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The effects of vessel noise on the antipredator behaviour of juvenile reef fishes

Thesis submitted by

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Statement of Contribution of Others

This thesis was conducted under the supervision of Mark McCormick and all chapters include his conceptual guidance, data analysis advice and editorial assistance. In **Chapter 2,** Eric Fakan participated in conceptual development, and technical and editorial assistance. In **Chapter 4**, Sophie Nedelec contributed conceptual guidance, data analysis advice and editorial assistance. In **Chapter 5,** Steve Simpson, Andrew Radford and Sophie Nedelec participated in conceptual development. Additionally, Sophie Nedelec provided data analysis advice. Peter Gatenby participated in technical assistance, data collection and data analysis. All co-authors provided editorial assistance.

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General Abstract

Vessel noise is increasing in marine environments as a result of human activities, changing marine soundscapes and having negative effects on the behaviour and physiology of some fish species. The recent recognition of this stressor as a pollutant by the World Health Organisation highlights the necessity of conducting research to provide governmental institutions with information for its management and regulation. Shipping is the biggest contributor of underwater noise, however, its effects on coral reef fishes are unknown. Coral reefs are particularly at risk from ship noise as many ports and shipping routes are located close to reefs. At the same time, coral reefs are often exposed to noise from small boats, which is better understood in terms of the impacts on behaviour and survival of reef fish. However, the long-term effects of small boats and whether reef fish can acclimate to exposure are unknown. In this thesis, I experimentally investigated the effects of ship and small motorboat noise on the antipredator behaviour of juvenile reef fishes. This thesis used three focal damselfish species as they are ecological important, common on coral reefs and there is knowledge about their hearing ranges. By conducting tank-based experiments, I was able to control for acoustic disturbances and environmental variation, to recreate a predator strike that was constant over trials (a startle stimulus) and to record in detail the response of individuals at high speed under different acoustic conditions. The tank was large (370 L) and the position of the experimental arena was optimized within the tank to minimise acoustic artefacts.

The noise produced by ships and small boats differs in frequency, intensity, duration and temporal structure due to differences in size and engine type. Although, the effects of 4-stroke outboard powered motorboats have been previously studied the effects of ship noise in juvenile reef fishes are unknown. My first objective was, therefore, to investigate the effects of ship noise playback on the antipredator behaviour of a juvenile reef fish and to compare its effects to that of 4-stroke powered motorboats. I exposed Whitetail damselfish juveniles (*Pomacentrus chrysurus*) to ship, 4-stroke motorboats or reef playback and recorded their routine swimming and escape response. Both 4-stroke motorboat and ship noise playbacks affected the routine swimming and escape response of Whitetail damselfish compared to individuals that were

exposed to the reef playback treatment, however, the magnitude of the effects differed. Fish exposed to ship noise moved shorter distances and responded more slowly (higher response latency) to the startle stimulus compared to individuals under the 4-stroke noise treatment. These results suggest that 4-stroke and ship noise can affect the activity and escape response of individuals to a simulated predation threat, potentially compromising their anti-predator behaviour. Additionally, these results indicate that the effects of ship noise and 4-stroke motorboats should not be generalised.

Habitat complexity has the potential to induce variations in the temporal structure of ship noise, yet the influence on behavioural outcomes has not been studied. Most studies have focused on individual's responses to abrupt noise, however, fish are more likely to be exposed to ramp-up noise in their natural environment as the noise source approaches from a distance. My second objective was, therefore, to compare the effects of ramp-up ship noise and abrupt ship noise on the anti-predator behaviour of juvenile Ambon damselfish (*Pomacentrus amboinensis)*. I found that ramp-up ship noise did not affect the routine swimming of juveniles, but their escape latency increased compared to juveniles exposed to reef sound. In contrast, abrupt ship noise affected both the routine swimming and escape response of juveniles, leading to a decrease in activity and an increase in response latency. These results indicate that ecologically important behavioural traits can be affected differently by ramp-up and abrupt ship noise and highlights the importance of including realistic temporal structures of noise in future studies. Additionally, these findings also highlight the importance of studying behavioural traits related to survival.

Fishes are likely to be exposed to multiple environmental stressors in their natural environment. Understanding how these stressors interact is critical, not only to understand their effects on marine communities but also for management. Ship noise and elevated temperature are two stressors likely to interact, as the speed of sound increases as temperature increases. My third objective was, therefore, to investigate the combined effects of elevated temperature and ship noise playback on the predator-prey interactions of two common coral reef fish species. I exposed prey (*Pomacentrus amboinensis*) and predators (*Pseudochromis fuscus*) to either a present-day control (29°C) or elevated temperature at levels

predicted for the end of the century (31°C). After 7 days, prey and predator interactions were recorded while being exposed to either reef playback or ship noise playback. I found that elevated temperature decreased prey escape speed. However, this did not result in higher capture success by the predator and behavioural traits of the predators were not affected by elevated temperature. These results suggest predators are more resilient to warming than prey. Additionally, I found no effects of ship noise playback and no evidence of an interaction between stressors. Thus in a scenario where elevated temperature and ship noise co-occur, elevated temperature is likely to be the dominant stressor.

Small boat noise has been found to affect the physiology, behaviour and survival of embryos, juveniles, and adults; however, it is currently not known whether effects persist beyond the brief period of noise exposure or are cumulative throughout life. My fourth objective was to evaluate the long-term effects of boat noise on growth and escape response of Spiny chromis (*Acanthochromis polyacanthus)*. Using a split-brood design, fish were exposed to playback of boat noise or reef sound prior to hatching (parents and embryos) and/or as juveniles for up to 78 days. Juveniles were tested for their escape response in the absence of noise and measured for growth. I found that individuals exposed to boat noise as juveniles were smaller compared to those exposed to reef sound, regardless of whether they were exposed to boat noise or reef sound prior to hatching. I also found that individuals exposed to boat noise prior to hatching and as juveniles were less likely to respond to a simulated predator attack than those that had experienced only reef sound, and when they did respond they were more likely to swim towards the predator. These results demonstrate that exposure to boat noise during early development has the potential to affect growth and disrupt the escape response of juveniles, even when the response occurs in quiet conditions.

The overall aim of this thesis was to examine the effects of ship noise and the long-term effects of small boat noise on the antipredator behaviour of juvenile reef fishes. Results indicated that ship noise can affect the antipredator behaviour of juveniles by reducing their activity and increasing their response latency. However, when prey and predators were exposed to ship noise I did not find effects on predator-prey interactions. Additionally, I found that small boat noise can have long-term effects on the growth and

escape response of juveniles. By testing different temporal structures of noise and exposing individuals to realistic exposure scenarios I increased the ecological significance of my results. These are the first studies to examine the effects of ship noise on coral reef fishes, how it might affect behavioural traits directly related to fitness and whether organisms might acclimate to long-term small boat noise exposure. Future studies should aim to test my findings in field conditions. Understanding how reef fishes respond to vessel noise remains a priority as it will allow for the development of more efficient management and mitigation policies.

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Chapter 1: General Introduction

1.1. Underwater sound and noise

Sound is possibly the most effective way of transmitting information underwater. Sound travels faster, further and is less affected by environmental variables compared to other sensory cues such as light and chemical odour (Urick 1983, Slabbekoorn et al. 2010). As a result, marine organisms have developed mechanisms to detect and produce sound, including whales, dolphins, fishes and invertebrates. Fishes are the most diverse vertebrate group in marine ecosystems and all fish species are believed to be able to detect and produce sound either voluntary or involuntary (Popper and Hawkins 2018). Fishes can detect sound by perceiving changes in particle motion or sound pressure (Urick 1983). Particle motion is detected by the inner ear and/or lateral line, while sound pressure is detected with gas-filled organs, such as the swim bladder, located next or close to the inner ear (**Table 1.1**; Popper and Fay 2011). Fishes can encounter both biotic and abiotic sounds (Pijanowski et al. 2011). Biotic sounds are those produced by living organisms. While abiotic sounds result from environmental phenomena, for example, wind, rain, thunder or geological activities (Popper and Hawkins 2018). Fish can produce involuntary sounds as the result of feeding, swimming, splashing or jumping (Rountree et al. 2018). Voluntary sounds are produced with organs such as the swim bladder, bones or muscles (reviewed in Putland et al. 2018), by drumming or stridulation (i.e., striking or rubbing body parts together; Kaatz and Lobel 1999, Amorim 2006). The ability to produce and detect sound indicates that it is a sensory cue actively used by fishes.

Table 1.1. Terminology used in this thesis. Modified from: Urick (1983) and Bass and Clark (2003).

Concept	Definition
Sound	Waveform that travels through a medium transferring energy
Sound pressure	Particles within the sound wave oscillate backwards and forwards moving closer together (i.e., compression) or further apart (i.e., rarefaction).
Particle motion	Individual particles do not travel with the wave but move backwards and forwards, transmitting their oscillatory energy to other particles.
Noise	Unwanted sounds that are considered unpleasant, loud, or disruptive.
Vessel noise	Noise produced by ships and recreational boats

Fishes use sound all throughout their life cycle and display multiple prominent acoustic behaviours. During the larval stage, sound is used for orientation and habitat selection, influencing the settlement success of individuals onto, for example, coral reefs (Gagliano et al. 2008, Vermeij et al. 2010). Juvenile fishes use sound for navigation (Radford et al. 2011), as well as to locate and migrate to new habitats (Simpson et al. 2008). Adult fishes produce sound to synchronise spawning aggregations or in courtship interactions (Mann and Lobel 1997, Amorim et al. 2015), for communication by alerting conspecifics about predators (Tricas and Boyle 2014), to defend territories (Tricas and Boyle 2014) or during antagonistic interactions towards conspecifics or heterospecifics (Mensinger 2014). Sound is therefore a key environmental cue used by fish throughout their life history and behaviour, and fish communities are strongly influenced by sounds occurring in their surrounding environments.

Human activities in marine ecosystems produce additional sound known as anthropogenic noise. Anthropogenic noise is often louder, and it directly overlaps with the frequency range of natural sounds and is within hearing ranges of most fishes (**Figure 1.1;** Hildebrand 2009). Sources of anthropogenic noise include aircraft noise, pile driving, seismic guns, tidal energy machinery, wind farms, ships and boats (reviewed in Rako-Gospic and Picciulin 2019). In the last 50 years, anthropogenic noise has increased significantly in marine ecosystems (Frisk 2012), compromising the role of underwater sound. In 2011, the World Health Organisation categorised anthropogenic noise as a form of pollution that has become omnipresent in marine ecosystems (WHO 2011), highlighting the need to understand its effects on marine organisms. While the number of studies that examine the impacts of noise on marine organisms has dramatically increased overall, we still have a very poor understanding of how noise affects population dynamics for any marine fish species (**Figure 1.2**). In Australia, the Great Barrier Reef Marine Park Authority considers noise pollution a threat, but the effects of some sources (e.g., shipping and motorboats) are poorly understood, as stated in the 2019 Outlook Report "little is known about the effects of noise on the Region's species" (GBRMPA 2019). In coastal areas, ships and small boats represent the main sources of noise pollution (Haviland-Howell et al. 2007, Bittencourt et al. 2014), therefore investigating their effects in the context of coral reefs is critical to develop effective mitigation and management strategies.

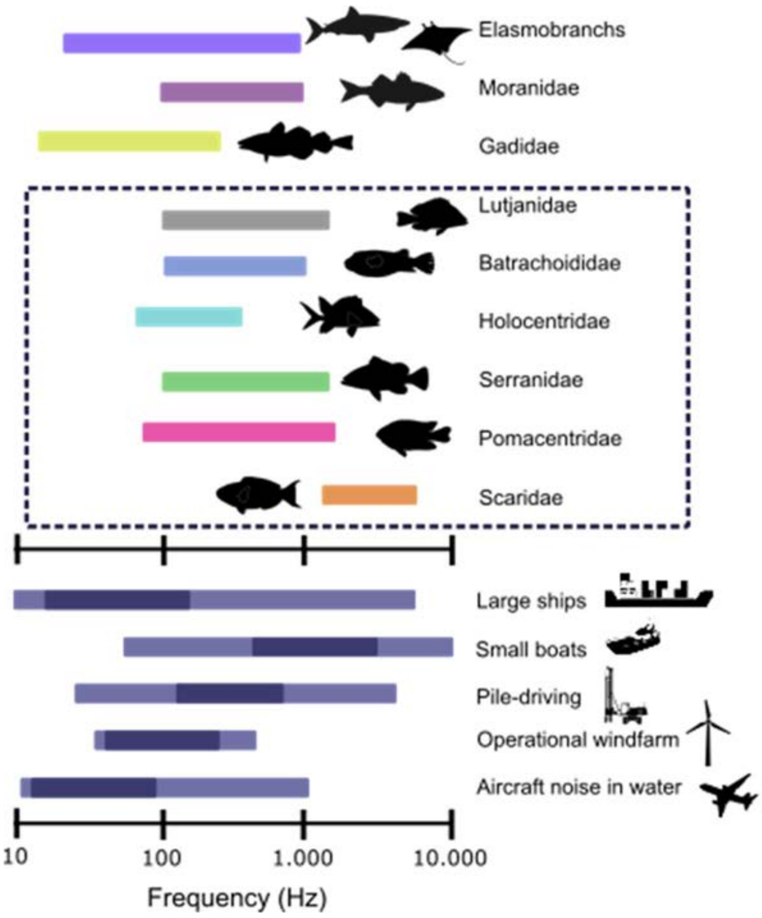

Figure 1.1. Hearing ranges of selected fish groups, dash box indicates reef fish families. Darker areas correspond to the acoustic energy of the dominant frequency range of each source (adapted from Slabbekoorn et al. 2010, Putland et al. 2018, Duarte et al. 2021).

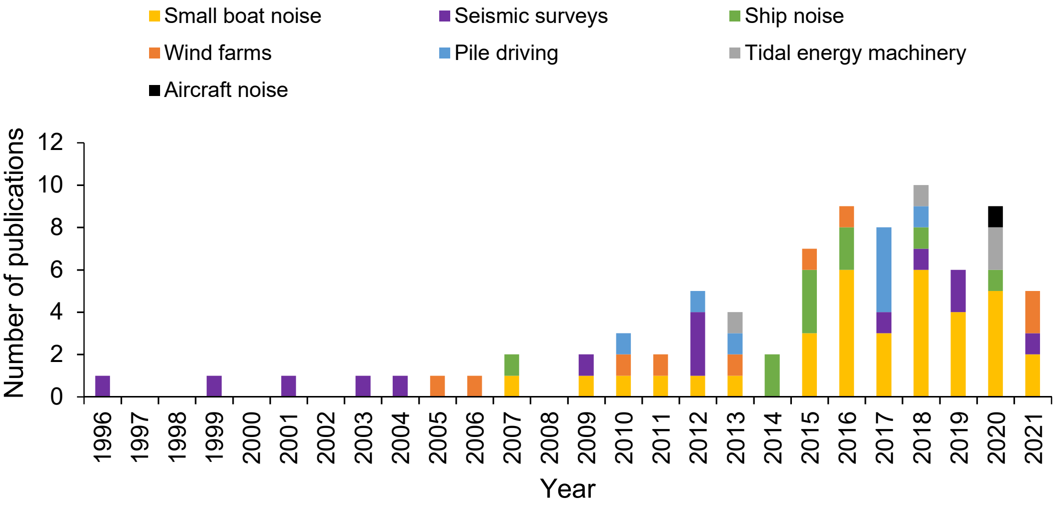

Figure 1.2. Number of publications examining the effects of different anthropogenic noise sources on marine fishes up to July 2021. Literature search was done in Web of Science using the keywords: "fish" AND "marine" AND "noise". Literature reviews and white/brown noise studies were excluded.

1.2. Sources of marine noise

Shipping is the biggest contributor of anthropogenic noise in marine environments (McKenna et al. 2012). Shipping is directly related to world economy and trade, and 80% of world merchandise trade is carried by sea. In 2019, approximately 11,076 millions of tons of cargo were transported by shipping and by early 2020 the world fleet amounted to 98,140 ships. Between 2019 and 2020, the world shipping fleet grew by 4.1%. Although the global economy was affected by Covid-19, this only resulted in a lower increase in global trading (0.5% increase; UNCTAD 2020). The increase in shipping in the last 50 years has led to an increase in ship sizes, ship numbers and longer transit routes (Kaplan and Solomon 2016, UNCTAD 2020). In 2014, the International Maritime Organisation released a set of guidelines for ships to reduce underwater noise, which included the design of more energy efficient propellers and required the reduction of noise on board ships for crew safety (IMO 2014). However, these guidelines are voluntary and not required by law to be included in the design of new ships (IMO 2014).

Table 1. 2. Ship arrivals and departures on the 11th of March 2021 in major ports located in areas with coral reef communities. Source: Marine Traffic 2021.

Country	Port	Departures (in 24 hours)	Arrivals (in 24 hours)	Ship transit per hour
China	Shanghai	1172	1260	101
Singapore	Singapore	1442	1450	120
Hong Kong	Hong Kong	737	890	68
Panama	Panama Pacific	81	75	7
Indonesia	Jakarta	62	71	6
Vietnam	Ho Chi Min	47	52	4
Indonesia	Batu Ampar	50	46	4
Australia	Townsville	35	34	3
Australia	Gladstone	18	19	1

Coral reefs are particularly vulnerable to ship noise, as many major ports and shipping routes are located close to coral reef areas. South East Asian nations contribute ~40% to overall maritime trade (UNDCAT 2020) and increases in ship traffic have led to the establishment of many large ports. Ports such as Shanghai, Singapore and Hong Kong receive more than 800 ships per day resulting in constant noise for coral reef communities located nearby (Goodkin et al. 2011, CSDHKSAR 2019, MPAS 2021). In Australia, shipping is a critical pillar of the economy (DITRDC 2020). Twelve ports are located near the Great Barrier Reef, with ships transiting two main routes, a route outside the coral reef lagoon, which runs along the edge of the continental shelf, and a shallower inner route that meanders around coral reefs. Ships that transit the reef enter using one of six major shipping channels, transiting in some cases just a kilometre away from coral reefs (**Figure 1.3**; AMSA 2021). It is worth noting that ship transits are much lower in Australia than in South East Asia, but there can still be transits of 1 to 3 ships each hour in some locations (**Table 1.2**), resulting in repeated noise exposure for nearby coral reef fish communities.

Coral reefs are also frequently exposed to noise from small motorboats. The growth of human settlements in coastal areas in the past decades has led to an increase in the use of such vessels, as indicated by boat registrations. For example, in the United States the number of small boats increased 1% per year between 1980 and 2017 (U.S. Department of Homeland Security 2018). In Queensland, Australia, the number of small boats registered increased by an average of 2.5% per year between 2005 and 2016, reaching 256,154 boats in 2016 (QLD Government 2016). Moreover, activities associated with the use of small boats, such as diving and fishing, are expected to continue to increase and they provide important benefits to the Australian economy (GBRMPA 2019).

Noise produced by ships and small motorboats can vary in intensity, frequency and duration, due to differences in vessel size and engine type. Noise produced by ships and boats is primarily associated with propulsion machineries, in particular propeller cavitation (Richardson et al. 1995). Cavitation occurs when a rotating propeller pushes water with its blades and forms collapsing bubbles which burst, resulting in noise (Jalkanen et al. 2018). Ships can produce sound levels of up to 205 dB re 1µPa in frequencies below 100 Hz (Richardson et al. 1995), while small boats produce quieter sound levels, up to 180 dB re 1µPa in frequencies ranging from 20 to 1000 Hz (Richardson et al. 1995). Ships also emit energy at higher frequencies, tens of kHz (Hermannsen et al. 2014, Veirs et al. 2016), whereas small boats have a broader frequency content and peak at high tonal frequencies (100 Hz to 6 kHz; **Figure 1.1**). The low frequency noise of ships can travel greater distances compared to the noise produced by small boats, as the low frequency components of ship noise suffer little attenuation by the environment (Hildebrand 2009). However, small boats can transit closer to or on top of coral reefs due to their smaller size. Additionally, although ships have a lower speed than small boats (on average 20 knots and 30 knots, respectively), ships transit longer routes resulting in longer periods of noise exposure.

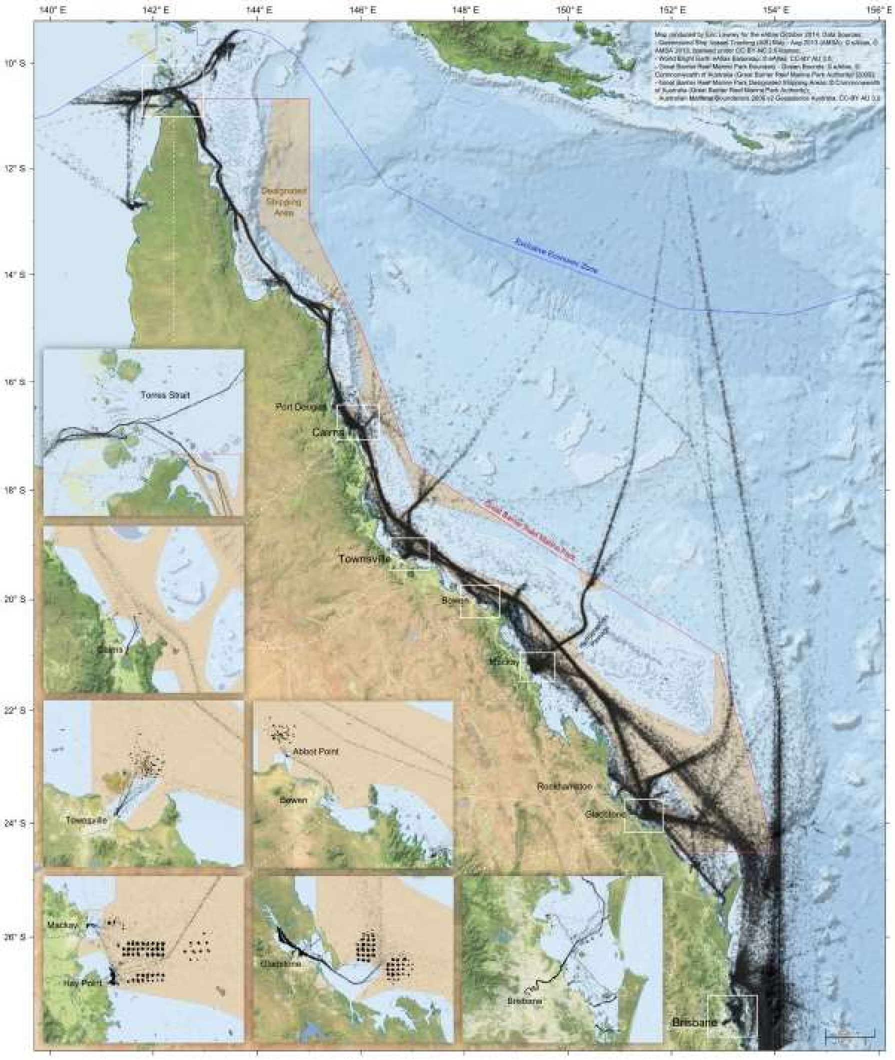

Figure 1.3. Ship traffic density along the Great Barrier Reef Marine Park in 2013. Designated shipping areas within the Marine Park are shown in orange. Each black dot represents a shipping vessel. Source: https://eatlas.org.au/

Studies evaluating the effects of ship noise have mostly focused on marine mammals (reviewed in Erbe et al. 2019). Our knowledge to date on marine fish is based on 9 studies on coastal fish species from around the world (8 species, **Figure 1.4**; see **Table A1** in **Appendix A** for details). Ship noise playback has been found to affect the physiology of Atlantic cod (*Gadus morhua*) embryos by altering the use of yolk sacs, hindering larval growth and disrupting their behavioural responses to predators (Nedelec et al. 2015). Ventilation rates of juvenile European seabass (*Dicentrarchus labrax*) were not affected by ship noise playback after one minute of exposure (Radford et al. 2016). For adults, ship noise has been found to affect communication of Lusitanian toadfish (*Halobatrachus didactylus*; Vasconcelos et al. 2007) and reduced the foraging success of Three-spined stickleback and European minnow (*Gasterosteus aculeatus* and *Phoxinus phoxinus,* respectively; Voellmy et al. 2014a). Also, ship noise has been found to disrupt the antipredator behaviour of Three-spined stickleback, causing individuals to respond faster to a simulated predator attack, while the antipredator behaviour of European minnow did not display any significant change (Voellmy et al. 2014b). Results from these studies suggest that ship noise can affect the behaviour of embryos and adults, however, the effects of ship noise on behavioural traits important for the survival of juvenile fishes are unknown. Moreover, to date the effects of ship noise on tropical coral reef fish species has not been studied.

Studies evaluating the effects of small motorboat noise have mostly focused on site-attached damselfishes (pomacentrids) inhabiting shallow coral reefs (**Figure 1.4**; **Table A1** in **Appendix A**). A total of 32 studies have been conducted, with 9 studies focused on small boats powered by 2-stroke outboard engines and 4 studies focused on 4-stroke engines (**Table A1** in **Appendix A**). During embryological development, boat noise playback has been found to stress embryos resulting in smaller yolk-sacs and higher heart rates (Jain-Schlaepfer et al. 2018, Fakan and McCormick. 2019). Boat noise has been found to affect offspring survival (Nedelec et al. 2017) and interfere with habitat selection when larvae are settling to coral reefs at the end of their larval phase (Simpson et al. 2005, Holles et al. 2013). After settlement, boat noise has been found to affect the boldness and activity of juveniles (Holmes et al. 2017) and to impair juvenile's antipredator behaviour resulting in higher mortality (Simpson et al. 2016). In adults, boat noise can disrupt the antipredator response (Harding et al. 2020), parental care (Nedelec et al. 2017) and reproductive behaviour of fishes (Nedelec et al. 2017). Most of these studies have looked at a single life stage in the presence of boat noise. Studies examining inter-generational exposure and carryover effects once boat noise has stopped are currently missing.

At present, ships and small boats are the most common sources of anthropogenic noise in coral reefs. Although previous studies have found that small boats can affect the antipredator behaviour of juvenile fishes the effects of ship noise on coral reef fishes are unknown. Based on the differences in engines and sizes between ships and small boats, it is likely that the effects of the two sources will differ. Therefore, it is important to study their effects individually to determine the extent to which impacts can be generalised across noise sources. It is only then that effective management measures can be enacted.

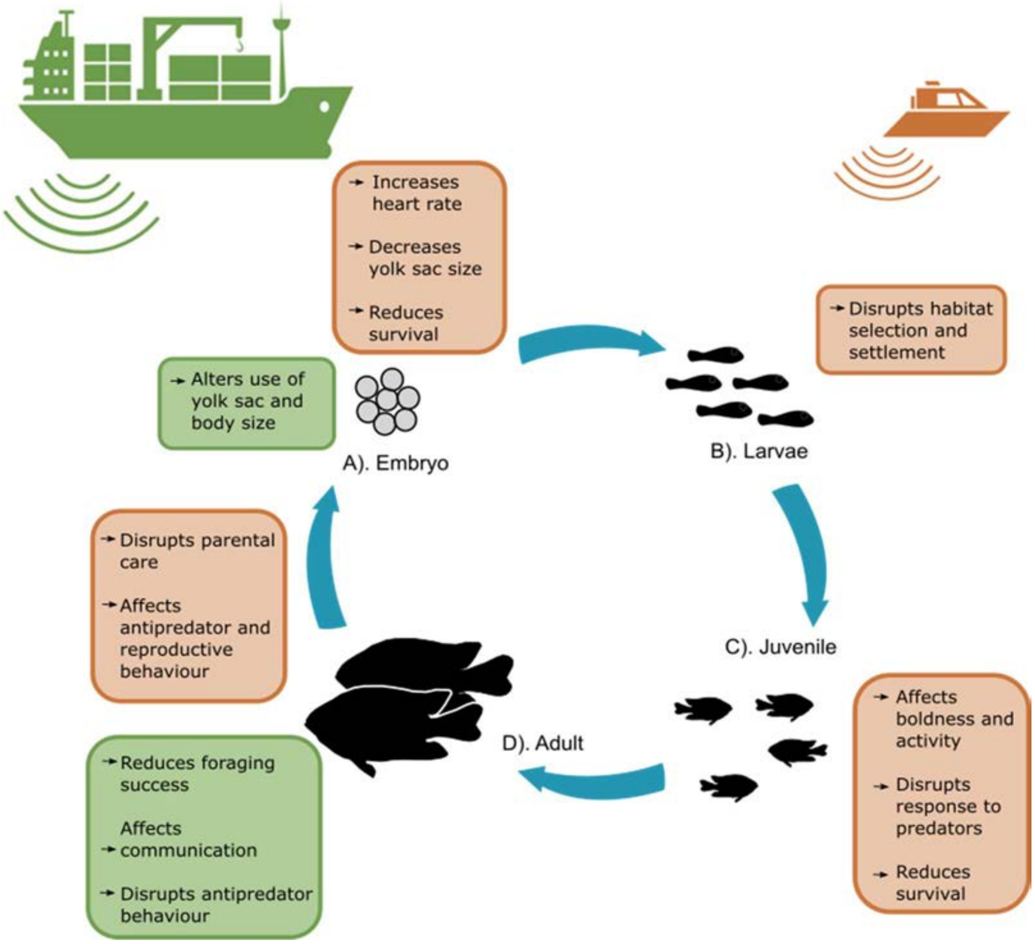

Figure 1.4. Effects of vessel noise on marine fishes at different life stages: A) Embryo, B) Larvae, C) Juvenile and D) Adult. Green boxes indicate effects of ship noise and orange boxes indicate effects of small boats. Based on **Table A1** in **Appendix A**.

