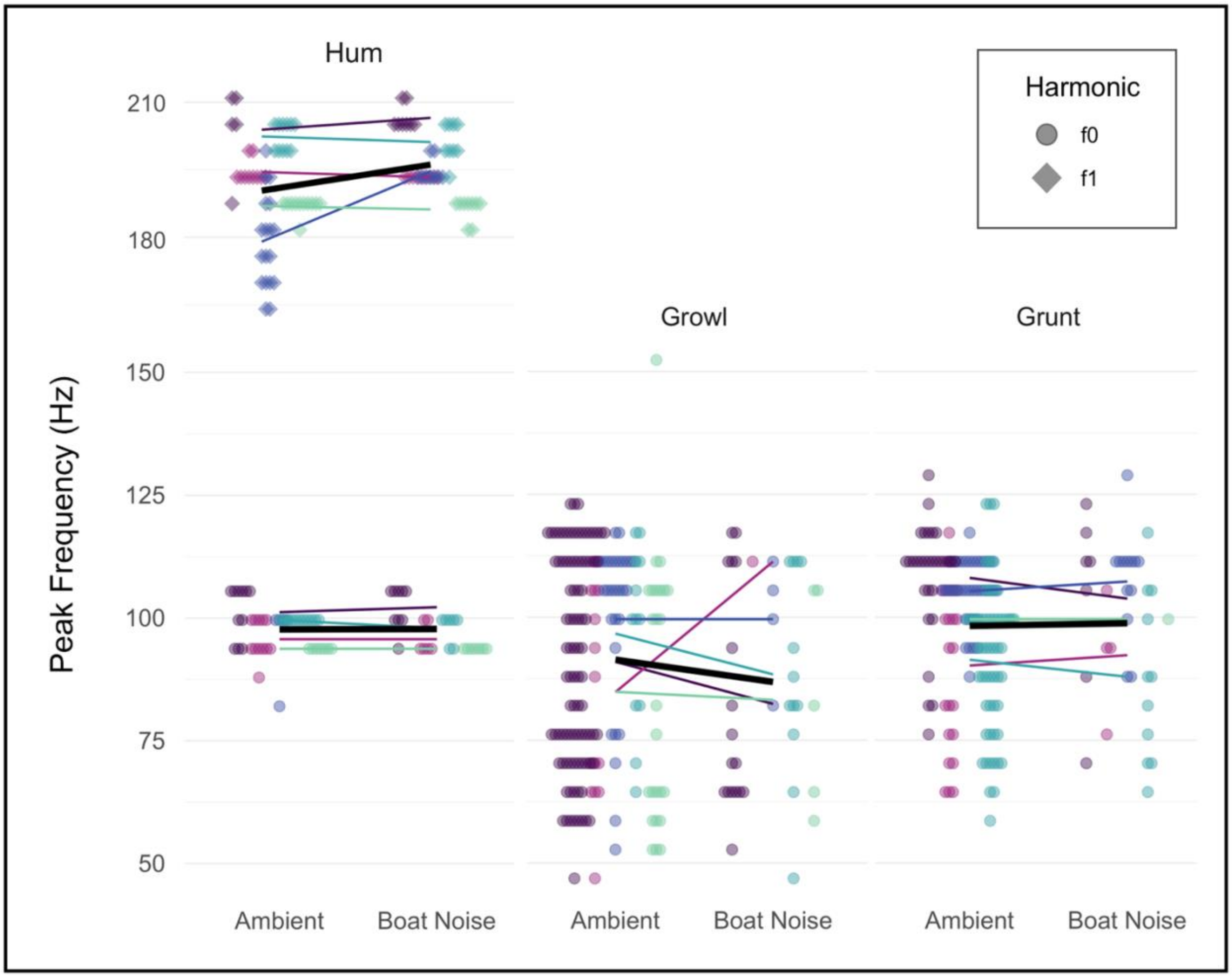

Figure 3.4 Peak frequency (in Hz) of the vocalizations produced by guarder male plainfin midshipman in each of five nest groups (represented by the different colours) during boat noise and ambient control trial periods. Both the fundamental frequency (F_0) and first harmonic (F_1) of mating hums are shown, and F_0 is shown for agonistic grunts and growls. Coloured lines show the mean peak frequency for each group within ambient and boat noise periods, and the thick black lines show the mean across all groups.

3.4.4 Amplitude of vocalizations

When examining the effect of boat noise on the peak power density (PPD; a measure of amplitude) of the first harmonic of midshipman vocalizations, we observed a significant effect of noise treatment ($\chi^2_1 = 14.43$, p = 0.0002; Figure 3.5), as well as a significant interaction between noise treatment and call type ($\chi^2_2 = 11.09$, $p = 0.004$), with grunts and growls both increasing in amplitude in the presence of boat noise. On average, grunts were 6.3 ± 1.4 dB (est. ± SE) louder during boat noise periods compared to ambient periods, while growls were 5.1 ± 1.3 dB louder during boat noise on average. We did not observe an effect of noise treatment on hum amplitude.

Call type was also a significant term in our model ($\chi^2_1 = 70.22$, $p < 0.0001$). Overall, hums were the loudest call, with a mean PPD of 106 dB, followed by growls with a mean PPD of 100 dB, with grunts being the quietest call, with a mean PPD of 97.2 dB (Table 3.1; Figure 3.5).

We measured the PPD of individual boat noise segments (by harmonic) in a subset of trials, taking measurements from the loudest (when the boat was passing closest to the nests) and the quietest (when the boat was at the most distant point of the loop) parts of the boat noise periods. The mean PPD of the boat noise measurements was 103.2 dB (F_0 mean = 104 dB, F_1 mean = 103 dB). The mean PPD of all three call types during boat noise periods (but not ambient periods) was higher or equal to the mean PPD of the boat engine (Table 3.1).

Table 3.1 Mean peak power density (PPD) for the first harmonic (F1) of each vocalization type and boat noise measurements from a subset of trials. The overall mean represents the mean across all periods, regardless of noise treatment. All amplitude measurements are in dB re 1 µPa.