1.3. Influence of the environment on noise received by fish

Sound transmission and propagation in shallow water are highly influenced by the environment (Bass and Clark 2003). Depth, seabed composition and environmental conditions such as temperature and salinity influence sound wave propagation (Simmons 2004) and in doing so alter the temporal structure of the noise received by organisms in their habitats. Coastal areas and coral reefs are highly complex habitats with sudden changes in depth, seabed composition and temperature (Graham and Nash 2013). As a result, sound waves are constantly interacting with the environment. When sound waves encounter a barrier they can be refracted, reflected, diffracted or absorbed (Bass and Clark 2003). Refraction occurs when waves move between media with different densities and change direction, for example, when a sound wave meets a thermocline resulting in the sound wave bending (Zhao et al. 2015). Reflection occurs when sound waves bounce off a medium with a different impedance. This can occur when sound waves encounter hard structures such as headlands, islands or reefs, resulting in a phenomenon known as acoustic shadows (Gerstein et al. 2005). Diffraction occurs when sound waves pass through a gap or around a barrier, for example, when low frequency sounds go around small structures such as a patch reef (e.g., coral bommie or boulder). Lastly, sound waves can also be absorbed depending on the physical characteristics of the barrier and the angle at which the wave meets the barrier (Duncan et al. 2009).

The impacts of noise on fish are not only determined by sound intensity, but also by temporal structure (Neo et al. 2014, 2016). Temporal structure refers to the variation in sound intensity and frequencies over time and how it is perceived by organisms (Hastings and Popper 2005). For example, ship noise could slowly increase in intensity if there are no barriers between the ship and the fish. This results in ramp-up noise. If, on the other hand, there is a barrier (e.g., an island or reef) the fish will only perceive the noise after the ship has passed the barrier, resulting in abrupt noise. Most studies evaluating the effects of vessel noise on fish have looked at responses during the highest sound intensity. However, ramp-up noise is more likely to occur in natural environments and this is particularly relevant when studying ship noise. Ships have lower speeds and the low frequency components of the noise they produce suggest that their noise can travel greater distances, reaching fish hundreds of metres away. Whether variations in the temporal structure of ship noise may affect coral reef fishes is unknown.

1.4. Interactive effects of noise with other stressors

Marine organisms are under threat from multiple environmental stressors (Côte et al. 2016). Climate change is a key driver of these stressors. Recent research shows that the ocean is conforming to predictions, with increasing water temperatures (Gleckler et al. 2012, Hoegh-Guldberg et al. 2018), reductions in pH (ocean acidification; Hoffman et al. 2011) and increased frequency of severe storms (Bhatia et al. 2019). These environmental stressors are likely to interact in unexpected ways. The combined effects of multiple stressors can be more (synergistic) or less (antagonistic) than their individual effects (Galic et al. 2018), resulting in 'ecological surprises' that can accelerate biodiversity loss (Brook et al. 2008) and disrupt ecosystem functioning (Folke et al. 2004). Vessel noise is likely to interact with other environmental stressors. To date, only one study has evaluated whether vessel noise interacts with other environmental stressors. In a tank-based experiment, McCormick et al. (2018a) found that elevated CO_2 and boat noise did not interact to influence predator-prey interactions of two common coral reef fish species, even though both stressors have been found to have effects when tested in isolation (Allan et al. 2015, Simpson et al. 2016). Interactive effects of vessel noise with other stressors, such as elevated temperature, are currently unknown. Global warming is the most imminent threat to marine ecosystems, leading to an increase in frequency, intensity and duration of marine heatwaves (Oliver et al. 2018). Heatwaves are abnormal periods of high sea surface temperature (Oliver et al. 2018) that have been found to affect the physiology and behaviour of fishes (Johansen et al. 2014, Allan et al. 2015, Schulte 2015). Sound propagation and attenuation are highly influenced by temperature (Bass and Clark 2003), suggesting that elevated temperature could potentially interact with vessel noise. Studying how multiple stressors interact will contribute to our ability to predict how environmental change may affect marine populations and communities.

1.5. Long term effects of vessel noise

Most studies investigating the effects of vessel noise have focused on a single life stage. However, coral reefs are likely to be exposed to noise repeatedly when multiple vessels visit or transit an area over long periods of time (**Table 1.2**; Leopold and Camphuysen 2008, Brandt et al. 2011). Organisms that experience a stressor over long periods of time can respond with different mechanisms that might allow them to cope and maintain their performance. One option is acclimation through phenotypic plasticity (Angilletta 2009), which can be permanent when developmental acclimation occurs during early ontogeny, or temporary as a response to daily or seasonal exposures. Acclimation can also occur across generations. Exposure of parents to a stressor can influence how their offspring might cope with the stressor, as a parent's condition strongly influences its offspring (reviewed in Green 2008). Transgenerational plasticity has been found to occur in fish exposed to elevated temperature (Donelson et al. 2012, Donelson and Munday 2015). Spiny chromis damselfish (*Acanthochromis polyacanthus*) parents exposed to elevated temperature altered the sex determination of their offspring, by reducing the proportion of females, compared to parents exposed to ambient temperature. Previous studies on effects of vessel noise have been conducted over too short periods of time to test for acclimation. However, phenotypic plasticity has been found to be more common in early life stages (Fox et al. 2019) suggesting that acclimation to vessel noise could be possible. No research has yet been conducted to explore the long-term effects of vessel noise exposure on fish.

1.6. Field, playback and laboratory experiments

Both field and tank-based experiments have been used to examine the effects of vessel noise on marine organisms (e.g., Simpson et al. 2016). Both types of studies have advantages and disadvantages. Field studies have the best representation of noise-source acoustics when a real source is used. However, they are more expensive, logistically challenging to conduct and often limit the type of organism variables that can be measured. Field acoustic studies also have less control over environmental variables (e.g., light) and human disturbance (e.g., passing boats or planes). On the other hand, tank-based experiments allow for a high control of environmental variables, including acoustics and light, and ensure that every individual receives the same stimulus. Unfortunately sounds are usually not well represented in tank environments due to boundaries that are highly reflective of sound (Rogers et al. 2015, Slabbekoorn 2016), making interpretation more problematic. Playback experiments also have disadvantages when conducted either in the field or in tanks. Underwater speakers can produce sound artefacts and increase particle motions levels in relation to sound pressure levels (Slabbekoorn 2016). Although differences in sound propagation between field and tanks have been acknowledged (Parvulescu 1964, Kaatz and Lobel 2001), some studies have found consistent results when conducting field and tank-based experiments simultaneously. For example, Simpson et al. (2016) found juvenile damselfish placed in a predator-prey scenario had increased mortality when exposed to real motorboats in the field and when exposed to motorboat noise playbacks in the laboratory. Simpson et al. (2015) also found similar effects of ship noise playback on the metabolic rates of European eels (*Anguilla anguilla*) when individuals were exposed in the field or in the laboratory. Lastly, Pieniazek et al. (2020) found that boat noise affected foraging and the swimming behaviour of multiple freshwater fish species in both field and laboratory studies. All studies assessing the effects of ship noise on fish have been conducted in the laboratory using playbacks, while 78% of studies on the effects of small boats have been conducted in the field (**Table A1** in **Appendix A**). While conducting field experiments represents the ideal scenario, tank-based experiments can provide important insights and can lead to predictions that future technology may allow to be tested in the field.

1.7. Thesis outline

The overall aim of this thesis was to investigate the effects of ship and motorboat noise on mechanisms that influence the survival of juvenile reef fishes. I focus my research on juveniles as this represents a critical life stage, where an average of 60% of individuals are preyed upon within two days of settling onto coral reefs (Almany and Webster 2006). Newly settled fishes represent the next generation entering the community and are prey for many predators. Additionally, I focused on Damselfishes (Pomacentridae), as they are one of the most abundant and diverse families on coral reefs and as planktivorous play a critical role transferring nutrients from the water column into coral reefs (Williams and Hatcher 1983, Pinnegar and Poluin 2006, Emslie et al. 2019). As juveniles, pomacentrids represent an important prey for predators and any factor that influences their survival can affect prey population dynamics and community structure, and can mediate cascading effects in food webs and influence the likelihood of rapid extinctions (Orrock and Fletcher 2014).

This thesis uses laboratory experiments and playback of boat and ship noise to address the detailed mechanics of how various aspects of vessel noise influence predator-prey dynamics. In addition to this introduction, this thesis is written as four stand-alone chapters that represent publications in journals (one published (Velasquez Jimenez et al. 2020), one submitted and two in preparation). In **Chapter 2,** I examine and compare the effects of ship noise playback and four-stroke motorboat playback on the antipredator behaviour of juvenile Whitetail damselfish (*Pomacentrus chrysurus),* providing evidence for the first time of the effects of ship noise on a coral reef fish species. In **Chapter 3,** I investigate the influence of different temporal structures of ship noise on the antipredator behaviour of juvenile Ambon damselfish (*Pomacentrus amboinensis)*. I compare two different ship noise temporal structures that are likely to occur in coral reefs: ramp-up noise and abrupt noise, and examine whether it influences the overall effects of ship noise on antipredator behaviour. In **Chapter 4,** I examine the interactive effects of ship noise and elevated temperature on predator-prey interactions. I use a common damselfish, Ambon damselfish (*Pomacentrus amboinensis)*, as prey and a common predator, Dusky dottyback (*Pseudochromis fuscus).* The elevated temperature treatment of 31ºC is projected to occur by the end of the century. Finally, in **Chapter 5,** I investigate the long-term effects of boat noise on growth and escape response of juvenile Spiny chromis (*Acanthochromis polyacanthus)*. Parents and embryos were exposed to either ambient or boat noise playback. After embryos hatched, juveniles were randomly split into ambient playback or boat noise playback groups and exposed for a further 41 days. Juveniles were subsequently measured. I then explore the potential carryover effects of boat noise on the escape response of juveniles by testing them in the absence of noise. This thesis advances our understanding of the potential effects of vessel noise on behavioural traits directly related to the fitness of reef fishes, particularly filling critical knowledge gaps on the effects of ship noise. Understanding the effects of this pollutant will be critical to prioritising and managing noise pollution in coral reefs.

Chapter 2: Vessel noise affects routine swimming and escape response of a coral reef fish

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Vessel noise affects routine swimming and escape response of coral reef fish

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2.1. Abstract

An increasing number of studies have shown that anthropogenic noise can negatively affect aspects of the anti-predator behaviour of reef fishes, potentially affecting fitness and survival. However, it has been suggested that effects could differ among noise sources. The present study compared two common sources of anthropogenic noise and investigated its effects on behavioural traits critical for fish survival. In a tank-based experiment we examined the effects of noise from 4-stroke motorboats and ships (bulk carriers > 50,000 tonnes) on the routine swimming and escape response of a coral reef fish, the Whitetail damselfish (*Pomacentrus chrysurus*). Both 4-stroke boat and ship noise playbacks affected the routine swimming and fast-start response of Whitetail damselfish, however the magnitude of the effects differed. Fish exposed to ship noise moved shorter distances and responded more slowly (higher response latency) to the startle stimulus compared to individuals under the 4-stroke noise treatment. Our study suggests that 4-stroke and ship noise can affect activity and escape response of individuals to a simulated predation threat, potentially compromising their anti-predator behaviour.

2.2. Introduction

Human activities are prevalent throughout marine environments. These activities contribute significant amounts of noise to marine soundscapes, thereby increasing overall ambient sound levels

(Hildebrand 2009, Slabbekoorn et al. 2010, Todd 2016). Recreational boating and commercial shipping are two of the most common sources of anthropogenic noise in marine ecosystems, particularly those found along the Australian coast (Small and Nicholls 2003). In 2014, there were 90,000 recreational motorboats registered in Queensland and 9,619 ships transited through the Great Barrier Reef. These numbers are projected to increase by 500 per cent and 250 per cent respectively by 2040 (GBRMPA 2019). Currently, the management of underwater noise in Australia is still in its early stages compared to international underwater noise regulations (GBRMPA 2017), which is partly due to a global lack of supporting scientific evidence. Therefore, research on the effects of vessel noise on marine organisms is required to develop effective management policies.

Sound is one of the most important sensory cues used by fishes to obtain information about their environment (Fay and Popper 2000). Fish use sound for predator avoidance, communication, navigation, orientation, reproduction and feeding (Fay and Popper 2000). Vessel noise can interfere with these functions in a number of ways, including acting as a distracting stimulus (Purser and Radford 2011), as a stressor (Smith et al. 2004, Wysocki et al. 2006, Nichols et al. 2015), or by masking important acoustic cues or signals (Codarin et al. 2009, Radford et al. 2014). Recent studies have found that interference with the ecological functions of fish by anthropogenic noise results in negative effects on the physiology and behaviour of at least some organisms regardless their life stage (Nedelec et al. 2017, Simpson et al. 2016, Neo et al. 2018). Therefore, anthropogenic noise is compromising the important role sound plays as an information source in marine ecosystems.

Distraction can have a critical effect on the outcome of predator-prey interactions (Wale et al. 2013). In a predator-prey scenario, the escape response of aquatic prey is usually represented as sudden changes in direction and acceleration as a consequence of a startle stimulus (i.e., predator strike) (Domenici and Blake 1997). Survival under natural conditions has been shown to be directly related to the speed of initiating an escape response (known as latency; McCormick et al. 2018b). Exposure to noise can affect the escape performance of individuals as a distracted prey may be less likely to respond to predators, which may reduce its likelihood of survival (Wale et al. 2013, Simpson et al. 2016). Moreover, since most marine fishes have complex life histories involving a dispersive larval phase and a reef-associated adult phase, any factor that influences mortality at critical life stages (Leis and McCormick 2002) such as metamorphosis and settlement, can lead to significant changes in the numbers reaching the next life stage (Houde 1987, Gordon et al. 2018).

This study presents results of a tank-based experiment that examined the routine swimming and detailed escape response of a juvenile reef fish, while investigating the effects of 4-stroke boat and ship noise in a controlled acoustic environment. Caution is needed when extrapolating results into the wild, as the use of tanks and speakers can result in sound fields that differ from the ones experienced by organisms in their natural environment (Rogers et al. 2015, Carroll et al. 2016). However, tank-based experiments allow for the control of confounding factors and detailed data collection (Slabbekoorn et al. 2016, Radford et al. 2016), and can contribute significantly to the understanding of anthropogenic noise (Wale et al. 2013, Simpson et al. 2015, Voellmy et al. 2014a). In our case it was possible to recreate a predator strike that was consistent over trials and examined in detail the effects of ship noise on behavioural traits critical for fish survival (McCormick et al. 2018b). Recent research suggests that the effects of anthropogenic noise and their magnitude can vary according to the source due to differences in their acoustic characteristics (e.g., frequency and power spectra; Radford et al. 2016, Jain-Schlaepfer et al. 2018, McCormick et al. 2018c). Therefore, our hypothesis was that ship playback would be more detrimental than 4-stroke motorboats playback due to differences in the sounds produced.

2.3. Materials and Methods

Study species and maintenance

The Whitetail damselfish, *Pomacentrus chrysurus*, is a common coral reef fish found throughout the Indo Pacific region. Typically found in shallow reef waters (<10 m), it has a bipartite life history with a planktonic larval stage maintained for 20-25 days before individuals settle onto a coral reef (Allen 1991, Ferrari et al. 2015). Previous research has shown that the hearing range of recently settled damselfishes is between 30 and 1000 Hz (Wright et al. 2010, 2011). Recently settled *P. chrysurus* juveniles (12.09 mm mean standard length) were collected overnight using light traps around Lizard Island Research Station (14.6680° S, 145.4638° E) and transported to the research station in 60 L tanks. Fish were identified to species level and placed in 30 L tanks for two days to acclimate. The flow-through seawater intake was placed below the surface to reduce ambient noise and no air-stone was present. Fish were fed twice a day with *Artemia spp*. Individuals were isolated and not fed for 24 hours prior to experimental trials in order to standardise for satiation.

All methods and research within this study were carried out in accordance with the animal ethics guidelines and regulations of James Cook University, and all protocols were approved by the James Cook University Animal Ethics Committee (approval number: A2408).

Soundscapes

Three different acoustic stimuli were used as treatments: ambient sound, 4-stroke motorboat and ship noise. Three recordings were made of each acoustic stimulus (9 in total) during the daytime at different locations around Lizard Island Research Station (see **Table B1** in **Appendix B** for details). The ambient reef recordings were collected on healthy reefs between 6 to 9 m depth. The 4-stroke boat recordings were collected from three different research station boats (5 m long aluminium hulls, Suzuki 4-stroke 30 hp DF30A, engine power 22.1 kW) travelling at a near constant speed at distances ranging from 5 to 25 m from the hydrophone. The ship recordings were made from different passing ships (~53,000 tonne bulk carriers, engines type MAN-B&W Diesel; engine power 13,501 kW) at distances ranging from 1.9 to 3.0 km from hydrophone. All sound recordings were made using SoundTrap 202 (Ocean Instruments, New Zealand) digital sound recorders with a 48 ksps sample rate (manufacturer's specifications of a flat response within ±3 dB between 20 Hz and 60 kHz).

Playback treatments were created from the field recordings using Audacity^{TM} 2.2.1 (http://www.audacityteam.org/). For each of the acoustic treatments three playback tracks were created. All tracks were 15 minutes in duration. Each playback consisted of 10 minutes of ambient playback followed by 5 minutes of the respective treatment (continued ambient playback, 4-stroke motorboat or ship noise). For the ambient playbacks a random section of the ambient reef recordings was used; for 4-stroke motorboat and ship playbacks, the chosen 5 minutes were from the maximum amplitude period of the replicate recording.

The 370 L acoustic treatment tank (50 x 65 x 115 cm) was positioned on top of bricks, with a 4 cm layer of foam between the base of the tank and the bricks to reduce acoustic artefacts caused by vibration transfer. Acoustic treatments were played using a J9 underwater speaker. J9 speakers are able to reproduce low frequency energy (i.e., peak spectral levels in the frequency band 10-50Hz; Bobber 1970). The J9 was kept in a fixed position by a bungee cable attached to a wood structure at one side of the tank. Water depth was 50 cm and the speaker was suspended 15 cm below the surface. Sound treatments were played placing the speaker 15 cm away from the experimental arena. The sound system used for playback of the treatments consisted of a 12v battery, MP3 player (SanDisk 8gb Clip Jam), amplifier (18 W, Kemo Langen Germany) and a J9 speaker (NUWC-USRD, Newport, RI, USA).

Prior to the experiment, acoustic tank dynamics were investigated in a 4x3 grid pattern (**Figure B1** in **Appendix B**). A calibrated tri-axial accelerometer (working frequency range 10 – 2000 Hz, [M20-040 from Geospectrum Technologies Inc, Dartmouth, Canada]) coupled to a Zoom F8 Multi-Track Field Recorder (Zoom Corporation) with a 96 kHz sampling rate was used to do independently measure particle acceleration and sound pressure at each position on the grid. The accelerometer was suspended from a series of vertical and horizontal beams to allow the sensor to be repositioned around the grid, maintaining a depth of 15 cm below the surface. Playback of sound, using ambient reef sound, 4-stroke noise and ship noise recordings was repeated for each of the grid circles. Sound analysis showed that the closest position central to the J9 speaker provided the most comparable sound pressure levels to the recordings from the field (**Figures B2- B5** in **Appendix B**). As a result, the experimental arena was located in this position for subsequent trials.

Experimental protocol

Individuals were carefully transferred using a jar from an acclimation tank into a circular experimental arena (diameter 20 cm; water level 4 cm) that was positioned inside a 370 L rectangular tank (50 x 65 x 115 cm). To minimise disturbance, the area above and around the arena was covered with an opaque white container to avoid laboratory disturbances and illuminated with LED lights (**Figure 2.1**). Water temperature during the experiment averaged 29.5°C.

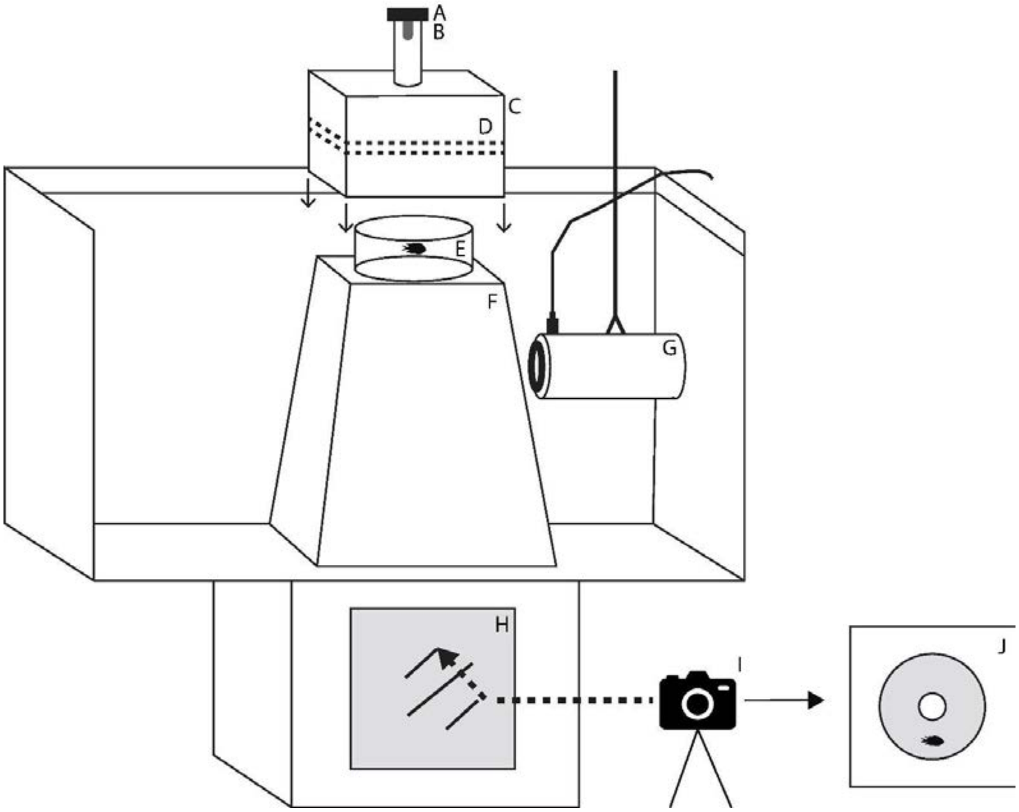

Figure 2.1. Schematic of the experimental set up. Experimental arena used for the recording of routine swimming and escape response of *Pomacentrus chrysurus*. Electromagnet **(A)**, tapered weight **(B)**, opaque white container **(C)**, LED lights **(D)**, experimental arena **(E)**, base of the experimental arena **(F)**, J9 speaker **(G)**, mirror **(H)**, camera **(I)** and image projected from the mirror **(J)**.

Randomly selected individuals were exposed to one of three treatments: ambient playback (n = 27), 4-stroke boat noise playback (n = 26), or ship noise playback (n = 26). Each individual was tested only once. Individuals were given nine minutes to habituate to the experimental arena. Routine swimming and escape response were recorded from below the tank with a camera (Casio EX-ZR1000) at 30 frames per second and 480 frames per second, respectively. Video recordings were later analysed using ImageJ software (https://imagej.nih.gov/ij/). Video analysis was based on the centre of mass of the fish, the point about which propulsive forces act (Webb 1976). To avoid observer bias, the video and audio recordings were analysed blind to the sound treatment. During the trials, individuals did not startle when either 4-stroke or ship noise started.

Routine swimming

The routine swimming of each individual was recorded one minute before and one minute after the start of the treatment to obtain an estimate of their space use and behaviour (**Figure 2.2**). Posterior analysis showed some video recordings were shorter than others, therefore analysis was standardised to 56 seconds before and 56 seconds after the start of the treatment. Speed, maximum speed and distance covered were recorded before and after the start of the treatments and the change between pre- and post-treatment calculated. Maximum speed was measured as the maximum speed reached at any point during the period of time evaluated (m/s). All variables were analysed by tracking the position of the individual every 0.5 s, which resulted in 112 data points per fish.

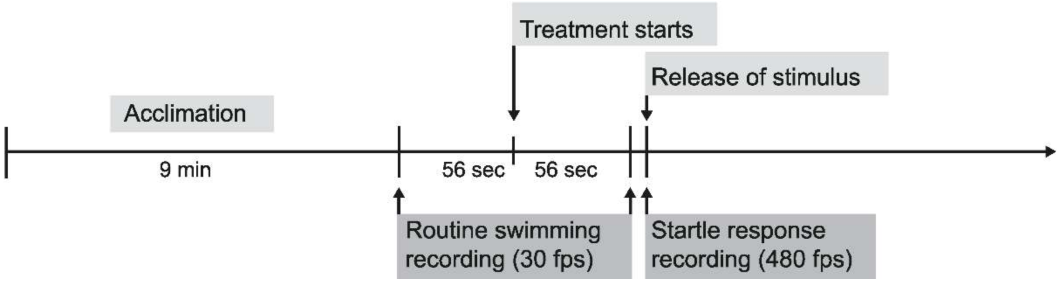

Figure 2.2. Experimental timeline. Each individual was placed in the experimental arena for nine minutes to acclimate. The routine swimming was recorded one minute before and one minute after the beginning of the sound treatment (ambient playback, 4-stroke noise playback, or ship noise playback). After recording routine swimming, a stimulus was released, and the fast-start escape response recorded.

Escape response

After recording routine swimming, an escape response was elicited from the fish by the release of a tapered weight above the water surface held in place by an electromagnet. The drop distance of the weight was controlled by a nylon string long enough to allowed it to just break the surface of the water. To prevent a visual warning of the falling weight, the weight was released through a PVC pipe (diameter 48.5 mm) suspended above the experimental tank, with the bottom edge at 10 mm above the water level (**Figure 2.1**).

The following escape response variables were measured:

1. Responsiveness was defined for each treatment as the proportion of individuals that responded with a sudden acceleration after being startled, out of the total number of fish.
2. Response latency (s) was measured as the time interval between the stimulus onset and the first detectable movement leading to the escape of the fish. The stimulus onset was defined as the moment the weight made contact with the surface of the water.
3. Speed (m/s) was measured as the distance covered within a fixed time (25 ms), which corresponds to the average duration of the first two flips of the tail (i.e., stages 1 and 2) (Domenici and Blake 1997). This period is considered crucial for avoiding predator ambush attacks (Domenici and Blake 1997, Walker et al. 2005, McCormick et al. 2018b)
4. Maximum speed (m/s) was measured as the maximum speed reached at any time during the escape response.
5. Response distance (m) was determined as the total distance covered by the fish from the stimulus onset to the end of the escape response.

Additionally, the length and distance of each individual from the stimulus was recorded as a potential covariate.

Statistical analyses

The effects of the three noise treatments on the routine swimming and escape response (excluding responsiveness) of *P. chrysurus* were examined using a one-factor multivariate analysis of variance

(MANOVA). Pairwise, sequential Bonferroni corrected MANOVAs were used to determine the nature of differences between treatments. Response latency was affected by the distance to the drop stimulus, although it was consistent among treatments (satisfying the assumption of homogeneity of slopes). To remove this confounding influence, analysis was undertaken on the residuals of the linear relationship between response latency and distance to the drop stimulus. A canonical discriminant analysis (CDA) was used to summarise, identify and display the nature of significant differences found by MANOVA. The CDA displays graphically the strength and importance of each of the original variables, by discriminating among treatment centroids. Data were transformed to satisfy the assumptions of the test (latency, natural log; speed, power 2; response distance, power 3).

Significant differences identified by MANOVA were further examined using planned comparisons to address two independent *a priori* hypotheses: 1) vessel noise affects the routine swimming and escape response of fish (i.e., comparing ambient playback to the grouped mean of the two noise treatments); and 2) the effects of exposure to noise depend on the noise source (i.e., comparing 4-stroke noise playback vs. ship noise playback). A logistic regression was used to analyse the effect of acoustic treatment on responsiveness. Model assumptions were assessed using residual plots, all of which were satisfactory. Statistical analysis was performed in the software Statistica version 13.4 and R version 3.6.1.

2.4. Results

Acoustic analysis indicated that playback sound pressure levels of ambient noise in the tank were lower than 4-stroke motorboat and ship noise playback levels (**Figure 2.3**). Playback sound pressure levels of ship noise at frequencies lower than 100 Hz were higher than sound pressure levels of boat noise, while at frequencies higher than 500 Hz the sound pressure levels of boat noise were higher (**Figure 2.3**). Playbacks differ to original recordings, probably due to near-field effects and interference caused by reflections and reverberations within the tank walls. Sound pressure in the experimental tank showed an increase in 20 dB and 30 dB for the 4-stroke motorboat and ship playbacks, respectively, compared to the field-recorded tracks (**Figure 2.3**). Despite the artefacts associated with confining sound in the tank, the relative differences between boat and ship noise across the acoustic power spectra were approximately maintained (**Figure 2.3.A** vs **B**). The ambient playback displayed a higher level of spectral distortion, resulting in almost 35 dB increase in sound pressure and several peaks (i.e., greater acoustic complexity) not present in the field-recorded tracks. Because the impact of the two vessel noise treatments is judged against the ambient control, this finding suggests that the results are likely to be conservative representations of the effects of vessel noise on the variables measured.

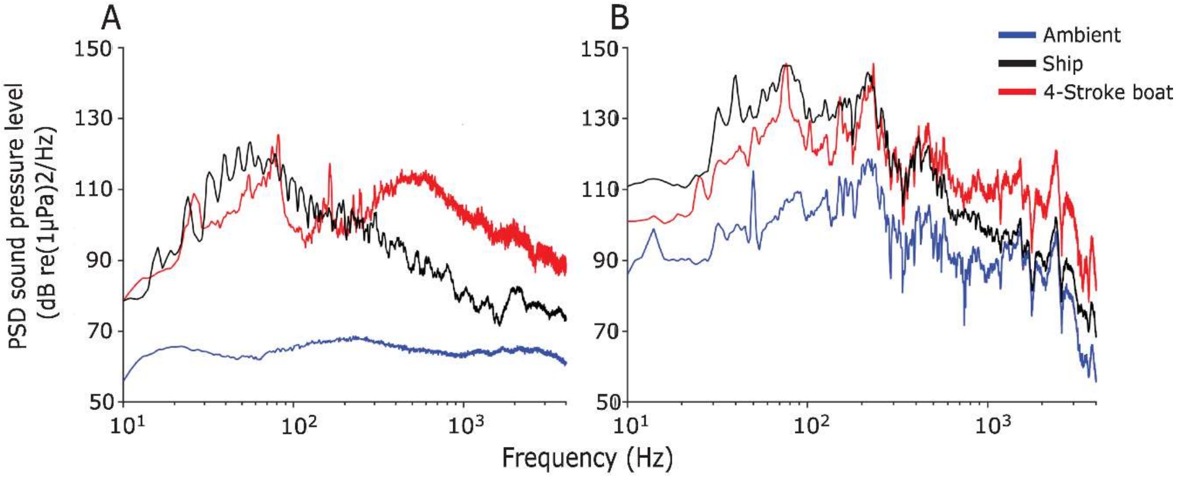

Figure 2.3. Power spectral densities (PSD) of acoustic treatments. Power spectral densities of **(A)** field and **(B)** playback of acoustic treatments. Mean PSD values were calculated from three individual samples of each noise source (Window type: 1 s Hamming, 50% overlap, frequency resolution of 1Hz). Duration of ambient, 4-stroke boat and ship playback samples for the sound analysis were approximately 30, 8 and 30s.

The routine swimming and escape response of *P. chrysurus* were significantly different among treatments (MANOVA, Pillai's trace = 0.60, $F_{12,106} = 3.84$, $p = <0.001$). For the routine swimming variables, the ambient playback was significantly different from 4-stroke and ship noise playbacks (Bonferroni-corrected MANOVA, p = <0.001, p = 0.0045 respectively), while there were no differences between 4-stroke and ship noise playbacks. For the escape response variables, the three treatments were significantly different from each other (Bonferroni-corrected MANOVA, $F_{2,64} = 14.77$, $p = <0.001$). A CDA showed that treatments were mainly discriminated by latency and change in distance (**Figure 2.4**). Fish exposed to ambient playback and ship noise playback were differentiated along both canonical axes. Fish exposed to ship and 4-stroke motorboat noise reduced their activity, as shown by a decrease in the routine swimming variable, change in distance. Treatments were also differentiated along the axis associated with a trend in fast-start latency with fish under a ship noise playback taking longer to respond to the stimulus (i.e., increased latency) compared to fish under 4-stroke noise and the ambient playback. In addition, fish exposed to 4-stroke playback had a lower maximum speed compared to fish exposed to ship noise and ambient playbacks.

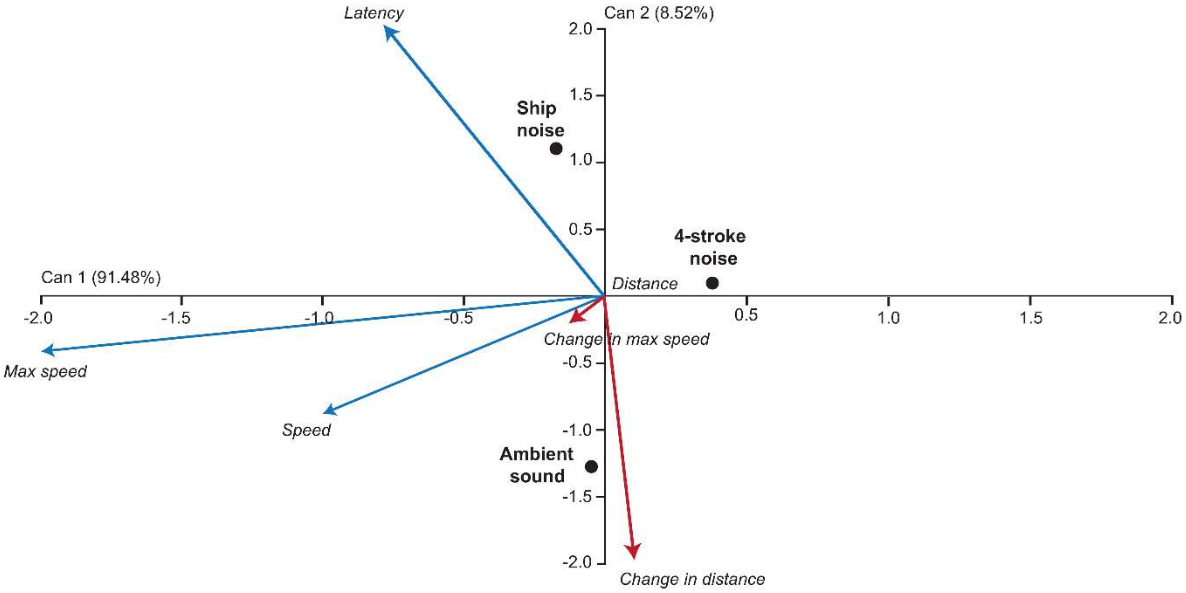

Figure 2.4. Canonical discriminant analysis. Canonical discriminant analysis displaying how the playback of noise from ships, 4-stroke powered motorboats and ambient affected the routine swimming (red line) and escape response (blue line) of juvenile *Pomacentrus chrysurus.* A canonical discriminant analysis displays the difference in routine swimming and escape response variables among acoustic treatments. The direction and importance of trends are indicated by the direction and the length of the vectors representing the original variables.

Univariate analyses on routine swimming and escape response confirmed the trends described by the CDA, however, there were significant differences only for latency and change in distance (**Figure 2.5** and **Figure B7** in **Appendix B**). Individuals exposed to ambient playback had almost 25% lower latency compared to individuals exposed to 4-stroke and ship noise playbacks (i.e., faster response to the drop stimulus) (Planned comparisons, SE = 0.097, *t* = -3.02, *p* = 0.004). While individuals exposed to 4-stroke playback had a shorter latency than those exposed to ship noise (Planned comparisons, SE = 0.109, *t* = -2.01, *p* = 0.048) (**Figure 2.5A**). Individuals exposed to 4-stroke and ship noise playback decreased their response distance by 15% and 30% respectively, while individuals exposed to ambient sound did not change their response distance (Planned comparisons, SE = 0.18, *t* = -2.51, *p* = 0.014). Fish exposed to boat and ship noise showed a statistically similar reduction in distance moved during routine swimming (**Figure 2.5B**, Planned comparisons, SE = 0.16, *t* = 1.17, *p* = 0.24). The proportion of individuals that responded to the stimulus was not significantly different among treatments (number displaying no reaction: ambient playback = 6; 4-stroke noise playback = 1; ship noise playback = 4; **Table B2** and **Figure B6** in **Appendix B**).

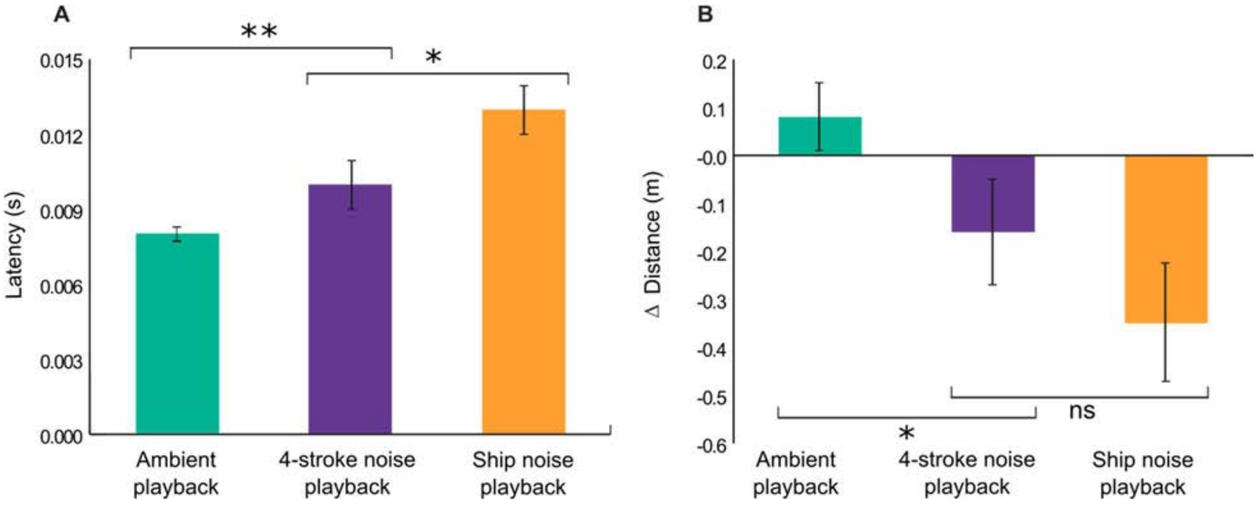

Figure 2.5. Effect of exposure to noise playbacks on routine swimming and escape response variables of *Pomacentrus chrysurus.* **(A)** Latency (mean ± s.e.) and **(B)** Change in distance (mean ± s.e.) covered between 56 s pre- and post- treatment. Data for latency were natural log transformed for analysis and standardised for distance to stimulus for analysis, but back-transformed covariate data are plotted. Asterisks above or below the bars represent significant differences between the planned comparisons (** = p<0.01; * = p<0.05; ns = no significant). Left to right: n = *21, 22, 25.*

2.5. Discussion

Examination of the effects of noise exposure on the escape response and routine swimming of marine organisms is a relatively new area of research (Ferrari et al. 2015, Simpson et al. 2016, McCormick et al. 2019). This study compared the effects on juvenile fish of noise from playbacks of 4-stroke motorboats and bulk-carrier ships, two of the most common sources of anthropogenic noise in inshore marine environments. Additionally, we used a J9 speaker, which are known to more accurately represent low frequency sounds compared to other speakers. This is noteworthy as early life-stages of many marine fishes are highly responsive to low frequency sound (Wright et al. 2011). Our results demonstrated that exposure to vessel noise can affect the routine swimming and escape response of the Whitetail damselfish. In specific, exposure to 4-stroke boat and ship noise playback decreased the total distance covered and increased the response latency of individuals compared to the ambient playback. However, the effect of ship noise was greater than the effect of 4-stroke noise indicating that different noise sources can affect fish to different degrees and in different ways. While the nature of our study question limited our methodology to a tank experiment, the results suggest that vessel noise may alter behavioural traits that have been shown to determine survival of juvenile fishes (McCormick et al. 2018b). This strongly suggests that further studies are warranted on the influence of ship noise on fish behaviour on coral reefs.

The response latency of *P. chrysurus* was longer for individuals exposed to ship noise compared to the other acoustic treatments. Previous tank-based studies have found contradictory effects of ship noise on the response latency of fishes. For example, Simpson et al. (2015) found that the response latency of European eels was 25% higher in individuals exposed to ship noise playback compared to individuals in ambient conditions, in keeping with the results of the current study. On the other hand, Voellmy et al. (2014b) found that Sticklebacks were faster to react to a predator simulated attack (i.e., lower latency) when exposed to ship noise, while Minnows did not show differences in their response latency. Studies examining the effects of 4-stroke motorboat noise have also found that this noise source can affect the escape response of fishes leading to longer escape latencies. In a field study, Simpson et al. (2016) found that individuals of Ambon damselfish (*P. amboinensis)* had a higher latency, when 4-stroke motorboats where passing compared to ambient conditions, supporting our observations. In a laboratory study, McCormick et al. (2019) also found that individuals of Whitetail damselfish (*P. chrysurus)* exposed to 4-stroke motorboat increased their latency by 50% compared to individuals exposed to ambient playback. These studies suggest that effects of vessel noise could be species specific. In our study, the observed increase in response latency caused by exposure to ship noise and 4-stroke noise means individuals are slower to respond, therefore less likely to avoid and escape from a predator (Domenici and Blake 1997, McCormick et al. 2018b).

In addition to the effects of noise on the fast-start response, our study provides evidence that exposure to vessel noise caused changes in space use. Analysis of routine swimming of Whitetail damselfish (*P. chrysurus)* prior to the startle stimulus showed that exposure to 4-stroke motorboat and ship noise playbacks reduced the total distance moved by 15% and 40% respectively, suggesting a decrease in the activity of individuals. Only one previous study has evaluated the effects of 4-stroke on routine swimming. McCormick et al. (2019) found that Whitetail damselfish (*P. chrysurus)* decreased the distance moved by 20% in the first 30 seconds of exposure to 4-stroke motorboat noise, supporting our results. Routine swimming is often used as a measure of activity and boldness, related to foraging, exploratory behaviour and vigilance (McCormick et al. 2018b). Previous studies have found that individuals that are more active and bolder in exploring their environment may learn information about their potential predators, have a greater appreciation of local threats and respond faster to predator strikes (Godin 1997, Manassa and McCormick 2013). Reduction in the time individuals allocate to these activities due to vessel exposure could theoretically increase mortality by predation (McCormick et al. 2019, Fuiman et al. 2010, Manassa and McCormick 2013), directly affect their fitness (Simpson et al. 2016) and population dynamics (Bracciali et al. 2012).

The routine swimming and escape response of Whitetail damselfish (*P. chrysurus*) were affected by 4-stroke motorboat and ship noise, however effects varied in their magnitude, with ship noise showing a greater effect. The different responses to 4-stroke motorboat and ship noise can be explained by the different acoustic properties of each treatment (e.g., rise time and frequency range). Ship noise was characterised by a rapid rise time to the highest pressure level at low frequencies (<100 Hz) that would have impacted fish without warning, while 4-stroke boat noise had slower rise time to its highest pressure level. In this portion of the frequency spectrum playback tracks correspond well with the noise levels originally recorded in the field (i.e., < 200 Hz). Moreover, low frequencies produced by ships overlap directly with the hearing ranges of recently settled damselfishes (30-1000 Hz; Kenyon 1996, Colleye et al. 2016, Slabbekoorn et al. 2010). Previous studies have found that different noise sources can have differential effects on fishes. For example, McCormick et al. (2018c) found that exposure to 2-stroke boat noise affected boldness and activity of juvenile Ward's damsel (*Pomacentrus wardi)* on patch reefs, while 4-stroke only affected activity. Moreover, while noise from 2-stroke engines prevented an effective antipredator response to alarm odours, no such effect occurred in response to noise from 4-stroke powered boats. Our findings suggest that ship noise is likely to have a similar, if not greater impact on risk assessment than noise from 4-stroke engines, compromising in theory their anti-predator behavioural in their natural environment (Simpson et al. 2016, McCormick et al. 2018b).

Complementary laboratory and field-based studies have been recognised as essential for the understanding of noise pollution and its effects on marine organisms (Slabbekoorm 2016, Simpson et al. 2016, Cox et al. 2018). Our experiment was conducted in a laboratory set-up that allowed for a detail examination of behavioural traits of individuals placed under identical acoustic conditions (Rogers et al. 2015). However, there are acoustic limitations to tank-based experiments (Carroll et al. 2016). The playbacks used in our study differed from the original recordings, in particular the ambient playback, therefore is possible that the effects of the acoustic treatments are under or overestimated. Still our results showed different effects from each of the acoustic treatments and suggest that some information was not lost (Cox et al. 2018). Our study contributes to the growing body of literature documenting the effects of anthropogenic noise on reef fish and represents an important stepping stone in the understanding of ship noise pollution and its effects on reef fishes. Further field-based studies are required to complement our findings and to determine the effects of long-term noise exposure and the capacity of fishes to acclimate or habituate to this disturbance.

Chapter 3: Variation in temporal structure influences the effects of ship noise playback on the behaviour of a juvenile reef fish

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3.1. Abstract

Ship noise can affect the behaviour of fishes, but although the temporal occurrence of this acoustic stressor has potential to influence behavioural outcomes, its importance has seldom been studied. Ships produce low frequency sounds that travel great distances and this can lead to the acoustic intensity slowly building up or occurring abruptly, as a result of acoustic shadows and sound reflection from seafloor topography. In a laboratory-based experiment, we compared the effects of ramp-up ship noise playback and abrupt ship noise playback on the routine swimming and escape response of a juvenile coral reef damselfish, *Pomacentrus amboinensis*. Our results showed that ramp-up ship noise playback and abrupt ship noise playback had different effects on juveniles. Exposure to ramp-up ship noise playback did not affect the routine swimming of juveniles, but their escape latency increased compared to juveniles exposed to reef sound, meaning they were slower to react to a simulated predator attack. In contrast, abrupt ship noise playback affected both the routine swimming and escape response of juveniles, leading to a decrease in their activity and an increase in their response latency. Our results indicate that ecologically important behavioural traits can be affected by both ramp-up and abrupt ship noise playbacks with potential implications for individual's survival. Additionally, our results suggest that temporal structure can influence the effects of noise on fish and should be included in future studies assessing the effects of ship noise on fishes.

3.2. Introduction

Shipping is the most prevalent source of anthropogenic noise in marine environments (Hildebrand 2009, Miksis-Old and Nichols 2016). Major ports are located close to coral reef areas resulting in a constant source of ship noise. For example, both Hong Kong and Singapore have coral reefs areas located close to shipping ports and these receive more than 160,000 and 130,000 ships per year (e.g., cargo vessels, bulk carriers, and tankers), respectively (Goodkin et al. 2011, CSDHKSAR 2019, MPAS 2021). In the case of Australia, shipping is critical for the economy as it is an island nation and 90% of goods arrive by shipping (DITRDC 2020). Along the Great Barrier Reef (GBR) there are 12 ports and more than 11,000 ship voyages per year (GBRMPA 2019), most of which transit the shallower inner GBR lagoon route. The continental shelf of the GBR has a high diversity of ecosystems including deep (>40 m depth) and shallow coral reefs (Pitcher et al. 2019). The low frequency noise produced by ships < 200 Hz (Ross 1976) can travel thousands of kilometres with little attenuation affecting remote locations (Urick 1983, Tournadre 2014). Although ship noise represents a potential threat to marine organisms, its effects on tropical reef fishes have been seldom studied (but see: Velasquez Jimenez et al. 2020). Ships are increasing in number (predicted to increase by 250% by 2040; GBRMPA 2014), therefore, understanding the effects of ship noise on the marine organisms of the GBR is essential.

Coastal habitats and coral reefs are highly complex habitats, with sudden changes in depth, seabed composition and environmental conditions (e.g., temperature and pressure). Sound propagation and attenuation are highly influenced by these environmental characteristics at large and small scales (Medwim and Clay 1997, Bass and Clark 2003). At a small scale, multiple acoustic telemetry studies have found that bathymetry can interfere with the detection efficiency of acoustic tags by limiting sound waves transmission (e.g., Welsh et al. 2012, Huveneers et al. 2015, Selby et al. 2016). At a large scale, large structures such as islands and headlands have been found to block sound waves resulting in acoustic shadows (Heaney et al. 2017). McWilliam (2018) found that the Lizard Island lagoon was the only area adjacent to a shipping route where ship noise was not detectable in long monitoring recordings, possibly related to the reef slopes and

Palfrey Island and South Island. Evidence from these studies, suggest that reefs act as sound barriers and could influence the temporal structure of noise received by organisms living within their environments. Abrupt noise can occur as a source comes out of the acoustic shadow of a reef or island. In contrast, noise levels from an approaching source can ramp-up slowly in intensity in an exposed site where sound waves travel through the open ocean with minimal attenuation. As a result, reef fishes can be exposed to abrupt or ramp-up noise in their natural environment.

Sound level is the most studied and reported attribute of anthropogenic noise. Most studies examine an individual's responses at consistent sound levels over time(e.g., Voemlly et al. 2014a, Radford et al. 2016, Velasquez Jimenez et al. 2020, but see: Nedelec et al. 2015). However, an organism's response to noise can also be determined by the temporal structure of the noise and few studies have examined individual responses while being exposed to ramp-up noise. This is particularly relevant when studying ship noise as ramp-up exposure is longer, compared to small motorboats, due low frequency noise suffering lower attenuation (Hildebrand 2009), longer travelling routes and slower travelling speeds. Studies evaluating the effects of ramp-up noise are mostly limited to mammals (e.g., Dolman et al. 2009, Miller et al. 2012, Cato et al. 2013). Only one previous study has examined the effects of ramp-up noise on fish. Neo et al. (2016) exposed temperate species European seabass (*Dicentrarchus labrax)* to brown ramp-up noise (tone band-passed: 200-1000 Hz) and found an effect on swimming behaviour, concluding that fish are sensitive to variation in noise temporal structure. To date, the effects of ramp-up ship noise on behavioural traits important for the survival of individuals are unknown. Moreover, previous studies have found that the effects of anthropogenic noise on fish are species- and context-specific (e.g., Voellmy et al. 2014b), therefore it is important to understand how differences in temporal structure of noise may influence the impact of ship noise on reef fishes.

The aim of this study was to examine the effects of ramp-up and abrupt ship noise on key behavioural traits during the juvenile stage of a common coral reef damselfish, *Pomacentrus amboinensis*. The study focused on the recently settled stage of a coral reef fish, which represents a critical bottleneck with mortality averaging 60% in the first two days after settlement (Almany and Webster 2006). Anything that adversely affects this early mortality trajectory can influence replenishment of local populations (Chamber and Trippel 1997). The current study focuses on the fast start escape response that has been found to be a critical behavioural trait for recently settled fishes, with those fish that elicit an appropriate and efficient fast start response having a higher probability of surviving in the field (e.g., Fuiman et al. 2010, McCormick et al. 2018b). Our experiment used playbacks of soundscapes in a custom-built tank, however because this study is laboratory-based it should be seen as hypothesis generating until methods can be developed that enable field-based assessments of Mauthner mediated fast-start responses. Our prediction based on previous research was that noise exposure with a ramp-up would not have an effect on behavioural traits as a result of acclimation to the gradual increase in noise levels (Neo et al. 2016).

3.3. Materials and Methods

Study species

The Ambon damselfish, *Pomacentrus amboinensis* (Pomacentridae), is a demersal coral reef fish species common to the Indo-Pacific (Allen 1991). Individuals have a pelagic planktonic larval phase that lasts for 18-23 days, before settling back onto reefs as juveniles (Kerrigan 1996). At settlement, *P. amboinensis* undergoes a rapid transformation limited to changes in colour (McCormick et al. 2002). Late-stage larval *P. amboinensis* (1.2 cm mean standard length) were collected overnight using light traps moored around Lizard Island Research Station (14° 40'S, 145° 28'E), northern Great Barrier Reef, Australia. In the morning of capture, individuals were transported to the research station in 60 L tanks. Fish were identified to species level and placed in 30 L tanks for two days to recover from the stress of capture. To reduce ambient noise in the holding tanks, seawater flow pipes were placed below the water surface and no air-stones were used. Fish were fed *Artemia spp.* twice a day to satiation. Individuals were isolated and not fed for 12 hours prior to experimental trials in order to standardise for satiation.

All methods and research within this study were carried out in accordance with the animal ethics guidelines and regulations of James Cook University, and all protocols were approved by the James Cook University Animal Ethics Committee (approval number: A2408).

Soundscapes

Three different acoustic stimuli were used as treatments: reef playback, abrupt ship noise playback and ramp-up ship noise playback. Playbacks were constructed from original sound recordings. Three daytime reef recordings were collected from three sites around Lizard Island, one per site, ranging from 6 to 9 m in depth. Ship noise was recorded from three different passing ships at distances ranging from 1.9 to 3.0 km from a hydrophone (see **Table B1** in **Appendix B** for details), one recording per ship. All sound recordings were collected using SoundTrap 202 (Ocean Instruments, New Zealand) digital sound recorders with a 48 ksps sample rate (manufacturer's specifications of a flat response within ±3 dB between 20 Hz and 60 kHz).

Playback treatments were created from field recordings using Audacity^{TM} 2.2.1 (http://www.audacityteam.org/). For each of the acoustic treatments three playbacks were created. The ambient reef playback consisted of 40 min of ambient reef sound. Two sets of ship noise playbacks were constructed, abrupt ship noise and ramp-up ship noise. The abrupt ship noise treatment consisted of 30 min of reef playback, followed by 10 min of maximum amplitude ship noise. The ramp-up treatment consisted of 5 min of ambient reef followed by 25 min of fade-in ship noise from ambient level to the same amplitude of the abrupt ship treatment, followed by 10 min of full amplitude ship noise (**Figure 3.1**). The fade in function in Audacity was used twice in each recording to create a uniform ramp-up of noise to mimic an approaching ship. The timing of the ramp-up treatment was based on a ship approaching at a speed between 7.4 ms^{-1} and 11 ms^{-1} (**Figure C1** in **Appendix C**; based on McKenna et al. 2012, McKenna et al. 2013). The sound system used for playback of the treatments consisted of a 12 v battery, MP3 player and a J9 speaker (NUWC-USRD, Newport, RI, USA). The J9 speaker is able to reproduce low frequency energy (frequency range 0.04-20 kHz; Bobber 1970), which is a major component of ship noise (Averson and Vendittis 2000, Miksis-Old and Nichols 2016).

An acoustic analysis was conducted to examine the effect of tank conditions on the acoustic treatments. Sound recordings were analysed using MATLAB. Mean power spectral density (PSD) levels were calculated from three individual samples of each noise source. Results indicated that playback tank recordings differed from field recordings due to tank-effects. While acoustic analysis showed that there were marked differences between the reef and the ship playback within the tank, the differences were not as large (**Figure C2** in **Appendix C**). The sound pressure levels of the reef treatment for both playback and original recordings were lower than for the ship noise treatment. The playback reef noise treatment showed a higher level of distortion at low frequencies, which resulted in an increase in 35 dB in sound pressure compared to the field recordings (**Figure C2** in **Appendix C**).

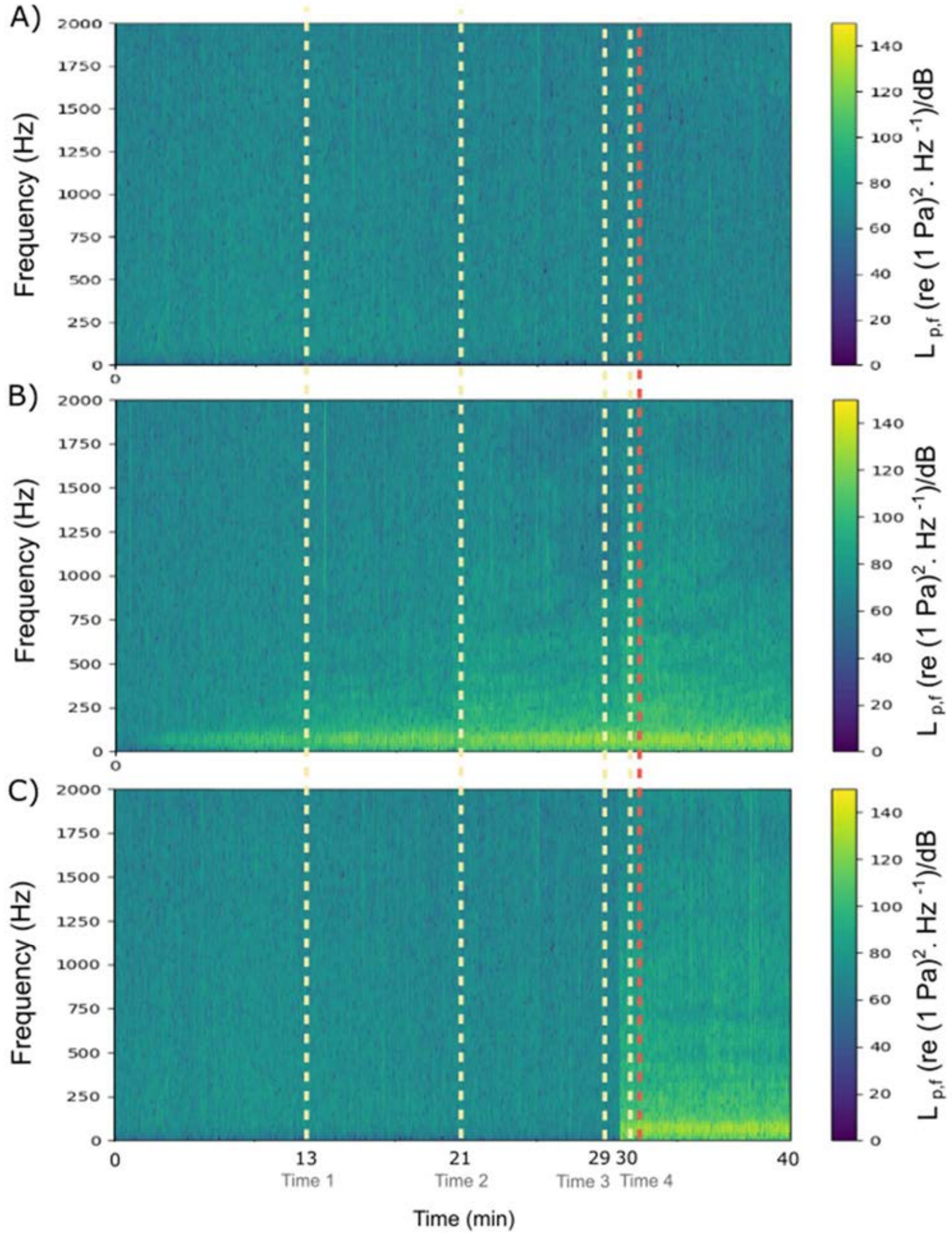

Figure 3.1. Spectrograms of the acoustic treatments: A) Reef sound playback, B) Ramp-up ship noise playback and C) Abrupt ship noise playback. Cream dash lines indicate timing of the routine swimming recordings. The red dash line indicates timing of the recording of the elicited escape response.