Call Type	Overall	Ambient Periods	Boat Noise Periods
Hum	106 dB	107 dB	106 dB
Growl	100 dB	99.7 dB	104 dB
Grunt	97.2 dB	96.5 dB	103 dB
Boat	*103 dB*	-	*103 dB*

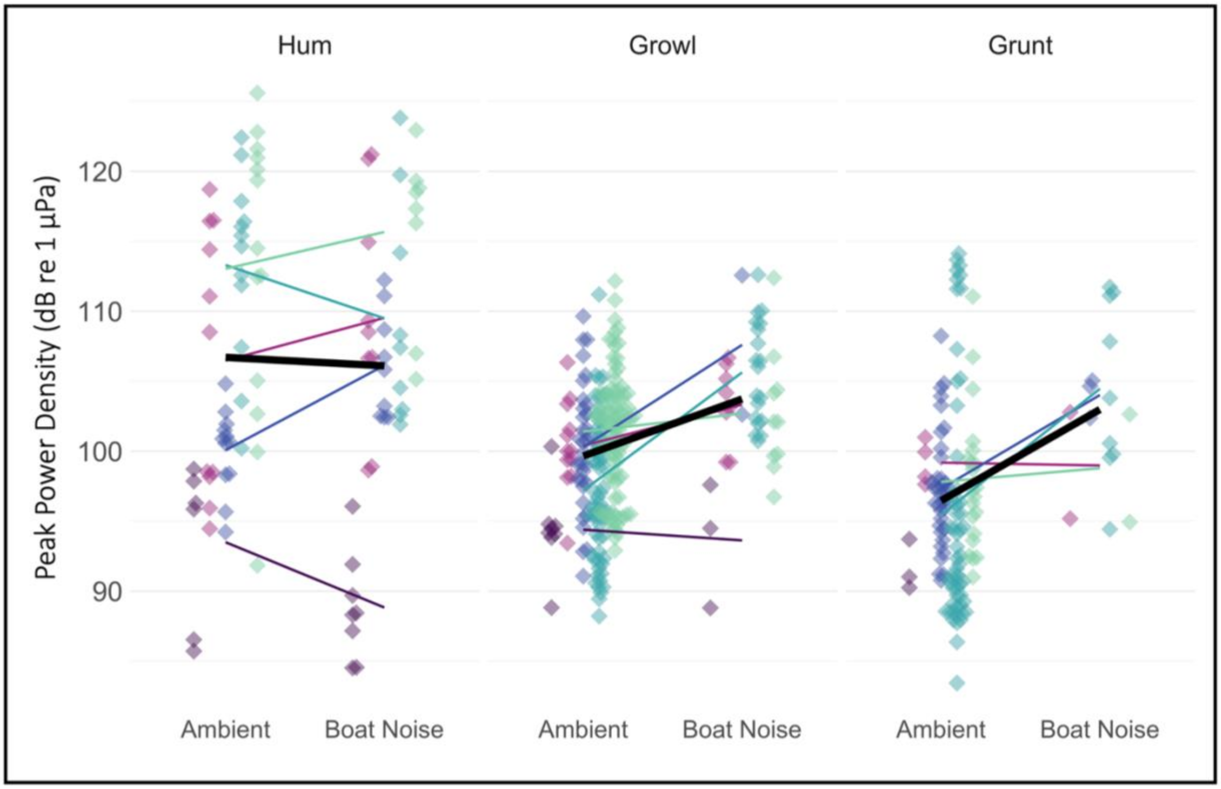

Figure 3.5 Peak power density (amplitude) of the first harmonic (F_1) of plainfin midshipman vocalizations during 10-minute periods of continuous boat noise and 10-minute ambient control periods. Colours denote the five nest groups, each tested on a different night. Coloured lines show the mean peak frequency for each group within ambient and boat noise periods, and the thick black lines show the mean across all groups.

3.4.5 Quantification of Boat Noise and Experimental Soundscape

Our boat noise trials caused large increases in underwater sound levels, including broadband sound and individual tonal signals (Figure 3.6; Figure 3.7). Power spectral density (PSD) for the control period show strong peaks at roughly 100, 200, 300, and 400 Hz, which are caused by midshipman humming, but underwater sound levels otherwise show a 30 dB width from 1st to 99th percentile, with decreasing power as frequency increases (Figure 3.6). Conversely, the boat noise periods show the same peaks from midshipman hums, but also includes multiple additional peaks from tonal boat noise, and in frequencies > 100 Hz, power has a 50 dB range that does not decrease with increasing frequencies (Figure 3.6). Moreover, PSD in the 200 to 1000 Hz range reaches > 90 dB in the boat noise period, whereas in the ambient control period, PSD only goes as high as 85 dB in the 200-500 Hz range and down to a maximum of 65 dB in the 800–1000 Hz range (Figure 3.6). PSD is therefore as much as 30 dB higher in the 800-1000 Hz range in boat noise periods compared to ambient control periods. The spectrograms in Figure 3.6c–d exemplify the broadband nature of the boat noise, compared to the strong harmonic structure of the midshipman hums, which have the highest amplitude in the lower frequencies (< 500 Hz). The vertical yellow bands spanning the entire frequency range in Figure 3.6d represent each boat pass as the boat was at the point of the loop closest to the experimental nests, while the horizontal yellow bands in Figure 3.6c–d represent midshipman hums, with the highest amplitude at ~200 Hz (hum F_1).

Sound pressure levels (SPL), which allow us to compare the sum of power across a broad frequency range, were significantly higher during boat noise periods compared to ambient control periods ($\chi^2_1 = 5.89$, $p = 0.015$), with a mean increase of 10.4 ± 4.3 dB (est. ± SE) during boat noise (Figure 3.7). The mean SPL in the ambient periods was 95.7 dB, while the mean SPL in the boat noise periods was 106 dB. Within each treatment (ambient and boat noise), the trends in SPL were relatively consistent across trial periods and between groups (Figure 3.7b). We did see a trend of increased sound levels later at night (darker coloured boxplots in Figure 3.7b), in both the ambient and boat noise treatments, though more pronounced in the ambient treatment, where noise levels were lower overall (Figure 3.7b). This temporal trend is likely due to more males humming later at night, as the hum chorus at midshipman breeding sites typically peaks around midnight (Halliday et al., 2018).

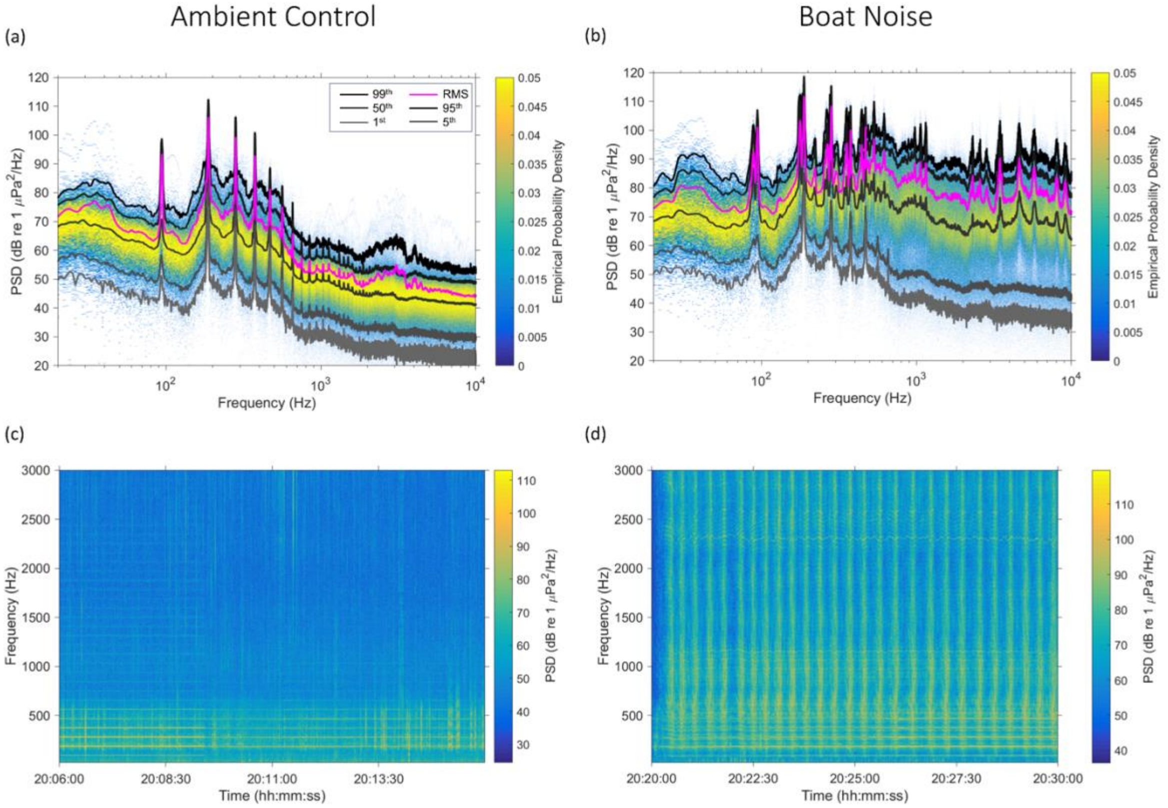

Figure 3.6 Power spectral densities (PSD) (a, b) and spectrograms (c, d) for example ambient control (a, c) and boat noise (b, d) trials. PSD plots are displayed as the 1st, 5th, 50th, 95th, and 99th percentiles and the root mean squared (RMS) average amplitudes across frequencies.

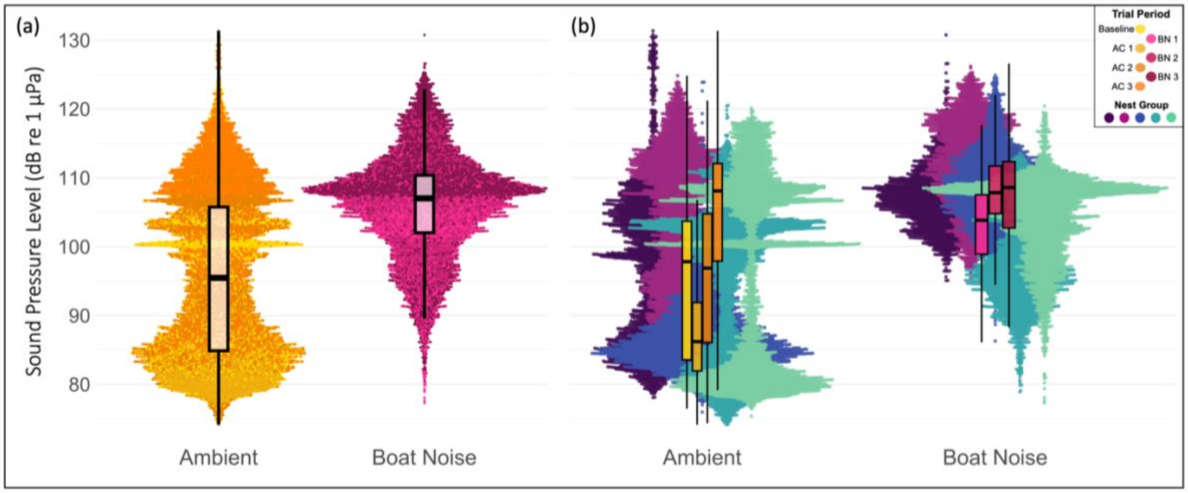

Figure 3.7 Sound pressure levels (SPL) in the 80 Hz octave band (71–141 Hz) during the four ambient control (yellow-orange) and three boat noise (pink-purple) periods of experimental trials. Each point represents one second of recording for each 10-minute trial period, for each of the five nest groups. (a) Boxplots represent the overall median and interquartile range for all data from each treatment, plotted on top of the raw data, coloured by trial period. (b) Boxplots show the median and interquartile range for each trial period, and are plotted on top of the raw data for each of the five nest groups.

3.5 Discussion

We found that wild nesting plainfin midshipman fish alter their vocalizations in the presence of boat noise. The midshipman fish in our study produced significantly fewer agonistic vocalizations and altered the acoustic characteristics of all three vocalization types in boat noise periods. We observed a frequency shift in the mating hum and an increase in amplitude in both grunts and growls in the presence of boat noise. Therefore, this study adds to the currently limited evidence of the Lombard effect in fishes, and it is the first *in situ* study using real motorboat noise to establish this effect.

3.5.1 Agonistic vocalization rate

During boat noise periods, we observed over four times fewer agonistic vocalizations (grunts and growls) compared to ambient control periods when the boat engine was off, thus supporting our first hypothesis that boat noise would decrease the rate of agonistic vocalizations. Though grunts were far more numerous than growls, we found the same trend in both vocalizations of an approximately four-fold decrease during boat noise. Further, we observed a decrease in grunt train length (number of grunts in a train) during boat noise periods. These results are consistent with those of Brown et al. (2021), who found a decrease in all three vocalization types when nesting plainfin midshipman were exposed to artificial noise. A reduction in agonistic vocalizations could compromise a male's ability to effectively defend his territory and offspring, leading to lower reproductive success. There is intense competition for nests during the midshipman breeding season, leading to frequent territory disputes and nest-takeovers, which often result in cannibalism of the eggs by the non-parental male who has won another male's nest (Bose et al., 2016b). Parental males also vocalize to defend their offspring from egg predators such as crabs and gunnels, in addition to lunging at and/or biting the intruders (Cullis-Suzuki, 2015; Woods et al., 2022). Therefore, the observed decreases in grunts and growls may increase offspring mortality and decrease the reproductive success of the resident male exposed to boat noise.