Experimental protocol

Randomly selected individuals were exposed to one of three treatments: reef playback, abrupt ship noise playback, or ramp-up ship noise playback. The experiment was conducted in the same experimental set-up described in detail in Velasquez Jimenez et al. (2020; **Chapter 2**). This study also describes the acoustic properties of the tank. Briefly, the experimental set-up consisted of an experimental arena (diameter 20 cm; height 7 cm; water level 4 cm) placed within a 370 L tank (50 x 65 x 115 cm). An opaque white container covered the experimental arena to avoid visual disturbance and LED lights were placed around the container to illuminate the arena (see **Figure 2.1** in **Chapter 2**). Individuals were transferred from the acclimation tank into the experimental area and given five minutes in silence to acclimate before the start of the acoustic treatment. At the designated time (**Figure 3.1**), routine swimming (Time 1, Time 2 and Time 3) and escape response (Time 4) were recorded from below the tank with a camera (CASIO EX-SR1000) at 30 frames per second and 480 frames per second, respectively. An average of 9 individuals were tested each day. Each individual was tested only once. After each trial, water in the experimental arena was mixed. To control for any potential effect, intra-day order of trial was included as a covariate in the statistical models. At the end of each day, the tank was completely drained and refilled. Trials were conducted between 8:00 and 17:00 hr over two summers (November- December 2018 and November-December 2019), with similar numbers of replicates each year. Water temperature averaged 28.7 °C during the experimental periods. Videos were analysed using the ImageJ software (Schneider et al. 2012). To avoid observer bias, videos were analysed blind to the acoustic treatment.

Routine swimming

Routine swimming recordings (30 s) were initiated for each individual at four different times: min 13 (Time 1), min 21 (Time 2), min 29:30 (Time 3) and min 30 (Time 4) (**Figure 3.1**). These times were selected to examine effects on routine swimming as the sound intensity of the ramp-up ship playback increased. Routine swimming was recorded for 30 seconds each time to obtain an estimate of space use and activity (following McCormick et al. 2019). The following routine swimming traits were measured: (1) distance (m), i.e., total distance covered) and (2) maximum speed (m/s, i.e., greatest speed reached). All variables were analysed by tracking the centre of mass of the individual every second, which resulted in 30 data points per fish per time. In order to compare the effects of abrupt and ramp-up ship noise, the change between pre- (i.e., Time 3) and post- (i.e., Time 4) treatment was calculated for both routine swimming variables.

Fast start response

After recording routine swimming, an escape response was elicited from each individual by the release of a tapered magnetic weight into the water surface (Time 4 in **Figure 3.1**). The drop distance of the weight was controlled by a nylon string long enough to allow it to just break the surface of the water. To prevent a visual warning the weight fell through a PVC pipe (diameter 48.5 mm) suspended 1 cm above the water surface. Video analysis focused on the first two kinematic stages that correspond to the critical period of the escape response (Webb 1976, Domenici and Blake 1997). The following escape response variables were measured:

1. Response latency (s): time interval between the stimulus onset and the first movement leading to the fish escape.
2. Speed (m/s): the distance covered within the first two flips of the tail (based on Domenici and Blake 1997) the time considered crucial for avoiding predator attacks (Webb 1976).
3. Maximum speed (m/s): the maximum speed reached at any time during the escape response.
4. Response distance (m): the total distance covered by the individual during the escape response. Additionally, the standard length and distance to the drop stimulus were also measured for each individual.

Statistical analysis

Separate linear mixed effects models (LMEs) fitted by maximum likelihood were used to examine the effect of ramp-up noise treatment and reef playback on routine swimming variables and to investigate whether acoustic treatment led to a change in routine swimming traits between pre- and post-acoustic treatment. Acoustic treatment, time, their interaction, standard length and order of trial were included as a fixed effects and fish identity and summer year were included as a random effect. A mixed effect logistic regression (GLMER) was used to examine the effects of acoustic treatment on responsiveness. Acoustic treatment, distance to the stimulus, standard length and order of trial were included as fixed effects and summer year was included as a random effect. We used a logit link function with a binomial family structure and a binary response variable (i.e., startled/ not startled). Linear mixed effect models fitted by maximum likelihood were used to test for the effect of acoustic treatment on latency, speed, maximum speed and distance on the escape response. Acoustic treatment, standard length and order of trial were included as fixed effects and summer year was included as a random effect. Escape speed was power of two transformed, and escape maximum speed, escape distance and escape latency were log transformed to meet distribution assumptions. For all models, the most parsimonious model was selected based on the lowest corrected Akaike Information Criterion (AIC). Model assumptions were assessed using residual plots, and all were found to be satisfactory. When models were significant, further differences were explored using post-hoc Tukey's tests. Statistical modelling was conducted in R version 4 using the packages lme4 (Bates et al. 2015), nlme (Pinheiro et al. 2014) and AICcmodavg (Mazerolle 2015).

3.4. Results

Routine swimming

The distance moved and maximum speed of *P. amboinensis* were not significantly affected by ramp-up ship playback compared with ambient reef sound at any of the three of the testing times, nor were there any significant interactions (**Figure 3.2**; see detailed statistics **Table C1** in **Appendix C**).

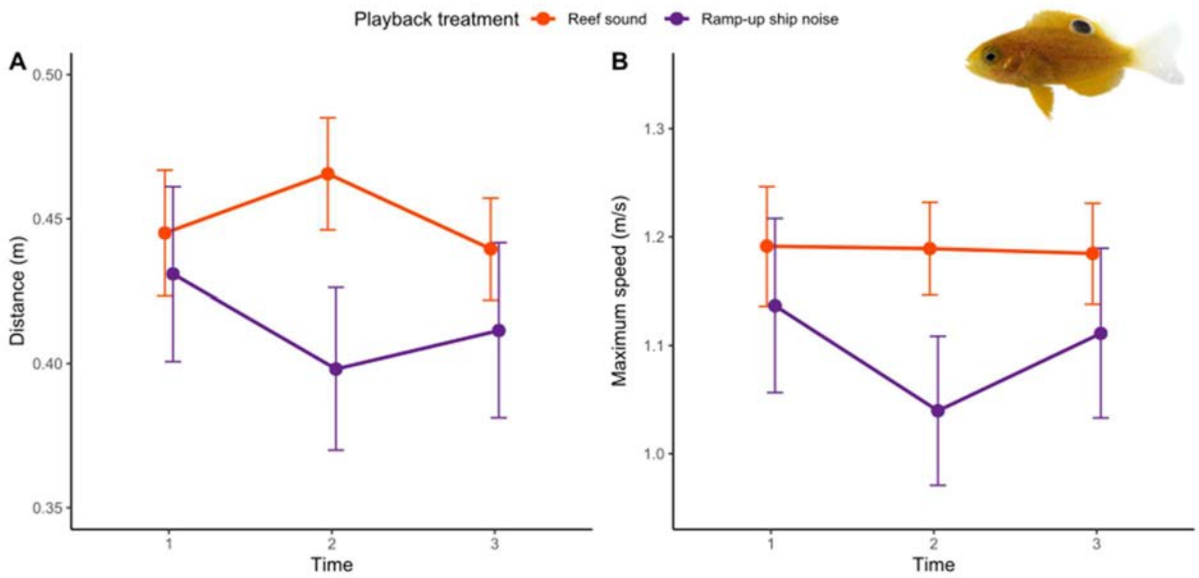

Figure 3.2. Effects of ramp-up ship noise on the routine swimming of *Pomacentrus amboinensis* in 30s intervals. (A) Total distance moved (mean ± s.e.) and **(B)** Maximum speed (mean ± s.e.) (Time 1 = 13 min, Time 2 = 21 min and Time 3 = 29 min). Reef playback: *n = 121*, Ramp-up ship playback: *n = 60*. Photo credit: M. McCormick.

Acoustic treatment caused a change in mean distances covered by *P. amboinensis* between time periods 3 and 4 (LM: F = 5.34, *p* = 0.005; **Figure 3.3A**). Individuals in the abrupt ship treatment decreased their distance covered by 11.6%, while individuals in the ramp-up ship treatment and reef treatment showed an increase in distance of 3.3% and 2.4%, respectively. The change in mean distance of individuals in the abrupt ship treatment was significantly different from individuals in the ramp-up ship playback (Tukey's: *t* = -2.91, *p* = 0.011) and reef playback (Tukey's: *t* = -2.76, *p* = 0.017), which did not differ from one another (Tukey's: *t* = 0.17, *p* = 0.984). There was no effect of acoustic treatment on changes in maximum speed (LM: F = 1.20, *p* = 0.302; **Figure 3.3B**).

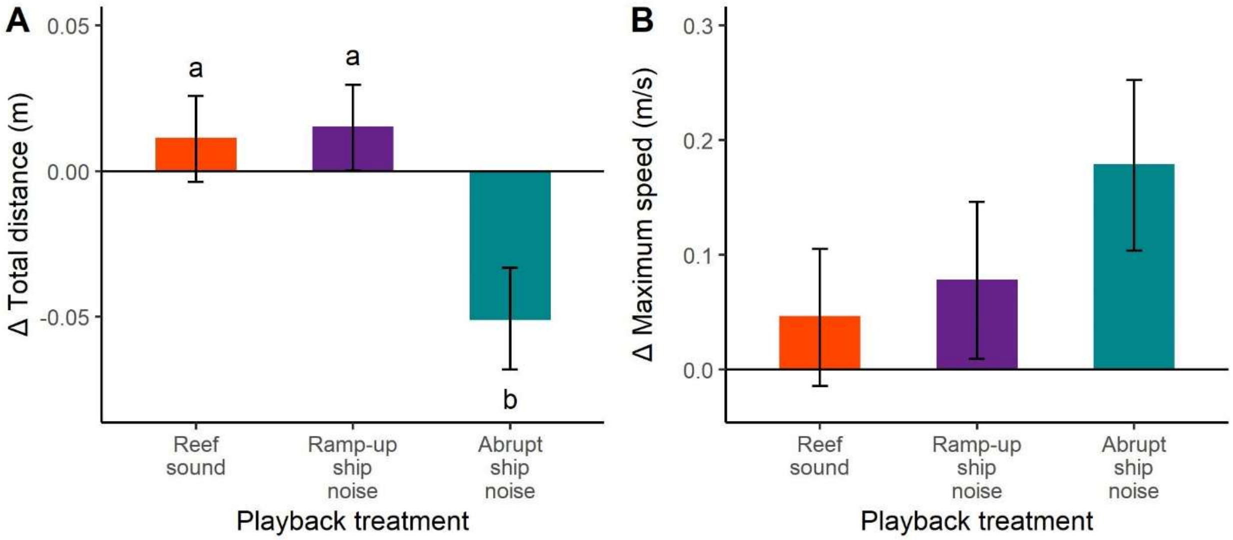

Figure 3.3. Changes in routine swimming behaviour of *Pomacentrus amboinensis* caused by acoustic treatments between time 3 and time 4 (see Figure 3.1, expressed as Time 4 – Time 3). (A) Change in distance (mean ± s.e.) and **(B)** Change in maximum speed (mean ± s.e.). Positive values indicate an increase in the respective variable and a negative value indicates a decrease. Letters denote post-hoc differences at P < 0.05. Bars without letters did not show significant differences. Left to right: *n = 63, 61, 59*.

Fast-start response

The response latency of *P. amboinensis* was affected by acoustic treatment (LM: F = 5.91, *p* = 0.003). Individuals exposed to abrupt ship noise and ramp-up ship noise had a significantly longer latency (i.e., time to initiate their escape) compared to individuals exposed to reef sound (Tukey: *t* = 4.02, *p* = <0.001; *t* = 2.52, *p* = 0.034, respectively; **Figure 3.4A**). There was no difference in the escape latency between individuals exposed to abrupt ship noise and individuals exposed to ramp-up ship noise (Tukey: *t* = 1.29, *p* = 0.402). Escape speed, maximum escape speed and escape distance of *P. amboinensis* were not affected by acoustic treatment (**Figure 3.4B, C, D**; see extended data **Table C1** in **Appendix C**).

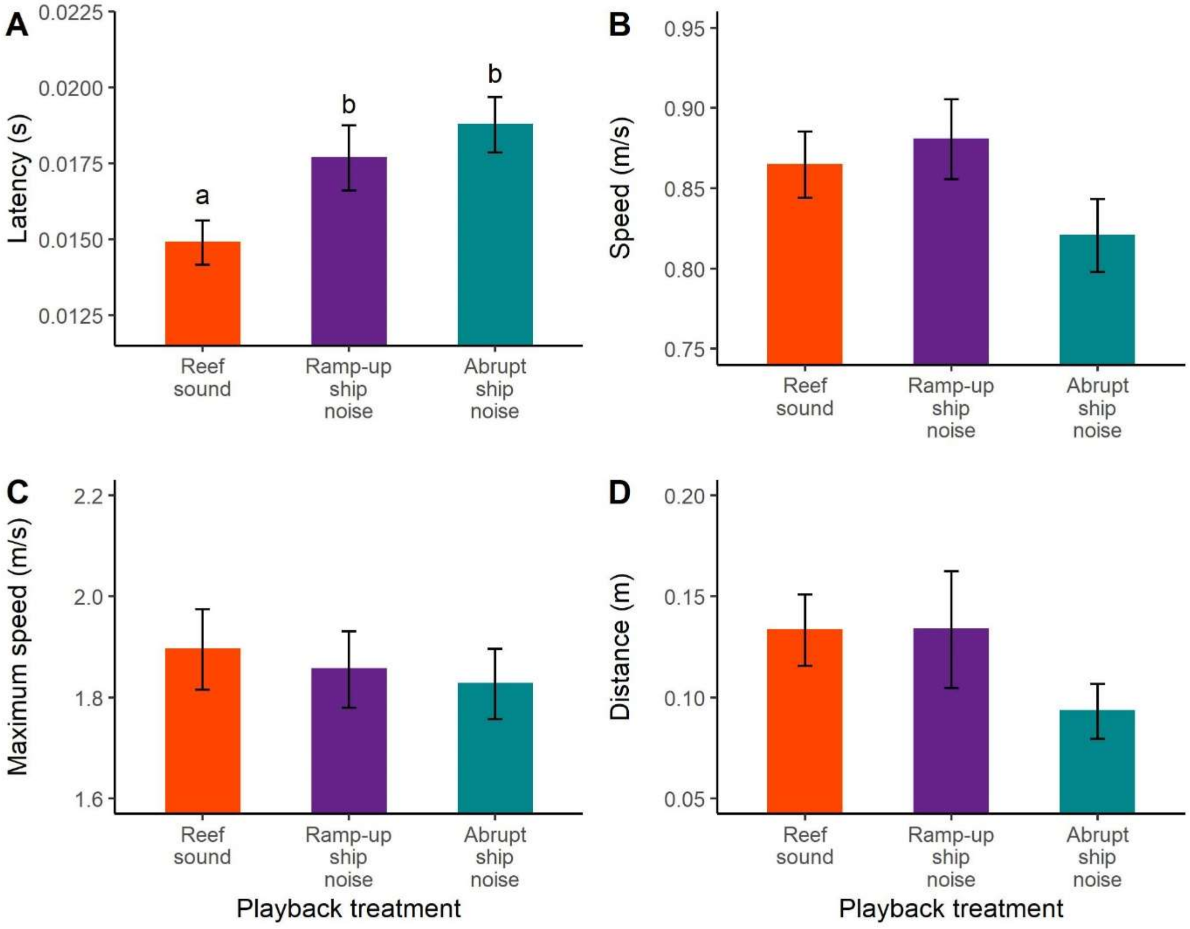

Figure 3.4. Effects of acoustic treatments on the fast-start response traits of *Pomacentrus amboinensis.* **(A)** Escape latency (mean ± s.e.), **(B)** Escape speed (mean ± s.e.), **(C)** Escape maximum speed (mean ± s.e.) and **(D)** Escape distance (mean ± s.e.). Letters denote post-hoc differences at $P < 0.05$. Bars without letters did not show significant differences. Left to right: *n = 44, 34, 39*.

3.5. Discussion

This is the first study to investigate the effects of ramp-up ship noise playback on the antipredator behaviour of juvenile reef fishes. Our results showed that exposure to ramp-up ship noise playback and abrupt ship noise playback can affect ecologically important behavioural traits in this critical life stage of *Pomacentrus amboinensis*. However, ramp-up ship noise had a different effect on juvenile fish behaviour to abrupt ship noise. Ramp-up noise did not affect the routine swimming of juveniles, but did cause an increase in the time to initiate an escape response (i.e., higher latency). In contrast, exposure to abrupt ship noise significantly reduced activity (i.e., distance covered), as well as increased latency. Our results go beyond reporting effects of ship noise by considering variations in temporal structure. This is highly relevant as organism inhabiting complex ecosystems, such as coral or temperate reefs, are likely to experience different temporal structures. We found variations in the temporal structure of ship noise can result in differing effects depending on the behavioural trait being measured and this is highly relevant when reporting the impacts of ship noise. Future studies assessing the effects of ship noise should use realistic temporal structures that are likely to occur in coral reefs ecosystems to increase the validity of their findings.

The routine swimming of *P. amboinensis* was not affected by ramp-up ship noise playback and did not significantly change as sound intensity increased over time. The lack of effect of the ramp-up noise treatment could be explained by the acoustic attributes of the treatment. The gradual increase in sound intensity of the ramp-up noise and the long noise exposure could allow fish to tolerate the noise and continue with their activity (Rankin et al. 2009, Von Benda-Beckmann et al. 2014, Neo et al. 2016). One previous study did find an effect of ramp-up noise on European seabass swimming behaviour. In a 2-5m depth field enclosure (depending on the tide), Neo et al. (2016) exposed adult European seabass to brown ramp-up noise and found that ramp-up noise caused diving behaviour within the first 5 min of exposure. However, there were no differences compared to the diving behaviour triggered by the abrupt noise treatment. Our results also showed that even when ramp-up noise reached its highest sound intensity routine swimming remained unchanged. Differences with this study could be related to species-specific responses and the acoustic treatment used. Our results suggest that ramp-up ship noise does not affect activity of *P. amboinensis*, however, whether ramp-up noise affects other behavioural traits should be evaluated in future studies.

Exposure to abrupt ship noise caused a change in routine swimming, while individuals exposed to ramp-up noise and reef sound did not display any change. Individuals exposed to abrupt ship noise playback decreased distance covered by 11.6%. This decrease in distance covered is in keeping with the typical response of fishes to a threat or stressor, with an increase in alertness resulting in individuals freezing or increasing vigilance (Morris-Drake et al. 2016). Abrupt ship noise playback has been previously found to decrease activity of juveniles Whitetail damselfish (*Pomacentrus chrysurus)* by 30% (Velasquez Jimenez et al. 2020). A decrease in distance moved suggests a decrease in activity and boldness, both of which have been found to contribute to survival of juvenile reef fishes in the field (McCormick et al. 2018b). Individuals that are more active and bolder, can learn information about their environment and the proximity and motivations of potential predators (Manassa and McCormick 2013, McCormick and Meekan 2010). It is likely that changes in activity at this life stage will affect an individual's knowledge of its environment and may limit its ability to make appropriate behavioural choices.

Escape latency was significantly affected by both ramp-up and abrupt ship noise playbacks. The response latency of individuals exposed to ramp-up noise and abrupt noise was almost double compared to individuals that were exposed to reef sound. Our results are in keeping with previous studies examining effects of ship noise playback on escape latency of fishes (e.g., Voellmy et al. 2014b, Velasquez Jimenez et al. 2020). For example, Velasquez Jimenez et al. (2020) found that congeneric species, Whitetail damselfish (*Pomacentrus chrysurus)*, increased their response latency by 25% when exposed to abrupt ship noise playback. On the other hand, Voellmy et al (2014b) found that temperate species Sticklebacks (*Gasterosteus aculeatus)* had a shorter latency while the response latency of European minnow (*Phoxinus phoxinus)* was not affected by abrupt ship noise playback. In the present study, individuals exposed to ramp-up noise playback also increased their response latency. While ramp-up noise potentially had a minimal effect on routine swimming, the continuous exposure to noise could have compromised the response of fish to an additional stimulus (i.e., simulated predator attack). Many studies conducted on other species and other stressors have found that continuous and long-term exposure to a first stressor can compromise the response of individuals to a second stressor (reviewed in Schreck 2000). Although, in the present study other escape response traits were not affected, the effects on escape latency are highly relevant (e.g., Simpson et al. 2016, McCormick et al. 2018b, 2019). McCormick at al. (2018b) conducted a field experiment where juvenile damselfishes were placed on reef patches for three days. Eighteen behavioural and morphological traits were recorded prior to release and survival was recorded. Fast-start escape latency was found to be the most important predictor of survival of juveniles. Our study shows that individuals increased their response latency when exposed to either abrupt or ramp-up ship noise, potentially increasing their vulnerability to predators.

Our results were obtained from a laboratory tank-based experiment. Sound analysis showed an increase in sound pressure levels for all the acoustic treatments while maintaining original trends. The reef playback showed the highest distortion with an increase of 35 dB, decreasing the difference in sound pressure between acoustic treatments. This suggests that our results are underestimating the effects of ship noise on fish and highlights the importance of conducting analogous field studies to validate our results. Although, tank-based experiments have acoustic disadvantages (described in: Rogers et al. 2015, Slabbekoorn 2016) it is unclear whether tank effects are significant enough to discredit behavioural responses (Cox et al. 2018). Tank based experiments have the advantage of allowing for detailed behavioural data collection, noise exposure over extended periods of time and control over environmental conditions that are not usually possible in field studies (Slabbekoorn 2016). Moreover, the aim of our study was to evaluate the effects of the slow increase in sound intensity of the ramp-up treatment and our results indicate that playback treatments retained sufficient acoustic attributes to trigger different effects on behavioural traits.

The present study examined the effects of two different temporal structures of ship noise, ramp-up vs. abrupt, both of which occur in coral reefs. Our results suggest that ramp-up noise may stress individuals, which can potentially compromise their response to future stressors or challenging situations, such as a predator attack. In a real scenario, prey and predator are likely to be exposed to noise simultaneously. However, previous studies have found that predators can respond to boat noise differently (e.g., Allan et al. 2015), suggesting our results are relevant during predator-prey interactions. It is vital to examine the effects of variations in temporal structure on the effects of noise and in particular fish behaviour as it is the behavioural decisions made by individuals under threat that influence their survival. Coral reefs are highly complex habitats where sound propagation may be altered by the structural characteristics of the environment; it is possible that acoustic shadows provided by large reef structures in shallow water, such as headlands and coral islands, might provide acoustic refuges where individuals may be able to avoid the negative effects of noise. However, our results suggest that if such shadowing leads to an abrupt exposure to noise when the ship moves and the corresponding shadow fades, effects on behaviour could be worse. Future studies should examine how habitat complexity of coral reefs, headlands and islands may facilitate the behavioural mediation of the detrimental effects of noise on marine organisms. Field studies in coral reefs are warranted not only to corroborate our results, but also to understand the complex soundscapes of coral reefs.

Chapter 4: Elevated temperature has a greater effect than ship noise playback on predator-prey interactions

In Preparation for Marine Ecology Progress Series

Velasquez Jimenez L, Nedelec SL, McCormick MI.

4.1. Abstract

Marine organisms are likely to be exposed to multiple stressors. Understanding how these multiple stressors interact is critical, not only to understand their effects on marine communities but also for management. In the present study, we investigated the combined effects of two current and growing environmental stressors: elevated temperature and ship noise playback (mean SPL 63.41) on the predator-prey interactions of two common species of coral reef fishes. We exposed prey (*Pomacentrus amboinensis*) and predators (*Pseudochromis fuscus*) to either present-day control (29°C) or elevated temperature at levels predicted for the end of the century (31°C). After 7 days, prey and predator interactions were recorded while being exposed to either reef playback or ship noise playback. We found that elevated temperature decreased prey escape speed. However, this did not result in higher capture success by the predator and the behavioural traits of the predator were not affected by elevated temperature. These results suggest that predators are more resilient to warming than prey. Additionally, we found no effects of ship noise playback and no evidence of an interaction between stressors. Thus, in a scenario where elevated temperature and ship noise co-occur, elevated temperature is likely to be the dominant stressor.

4.2. Introduction

Multiple anthropogenic stressors co-occur in aquatic and terrestrial environments threatening organisms (Hewitt et al. 2015, Poloczanska et al. 2016). These stressors repeatedly interact with one another in complex and unexpected ways that are not easy to predict (Côté et al. 2016). Previous research has found that the combined effects of multiple stressors can be more than (synergistic) or less than expected (antagonistic) based on their individual effects (Galic et al. 2018). Elevated temperature and anthropogenic noise are two stressors that are likely to interact in marine environments. Average sea surface temperatures are predicted to increase as a result of global warming. Projections show that sea surface temperatures will undergo a 2.64 ± 0.08°C increase by the end of the century (IPCC 2014, CSIRO and Bureau of Meterology 2015, Ruela et al. 2020). Simultaneously, the growth of coastal populations and the expansion of world trade networks have increased marine traffic resulting in an increase to noise levels in marine environments (Shannon et al. 2015, GBRMPA 2017, Erbe et al. 2019). Although the effects of elevated temperature and anthropogenic noise on predator-prey interactions have been examined in isolation before (e.g., Allan et al. 2015, Allan et al. 2017, McCormick et al. 2018a), their combined effects are unknown. Moreover, studies of interactive effects that combine globally and locally managed stressors (e.g., ocean warming and anthropogenic noise, respectively) are directly useful for management (Côté et al. 2016).

Predator-prey interactions play a critical role in marine fish communities and population dynamics (Huniscker et al. 2011). Most coral reef fishes have a bipartite life cycle, with a pelagic larval phase and a juvenile-adult benthic associated phase. At the end of their larval phase individuals settle from the open water column to coral reef habitats. Predation can represent a critical driver of population dynamics in the days following settlement, with up to 60% on average of the individuals being preyed upon in the first two days (Almany and Webster 2006, Cowen and Sponaugle 2009). Alterations of predator-prey interactions can influence the abundance of predators and prey and therefore community structure leading to trophic cascades or ecosystem shifts into alternate states (Frank et al. 2005). Given the importance of predator-prey interactions in governing the distribution and abundance of marine fishes (Hunsicker et al. 2011), it is important to understand how multiple stressors may affect these fundamental community interactions.

Fishes are ectotherms, meaning that they are strongly sensitive to changes in ambient temperature (Isaak and Rieman 2013). Changes in temperature can influence fishes' metabolic demands, and lead to changes in their nutritional needs (Deutsch et al. 2015). Sea surface temperature can naturally fluctuate in coral reefs between seasons, days or tidal cycles (Kaplan et al. 2003, Chollett et al. 2012). However, global warming has led to an atypical increase in global average temperatures that exceeds natural variations (IPCC 2014, CSIRO and Bureau of Meterology 2015, Ruela et al. 2020), resulting at the same time in an increase in frequency, intensity and duration of heatwaves (Oliver et al. 2018). Many experimental studies have documented the effects of elevated temperature on physiology (e.g., metabolism, growth, and reproduction) and its implications for behaviour (e.g., swimming performance and kinematics of fishes; Lea et al. 2016, reviewed in Domenici et al. 2019). During predator-prey interactions, elevated temperature has been found to be more detrimental for some prey fish, while predators may increase their capture success (Allan et al. 2015). Recent studies have examined the interactive effects of elevated temperature with ocean acidification on predator-prey interactions (reviewed in Domenici et al. 2019), however interactive effects with local stressors, such as anthropogenic noise, are unknown.

Ships are the biggest contributors of anthropogenic noise in marine ecosystems and represent a major threat to fishes due to their ubiquitous presence (Miksis-Olds and Nichols 2016, Erbe et al. 2019). The Great Barrier Reef hosts the most transited shipping route in Australia, with over 11,000 ships transiting per year (GBRMPA 2019, AMSA 2021). However, the effects of ship noise on coral reefs have been seldom studied. To date only one study has examined effects of ship noise on a coral reef fish (Velasquez Jimenez et al. 2020), while other studies have focused on temperate marine species (Voellmy et al. 2014a, Radford et al. 2016) and freshwater species (Wysocky et al. 2006). Ship noise has been found to cause stress (Wysocky et al. 2006), disrupt communication (Vascocelos et al. 2007) and affect anti-predator behaviour of fishes (Velasquez Jimenez et al. 2020). The effects of ship noise on antipredator behaviour have been found to differ between species, with some studies finding an effect (Velasquez Jimenez et al. 2020, Voellmy et al. 2014b) and other finding no effect (Voellmy et al. 2014b, Radford et al. 2016). These studies suggest that not all fish species are equally affected by ship noise, highlighting the importance of investigating their effects during predator-prey encounters. Moreover, it is unknown how ship noise may interact with other stressors.

The present study examined the combined effects of elevated temperature and ship noise playback on predator-prey interactions. A common tropical prey fish (Ambon damselfish; *Pomacentrus amboinensis*) and predator (Dusky dottyback; *Pseudochromis fuscus*) were exposed either to average summer temperature for the study site (29°C) or elevated temperature (31°C; projected 100 year future temperature; Collins et al. 2013). During the predator-prey trials, fishes were exposed one time to either reef playback or ship noise playback. This study was a laboratory-based experiment due to the logistic constrains of manipulating temperature in the field, and the benefits of recording detailed kinematics in a controlled environment that are only possible in a laboratory scenario.