These results also support the findings of our previous study, Woods et al. (2023), which examined the effect of boat noise on nesting plainfin midshipman fish at the level of the individual using an experimental design similar to the present study, and observed a decrease in agonistic vocalizations on trial days compared to days with no boat noise. Additionally, several other studies have found decreased calling rates in fishes (Ladich, 2019). In the field, oyster toadfish (*Opsanus tau*), a species closely related to plainfin midshipman, decreased their call rates by 32% following exposure to noise from an idling boat engine, compared to pre-exposure call rates (Mackiewicz et al., 2021). In the lab, male two-spotted gobies (*Gobiusculus flavescens*) and painted gobies (*Pomatoschistus pictus*) both decreased their courtship vocalization rates in response to artificial noise, which may have led to the observed decrease in spawning success in painted gobies under noisy conditions (de Jong et al., 2018a).

We predicted that grunts and growls would decrease during boat noise because they were more likely to be masked, and therefore, it might be beneficial for the males to wait to vocalize until after the boat noise had stopped, rather than wasting energy on vocalizations that would not be received. However, several studies in other species (primarily birds and mammals) have found an increase in vocalization rate to increase signal redundancy (Brumm and Zollinger, 2011; Shannon et al., 2016). This includes one study in fish, which observed an increase in pulse rate in Brown meagres (*Sciaena umbra*; Picciulin et al., 2012). It is possible that we would have seen this opposite effect of increased redundancy, both in number of agonistic vocalizations and in grunt train length, if our noise stimulus had been chronic or lower amplitude. Because our boat noise only lasted 10 minutes at a time and ceased completely within an hour, and because the noise was loud enough to heavily mask agonistic vocalizations, the males in our study may have been able to rely on other methods of territory and offspring defence, such as lunging and biting, during those short periods. In a lab study examining the effects of boat noise on territory disputes in red mouthed gobies (*Gobius cruentatus*), the resident males decreased their agonistic vocalizations, the intruder males were less submissive, and both residents and intruders increased the duration of their agonistic circling behaviours during the noise treatment. This resulted in the intruder males winning significantly more disputes in the noise treatment compared to quiet conditions (Sebastianutto et al., 2011) and may have been an example of the males increasing other defensive behaviours to compensate for reducing their agonistic vocalizations during noise. Future field studies should be done using both cameras and acoustic recorders to assess whether a decrease in agonistic vocalizations is associated with an increase in other agonistic behaviours as a potential method of compensation.

3.5.2 Duration of agonistic vocalizations

While both the number of individual grunts and grunts per grunt train decreased, we did observe an increase in individual grunt duration. Increased duration of short vocalizations (below a few hundred milliseconds, a range encompassing midshipman grunts but not growls) has been identified as a mechanism to increase signal recognition in noisy conditions in other animals, including humans (Brumm and Slabbekoorn, 2005). We did not see this effect on growls, which are much longer-duration vocalizations than grunts; in fact, we observed a decrease in growl duration during boat noise. The observed decrease in growl duration may be related to the decrease in agonistic call rate in our study; agonistic vocalizations may not have been as effective during boat noise periods, so it is possible the males conserved energy with fewer vocalizations and shorter growls, and potentially relied upon other methods of nest defence during those noisy periods.

3.5.3 Frequency of vocalizations

Another way that animals avoid masking of their vocalizations is to shift the frequency of their calls away from the dominant frequencies of the noise (Brumm and Slabbekoorn, 2005). We observed this effect in the first harmonic (F_1) of the mating hums, supporting our second hypothesis. The boat noise was typically slightly lower frequency than the hums at each harmonic, and we observed a mean increase of approximately 4 Hz in the hum F_1 during boat noise periods, shifting it farther away from the boat noise F_1. To our knowledge, ours is the second study to date to observe a frequency shift in vocalizations in response to noise in fishes, as it was previously thought that fish were not capable of these sorts of vocal adjustments (Ladich, 2019). Brown et al. (2021) observed a decrease in the fundamental frequency of the mating hum in nesting plainfin midshipman, opposite to the increase in hum frequency observed in the present study. Increases in vocalization frequency are a commonly observed vocal adjustment in noisy environments in other species (Brumm and Slabbekoorn, 2005), rendering our findings consistent with information theory and the Lombard effect (Brumm and Zollinger, 2011; Shannon and Weaver, 1949). However, both our study and Brown et al. (2021) were conducted at the group level rather than on individual fish, meaning that the changes in frequency could have either been true vocal adjustments or individual fish joining or leaving the hum chorus.

3.5.4 Amplitude of vocalizations

Both grunts and growls significantly increased in amplitude (peak power density) in the presence of boat noise, thereby exhibiting the Lombard effect and supporting our final hypothesis. On average, grunts were six decibels (dB) louder and growls were five dB louder in boat noise periods compared to ambient periods. To date, there have only been three other studies that have found the Lombard effect in fishes (Brown et al., 2021a; Holt and Johnston, 2014; Luczkovich et al., 2016). When exposed to white noise playbacks in aquaria, blacktail shiners (*Cyprinella venusta)* increased the amplitude of burst vocalizations (Holt and Johnston, 2014). In the field, oyster toadfish increased the amplitude of their vocalizations during and after noise playbacks (Luczkovich et al., 2016), and plainfin midshipman fish increased their hum amplitude in response to a low-amplitude noise stimulus (Brown et al., 2021a).

In our study, grunts and growls both increased in mean peak power density during the boat noise periods to a level higher (growls) or equal to (grunts) the boat noise, from a level lower than our boat noise stimulus during ambient control periods, further supporting our hypothesis that plainfin midshipman do exhibit the Lombard effect to overcome masking by anthropogenic noise.