4.3. Materials and Methods

Study species and fish handling

Ambon damselfish *(Pomacentrus amboinensis*, Pomacentridae) juveniles were used as prey (Standard length: 9.04 ±0.07mm, mean ± s.e.). Ambon damselfish is a common species found across shallow reefs in the Indo-Pacific (Allen 1991). They have a bipartite life history with a planktonic phase maintained for 15 to 20 days before settling into coral reefs during the summer months (Kerrigan 1996). The hearing range of recently settled damselfishes is between 30 and 1000 Hz (Wright et al. 2010, 2011). The Dusky dottyback (*Pseudochromis fuscus)* was used as the predator (Standard length: 42.8 ±0.35mm, mean ± s.e.). Dusky dottybacks are small carnivorous species, widely distributed throughout the Indo-pacific reefs (Michael 2004). Dusky dottybacks inhabit complex habitats, such as rubble and live coral, and are voracious predators of newly settled fishes, including *P. amboinensis* (Feeney et al. 2012). Prey (n = 152) and predators (n = 81) were collected from reefs adjacent to Lizard Island Research Station (14°40´S, 145°28´E), northern GBR, in October and November 2019. Newly metamorphosed *P. amboinensis* were collected using light traps moored on the reef edge overnight (Meekan et al. 2001). Upon collection, juveniles were transported to the research station, where they were identified to species level and placed in 30L tanks. Dusky dottybacks were collected from reefs located in the Lizard Island lagoon by SCUBA divers using hand nets and a mild anesthetic clove oil solution. Upon collection, predators were transported to the research station and kept in individually at ambient temperature in porous 1 L containers in a 60 L tank.

Experiment overview

Predators and prey were assigned to control (29°C; AIMS 2014) or elevated temperature (31°C; projected 100 year future temperature; Collins et al. 2013) treatments and maintained in them for seven days. During interaction trials, predator and prey were either exposed one time to reef playback or ship noise playback resulting in a full factorial design (29°C– reef playback, n = 34; 29°C- ship noise playback, n = 37; 31°C- reef playback, n = 34; 31°C- ship noise playback, n = 44).

All methods and research within this study were carried out in accordance with the animal ethics guidelines and regulations of James Cook University, and all protocol were approved by the James Cook University Animal Ethics Committee (approval number: A2408).

Thermal conditioning

Prey and predators were randomly allocated to control (n = 71; present-day temperature, ~29.2 ± 0.04°C) and treatment groups (n = 78; elevated temperature, ~31.1 ± 0.03°C). Juvenile *P. amboinensis* were conditioned in three 30 L tanks per temperature treatment. Dusky dottybacks were placed individually in porous 1 L containers in groups of 20 in two 60 L tanks per temperature treatment (**Figure D1** in **Appendix D**). Water was fed through a header tank with temperatures maintained using 1000 W titanium heaters with digital thermostats, with 300 W batten heaters placed in each of the individual conditioning tanks (**Figure D1** in **Appendix D**). The water flow of each tank was placed below the surface to reduce noise and no air-stones were used. For the fishes in the elevated temperature treatment, temperature was increased 1°C per day until reaching 31°C (Allan et al. 2015, 2017) and then maintained at this temperature for seven days. Once treatment temperatures were reached, water temperature was monitored at least twice per day. Previous studies have found reef fishes can acclimate to changes in temperature in seven days (Nilsson et al. 2010). Juvenile *P. amboinensis* were fed *Artemia spp.* twice a day to satiation. Dusky dottybacks were fed two euthanized juvenile reef fish morning and night. Prey were not fed 12 hr prior to the trials and predators were not fed 24 to 48 hr prior to the behavioural trials to standardize satiation. A 12 hr light:12 hr dark regime was used.

Acoustic treatments

Two acoustic stimuli were used during the interaction trials: reef playback (i.e., control; n = 70) and ship noise playback (n = 82). Three sets of recordings were made for each of the acoustic stimulus (6 in total). Reef recordings were collected during the daytime from three different healthy reefs located around Lizard Island Research Station when there were no boats or ships passing. Ship recordings were made from different passing ships (~53,000 tonne bulk carriers, engines type MAN-B&W Diesel; engine power 13,501 kW) at distances ranging from 1.9 to 3.0 km from the recorder (as per Velasquez Jimenez et al. 2020; **Chapter 2**). All sound recordings were made using SoundTrap 202 (Ocean Instruments, New Zealand) digital sound recorders with a 48 ksps sample rate (manufacturer's specifications of flat response within ± 3 dB between 20 Hz and 60 kHz).

Playbacks were constructed from original field recordings using the software Audacity^{TM} 2.2.1 (http://www.audacityteam.org/). Each playback was 30 min long and consisted of 15 min of ambient reef noise playback followed by the respective treatment (i.e., reef playback (mean SPL 51.26) or abrupt ship noise playback (mean SPL 63.41)). The circular holding tank (112.5 cm diameter x 40 cm height) was positioned on top of bricks, with a 4 cm, layer of foam between the base of the tank and the bricks to reduce acoustic artefacts caused by vibrations transmitted through the ground. Acoustic treatments were played using a MP3 player (SanDisk 8 GB Clip Jam), an amplifier (18W, Kemo Langen, Germany) and a J9 speaker (NUWC-USRD, Newport, RI, USA). The J9 speaker was kept in a fixed position by a bungee cable attached to a wood structure on top of the tank. Water depth was 30 cm and the speaker was suspended 10 cm below the surface and 30 cm away from the interaction arena.

Acoustic conditions were assessed in the center of the interaction arena (**Figure 4.1**). Sound recordings of playbacks were made using an accelerometer with an inbuilt hydrophone (M20-040 Geospectrum Technologies, Dartmouth, Canada, sensitivity following a curve from 10 to 300 Hz, calibrated by manufacturers) connected to a digital eight-track recorder (Zoom F8 field recorder, sampling rate 48 kHz, Zoom Corporation, Tokyo, Japan; **Figure D2** in **Appendix D**).

Experimental set-up

Experimental trials were undertaken in an elliptical interaction arena placed within an outer tank (Nally IH051, 645 x 413 x 276 mm, 10 cm water height; 2.5 mm wall thickness). At the same time, the outer tank and the speaker were contained in a circular holding tank (as described above). LED lights placed outside the outer tank illuminated the interaction arena. During behavioural trials, white corflute (plastic sheet) covered the interaction arena to minimise visual disturbance. The corflute had a 3 cm diameter hole where the camera was placed to record the interaction trials (**Figure 4.1**).

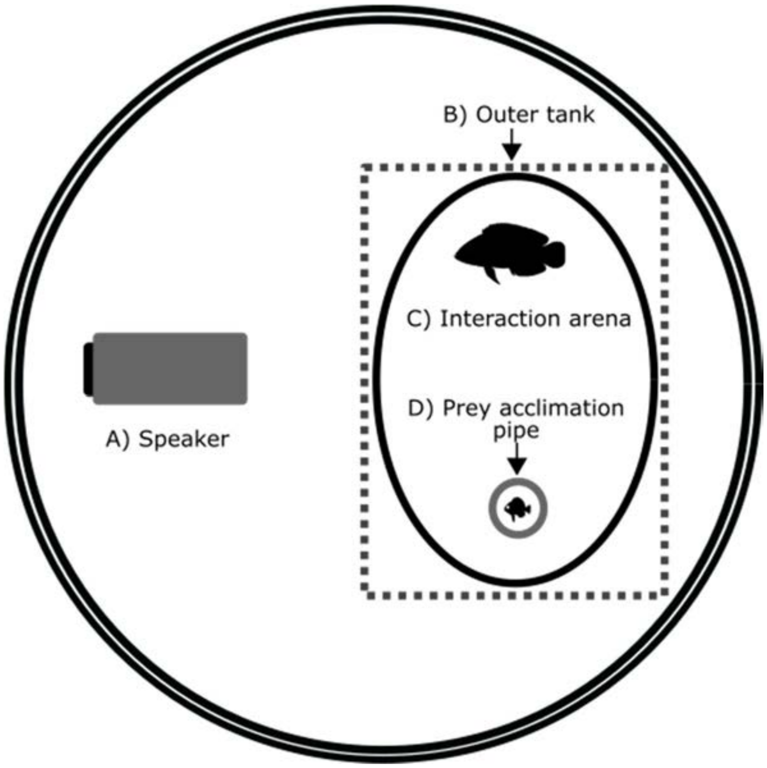

Figure 4.1. Experimental set-up used to examine the effects of elevated temperature and ship noise on predator-prey interactions. The setup was contained in a round holding tank. The setup included **(A)** a J9 speaker, **(B)** an outer tank, **(C)** the interaction arena where the predator *Pseudochromis fuscus* was placed and **(D)** the acclimation pipe where the prey *Pomacentrus amboinensis* was placed. The interaction trials started by lifting the pipe and releasing the prey.

Interaction trials

Predator-prey interactions were examined using the protocol described by Allan et al. (2015, 2017). In summary, it involved placing randomly selected prey in the acclimation pipe and randomly selected predator in the interaction arena. Before the start of the trial, the interaction arena was covered to avoid visual disturbances. Fish were given 15 min of acclimation in the interaction arena while reef playback was playing. The trial started by carefully lifting the pipe enclosing the prey. The interaction between the predator and prey was recorded using a digital camera (Casio EX-ZR1000) at 240 frames per second for 10 min or until the prey was consumed. To standardize for predator position, trials only commenced when the predator was at least 10 cm away from the prey acclimation pipe. Water within the experimental arena was the same temperature as the respective conditioning tanks (i.e., either 29°C or 31°C). At the end of each trial, the seawater in the interaction arena was replaced with fresh seawater and brought up to the appropriate temperature for the next trial.

Videos were analysed using the software ImageJ 1.53f and manual tracking plugin (https://imagej.nih.gov/ij/). Kinematic variables were analysed based on the centre of mass (COM) of the fish when straight (Webb 1976). All variables, except attack rate, predation rate and capture success, were measured only during the first predator attack of the interaction trial. This was to control for anaerobic stress from either the predator or the prey due to prolonged attacks. Prey was used only once to avoid acclimation to the experimental procedure. Due to the low number of *P. fuscus* collected, predators were used twice.

The following variables were measured from the videos:

Prey

1. Mean prey speed (m/s): distance covered by prey within the first two tail flips (24 ms). The first two tail flips (24 ms) is consider the crucial time to avoid ambush predator attacks (Webb 1976, Domenici and Blake 1997).

2. Prey reaction distance (m): distance between the prey and the tip of the predator's snout at the onset of the escape response to a predator attack.
3. Prey distance travelled (m): the straight-line distance between the prey COM at the onset of the escape response and at the end of the escape response (i.e., when the prey came to a halt).

Predator

4. Predator attack distance (m): the straight-line distance between the predator COM at the time the attack commenced and the end of the attack (end is defined as when the predator came to a halt).
5. Maximum attack speed (m/s): the top speed achieved by the predator at any point in time during the attack, based on the predator COM.
6. Attack rate (# attacks/min): total number of attacks per minute for the duration of the interaction, measured for each interaction (end of the trial defined when the prey is consumed or after 15 min).
7. Capture success: whether prey was consumed or not by the predator (i.e., binary response, 1 when prey was consumed and 0 when it was not).

Additionally, prey length and predator length were recorded from the videos and included as covariates. Interaction trials where predators did not attack the prey were excluded from the analysis **(**29°C– reef playback, n = 9; 29°C- ship noise playback, n = 5; 31°C- reef playback, n = 9; 31°C- ship noise playback, n = 5**)**, statistical analysis indicated that there were not significant differences in whether predators attack or not between trials treatments (**Table D1** in **Appendix D**).

Statistical analysis

Separate linear mixed effect models (LMERs) were used to examine the effects of temperature treatment, acoustic treatment and their interaction on prey and predator kinematics, while controlling for the random effect of predator identity. A mixed-effect logistic regression (GLMER) was used to examine the effects of temperature treatment, acoustic treatment and their interaction on capture success, while controlling for the random effect of predator identity. For all models, temperature treatment, acoustic treatment and their interaction were included as fixed effects, with prey length, predator length, food depravation time and number of trial as covariates. The minimal model was obtained by sequentially removing fixed affects and their interactions when found non-significant (interactions being removed before individual fixed effects). Significance was tested by likelihood radio model comparisons of the maximal model with the nested model where the effect in question was removed. Chi-squared statistic and p-values for fixed effects were obtained by likelihood ratio tests comparing the minimal model with a model excluding the fixed effect included in the minimal model. Model assumptions were assessed using the check model function from the Performance package (Lüdecke et al. 2021). Post-hoc pairwise comparisons were performed using the Tukey method. Statistical models were made in R version 4 using the lme4 package (Bates et al. 2015).

4.4. Results

Prey escape speed was affected by elevated temperature (LMER: $X^2 = 14.33$, $p = <0.001$, predator id: variance = 3696, s.d. = 60.79), but not by acoustic treatment ($X^2 = 0.18$, $p = 0.665$) or by the interaction of temperature and acoustic treatment ($F = 0.72$, $p = 0.395$). Prey exposed to elevated temperature had a 36.5% lower escape speed compared to individuals in the control temperature treatment, regardless of the acoustic treatment they were exposed to (**Figure 4.2A**). Additionally, prey escape speed was affected by predator length ($X^2 = 4.79$, $p = 0.028$; **Figure D3A** in **Appendix D**) and food depravation time ($X^2 = 13.01$, $p = 0.011$; **Figure D3B** in **Appendix D**). When interactions occurred with bigger predators, prey displayed a higher escape speed, while as the food depravation time increased prey escape speed decreased (**Figure D3** in **Appendix D**). Prey reaction distance and prey escape distance were not affected by temperature treatment, acoustic treatment nor their interaction (**Figure 4.2B**, **Figure D4A** in **Appendix D**, respectively; see **Table D1** in **Appendix D** for statistic details).

Predator attack distance and predator maximum speed were not affected by temperature treatment, acoustic treatment, their interaction or any of the covariates (**Figure D4B** and **C;** and **Table D1** in **Appendix D** for complete statistical results). Attack rate was not affected by temperature treatment, acoustic treatment or their interaction (**Figure 4.2C**), but it was affected by predator length (LMER: X^2 = 32.24, p = <0.001; predator id: variance = 0, s.d.= 0; **Figure D5A** in **Appendix D**) and predator food depravation time (X^2 = 11.07, p = <0.001; **Figure D5B** in **Appendix D**). Bigger predators displayed a higher attack rate, while as the food depravation time increased attack rate decreased (**Figure D5** in **Appendix D**). Capture success was not affected by temperature treatment, acoustic treatment or their interaction (**Figure 4.2D**), but it was affected by prey length (GLMER: X^2 = 23.33, p = <0.001; predator id: variance = 2.62, s.d. = 1.61). As prey length increased they were more likely to be captured by the predator (**Figure D6** and **Table D1** in **Appendix D** for details).

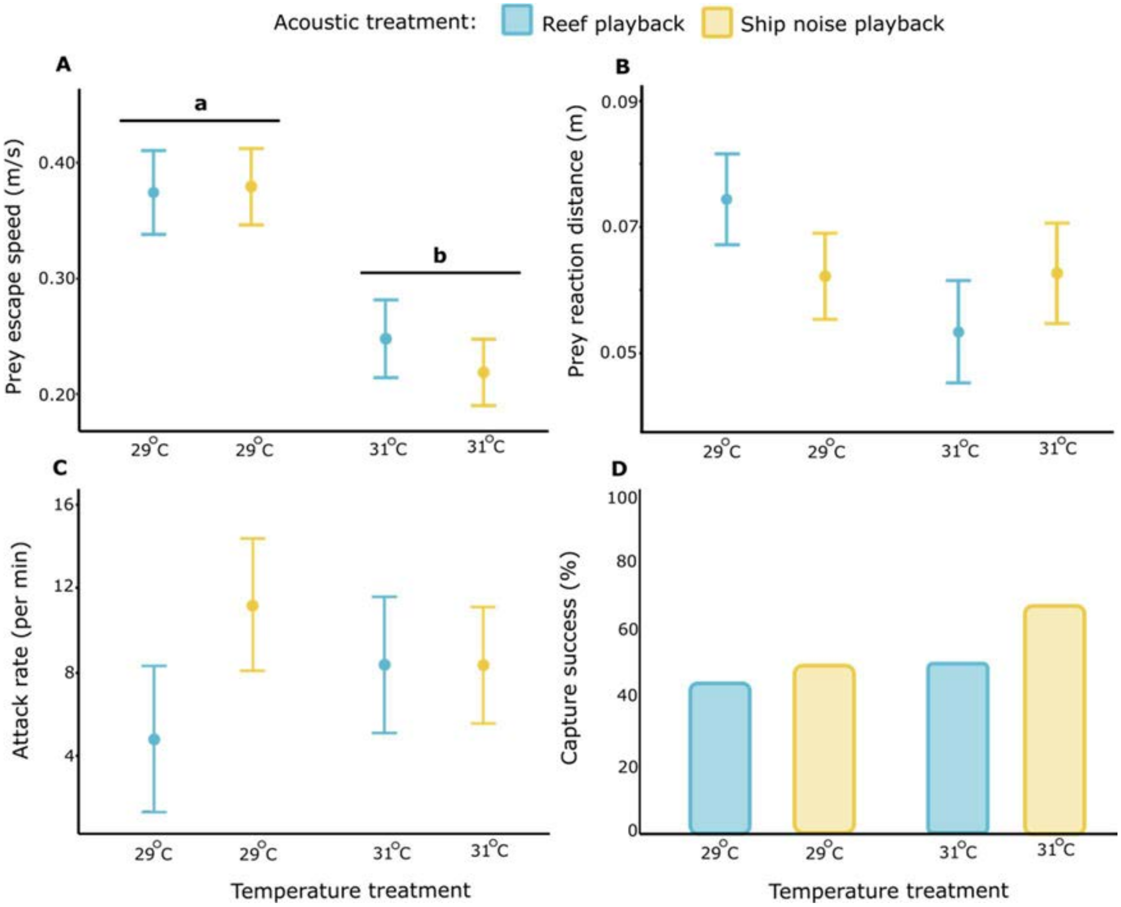

Figure 4.2. **Effect of elevated temperature and ship noise playback on predator-prey interactions of juvenile *Pomacentrus amboinensis* and predator *Pseudochromis fuscus*.** **(A)** Prey escape speed (mean ± s.e.), **(B)** Prey reaction distance (mean ± s.e.), **(C)** Attack rate (attacks per 10 min) and **(D)** Capture success. Lowercase letters represent Tukey's HSD post-hoc means comparison groups. Adjusted means plotted. Left to right: *n = 22, 26, 25, 37*.

4.5. Discussion

Marine communities are and will continue to be exposed to multiple anthropogenic stressors. Our study is the first to investigate the combined effects of elevated temperature and ship noise playback on predator-prey interactions. We found that elevated temperature affected prey, whereas ship noise playback had no detectable effects on predator-prey interactions. In specific, we found that elevated temperature caused prey to decrease their escape speed, however, this did not result in a higher capture success by the predators. Furthermore, there was no evidence of an interaction between both stressors. Our study highlights the challenges associated with predicting interactive effects of environmental stressors and the importance of examining their effects on ecological interactions. Our study provides useful knowledge for management and suggests that in the scenario where elevated temperature and ship noise co-occur, elevated temperature would be the dominant stressor.

Elevated temperature led to a decrease in prey escape speed, however, predator behavioural traits and mortality of prey were not affected. Studies examining the effects of elevated temperature on juvenile performance and predator-prey interactions have found prey to be negatively affected while predators increase their success. Allan et al. (2015) found that when exposed to elevated temperature (+ 3°C), damselfish prey (*Pomacentrus wardi)* displayed slower swimming speeds and shorter escape distances, while Allan et al. (2017) found that prey (*P. wardi*) reaction distances were shorter. Additionally, both studies found that predators (*P. fuscus)* increased their attack speeds, attack rates and had higher predation rates. Differences could be related to different experimental temperatures and acclimation periods. The experimental temperatures used in these studies were lower than the ones used in the present study (Control: 26.7°C and 27°C, elevated temperature: 29.6°C and 30°C). Additionally, although prey were exposed to temperature treatments for seven days some predators in the present study were used twice, meaning they spent one or two more days exposed to the temperature treatment and could have potentially acclimated to the elevated temperature. Previous studies have found that predator pressure increases with temperature up to a point and then declines. For example, Scott et al. (2017) found that foraging frequency of Coral trout

(*Plectropomus leopardus)* increased with temperature but declined at temperatures higher than 30°C degrees. While Grigaltchik et al. (2012) found a similar pattern in freshwater species. These studies suggest that after predators have reached their performance optima motivation to attack decreases, which could potentially explain the lack of effects in predator behavioural traits in our study.

Ship noise playback did not affect predator-prey interactions. To date there have been no studies examining effects of ship noise on predator-prey interactions, but one previous study found that in isolation ship noise playback affected the anti-predator behaviour of a congeneric species. In a tank-based experiment, Velasquez Jimenez et al. (2020) found that ship noise playback increased the latency response and decreased the activity of juvenile Whitetail damselfish (*P. chrysurus*), resulting in a slower response to predator attacks. In the present study, ship noise did not affect the escape response of prey. This could be due to differences in the experimental set up used. However, a variety of responses to ship noise have been found when studying temperate species. Voellmy et al. (2014b) found that the response latency of the Three-spined stickleback (*Gasterosteus aculeatus*) decreased when exposed to ship noise playback, while the response latency of European minnow (*Phoxinus phoxinus*) was not affected. These differences between studies could be related to species-specific hearing ranges, which are determinant of their response to noise. Marked differences between taxa exposed to the same acoustic treatment have been previously shown (e.g., Voellmy et al. 2014a, 2014b, Pieniazek et al. 2020). In the present study we observed that attack rates were higher when predators were exposed to ship noise playback. However, analysis showed that this pattern was driven by predator' size and the time predators were food deprived. Predators attacked more when they were bigger and when the food depravation time was shorter. Another potential explanation for differences among these studies are differences in acoustic treatments. In our study, the playback of ambient reef noise was noisier than the original recordings and the ship noise playback was quieter than the original ship recordings, resulting in smaller differences in soundscapes between ambient and ship noise treatments. This may have led to a conservative response to ship noise in the current study.

We found no evidence for interactive effects between elevated temperature and ship noise playback on predator-prey interactions. Moreover, the combined effects of these two stressors were less negative than expected. For example, in elevated temperature conditions capture success by Dusky dottybacks was found to increase up to 41% (Allan et al. 2015), whereas we found only a 6% increase when exposed to elevated temperature and a 23% increase in the additive treatment with ship noise. Overall, we only found elevated temperature had a greater effect than ship noise playback. This could be related to the nature of the effect of each stressor and their levels. Elevated temperature affects physiological processes (reviewed in Domenici et al. 2019) while anthropogenic noise causes stress and impairs sensory abilities and inhibits attention (e.g., Purser and Radford 2011, Holmes et al. 2017), suggesting that the response to one stressor can influence the individual's response to the second stressor (Galic et al. 2018). However, we did not test this directly and future studies are required to corroborate this possibility.

Examining the effects of multiple stressors is challenging as they can interact in complex ways (Jackson et al. 2016), effects can be context dependent (Belarde and Railsback 2016) and experiments can be logistically challenging to conduct. Our study provides a first attempt to examine the interactive effects of elevated temperature and ship noise playback on a key ecological process such as predator-prey interactions. We found that prey were detrimentally affected by elevated temperature, making them more vulnerable to predation, however this did not translate to a higher capture success by the predator. The temperature exposure time in our study was relatively short (seven days), simulating a heatwave which is currently ecologically relevant (Warren et al. 2016), however in future climate change scenarios fish will likely be exposed to elevated temperature for longer periods of time which may influence how they acclimate over time (e.g., Warren et al. 2016). Nevertheless, previous studies have found results cannot be extrapolated as effects are highly variable between species and noise sources (e.g., Voellmy et al. 2014b, McCormick et al. 2019, Pieniazek et al. 2020). Understanding the effects of anthropogenic noise and its interactions with other stressors is valuable for management, since noise pollution management is easier to achieve than the management of global stressors such as elevated temperature (i.e., climate change, Côté et al. 2016).

Chapter 5: Prenatal exposure to boat noise playback has carryover effects on escape response, but recent noise history affects fish growth

In preparation for Oikos

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5.1. Abstract

Small motorboats produce noise that has been shown to have detrimental effects on fishes. Boat noise has been found to affect the physiology, behaviour and even survival of embryos, juveniles and adults; however, it is currently not known whether effects persist beyond the brief period of noise exposure or are cumulative throughout life. Here we evaluated the long-term effects of boat noise playback on the escape response and growth of a common reef damselfish, *Acanthochromis polyacanthus*. In a tank-based experiment, using a split-brood design, fish were exposed to playback of boat noise or ambient sound prior to hatching (parents and embryos) and/or as juveniles for up to 78 days and were subsequently tested for their escape response in the absence of noise and measured for growth. We found that individuals exposed to boat noise prior to hatching and as juveniles were less likely to respond to a simulated predator attack than those that had experienced only ambient sound, and when they did respond they were more likely to swim towards the predator. We also found that individuals exposed to boat noise as juveniles were smaller compared to those exposed to ambient sound, regardless of whether they were exposed to boat noise or ambient sound prior to hatching. Our results demonstrate that exposure to boat noise during early development has the potential to affect growth and disrupt the escape response of juveniles, even when the response occurs in quiet conditions. Limiting motorboat traffic close to reefs during the breeding season may mitigate the adverse effects of boat noise on reef fishes during the vulnerable early life stages.

5.2. Introduction

Anthropogenic noise in marine environments is now recognised as a pollutant that can affect key indicators of ecological performance, including behaviour (e.g., communication, foraging, anti-predator behaviour), physiology (e.g., heart rate, growth), survival and reproduction (e.g., Simpson et al. 2016, Nedelec et al. 2017, Fakan and McCormick 2019). One of the most prevalent sources of anthropogenic noise in marine ecosystems is small motorboats, which are predicted to increase markedly in coming years (GBRMPA 2019, UNCTAD 2020). To date, most studies evaluating the effects of motorboat noise have examined direct responses to short-term or single noise exposures (e.g., Holmes et al. 2017, McCormick et al. 2019). Few studies have examined the long-term effects of repeated exposure (e.g., Nedelec et al. 2016a, Radford et al. 2016, Neo et al. 2018), which is likely to occur in natural environments when multiple boats visit or transit an area. The impact of boat noise on fishes has been considered to be potentially "short lived", meaning that once the noise source moves away or is removed there are no remaining effects (Bruintjes et al. 2016, McCormick et al. 2019). However, some studies suggest the effects of boat noise can persist after noise exposure has stopped (e.g., Amoser and Ladich 2003, Mills et al. 2020). Currently, it is unknown whether repeated exposure to boat noise of parents and their embryos, and/or juveniles has detrimental effects that persist beyond the actual period of noise exposure to influence subsequent life stages.

Most studies that have evaluated the effects of boat noise have focused on a single life-stage (e.g., Holmes et al. 2017, Mills et al. 2020, but see: Fakan and McCormick 2019). However, different life-stages are likely to be affected differently due to differences in acoustic sensitivity and energy allocation (Kenyon 1996, Wright et al. 2011). Previous studies have found that boat noise can affect embryos during development. For example, in a field study Jain-Schlaepfer et al. (2018) found that embryonic exposure to boat noise affected the heart rate of Staghorn damselfish (*Amblyglyphidodon curacao*). While in a tank-based study Fakan and McCormick (2019) also found that boat noise led to an increase in heart rate in Spiny chromis (*Acanthochromis polyacanthus*) and the Fire clownfish (*Amphiprion melanopus*). In the same study, Fakan and McCormick (2019) found that after hatching Spiny chromis (*Acanthochromis polyacanthus*) juveniles previously exposed to boat noise were larger than individuals exposed to ambient playback. Exposure to boat noise during larval development has been found to disrupt behaviour but not affect growth of Atlantic cod (Nedelec et al. 2015). Whether the detrimental effects of noise during embryonic development extend into the juvenile stage, or result in ontogenetic cumulative effects, is currently unknown.

Many studies have found important intergenerational effects of stressors, where the conditions experienced by the parents influence the potential fitness of their offspring through nutritional, physiological or epigenetic effects (e.g., Donelson et al. 2009, McGhee and Bell 2014, Stratmann and Taborsky 2014). If parents have been exposed to the acoustic stress of boat noise does that make their juvenile offspring more or less resilient to the stress of similar noise? The strong physiological, developmental, behavioural and performance links among fish life-stages suggest that carryover and cumulative effects are fundamental in the shaping of existing populations and communities (McCormick and Gagliano 2008, O`Connor et al. 2014). Given the adverse effects of boat noise on adult behaviour and physiology (e.g., Nedelec et al. 2017), and its direct effects on juveniles (McCormick et al. 2019), it is of interest to know the relative importance of the prenatal acoustic influences stress (i.e., through parents) versus direct juveniles effects and how acoustic stress during these two life stages may combine to influence juvenile fitness. Studying these cross life-stage effects can help us understand the long-term effects of noise pollution and its consequences for population fitness (Lara and Vasconcelos 2021), which may be underestimated in short-term or discrete exposure studies.

The aim of the present study was to investigate the relative influences of exposure to boat noise prior to hatching or during juvenile life on growth and escape response of a juvenile reef fish. Using a split-brood design, breeding pairs of Spiny chromis, *Acanthochromis polyacanthus*, were maintained in ambient sound or boat noise playback treatments, and then their broods of newly hatched juveniles were split and reared either in reef sound or boat noise playback treatments. The growth and predator escape response of juveniles were then measured eleven-weeks later. From previous research we predicted that parental influences prior to hatching may set the initial growth trajectories of juveniles, that would be emphasised through development and influence their subsequent performance and responsiveness to boat noise.

5.3. Materials and Methods

Study species

The Spiny chromis, *Acanthochromis polyacanthus*, is a widely distributed damselfish species found across the Indo-Pacific, with adults forming pairs and breeding during the summer months (**Figure E1** in **Appendix E**; Robertson 1973). The species has a direct development (i.e., lacks a planktonic larval stage) and parental influences extend to offspring care as, like many demersal fishes (Thresher 1984), they guard clutches of embryos prior to them hatching into independent offspring (Kavanagh 2000). For this species, the influences due to epigenetics, physiology and nutrition are inseparable from the influences of nest tending behaviours under natural conditions.

In the present study, *A. polyacanthus* adults were collected using barrier nets and hand nets from shallow backreef habitat around Lizard Island (14°41′S, 145°27′E), northern Australia, during November 2016. They were then transported to indoor aquarium facilities at James Cook University, Townsville, Australia, placed into breeding pairs within 200 L tanks and maintained at 28.5℃, with a light cycle that matched natural summer conditions (12 hr:12 hr light:dark). The present study was conducted from March to July 2018 on these original pairs.

All methods and research within this study were carried out in accordance with the animal ethics guidelines and regulations of James Cook University, and all protocols were approved by the James Cook University Animal Ethics Committee (approval numbers: A2408 and A2361).

Sound treatments

Sound recordings were made at sandy-bottom locations around Lizard Island back reef in 2013. Ambient reef sounds were recorded using a hydrophone during the day adjacent to healthy reefs. Boat noise was recorded from five different research station motorboats (aluminium hull with 30 horsepower 4-stroke Suzuki outboard motors). Each boat cruised at various speeds between 10 to 200 metres away from the hydrophone, simulating typical boat traffic experienced around coral reefs. Recordings were taken 1 metre above the seabed. Acoustic pressure was measured using a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier; sensitivity flat across the frequency range 2 Hz–30 kHz; -167 dB; calibrated by manufacturers, High Tech Inc., Gulfport MS). Particle acceleration was measured using a calibrated triaxial accelerometer (M20-040; sensitivity following a curve over the frequency range 0–3 kHz; calibrated by manufacturers, Geospectrum Technologies, Dartmouth, Canada) and a digital recorder (Boss BR-800, 44.1 kHz sampling rate, Roland Corporation, Los Angeles, CA).

Ambient sound and boat noise playbacks were created from field recordings using Audacity™ version 2.2.0 (http://www.audacityteam.org/). Five sets of each sound treatment were created by compiling ambient and boat recordings from different reefs and boats, to minimise pseudo-replication. The ambient playbacks were 12-hour sections of ambient reef sound (played during daylight hours: 06:00–18:00). The boat noise playbacks were identical with the addition of a random number (minimum 3, maximum 6) of 20-minute sections of boat noise spliced into each 12-hour section of ambient sound. The timing of boat noise sections was random within daylight hours. Silence was played during night-time hours. To control for the potential impact of the soundtrack changing from one playback track type to another in the boat noise treatment (e.g., if any artefacts were created in the sounds as a result of combining files), ambient sound playbacks also had different ambient sound sections with the same duration and timing spliced in to match the boat recordings in timing. The sound system used for playback of the treatments consisted of a battery pack (Cygnett Incharge 2500), an MP3 player (SanDisk 8 GB Clip Jam), an amplifier (18 W, Kemo Langen Germany) and an underwater loudspeaker (Lubell UW-30, University Sound, Whitehall, OH, USA, frequency range: 0.1–10 kHz).

Acoustic pressure and particle acceleration of playbacks were recorded in one parental tank, while only acoustic pressure was measured in one juvenile tank, due to time restrictions (note that all parental and all juvenile tanks had the same dimensions). Recordings were made using the same hydrophone and accelerometer as detailed above. The digital recorder used was different (Zoom F4, Zoom Corporation, Tokyo, Japan), but was calibrated in the same way (pure sine waves measured with an oscilloscope). The sound levels recorded in the field, the parent tanks and the juvenile tanks were analysed using paPAM software (Nedelec et al. 2016b). Power spectral density across the frequency range 0–2 kHz (hearing range of juvenile reef fishes; Wright et al. 2010) was calculated from 30-second clips for all situations and both sound treatments using Fast-Fourier Transformation (see **Figure 5.2** for the acoustical representation of the field and the playback recordings).

Experimental design

Breeding pairs (n = 23) were provided with half a terracotta pot as a shelter and a spawning site; an underwater loudspeaker was placed at the side of the tank, facing the shelter (**Figure D2A** in **Appendix D**). Pairs were fed twice daily with O.range NRD G12 fish hatchery diet. Each pair was randomly assigned to ambient sound playback (n = 14; mean standard length: 10.73 ± 0.11 cm) or boat noise playback (n = 11; 10.77 ± 0.12 cm). Tanks were checked daily for the presence of a new clutch. All pairs were exposed to treatments for a minimum of 4 days before the first egg clutch; maximum exposure before laying was 26 days (**Figure 5.1**). Embryos were kept with the parents, meaning they were exposed to the same acoustic treatment, as a result parental and embryological effects cannot be isolated with the current design. Nevertheless, this is the natural situation in the current species, where embryos are guarded, oxygenated and maintained by the parents (Robertson 1973).

On the day of hatching, juveniles were separated evenly into separate 5 L tanks (**Figure E2B** in **Appendix E**). The number of individuals placed into each treatment varied according to the survival within each brood (final numbers **Table E1** in **Appendix E**). Juvenile tanks were exposed either to ambient sound playback or boat noise playback resulting in four treatments (prenatal – juvenile treatment): AA = Ambient–ambient, AB = Ambient–boat, BB = Boat–boat and BA = Boat–ambient (see **Figure 5.1**). Juveniles were initially fed twice a day with *Artemia spp*. nauplii and after three weeks, individuals were fed twice a day with O.range WEAN-S fish hatchery diet. Juveniles were housed in buckets contained in four 400-L cylindrical tanks (110 x 40 cm). A loudspeaker was placed in the centre of the tank, facing upwards (assuring a symmetrical sound field within the tank), and the juvenile 5-litre buckets were sitting on terracotta pots around the outside of the tank, forming a circle around the loudspeaker, to maximise depth for the sound field while keeping juveniles in suitably sized subdivisions (**Figure E2.B** in **Appendix E**). Individuals were exposed to their respective treatment for 41 days, after which they were tested for their escape response and measured for length.

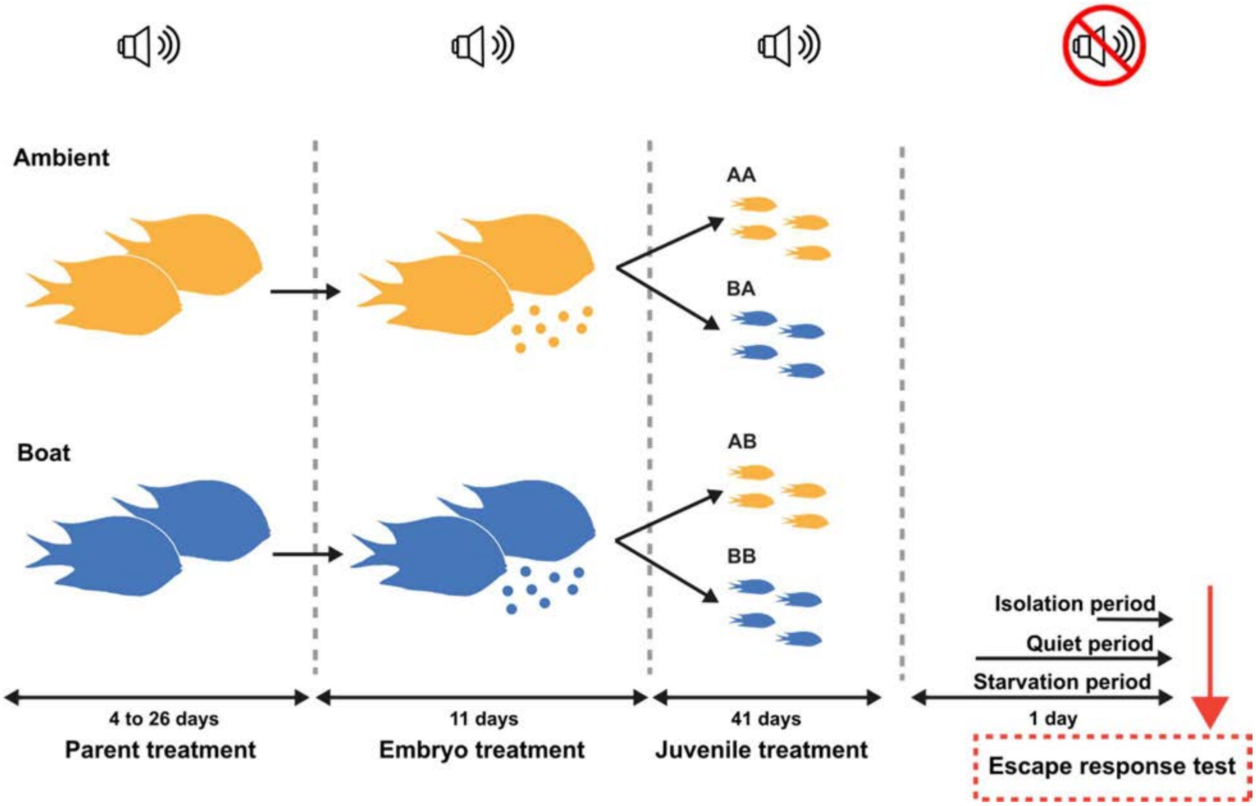

Figure 5.1. Breeding and rearing design to examine the long-term effects of boat noise exposure on *Acanthochromis polyacanthus*. Breeding pairs of *Acanthochromis polyacanthus* and embryos were exposed to either ambient sound playback (yellow, *n = 14*) or boat noise playback (blue, *n = 11*). Offspring from each brood were divided randomly between juvenile ambient sound (yellow) and boat noise (blue) treatments. Abbreviations: AA = Ambient–ambient (*n = 92*), AB = Ambient–boat (*n = 96*), BA = Boat–ambient (*n = 54*) and BB = Boat–boat (*n = 57*). Samples sizes correspond to the final number of individuals tested.

Escape-response protocol

The testing arena consisted of a circular experimental arena (diameter 20 cm) contained within a large opaque-sided plastic tank (38 cm x 58 cm x 4 cm water height) with a transparent bottom to allow responses to be recorded from below the tank. Water temperature was 27.5°C. The tank containing the experimental arena was covered with an opaque white lid, to avoid laboratory disturbances, and was illuminated with a strip of LED lights around the tank, placed above the water surface (**Figure E3** in **Appendix E**).

Individuals were deprived of food for 24 hours prior to the start of the trials to standardise for satiation. Additionally, individuals were isolated from playback noise for approximately 14 hours (overnight and the morning of the test) and isolated from conspecifics for 2 hours (on the morning of the test) before the trial to allow them to acclimatise to being alone (**Figure 5.1**). For each trial, a single fish was introduced to the testing arena via a water-filled sample jar (an attempt to minimizing stress associated with the movement from the tank to the arena).

After a 10-min acclimation period, a tapered weight was released into the tank from above the water surface. This was accomplished by turning off an electromagnet to which the weight was attached via a metal disc (15 mm in diameter); the stimulus was released through a white PVC tube (diameter 48.5 mm) suspended above the experimental tank, with the bottom edge at 10 mm above the water level, to provide a sudden stimulation. Responses to the stimulus were recorded on video at 480 frames per second with a camera (CASIO EX-ZR1000). Water in the experimental arena was stirred between trials to homogenize potential olfactory cues and it was changed completely every four trials. Escape response variable analysis was restricted to the first two kinematic stages of the escape response (the first two axial bends, i.e., stages 1 and 2 defined based on Domenici and Blake 1997), which is the period considered crucial for avoiding predator ambush attacks (Domenici and Blake 1997).

Escape response videos were analysed using ImageJ software (https://imagej.nih.gov/ij/), blind to the sound treatment. Distances in videos were calibrated using the diameter of the PVC tube as a reference.

The distance to the stimulus at the onset of the response was recorded as a potential covariate. Variables were measured based on the centre of mass of the fish when straight (based on Webb 1976).

Escape response variables were defined as follows:

Non-locomotor variables:

1. Responsiveness: whether individuals responded with a sudden acceleration to the stimulus (i.e., binary response: 1 = startled, 0 = not startled; Fuiman et al. 2006).
2. Directionality: whether the first detectable movement of the head during a response was orientated away or towards the stimulus (Domenici 2010).
3. Response latency (s): the time interval between the stimulus onset (the moment the stimulus made contact with the surface of the water) and the first detectable movement of the fish (Domenici 2010).

Locomotor variables (defined based on Domenici and Blake 1997):

4. Response distance (m): the total distance covered by the fish during the escape response.
5. Mean escape speed (m/s): the distance covered within the first two tail flips (24 ms). The first two tail flips are considered the crucial time to avoid predator attacks.
6. Maximum escape speed (m/s): the maximum speed reached at any time during the escape response.

After the trials, individuals were humanely euthanized according to ethical procedures permitted by James Cook University. A photograph of each individual was taken using a Casio EX-ZR1000 camera next to a ruler as scale. Length measurements were conducted blind to the acoustic treatment using ImageJ version 152.d (https://imagej.nih.gov/ij/).

Statistical analyses

Mixed-effects logistic regressions were used to analyse the effect of prenatal treatment, juvenile treatment, their interaction and days of parental exposure on juvenile responsiveness and directionality, while controlling for the random effects of clutch and juvenile tank. Standard length was included as a covariate. Linear mixed-effects models fitted by maximum likelihood were used to test for the effect of embryo treatment, juvenile treatment, their interaction and days of parental exposure on speed, maximum speed, distance, latency and length, while controlling for the random effects of clutch and juvenile tank. For all models, the minimal model was obtained by sequential removal of fixed effects and their interactions when found to be non-significant. Significance was tested by likelihood ratio model comparisons of the maximal model with the nested model where an effect in question was dropped. Chi-squared statistic and p-values for fixed effects were obtained by likelihood ratio test comparing the minimal model with a model excluding the fixed effect included in the minimal model. Distance and speed were power of 2 transformed to meet normality assumptions. Model assumptions were assessed using residual plots, all of which were satisfactory. Post-hoc pairwise comparisons were performed using the Tukey method. The assumptions of normality and homogeneity of variance were examined graphically and found to be satisfied for maximum speed and latency. Statistical modelling was performed in the software R version 4 using the lme4 package (Bates et al. 2015).

5.4. Results

Playback tank recordings differed from field recordings due to near-field effects and interference caused by reflections and reverberations within the tank walls. However, acoustic analysis showed a clear difference between the boat noise treatment and the ambient sound treatment in both the parent and juvenile tanks. The sound-pressure and particle-acceleration levels of the ambient sound treatment were lower than the boat noise treatment levels. Sound-pressure and particle-acceleration levels in the experimental tanks were greater than values in field recordings, especially in low frequencies (<300 Hz) for both acoustic treatments. The juvenile tank playbacks had a higher level of spectral distortion than field recordings, resulting in almost 40 dB greater sound-pressure levels in frequencies under 500 Hz (**Figure 5.2**).

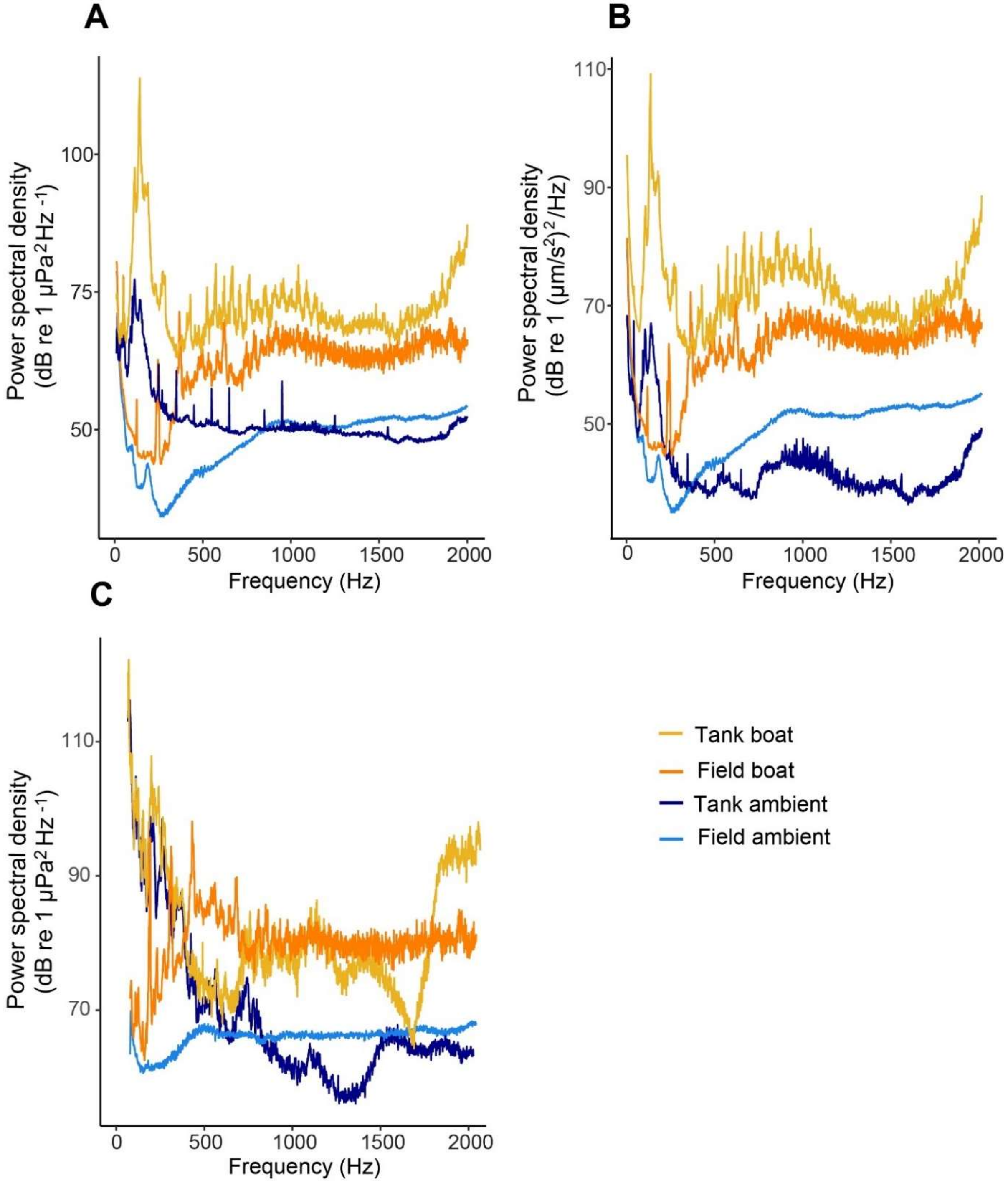

Figure 5.2. **Acoustical representation of the field and the playback recordings. (A)** Power spectral density for sound-pressure levels and **(B)** Triaxial particle-acceleration levels in parent tanks compared to field recordings. **(C)** Power spectral density sound-pressure levels in the juvenile tanks compared to field recordings.

A. polyacanthus juvenile length was significantly affected by juvenile treatment (LME: $X = 4.47$, $p = 0.034$; clutch: variance = 0.006, s.d. = 0.08; juvenile tank: variance = 0.008, s.d. = 0.09) and days of parental exposure ($X^2 = 5.09$, $p = 0.024$). Juveniles exposed to boat noise playback after hatching were 7% shorter compared to individuals exposed to ambient sound playback (Tukey, $t = 2.18$, $p = 0.042$; **Figure 5.3**). Additionally, as days of parental exposure increased individuals were smaller (**Figure E4** in **Appendix E**). However, there was no significant effect of prenatal treatment ($X^2 = 2.57$, $p = 0.108$) or a treatment interaction ($X^2 = 0.12$, $p = 0.728$).

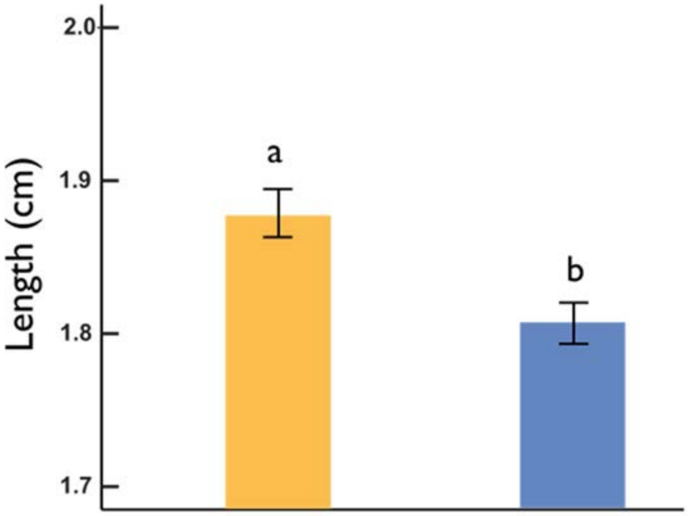

Figure 5.3. **Standard length (mean ± s.e.) of *Acanthochromis polyacanthus* juvenile exposed to boat noise (blue) or ambient sound (yellow) after hatching.** Bars with the same letter did not differ significantly according to the Tukey's HSD post-hoc means comparisons.

Responsiveness of *A. polyacanthus* juveniles to the startle stimulus was significantly affected by the interaction between prenatal treatment and juvenile treatment (GLMER: $X^2 = 9.35$, $p = 0.002$; clutch: variance = 1.11, s.d. = 1.05; juvenile tank: variance = 0.72, s.d. = 0.85; prenatal treatment: $X^2 = 0.11$, $p = 0.738$; juvenile treatment: $X^2 = 1.08$, $p = 0.298$; days of parental exposure: $X^2 = 0.08$, $p = 0.769$). The significant interaction was driven by a difference in responsiveness between juveniles that had prenatal experience with boat noise and then went on to experience either boat noise playback, and those that experienced ambient sound playback as juveniles (**Figure 5.4A**). A significantly lower proportion of juveniles in the boat–boat treatment responded to the stimulus (68%) compared to individuals in the boat–ambient treatment (83%; Tukey, z = 2.81, *p* = 0.025). However, there were no differences in the proportion of individuals that responded to the stimulus between the ambient–ambient and ambient–boat treatments (Tukey, z = 0.72, *p* = 0.88; z =1.45, *p* = 0.462 respectively; **Figure 5.4A**).

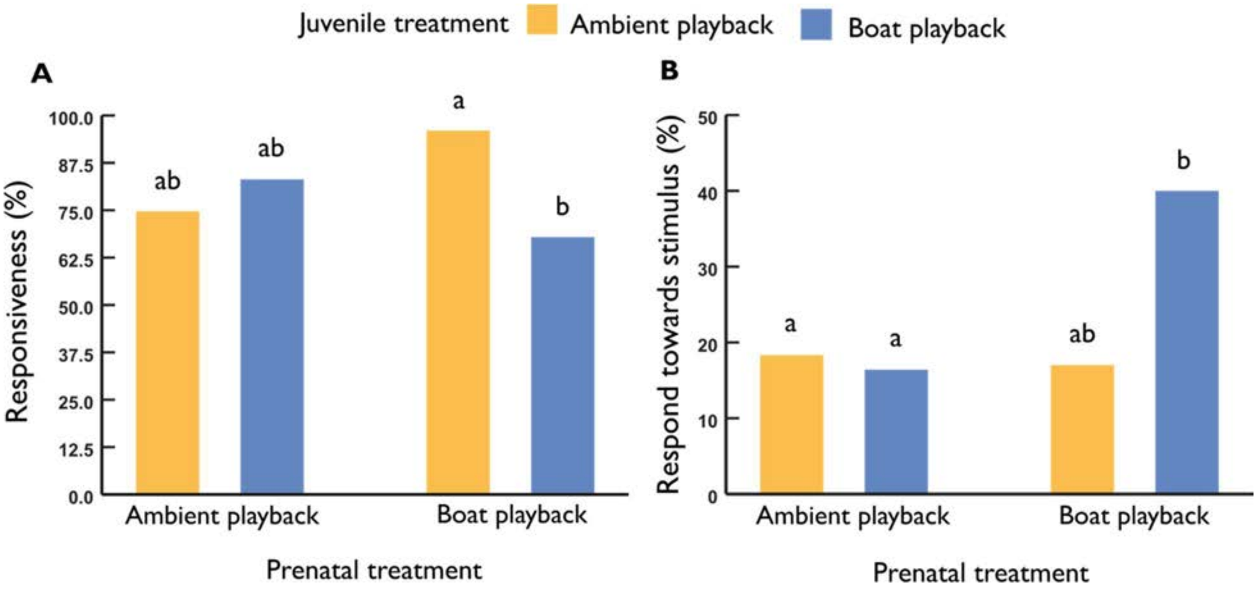

Figure 5.4. **Non-locomotor variables of escape response of *Acanthochromis polyacanthus* juvenile prenatally exposed to boat or ambient reef sound playback and then exposed to boat or ambient reef sound after hatching. (A)** Proportion of individuals that responded to the simulated predator attack, **(B)** proportion of individuals that swam towards the stimulus when startled. Bars with the same letter did not differ significantly according to the Tukey's HSD post-hoc means comparisons.

Direction of the escape response was significantly affected by the interaction of prenatal treatment and juvenile treatment (GLMER: interaction: $X^2 = 4.52$, $p = 0.033$; clutch: variance = $4e^{-14}$, s.d. = $2e^{-07}$; juvenile tank: variance = 0, s.d. = 0; prenatal treatment: $X^2 = 4.08$, $p = 0.043$; juvenile treatment $X^2 = 1.43$, $p = 0.231$; days of parental exposure: $X^2 = 4.97$, $p = 0.025$). Individuals that experienced prenatal boat noise and then experienced boat noise playback had a significantly higher proportion of juveniles swimming towards the startle stimulus compared to individuals in all the other treatments. A significantly higher proportion of juveniles from the boat–boat treatment swam towards the stimulus (40%) compared to individuals in the ambient–boat treatment (16.4%; Tukey, z = 3.01, *p* = 0.013) and the ambient–ambient treatment (18%; z = 2.56, *p* = 0.051), but not compared to the boat–ambient treatment (17%; z = 2.44, *p* = 0.069; **Figure 5.4B**).

Response latency, average speed, maximum speed and response distance of *A. polyacanthus* juveniles were not significantly affected by prenatal treatment, juvenile treatment, their interaction or length (see extended data **Table E2** and **Figure E5** in **Appendix E**).

5.5. Discussion

We found that long-term exposure to boat noise can affect growth and escape response of a juvenile fish. We found that individuals exposed to boat noise as juveniles were smaller compared to juveniles exposed to ambient playback, independent of their prenatal acoustic treatment. Moreover, prenatal and juvenile exposure to boat noise playbacks for up to 78 days caused lasting effects on behavioural traits related to survival. Juveniles exposed to boat noise both prior to hatching and as juveniles were less likely to respond to a simulated predator attack and when they did respond they were more likely to swim towards the simulated predator strike. Individuals exposed to boat noise either prenatally or as juveniles, but not during both time periods, showed no lasting effects on any aspect of their escape response, suggesting that carryover effects on escape response were the cumulative result of exposure prior to hatching and as juveniles. These findings of cumulative effects are particularly pertinent to the study species as they brood their young (Kavanagh 2000), and therefore a combination of parental, embryonic and juvenile exposure to boat noise is likely to occur in high traffic areas. It is in these areas that we would predict lower juvenile survival. Additionally, our results suggest that long-term effects of boat noise can vary depending on the life stage of the individual and the duration of noise exposure.

Individuals exposed to boat noise as juveniles were smaller compared to juveniles exposed to ambient sound. Previous studies have found contradictory effects of noise on growth. For example, Nedelec et al. (2015) found that after two days of exposure to ship noise Atlantic cod larvae were smaller to those exposed to ambient, however after 16 days their length converged between treatments. While Davison et al. (2009) found that the noise from aquaculture systems affected growth of rainbow trout only in the first month of exposure with growth converging after five months of exposure. In our study, after 41 days of exposure individuals exposed to boat noise were smaller. Stress is likely to be the underlying mechanism explaining the effects on growth. Stress causes changes in energy allocation and resources that were supposed to be invested in growth (Bonga 1997) are allocated to cope with the stressor (i.e., boat noise). Size is a critical trait in behavioural interactions in vertebrates (Werner and Gilliam 1984), particularly fishes (Saenz-Agudelo et al. 2015, McCormick 2016), with bigger individuals being dominant and having better access to food (Coates 1980), and with these advantages being accentuated through time to affect fitness and survival (Walker et al. 2007, McCormick et al. 2010, McCormick et al. 2018b). Moreover, modified growth has been found to be a key parameter that can carryover between life-history stages, particularly in fishes (Gagliano et al. 2007). Together, this research suggests that the effects of repeated boat noise on juveniles has detrimentally affected growth trajectories, which may be accentuated through time to cause higher mortality through the correlations between low growth, small size-at-age, social status and vulnerability to predation (McCormick and Gagliano 2008, McCormick 2016).

Long-term exposure to boat noise resulted in carryover effects on non-locomotor traits of the escape response, where both exposure prior to hatching and during the juvenile stage combined to detrimentally affect responsiveness and directionality - two key traits for an effective escape response (Walker et al. 2005, Fuiman et al. 2006). An unresponsive fish or a fish that startles towards the predator is less likely to survive a predator attack (Metcalfe et al. 1987, Domenici 2010). There are three non-exclusive potential explanations for the detrimental effects of ongoing exposure to boat noise from parents through to juveniles. Firstly, boat noise playback may have affected the parent's condition and lead to negative effects on the offspring; second boat noise playback had a direct effect on embryos; and lastly, boat noise playback had a direct effect on juveniles.