Mating hums, which are typically longer and louder than grunts and growls, were the only vocalization for which we did not observe an effect of boat noise on vocalization amplitude. Brown et al. (2021) tested the effect of a low-amplitude noise stimulus on the vocalizations of nesting plainfin midshipman fish and observed an increase in hum amplitude. However, due to the low amplitude and acoustic similarity to plainfin midshipman mating hums, their stimulus may have elicited a response based on competition rather than masking. Our study aimed to elucidate whether plainfin midshipman males would respond in the same way to boat noise (*i.e.* a realistic anthropogenic noise stimulus). As we did not see an increase in hum amplitude, our results lend support to the idea that the fish in Brown et al. (2021) may have been trying to compete with an “additional male”. The boat noise in our study heavily masked the mating hums, especially the fundamental frequency. Because of this, it may not have been worth the potential physiological costs of humming louder to compete with (*i.e.* avoid being masked by) such a loud stimulus, unlike the low-amplitude noise stimulus in Brown et al. (2021). This hypothesized explanation is further supported by the fact that Brown et al. (2021) did not see an increase in the amplitude of agonistic grunts and growls, while our study did. Increasing the amplitude of short duration vocalizations necessary for nest defence may be a viable strategy to maintain territory and protect offspring during loud boat noise; however, it may not have been necessary in response to the quieter stimulus used in Brown et al. (2021), as that stimulus was more akin to an additional male humming and unlikely to mask agonistic vocalizations.

Further, it is difficult to measure the amplitude of hums at the group level because many males chorus together, creating a loud, additive mating hum chorus. Due to this additive nature, it is hard to parse apart amplitude changes from certain males in the group starting and stopping humming, thereby altering the amplitude of the hum chorus. Therefore, agonistic grunts and growls provided an easier way to examine the effect of boat noise on individual vocalizations.

We saw the largest effect of boat noise on vocalization amplitude in grunts, which are the quietest midshipman vocalization, meaning that grunts would have the lowest signal-to-noise ratio during boat noise periods (and ambient periods). In a meta-analysis on the Lombard effect, Kunc et al. (2022) hypothesize that signal-to-noise ratios may be the key factor in explaining the variation in whether or not the Lombard effect was observed across studies, with lower signal-to-noise ratios being more likely to elicit the Lombard effect. In reptiles and amphibians, studies that did not observe the Lombard effect had significantly higher signal-to-noise ratios than those that did establish an effect (Kunc et al., 2022). Hums would have had the highest signal-to-noise ratio in our study, which may explain why we observed the Lombard effect in the lower amplitude grunts and growls but not the louder mating hum.

3.5.5 Conclusion

We found that real world motorboat noise affects plainfin midshipman vocalizations in a variety of ways, including decreasing the rates of their agonistic vocalizations, while increasing the frequency of their mating hums and the loudness of their agonistic vocalizations. This study is the fourth ever to establish the Lombard effect in fishes, and it is the first study to establish this effect using a real motorboat as a noise stimulus. Our study reinforces that additional studies in other fish species need to be conducted to examine whether this effect is more widespread than originally thought. Further field studies using realistic noise stimuli will be instrumental in determining how anthropogenic noise is affecting wild populations.

3.6 References

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Chapter 4: Conclusion

Noise pollution is rapidly changing underwater soundscapes, leading to a variety of consequences for marine life (Duarte et al., 2021). While investigating the effects of noise on marine mammals has become a widely studied subject, the effects of noise on fishes are not nearly as well documented. Further, few field studies using realistic noise stimuli have been conducted, and the results of laboratory studies are often applied to animals in their natural habitats, without taking testing context into account. This thesis uses a combination of laboratory and field studies to examine how noise affects the behaviour and vocalizations of nesting plainfin midshipman fish. It also determines how testing context impacts experimental outcomes.

In Chapter 2, I used laboratory and field experiments to address my first two objectives, which were to assess 1) how anthropogenic noise affects plainfin midshipman fish in different contexts and testing environments, and how those effects of context translate to real-world effects, and 2) how noise impacts parental care and nest defence. I first tested how nest-guarding males in the lab responded to an egg-predator (black clawed crab) intruding their nest in the presence and absence of a boat noise playback. I found that males were more aggressive and increased their defensive behaviours during boat noise playbacks. I then conducted a similar field experiment that had a few key differences from the laboratory experiment, thereby testing the males in different contexts. First, while the nests all had eggs in the field experiment, the males were guarding empty nests in the laboratory. Second, the noise stimulus used in the field experiment was a vibrating device called a Noise Egg, which was much quieter than the boat noise playbacks in the laboratory (~8 times quieter at the respective peak frequencies). Finally, the testing environments differed; field work was conducted in their natural breeding habitat in the intertidal zone, while the laboratory work occurred in glass aquaria. In contrast to the laboratory experiment where the males were more defensive during noise playbacks, I did not observe a difference in defensive behaviours or parental care during the noise treatment in the field experiment.

Because there were multiple differences across experiments (presence of eggs, noise stimulus, and lab vs. field), it is challenging to determine the cause for the different results in the lab vs. in the field. I hypothesize that the louder noise stimulus in the lab was more likely to elicit a response than the Noise Eggs, which did not resemble boat noise and may have been too quiet a stimulus. Further, the presence of eggs likely caused males in the field to be more defensive overall, regardless of noise treatment. This second chapter demonstrates that testing context is important and should be considered both when designing studies and when interpreting laboratory study results and translating them to impacts of noise in the wild.

Further research is needed to determine how noise affects nest defence and parental care in the plainfin midshipman fish, as the results of Chapter 2 were somewhat inconclusive. In a study concurrent to that in Chapter 3, I performed an experiment to investigate how boat noise affects parental care behaviour, using a much larger sample size and real motorboat noise. However, those data are still being analyzed and that experiment did not examine the effects of noise on nest defence. Because there were multiple differences between the laboratory and field experiments in Chapter 2, it would also be beneficial to conduct further studies comparing lab and field results directly, keeping all other variables consistent across studies. This would allow us to determine whether the differences observed between the experiments in Chapter 2 were due to testing environment (i.e. lab vs field), or if the different stimuli or presence/absence of eggs contributed to the different results. If all other variables were consistent and the results of paired laboratory and field studies still differed, this would suggest that laboratory studies may not be a useful way to determine how fish are being affected by noise in the wild. Additionally, it would be useful to conduct further studies comparing presence vs. absence of eggs and comparing stimulus type (boat playbacks vs. tonal Noise Eggs) and intensity, each individually, by changing only one variable at a time.

I used the field study in Chapter 3 to address my third research objective, which was to assess if and how noise alters vocalizations, and test whether plainfin midshipman exhibit the Lombard effect in response to boat noise. Because I hypothesized that we may not have seen an effect in the field in Chapter 2 because the Noise Egg was too weak a stimulus, I decided to use a louder, much more realistic stimulus in Chapter 3. This noise stimulus was a recreational-sized motorboat, similar to those that are abundant in nearshore areas adjacent to midshipman breeding sites, especially during the summer breeding months. In this new vocalization experiment, I found that motorboat noise significantly reduced the rates of agonistic vocalizations, increased the frequency (pitch) of their mating hum, and increased the amplitude (loudness) of their agonistic grunts and growls.

Adjusting one’s vocalizations in noisy environments is a widespread phenomenon across the animal kingdom (Kunc et al., 2022), yet it has scarcely been studied in fish. The experiment in Chapter 3 is the first study to document the Lombard effect (vocalizing more loudly or altering the frequency of vocalizations in the presence of noise) in fish using experimentally introduced noise from a real motorboat, and only the fourth to ever document the Lombard effect in a fish. As this vocalization study was conducted at the group level (i.e. using recordings from a group of fish in different nests, meaning they contained vocalizations of multiple fish from the group), a useful next step for this research would be to conduct a similar, large-scale experiment on individual vocalizing fish. Recording the vocalizations of individual fish exposed to experimental boat noise would give us more precise information about the vocal adjustments the individual fish are making. It would also allow us to better analyse how noise affects the mating hum, as midshipman males hum simultaneously and chorus together, making it difficult to determine whether changes in amplitude or frequency in the chorus are due to vocal adjustments of individual fish, or just due to some fish in the chorus stopping or starting humming.

I conducted a small-scale study on four individual fish while I was collecting data for Chapter 3, and observed an overall decrease in the number of agonistic vocalizations on trial days compared to control days with no boat noise, but I did not see an effect of boat noise between noise treatments within trial days (Woods et al., 2023). However, because we only measured vocalizations from four fish, I suggest further research is warranted to determine how noise affects the vocalizations of individual fish using a larger sample size.

The overarching goal of my MSc research was to contribute to our understanding of how boat noise affects fish and other marine life so that policy makers can implement effective solutions to negate the harmful impacts of noise pollution. Therefore, it would be useful for future studies to incorporate a noise mitigation treatment, which could help us determine what type of regulations might benefit the organisms facing detrimental effects of noise pollution. For example, two recent field studies examined the effects of motorboat noise on fishes and included a mitigation treatment, where the same motorboat used in the noise treatment was then driven more slowly and farther away from the experimental subjects (McCloskey et al., 2020; Mills et al., 2020). In both studies, there was a negative effect of noise but no significant difference between the ambient and mitigated-noise treatments.

In summary, my thesis results add to the growing evidence that noise negatively affects fishes by demonstrating that plainfin midshipman fish can alter their nest defence (Chapter 2) and vocalizations (Chapter 3) in response to boat noise. I show the importance of considering testing context when designing noise studies, as the results of the lab and field studies in Chapter 2 differed. I also provide one of the first documentations of vocal adjustments in response to noise in fishes, and I am the first to document amplitude and frequency changes in wild fish vocalizations using a real motorboat as a noise stimulus. These results, along with future studies on other fish species, will help solidify our understanding of how noise affects fishes and guide policy and suggest possible solutions to mitigate the harmful effects of noise pollution on marine life.

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Appendices

Appendix A: Supplemental materials for Chapter 2

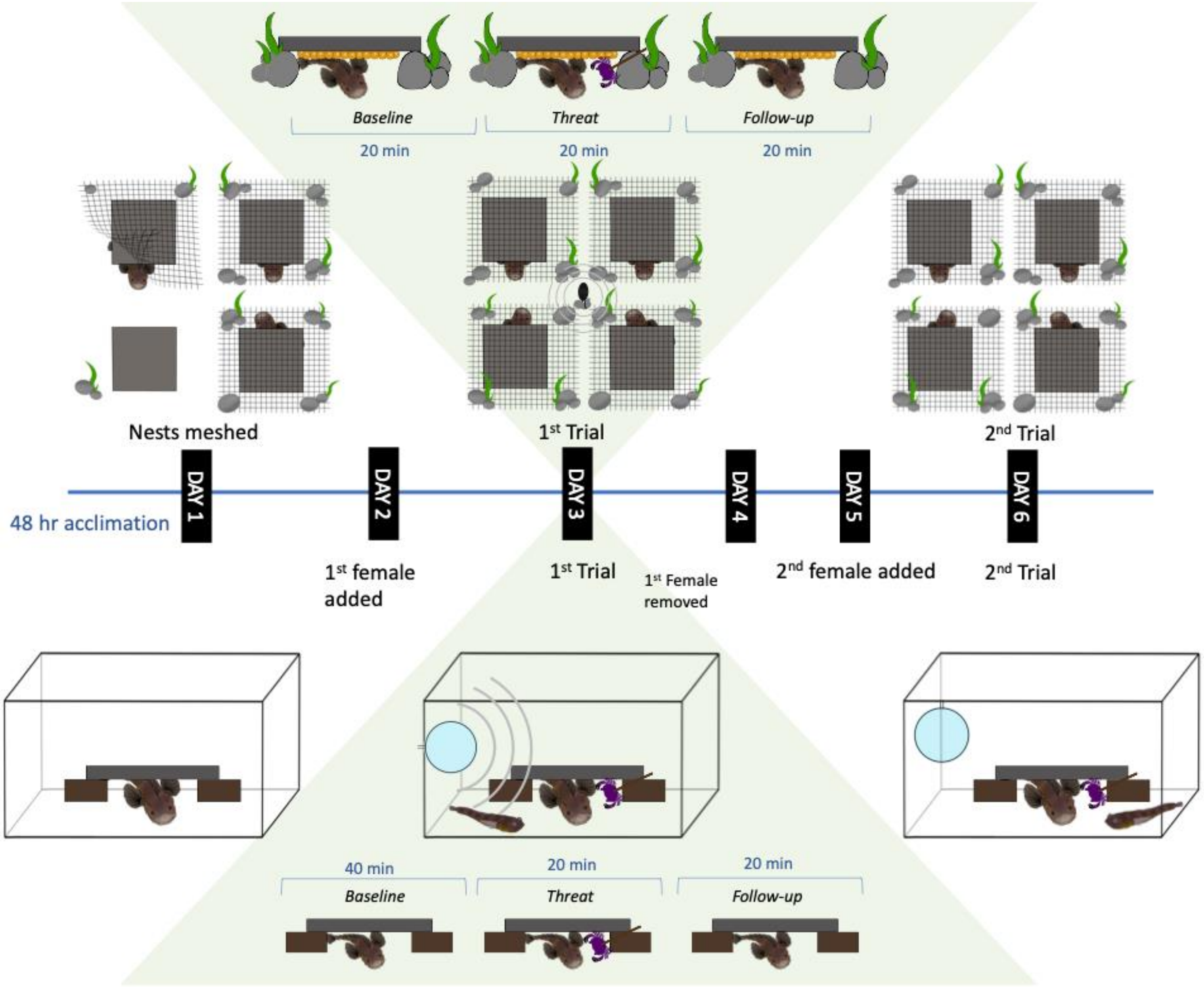

Figure S2.1 Schematic methods diagram showing the six-day trial sequence and three-period experimental trials in the field (top) and laboratory (bottom) experiments. The order of noise and ambient treatments was determined randomly for each fish; in this figure, the first trial (Day 3) is shown as a noise trial and the second trial (Day 6) is shown as an ambient trial.