The role of parents in preparing offspring for the conditions within their natal environment is well known (Uller 2008). In the current study parents were exposed to boat noise from 4 to 26 days before eggs were laid. We found a significant effects of days of parental exposure on direction of the escape response. It is possible that individuals inherited a negative influence from parents exposed to boat noise, which could have increased their susceptibility to boat noise as juveniles. Previous studies have found that boat noise can lead to elevated stress, affecting energetic and physiological condition of adult fishes (Bracciali et al. 2012, McCloskey et al. 2020), which can lead directly to lower condition offspring (e.g., McCormick 1998, 2006). For example, Donelson et al. (2008) found that *A. polyacanthus* offspring that came from parents with a good body condition tend to be bigger at hatching, have bigger yolk sacks and have higher survival. Boat noise may also have disrupted parental behaviour (e.g., Nedelec et al. 2017). Parental care has also proven to be important for offspring development and survival (Sabat 1994, Sargent 1997). For example, McGhee and Bell (2014) found that Three-spined stickleback juveniles deprived of parental care had higher anxiety, which resulted in juveniles being attacked and captured by a predator faster compared to individuals that received parental care. In the current study, parents exposed to boat noise reduced the time spent fanning their eggs (See Nedelec et al. *submitted* for details), which would reduce oxygen circulation around the eggs and hamper offspring development (Green and McCormick 2004, Green et al. 2006). A reduction in the time fanning may also have led to a disruption of sensory and neural development (e.g., the lateral line, Lefrancois et al. 2005, Mirjany and Faber 2011, Mirjany et al. 2011), which could explain the effects we found on direction of the escape response. Although parental condition and behaviour can influence offspring through indirect genetic effects or transgenerational effects (McGhee and Bell 2014), our experimental design does not allow us to separate parental from embryological effects and future studies are required to isolate these effects.

Prenatal exposure to boat noise may have had a direct effect on embryo development. Fish embryos within benthic clutches have been found to be affected by anthropogenic noise (Simpson et al. 2005). Developing in a stressful environment may disrupt the development or damage sensory organs such as the lateral line. In a laboratory experiment, Uribe et al. (2018) found that white noise caused damage to the lateral line hair cells of *Danio rerio* after 80 min of exposure. In our study, embryos were exposed to boat noise for 11 days suggesting that possible their lateral line hair cells were damage. The lateral line provides inputs to the Mauthner cells which trigger the escape response in fish (Mirjany et al. 2011, Medan and Preuss 2014). It is possible that boat noise damage hair cells in the lateral line of the embryos compromising their escape response. Another possible explanation is that, by developing in a stressful environment offspring reduced the energy they allocate for neural, muscular or morphological development (Roussel 2007, McCormick 2009, Besson et al. 2020). Fakan and McCormick (2019) found that *A. polyacanthus* embryos exposed to boat noise had a higher heart rate and smaller yolk sacks compared to individuals developed in reef noise conditions. Besson et al. (2020) found that stress caused by elevated temperature and pesticides interfered with the development of olfactory, visual and mechanosensory structures, and resulted in higher predation. While there are many mechanisms through which boat noise may affect offspring performance, further studies are required that isolate parental from embryo-environment effects.

Boat noise may have also affected juveniles directly. Juveniles exposed to boat noise displayed reduced responsiveness and poor directionality choices. Effects on responsiveness and directionality could also be explained by stress and effects of noise on sensory development. Responsiveness is determined by the strength of the threat and the environmental context (Domenici 2010, Domenici and Hale 2019). While we controlled for the strength of the threat of the simulated predatory attack (i.e., the stimulus was the same across trials), exposure to boat noise during juvenile development could have resulted in a stressful environment disrupting juveniles' decision making (i.e., economic model theory, Ydenberg and Dill 1986). Moreover, directionality is determined by behavioural and neurophysiological mechanisms. Whether the lateral line continues developing after the eggs hatch is unknown, but a previous study found that olfactory and skeletal ossification development of *A. polyacanthus* continued after hatching (Kavanagh and Alford 2003), suggesting that exposure to boat noise could disrupt the development of other sensory organs.

Tank based studies allow for long term exposure experiments and the recording of behavioural responses in detail, while controlling for environmental variables such as noise and light. Recent studies have found that individuals display similar behavioural responses when conducting field and tank based experiments simultaneously (e.g., Simpson et al. 2016, Pieniazek et al. 2020). However, tank based experiments have acoustic disadvantages (as discussed by Rogers et al. 2015, Slabbekoorn 2016). In the present study, acoustic treatments were distorted due to reverberations and speakers effects. These distortions were more significant for the reef sound treatment, were sound pressure was increased by 20 to 40 dB in frequencies under 500 Hz. Nevertheless, individuals displayed different behavioural responses to each acoustic treatment suggesting our results are conservative and in conditions were reef sound is quieter the impact of boat noise would be higher.

The effects of boat noise are generally assumed to be transient and only manifested during the period when fish are exposed to noise. However, we found that a history of exposure to the playback of boat noise disrupted the ability of juveniles to react to a simulated predator attack, even when the escape response of juveniles occurred in the absence of noise. These carryover effects lasted for at least 14 hours demonstrating that impacts of noise do not diminish in the absence of noise. Moreover, we found juveniles exposed to boat noise were smaller compared to juveniles exposed to ambient reef sound. The observed effects on anti-predator responses and growth have the potential to be fatal. Long-term effects of boat noise on escape response can have future implications for population and community dynamics, by increasing the vulnerability of juveniles to predation and reducing the numbers of individuals that survive to the next life stage. Noise pollution represents a recently recognised stressor in marine environments (Williams et al. 2015), but compared to other stressors such as ocean warming, its mitigation and management is easier. Our results suggest that fishes inhabiting busy boat areas are more likely to be affected due to long-term exposure and suggests that protection from boat noise during the breeding and recruitment season (October-December) could reduce the impact of boat noise on reef fish populations.

Chapter 6: General Discussion

Research into the effects of vessel noise on fish has increased in the last 30 years. Most of this research has focused on a small group of noise sources and in a narrow diversity of species. Recent studies have found that the effects of noise can be source- and species- dependent, highlighting the importance of conducting more research that can allow us to understand this recently recognised pollutant. Coral reefs have a high concentration of organisms that use and produce noise (Parsons et al. 2016, McWilliam 2018). At the same time, proximity to shipping routes and the increase in boat-associated activities make coral reefs particularly vulnerable to vessel noise. The overall aim of this thesis was to investigate the effects of ship and small motorboat noise on the antipredator behaviour of juvenile reef fishes. This was achieved by conducting four studies that, for the first time, provide evidence of the effects of ship noise and the long-term effects of small boats on the antipredator behaviour of juvenile coral reef fishes.

Juvenile reef fishes suffer one of the highest mortality rates of vertebrates with an average of 60% of individuals likely to be preyed upon within two days of settling to coral reefs (Almany and Webster 2006, Hamilton et al. 2008). Juvenile fishes are prey for a variety of predators (Hixon and Webster 2002, Webster 2002) and any changes in mortality trajectories during early life can have major repercussions for the numbers entering later adult life stages. Therefore, any additional reductions to juvenile survival by environmental stressors can affect population dynamics and community structure. In **Chapter 2** and **Chapter 3,** I found that abrupt ship noise playback caused a decrease in the activity of juvenile Whitetail damselfish (*Pomacentrus chrysurus)* and Ambon damselfish (*Pomacentrus amboinensis)*. This decrease in activity could be the result of an increase in alertness and freezing behaviour. While this response has not been reported in response to ship noise, it has been found when European seabass (*Dicentrarchus labrax*; Herbert-Read et al. 2017) and Atlantic cod (*Gadus morhua*; Poulton et al. 2016) are exposed to pile driving. A decrease in activity suggests individuals will reduce the time they spend exploring their environment and learning about potential predators (Godin 1997, Manassa and McCormick 2013), which is critical in a natural setting. My research also showed that ship noise playback causes an increase in response latency

(**Chapter 2** and **Chapter 3)**. Effects on response latency may be driven through alterations to the Mauthner cells that trigger the escape response (Kohashi and Oda 2008). Effects of ship noise on response latency have differed among fish species, with some increasing their response latency (Voellmy et al. 2014b), some decreasing (Simpson et al. 2015), and others being unaffected by noise (Voellmy et al. 2014b). Differences among studies could be explained by a number of factors including: differences in acoustic treatments (e.g., Simpson et al. 2015), experimental set-ups (e.g., Simpson et al. 2015), prior acoustic environment of the fish (reviewed in Harding et al. 2019), differences in antipredator responses (freezing vs fleeing) (Blaxter and Fuiman 1990, Williams and Brown 1991) and evolutionary history of the studied species (e.g., Voellmy et al. 2014b). Nevertheless, an increase in response latency for my study species is concerning because an individual that takes longer to react has been shown to be less likely to survive a predator attack in the wild (McCormick et al. 2018b). Activity and response latency are two of the most important behavioural traits that can determine the survival of juvenile damselfishes in their natural environment (McCormick et al. 2018b). Findings from **Chapter 2** and **Chapter 3** suggest that ship noise has the potential to compromise antipredator behaviour of juvenile reef fishes and alter their survival in the wild; however, an understanding of how predators will be directly affected by ship noise is also required.

Predator-prey interactions play a critical role shaping marine communities by regulating the abundance and distribution of species (Hunsicker et al. 2011). My studies show that ship noise is consistently having negative effects on the antipredator behaviour of juvenile reef fish that are often prey (**Chapter 2, Chapter 3**). One previous study found that ship noise reduced the foraging success of two predator species. In a laboratory-based study, Voellmy et al. (2014a) found that when exposed to ship noise the Three-spined stickleback (*Gasterosteus aculeatus*) consumed less prey while the European minnow (*Phoxinus phoxinus)* stopped foraging. However, studies examining effects of ship noise on predator-prey interactions are lacking. Therefore, in **Chapter 4**, I investigated the effects of ship noise playback on predator-prey interactions of a common coral reef system. Surprisingly, I did not find any effects of ship noise on prey (i.e., prey speed, reaction distance or distance travelled) or predator traits (i.e., predator attack distance, attack rate or capture success). However, the possible effects on predator-prey interactions of a louder noise (e.g., a ship transiting closer by) or noise from a different source (e.g., small motorboat) than the one used in this experiment should not be discarded. The sound intensity of the ship noise in this experiment was lower than those used in **Chapter 2** and **Chapter 3**, while the sound intensity of the reef playback was higher. These alterations in the acoustic treatments are the result of tank effects (Kaatz and Lobel 2001, Campbell et al. 2019) and the presence of a water heater within the tank, and they potentially contributed to a decrease in the acoustic difference between both treatments. Fish communities are likely to be exposed to ship noise multiple times over longer periods and it remains unknown whether predator-prey interactions could be affected after a longer period of noise exposure. Future studies evaluating different sound intensity levels and repeated exposure would greatly contribute to our understanding of the effects of ship noise on predator-prey interactions.

Another understudied aspect of vessel noise is how its temporal structure may affect fishes. Most studies evaluate responses to abrupt noise or make observations during the highest sound intensity periods of the acoustic treatment. However, variations in sound intensity and timing have been found to influence the response of some fish species (e.g., Nedelec et al. 2015, Neo et al. 2016). Noise produced by ships can travel great distances (>100 km; Hildebrand 2009), meaning that fish communities can receive ramp-up noise, but as the distance between the noise source and the receiver (i.e. fish) increases, the influence of the environment on sound increases as well (Forrest et al. 1993). In **Chapter 3**, I investigated two different temporal structures that could occur close to coral reefs: ramp-up and abrupt noise. The ramp-up noise treatment simulated a 54,000 tonne bulk carrier approaching at 9.2 ms^{-}1 (17.9 knots; based on McKenna et al. 2012, McKenna et al. 2013), while the abrupt noise treatment simulated a bulk carrier coming out of an acoustic shadow (Gerstein et al. 2005) caused by, for example, an island. I found that while both acoustic treatments had the same effect on escape response (increased response latency) they differed in their effect on activity, with abrupt ship noise reducing activity and ramp-up ship noise causing no change (**Chapter 3**). These results highlight that temporal structure can influence the effects of ship noise on fish. Many other variations of vessel noise temporal structures remain to be studied. The structural complexity of coral reefs result in sound waves being constantly reflected, diffracted, scattered and attenuated (Katsnelson et al. 2012) ensuring highly complex sound fields. Acoustics of shallow water are known to be more complicated than deep water (Katsnelson et al. 2012), nevertheless, understanding how the environment influences the temporal structure of vessel noise is a critical step towards comprehending the effects of vessel noise on coral reef communities.

Vessel noise is likely to co-occur with other environmental stressors in marine environments and to be influenced by them, as sound propagation is highly influenced by environmental characteristics (Bass and Clark 2003, Katsnelson et al. 2012, Côte et al. 2016). In **Chapter 4**, I tested for the effects of elevated temperature and ship noise playback and found no interactive effects on prey or predator kinematics or capture success. Elevated temperature and boat noise have been previously found to disrupt predator-prey interactions in isolation. Elevated temperature has been found to reduce prey performance (i.e., reduced prey speed) and increase predator success (i.e., capture success; Allan et al. 2015, 2017). Noise from small motorboats has been found to decrease predator attack speed and predator attack distance but not to affect capture success (McCormick et al. 2018a). Differences in experimental set ups, temperature treatments and noise source are likely to be driving differences in results. While my results could suggest limited concern for the combined effects of temperature and ship noise, many other potential interactions with other noise sources, such as small motorboats, remain to be studied. Other environmental stressors are also likely to have interactive effects with vessel noise. For example, habitat degradation could influence sound propagation in coral reefs because in a less structurally complex environment sound propagation is likely to improve. Clearly, more studies examining the interactive effects of vessel noise and other environmental stressors are needed.

Most studies assessing the effects of noise used short or single exposures to noise (e.g., Holmes et al. 2017, Voellmy et al. 2014a), however, fish communities are likely to be exposed to noise for longer periods of time and multiple times. As a result, previous studies may over- or under- estimate the effects of vessel noise on fishes. Research conducted on other stressors (e.g., elevated temperature, elevated CO_2) has found that after long-term exposure organisms may develop mechanisms within a life stage, across life stages or across generations, to cope with these stressors (e.g., Donelson 2008, Donelson and Munday 2015, Leggea et al. 2016). Studies examining cross-life stage effects of boat noise are rare (but see Fakan and McCormick 2019). In **Chapter 5**, I exposed parents, embryos and juveniles of the Spiny chromis, (*Acanthochromis polyacanthus)* to small boat noise for up to eleven-weeks. When parents, embryos and juveniles were exposed to boat noise, the ability of juveniles to respond to a simulated predator attack and the direction of their escape response was affected even after noise exposure had ended. This research also showed that juvenile treatment affected body length, with juveniles exposed to boat noise being smaller. The observed effects on growth showed that boat noise had a direct effect on juveniles. Whether the effects on escape response are possibly the result of parental or embryological effects, or a combination of the two. Parental effects have been found to facilitate the acclimation to environmental stressors between generations (Burton and Metcalfe 2014, Donelson and Munday 2015, Chirgwin et al. 2018). Through transgenerational plasticity parents are able to modify their offspring phenotype to better match the environment that offspring are likely to experience (Marshall 2008, Latzel et al. 2010). However, transgenerational acclimation may not occur equally on all traits (e.g., Allan et al. 2014) or might be non-adaptive (e.g., Welch et al. 2014). Future studies should isolate parents from their offspring to test whether parents can influence their offspring when they reproduced in noisy conditions. Inter-generational studies are highly relevant as they can provide insights into the mechanisms individuals might develop to cope with stressors in their environment.

This thesis represents the first series of studies examining the effects of ship noise and the long-term effects of small motorboat noise on juvenile reef fishes. Throughout this thesis I used juvenile damselfishes as the study species, and while these findings are specific to the taxonomic group studied (i.e., Pomacentridae), conclusions may be generalised to other species that share similar hearing ranges (Wright et al. 2011) and life history behaviours (e.g., ‘dispersive’ planktonic larval stage; Leis and McCormick

2002). The study species used in **Chapter 5** *Acanthochromis polyacanthus*, is considered 'rare' in the sense that it provides parental care for many weeks after hatching (Kavanagh 2000), yet the effects on escape response could be applicable to other species where offspring and parents share the same environment, for example mouthbrooders (e.g., cardinalfishes; Barnet and Bellwood 2005). Results from this thesis are particularly relevant to species that suffer high predation pressure at early life stages (Almany and Webster 2006), as any effects on their ability to respond to a predator attack could result in an increase in their mortality. Whether these results are applicable to species with different hearing ranges (e.g., parrotfishes; Colleye et al. 2012) is unknown and warrants further study.

While logistical and economic challenges have limited the study of ship noise on coral reefs fishes, this thesis provides evidence of the effects of ship noise on the antipredator behaviour of juvenile reef fishes. Throughout this thesis, I used tank experiments to investigate in detail the effects of ship noise on the kinematics of the escape response of individuals, effects that are currently almost impossible to measure in the field. The present studies would benefit from future studies, particularly those aimed at examining effects in the field and incorporating realistic noise exposures (i.e., repeated exposure). Because of the well-known effects of tanks on sound propagation (Kaatz and Lobel 2001, Campbell et al. 2019), the results presented in this thesis should not be extrapolated or generalised. Tank walls act as sound reflectors resulting in hundreds of sound waves that cancel each other out, resulting in complex sound fields (Akamatsu et al. 2002, Rogers et al. 2015). Despite these problems, previous studies examining the effects of noise from small motorboats have found similar effects when field and tank studies have been conducted simultaneously (e.g., Simpson et al. 2015, 2016, Pieniazek et al. 2020). Whether this is the case for ship noise is currently unknown. Acoustic analyses in all of my chapters showed that the tanks led to an increase in the sound pressure of the ambient reef sound treatment, suggesting that my results are an underestimation of the effects of the ship noise treatment. Field studies assessing the effects of ship noise on fish will be important in ascertaining the validity of my results. Such field studies may potentially allow the boundaries of utility for tank studies to be better defined, potentially saving research resources.

Small boats and ships are the main two sources of anthropogenic noise on coastal habitat, including coral reefs (Haviland-Howell et al. 2007, Bittencourt et al. 2014), and often their effects are generalised (e.g., Duarte et al. 2021). This thesis provides evidence that the effects of small boats and ships can differ in magnitude (**Chapter 2**). In **Chapter 1**, I illustrated how most research on coral reefs has focused on small boats. This thesis provides evidence on the effects of ship noise on juvenile fishes and, as previously mentioned, more studies investigating long-term effects are needed for the development of mitigation and management strategies. Vessel noise regulation in Australia is in its infancy, limiting the management of ships to the establishment of designated transit areas which only protect 20% of the Great Barrier Reef Marine Park from ship noise (GBRMPA 2014, AMSA 2021). While the Great Barrier Reef Marine Park Authority recognises ship noise as a pollutant (GBRMPA 2019), little has been done to understand the effects of ship noise on fishes. This thesis represents a first step at filling this critical knowledge gap. Conducting research into the effects of ship noise in Australia can also provide important information for coral reef areas that are subject to much higher (e.g., Singapore and Indonesia) or much lower (e.g., Pacific islands) shipping traffic.

Through this thesis, I created a progression from short (one time) exposure to repeated long-term exposure; from one life stage to two life stages; and from studying the behaviour of prey to studying both prey and predators. The main objective of these progressions was to identify particular mechanisms driving the responses of individuals and to see how they changed as the complexity of the study system increased. My results showed that as complexity was added the effects were not as expected. Vessel noise is a complex stressor and its effects on fish can vary according to the source (**Chapter 2**), temporal structure (**Chapter 3**), behavioural traits measured (**Chapter 4**), duration of the exposure and life stage (**Chapter 5)**. This complexity makes predictions of the effects of vessel noise on marine communities more challenging. Research into the effects of anthropogenic noise is increasing globally (Duarte et al. 2021), yet much remains to be studied in tropical areas, including the Great Barrier Reef. This thesis provides a stepping-stone towards the understanding of the impacts of vessel noise on coral reef fishes and encourages further research that contributes to the development of management policies.

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Appendix A: Supporting Information for Chapter 1

Table A1. Studies examining the effects of vessel noise on fishes. NA = not applicable

Authors	year	noise source	*species*	Set-up	Playback/ Real source	Engine	Region	Life stage
Bruintjes R et al.	2015	Ship noise	*Anguilla anguilla*	Laboratory	Playback	NA	Temperate	Adult
			Dicentrarchus labrax	Laboratory	Playback	NA	Temperate	Adult
Putland et al.	2018	Ship noise	*Pempheris adspersa*	Field	Real	NA	Temperate	Adult
Ivanova et al.	2020	Ship noise	*Boreogadus saida*	Field	Real	NA	Artic	Adult
Purser et al.	2016	Ship noise	*Anguilla anguilla*	Laboratory	Playback	NA	Temperate	Adult
Radford et al.	2016	Ship noise	*Dicentrarchus labrax*	Laboratory	Playback	NA	Temperate	Adult
Simpson et al.	2015	Ship noise	*Anguilla anguilla*	Laboratory	Playback	NA	Temperate	Adult
Vasconcelos et al	2007	Ship noise	*Halobatrachus didactylus*	Field	Playback	NA	Temperate	Adult
Voellmy et al.	2014	Ship noise	*Phoxinus phoxinus*	Laboratory	Playback	NA	Temperate	Adult
Voellmy et al.	2014	Ship noise	*three-spined stickleback*	Laboratory	Playback	NA	Temperate	Adult
Nedelec et al.	2015	ship noise	*Gadus morhua*	Laboratory	Playback	NA	Temperate	Larvae
Barcelo-Serra et al.	2021	Small boat noise	*Serranus scriba*	Field	Real	not specified	Temperate	Adult
Celi et al.	2016	Small boat noise	*Sparus aurata*	Laboratory	Playback	not specified	Temperate	Adult
Codarin et al.	2009	Small boat noise	*Chromis chromis*	Field	Playback	163 HP inboard diesel engine	Subtropical	Adult
			Sciaena umbra					
			Gobius cruentatus					
Dinh et al.	2018	Small boat noise	*Community*	Field	Real	not specified	Tropical	NA
Fakan and McCormick	2019	Small boat noise	*Amphiprion melanopus*	Laboratory	Playback	2-stroke	Tropical	Juvenile
			Anthochromis polyacanthus					Juvenile
Ferrari et al.	2018	Small boat noise	*Pomacentrus amboinensis*	Laboratory	Playback	2-stroke	Tropical	Juvenile

Authors	year	noise source	*species*	Set-up	Playback/ Real source	Engine	Region	Life stage
Handegard et al.	2015	Small boat noise	*Clupea harengus*	Field	Playback	not specified	Temperate	Adult
Harding HR et al.	2020	Small boat noise	*Chromis viridis*	Field	Real	2-stroke	Tropical	Adult
Holles et al.	2013	Small boat noise	*Apogon doryssa*	Field	Playback	not specified	Tropical	Larvae
Holmes et al.	2017	Small boat noise	*Pomacentrus amboinensis*	Field	Real	2-stroke	Tropical	Juvenile
Jain-Schlaepfer et al.	2018	Small boat noise	*Amblyglyphidodon curacao*	Field	Real	2-stroke 4-stroke	Tropical	Juvenile
La Manna et al.	2016	Small boat noise	*Sciaena umbra*	Field	Real	40 to 270hp	Subtropical	Adult
Mascolino et al.	2019	Small boat noise	*Chromis chromis*	Field	Real Playback	Marine traffic	Subtropical	Adult
McCloskey et al.	2020	Small boat noise	*Pomacentrus amboinensis*	Field	Real	4-stroke	Tropical	Juvenile
McCormick et al.	2018	Small boat noise	*Pomacentrus wardii*	Field	Real	2-stroke 4-stroke	Tropical	Juvenile
McCormick et al.	2019	Small boat noise	*Pomacentrus chrysurus*	Laboratory	Playback	2-stroke 4-stroke	Tropical Tropical	Juvenile
McCormick et al.	2018	Small boat noise	*Pomacentrus amboinensis*	Laboratory	Playback	4-stroke	Tropical	Juvenile
Mensinger et al.	2018	Small boat noise	*Chrysophrys auratus*	Field	Real	4-stroke	Subtropical	Adult
Mills et al.	2020	Small boat noise	*Amphiprion chrysopterus*	Field	Playback	25 hp	Tropical	Adult
Nedelec et al.	2017	Small boat noise	*Acanthochromis polyacanthus*	Field	Playback	2-stroke	Tropical	Juvenile
Nedelec, et al.	2016	Small boat noise	*Dascyllus trimaculatus*	Field	Playback	25 hp	Tropical	Juvenile
Nedelec et al.	2017	Small boat noise	*Labroides dimidiatus*	Field	Real	25 hp	Tropical	Adult
Nichols et al.	2015	Small boat noise	*Heterostichus rostratus*	Laboratory	Playback	4-stroke	Temperate	Adult
Pena, M	2019	Small boat noise	*Community*	Field	Real	Marine traffic	Temperate	Adult

Authors	year	noise source	*species*	Set-up	Playback/ Real source	Engine	Region	Life stage
Picciulin et al.	2012	Small boat noise	*Sciaena umbra*	Field	Real	163-hp, 40-hp. 130-hp	Subtropical	Adult
Picciulin et al.	2010	Small boat noise	*Chromis chromis*	Field	Playback	40hp	Subtropical	Adult
Pyc et al.	2021	Small boat noise	*Sanopus splendidus*	Field	Real	Marine traffic	Tropical	Adult
Sara et al.	2007	Small boat noise	*Thunnus thynnus*	Field	Real	2000 hp, 100hp	Subtropical	Adult
Sebastianutto et al.	2011	Small boat noise	*Gobius cruentatus*	Laboratory	Playback	40 hp	Subtropical	Adult
Simpson et al.	2015	Small boat noise	*Community*	Field	Playback	2-stroke	Tropical	NA
Simpson et al.	2016	Small boat noise	*Pomacentrus amboinensis*	Field	Real	30 hp	Tropical	Juvenile
				Laboratory	Playback			Juvenile
Staaterman et al.	2020	Small boat noise	*Halichoeres bivittatus*	Field	Playback	not specified	Tropical	Adult

Appendix B: Supporting Information for Chapter 2

Table B1. Details of ambient, 4-stroke boats and ship recordings used in playback experiments

Treatment	No.	Location	Distance to receiver	Receiver depth	Vessel	Tonnage	Engine type	Engine power (kW)
Ambient	1	Lagoon		6 - 9 m				
	2	Palfrey inner		6 - 9 m				
	3	Inner Bird		6 - 9 m				
Ship	1	Big V outer	1.9-2.5 km	16-19 m	RTM Twarra	53988	MAN-B&W Diesel	13,501
	2	Eagle	1.9-3.2 km	17-20 m	RTM Gladstone	53988	MAN-B&W Diesel	13,501
	3	Eagle	2.0-3.0 km	17-20 m	RTM Piiramu	53988	MAN-B&W Diesel	13,501
4-stroke	1	Lagoon middle ST	1 - 25 m	2 m	Research boat		Yamaha 4-stroke 30 hp	22
	2	Lagoon middle ST	1 - 25 m	2 m	Research boat		Yamaha 4-stroke 30 hp	22
	3	Lagoon - close to reef	1 - 25 m	2 m	Research boat		Yamaha 4-stroke 30 hp	22

Table B2. Summary of logistic regression comparing the number of responsive individuals among acoustic treatments.

Predictor variable	Estimate	SE	Z value	P value
Ambient playback	1.2528	0.4629	2.706	0.0068
Ship noise playback	0.4520	0.7140	0.633	0.5267
4-Stroke noise playback	1.9661	1.1196	1.756	0.0791

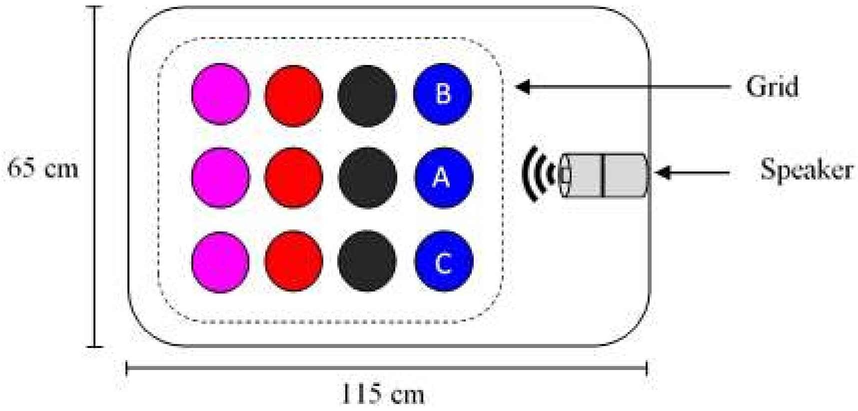

Figure B1. Grid design to investigate the sound pressure and particle motion in experimental tank. Colour and letters indicate position where posterior sound recordings were made within the grid.