Appendix B: Supplemental materials for Chapter 3

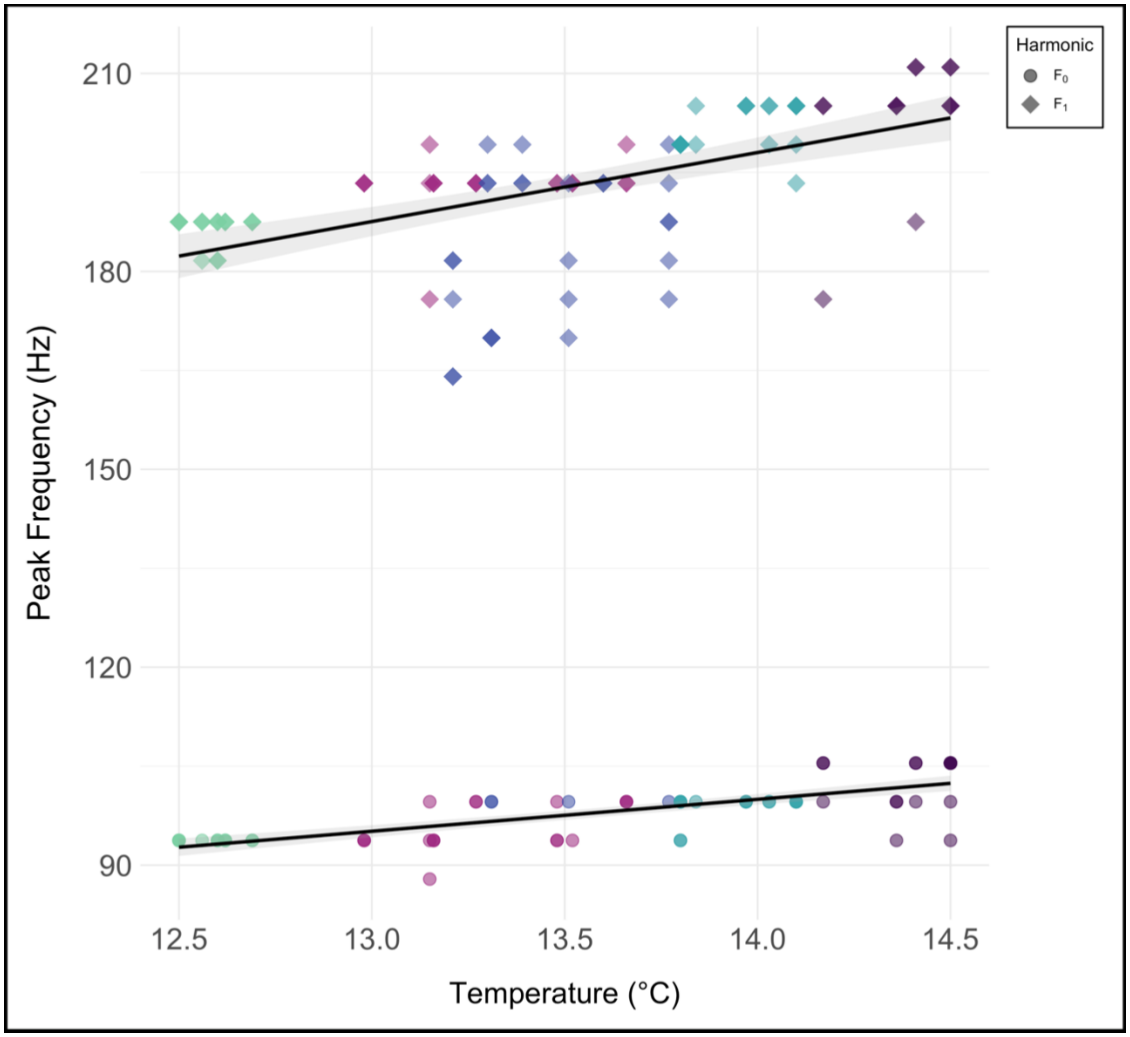

Figure S3.1 Hum peak frequency vs. temperature. Relationship between water temperature (avg. temperature over each 10-min trial period) and the fundamental frequency (F0) and first harmonic (F1) of all the mating hum. Colours represent the five nest groups. Black trendlines represent linear models and standard error for harmonic.

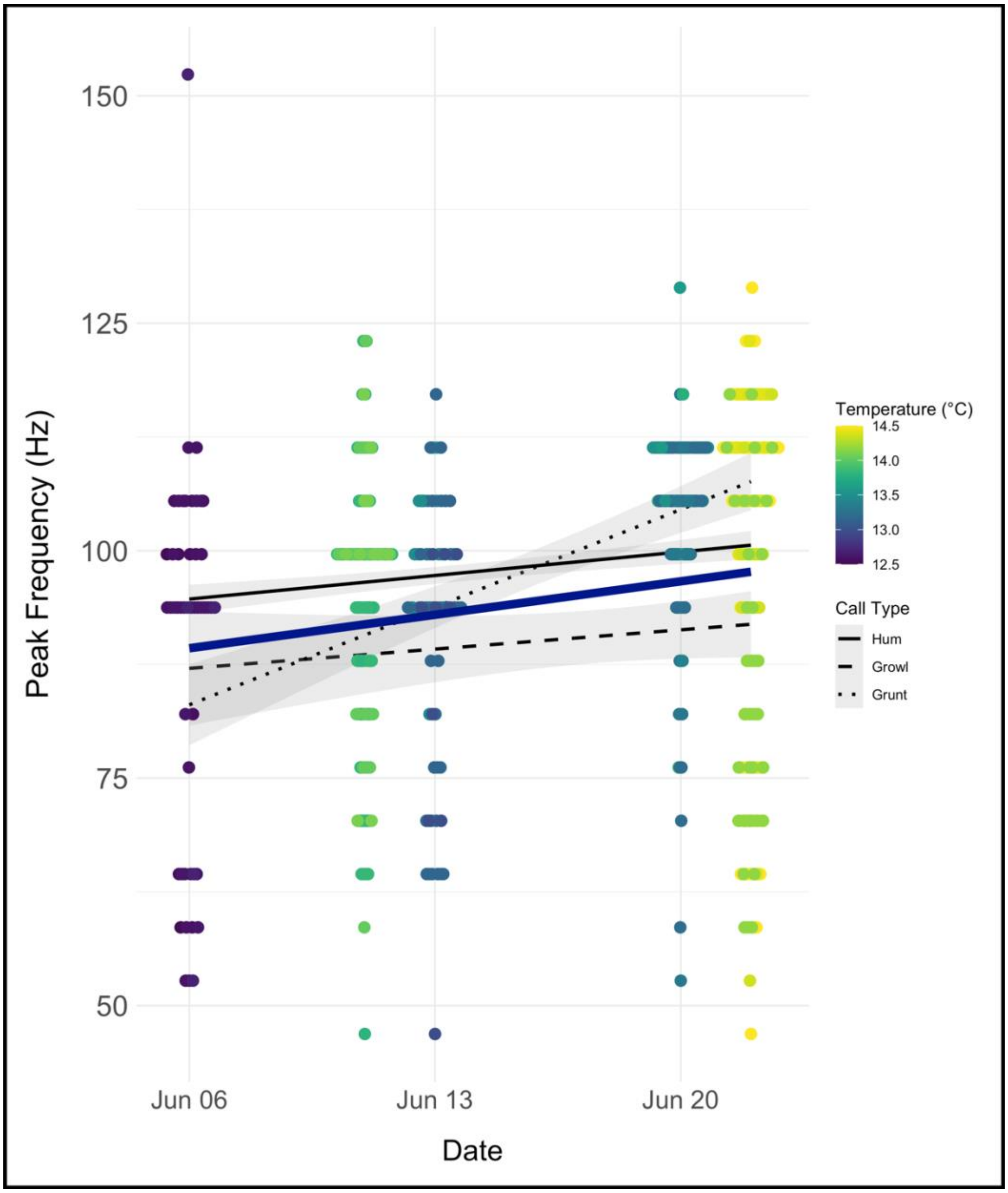

Figure S3.2 Peak frequency and temperature by date. Relationship between trial date, water temperature (avg. temperature over each 10-min trial period), and the fundamental frequency (F_0) of all three call types. Hum, growl, and grunt trendlines represent linear models with standard error and the thick blue trendline represents the overall relationship between all vocalizations and peak frequency. Each of the five nest groups was tested on a different date, ranging from June 6th to June 22nd, 2022.

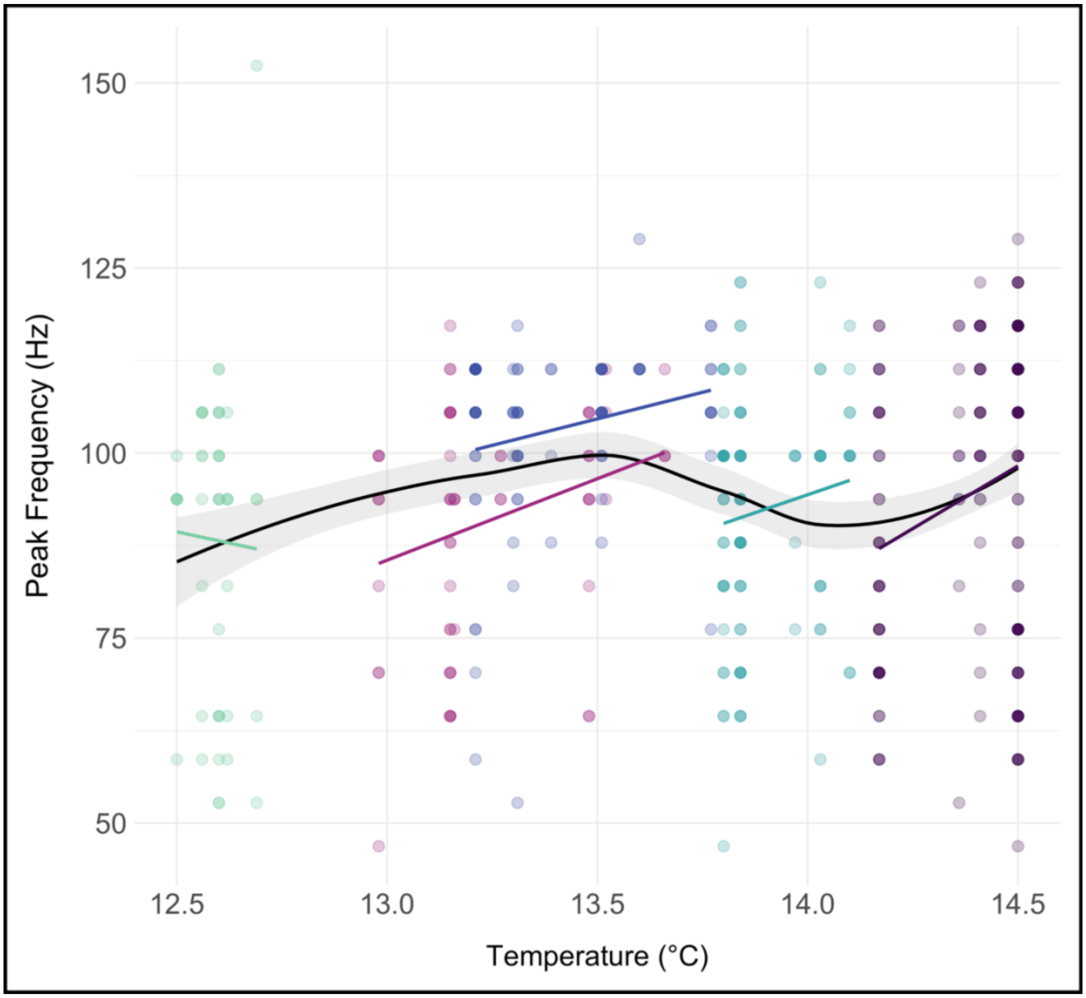

Figure S3.3 Vocalization F_0 peak frequency vs. temperature. Relationship between water temperature (avg. temperature over each 10-min trial period) and the fundamental frequency (F_0) of all three call types. Colours represent the five nest groups. Coloured trendlines represent linear models for each nest group and the black overall trendline = geom_smooth with standard error.