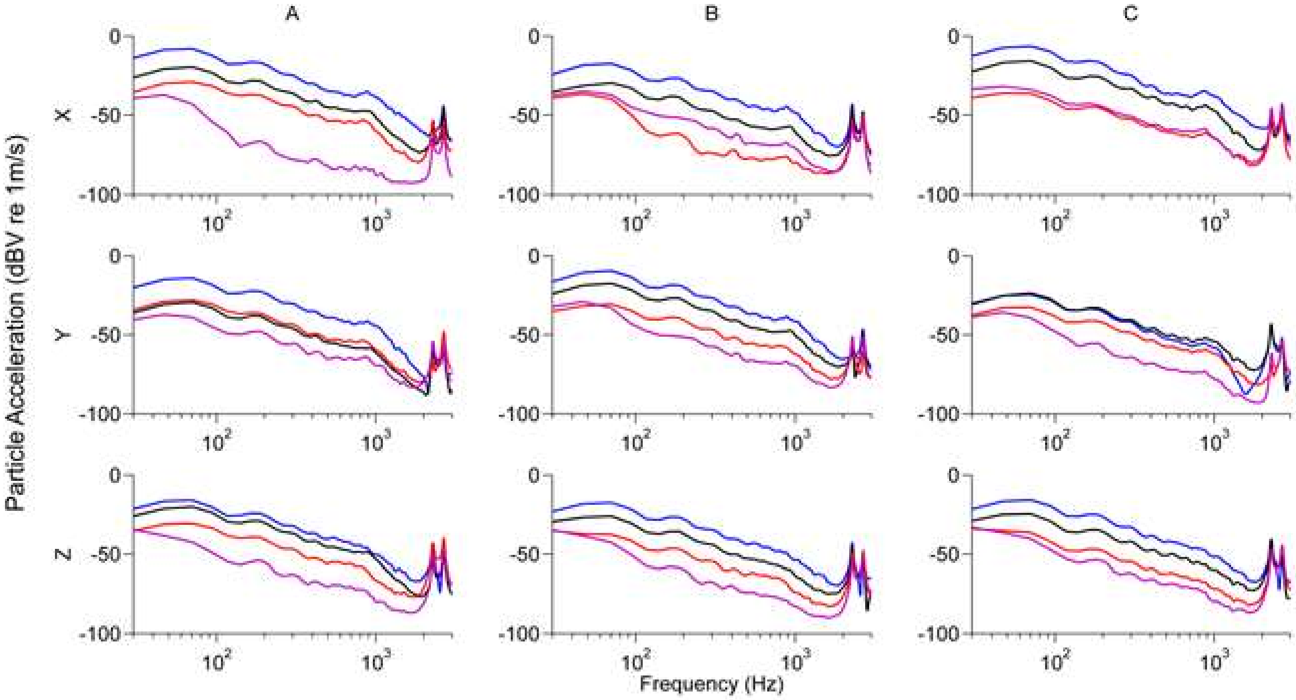

Figure B2. Particle acceleration measurements from tank playback grid experiment. Colour plots represent longitudinal grid position in relation to proximity to the speaker (blue – 1st, black – 2nd, red – 3rd, purple – 4th). Letters represent transverse grid position (A – centre, B – right, C – left).

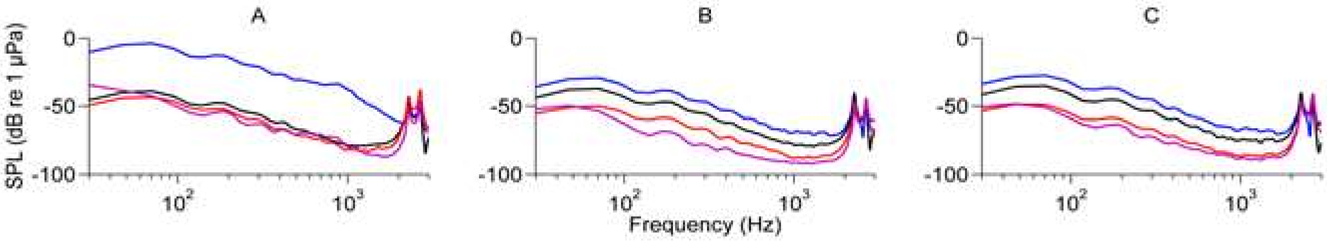

Figure B3. Sound pressure measurements from tank playback grid experiment. Colour plots represent longitudinal grid position in relation to proximity to the speaker (blue – 1st, black – 2nd, red – 3rd, purple – 4th). Letters represent transverse grid position (A – centre, B – right, C – left).

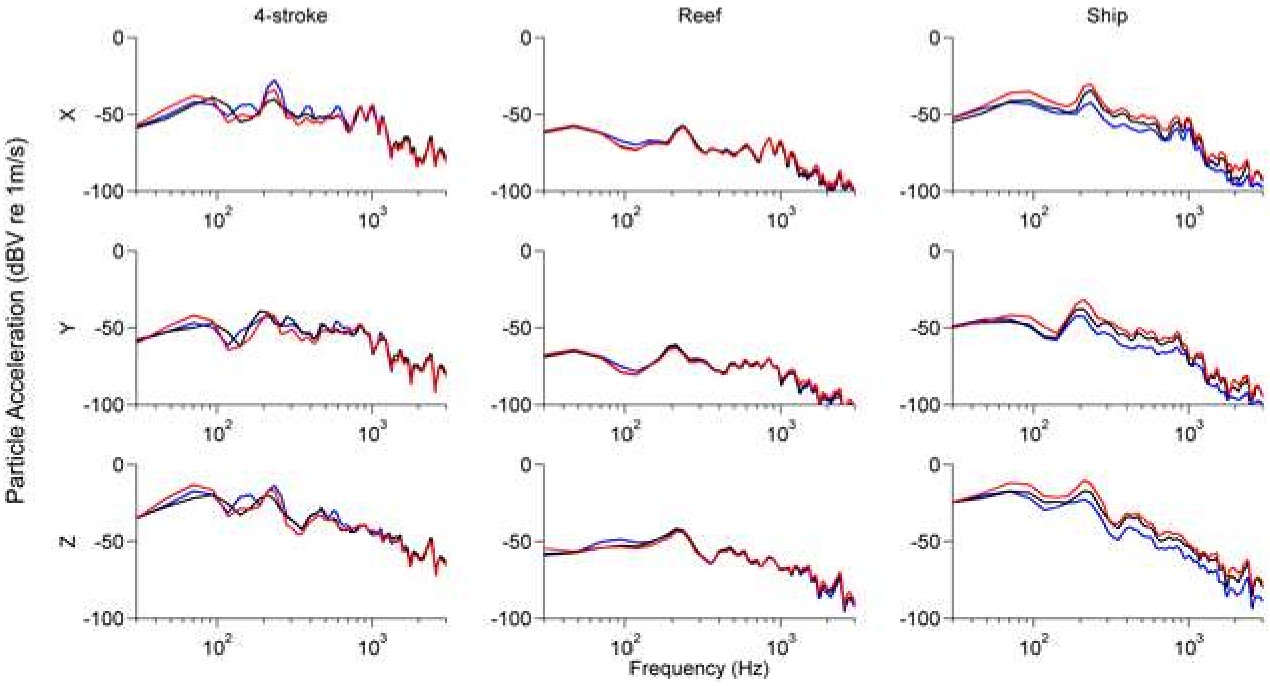

Figure B4. Particle acceleration measurements from tank playback experiment. Colour plots represent playback samples (n = 3 separate sound tracks)

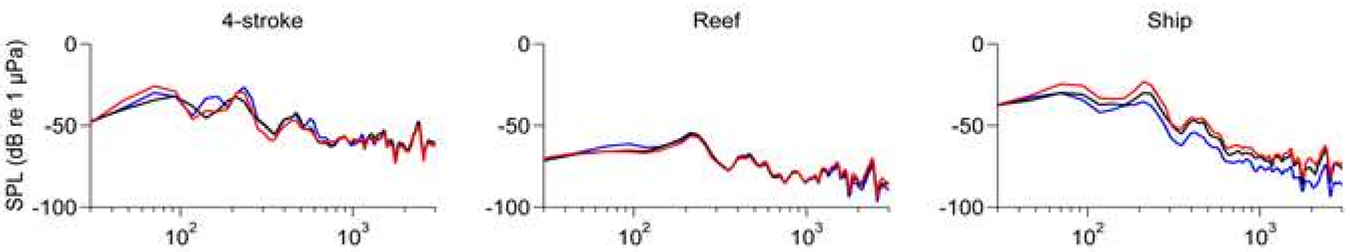

Figure B5. Sound pressure measurements from tank playback experiment. Colour plots represent playback samples (n = 3 separate sound tracks)

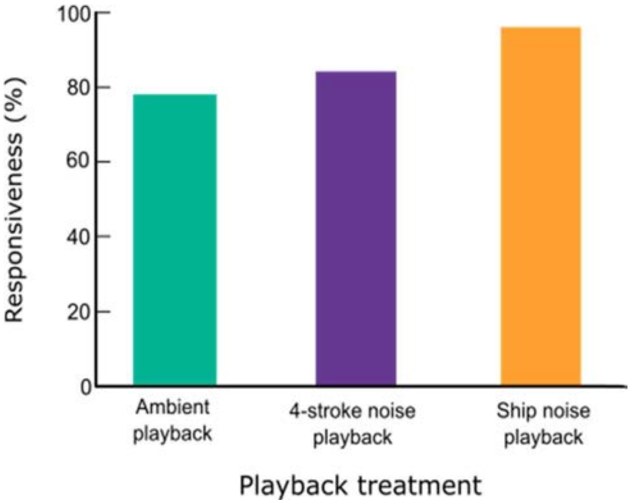

Figure B6. Proportion of individuals that performed an escape response among treatments

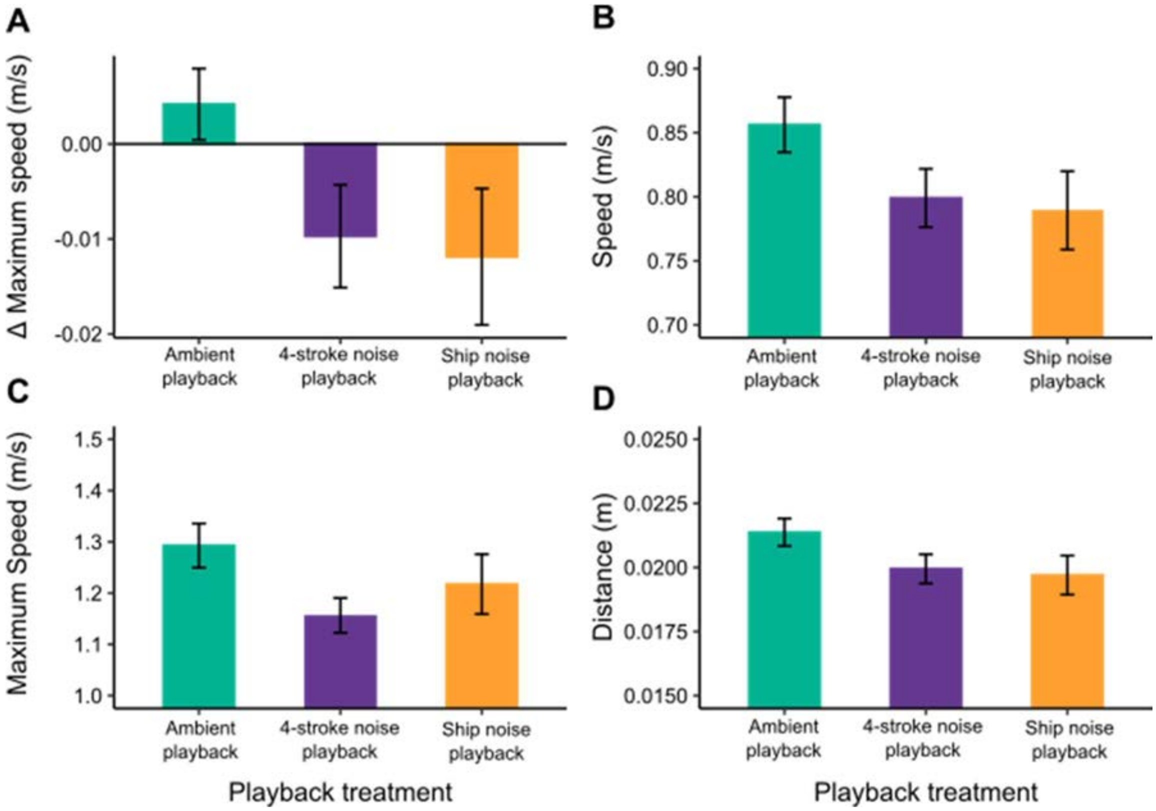

Figure B7. Effect of noise exposure on routine swimming and escape response variables ***Pomacentrus chrysurus.*** **(A)** Change in maximum speed (mean ± s.e.), **(B)** Speed (mean ± s.e.), **(C)** Maximum speed (mean ± s.e.) and **(D)** Distance (mean ± s.e.). Back-transformed data are plotted.

Appendix C: Supporting Information for Chapter 3

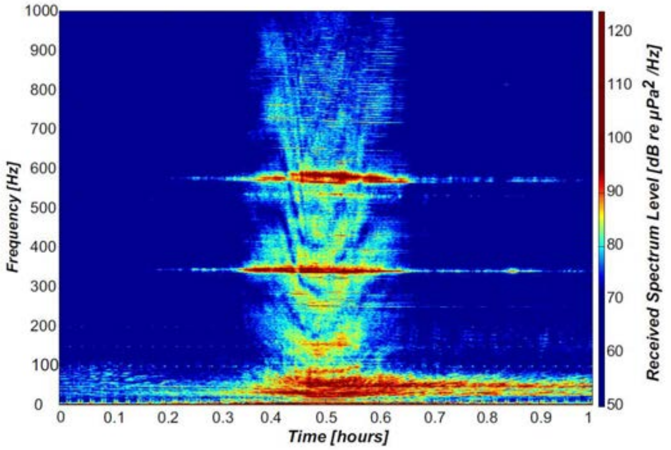

Figure C1. Spectrogram of a ship passing. Source: McKenna et al. 2013

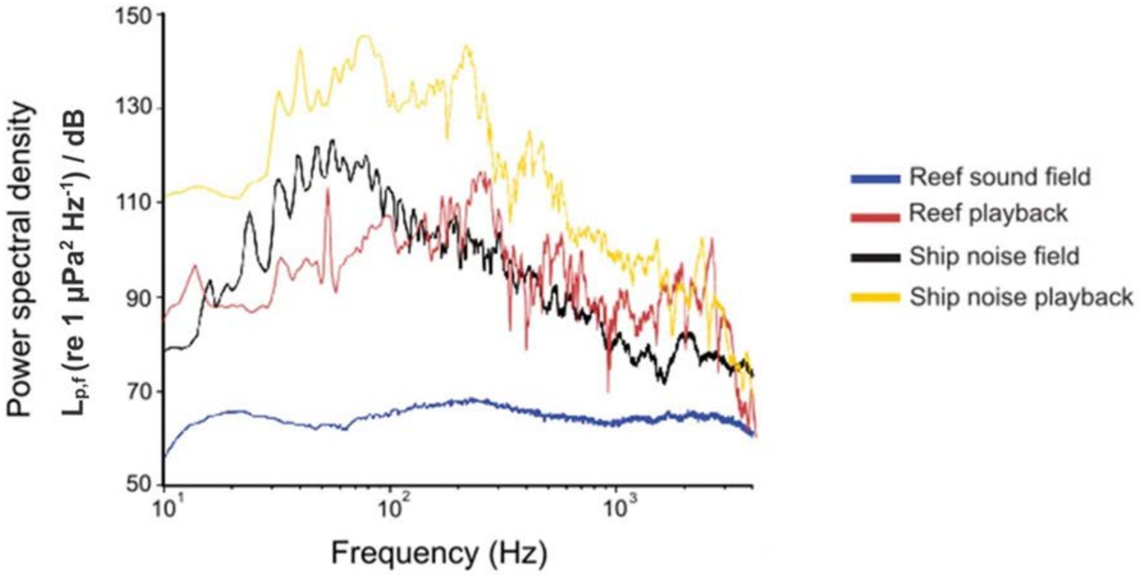

Figure C2. Power spectral density level of field and playback treatments. Playback recordings were done in the middle of the experimental arena. Mean PSD values were calculated from the recordings for each acoustic treatment (1s Hamming, 50% overlap, 1 Hz frequency resolution). Duration of reef and ship samples were approximately 30 and 8 s.

Table C1. Summary output of final models fitted

Response variable	Model used	Predictor variable	Estimate	SE	z/t value	P value
Maximum speed	Linear mixed effect model	Ramp noise	-0.083	0.047	-1.77	0.077
		Time 2	-0.032	0.054	-0.593	0.553
		Time 3	-0.014	0.054	-0.256	0.797
		Standard length	1.103	0.281	3.917	<0.001
Distance	Linear mixed effect model	Ramp noise	-0.038	0.029	-1.300	0.195
		Time 2	0.002	0.012	0.210	0.833
		Time 3	-0.010	0.012	-0.792	0.429
Change in maximum speed	Linear mixed effect model	Ramp-up noise: ship noise	-0.113	0.089	-1.267	0.206
		Reef sound	-0.145	0.088	-1.641	0.102
		Standard length	-1.316	0.438	-3.001	0.003
Change in distance	Linear mixed effect model	Ramp-up noise: ship noise	0.065	0.022	2.934	0.003
		Reef sound	0.061	0.022	2.788	0.005
Latency	Linear mixed effect model	Ramp-up noise: ship noise	-0.116	0.076	-1.518	0.131
		Reef sound	-0.289	0.071	-4.029	<0.001
		Distance to stimulus	0.009	0.001	5.086	<0.001
Escape speed	Linear mixed effect model	Ramp-up noise: ship noise	0.098	0.054	1.824	0.070
		Reef sound	0.078	0.050	1.548	0.124
Escape maximum speed	Linear mixed effect model	Ramp-up noise: ship noise	0.072	0.049	1.469	0.144
		Reef sound	0.044	0.046	0.964	0.337
Escape distance	Linear mixed effect model	Ramp-up noise: ship noise	0.154	0.197	0.782	0.436
		Reef sound	0.262	0.185	1.411	0.161

Appendix D: Supporting Information for Chapter 4

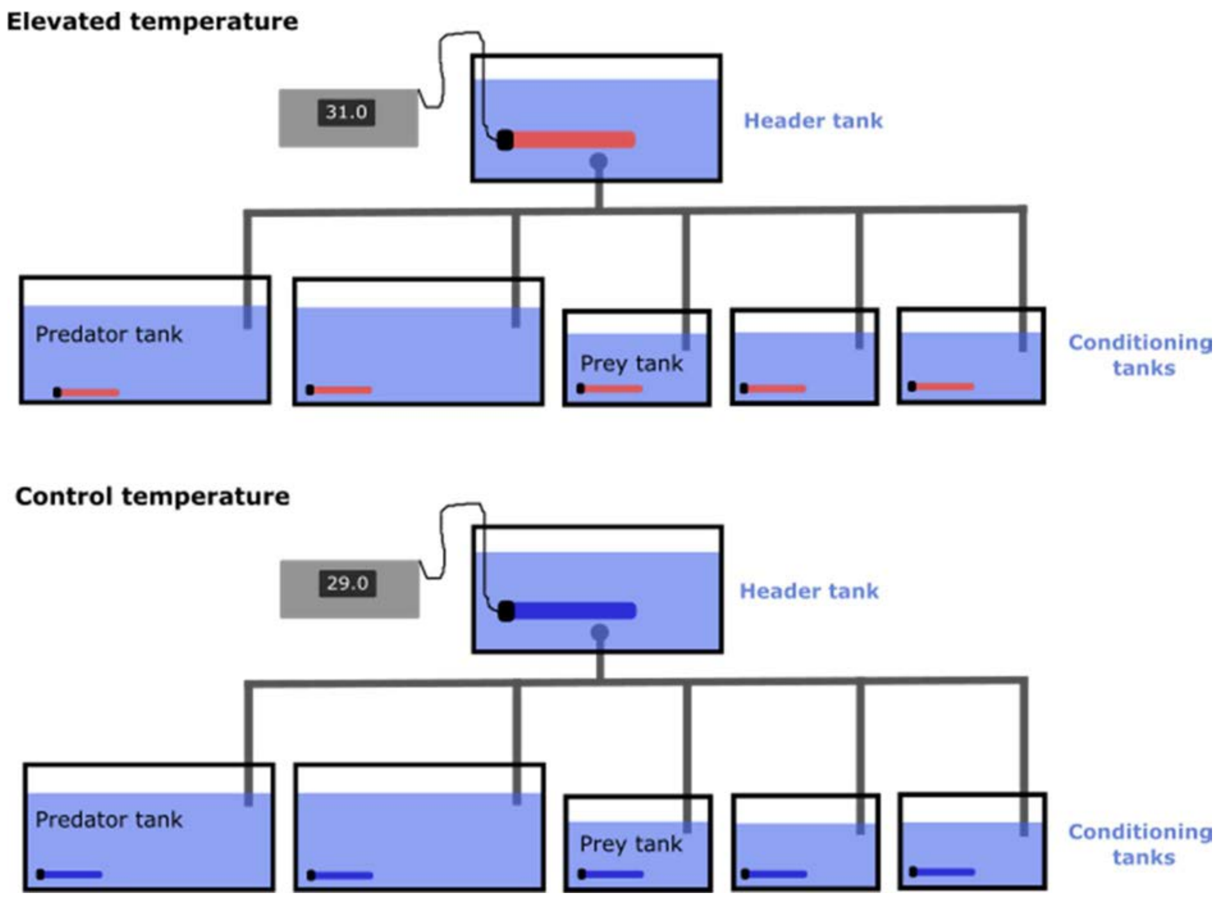

Figure D1. Diagram of tank organisation. Predator and prey conditioning tanks were fed from a heater tank.

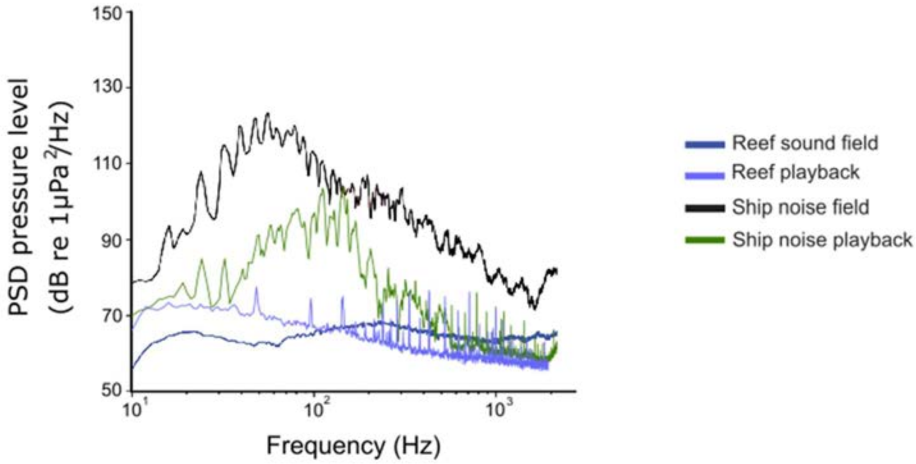

Figure D2. Power spectral densities of sound pressure levels of ship noise playback (green line) and reef playback (blue line). Playback recordings were affected by near-field effects and by the speaker performance as a result some frequencies were louder and others quieter. Overall, ship noise was louder than ambient sound and ship noise playback was louder that ambient sound playback. Playback recordings contained harmonic noise at 50 Hz intervals; this was an artefact of the water heater placed in the holding tank.

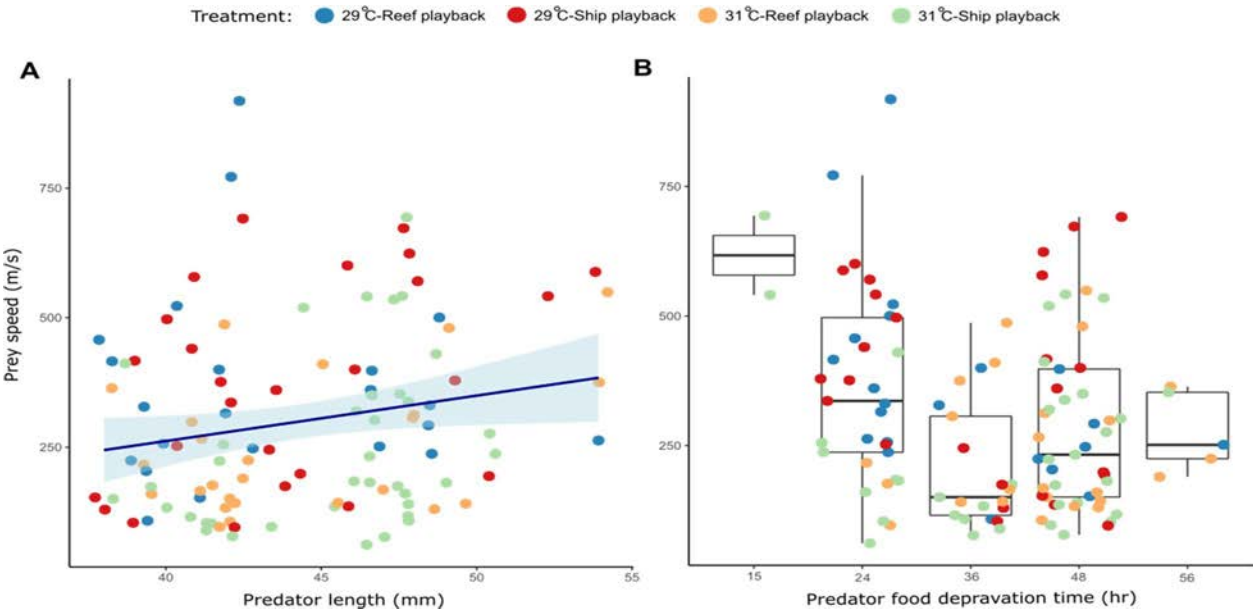

Figure D3. Relationship between prey speed and (A) Predator length and (B) Predator food depravation time (mean ± s.e.). Blue line in panel (A) indicates the nature relationship between both variables.

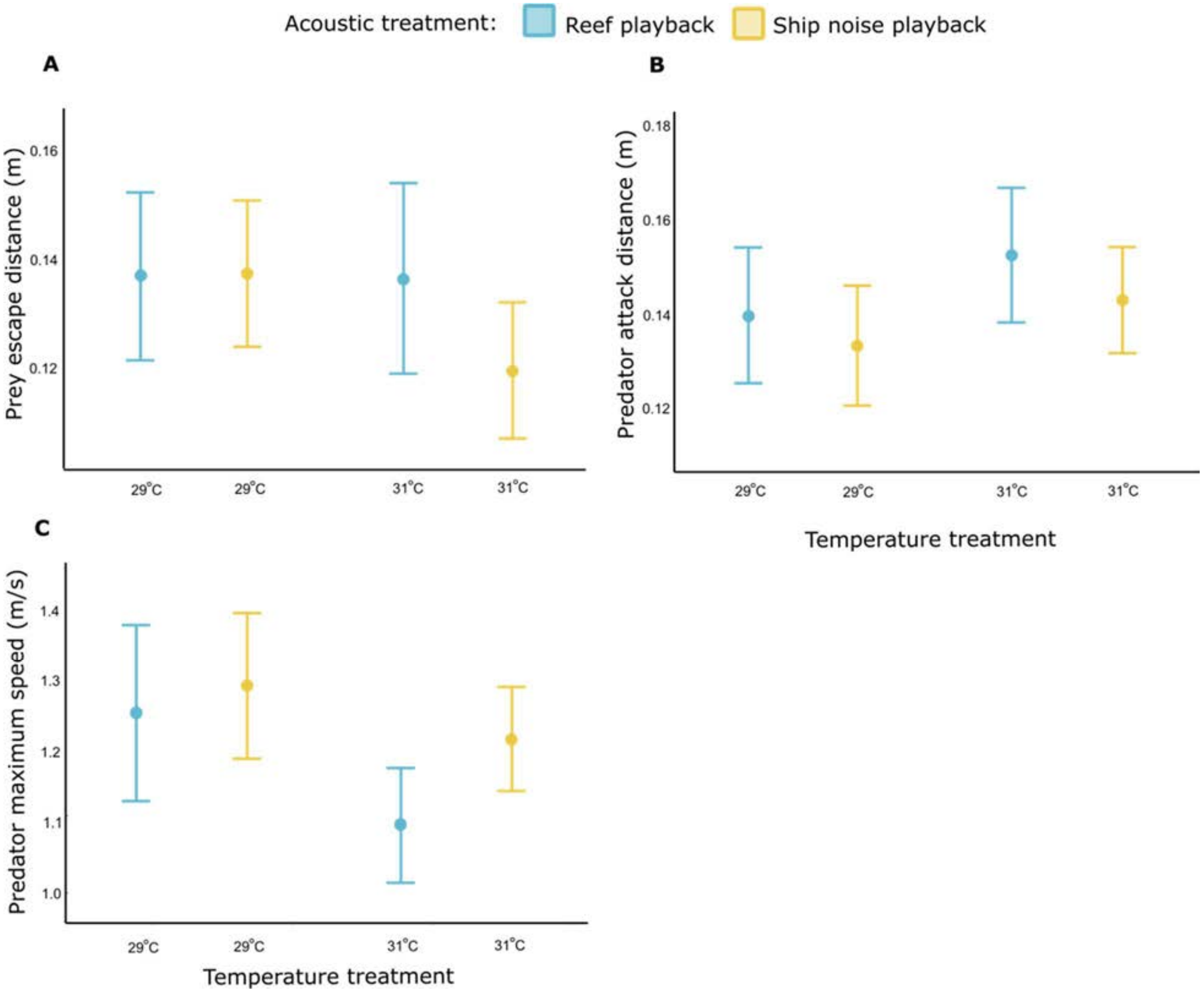

Figure D4. Effect of elevated temperature and ship noise playback on predator-prey interactions of juvenile *Pomacentrus amboinensis* and predator *Pseudochromis fuscus.* **(A)** Prey escape distance (mean ± s.e.), **(B)** Predator attack distance (mean ± s.e.), **(C)** Predator maximum speed (mean ± s.e.). Adjusted means plotted. Left to right: *n = 24, 28, 25, 37.*

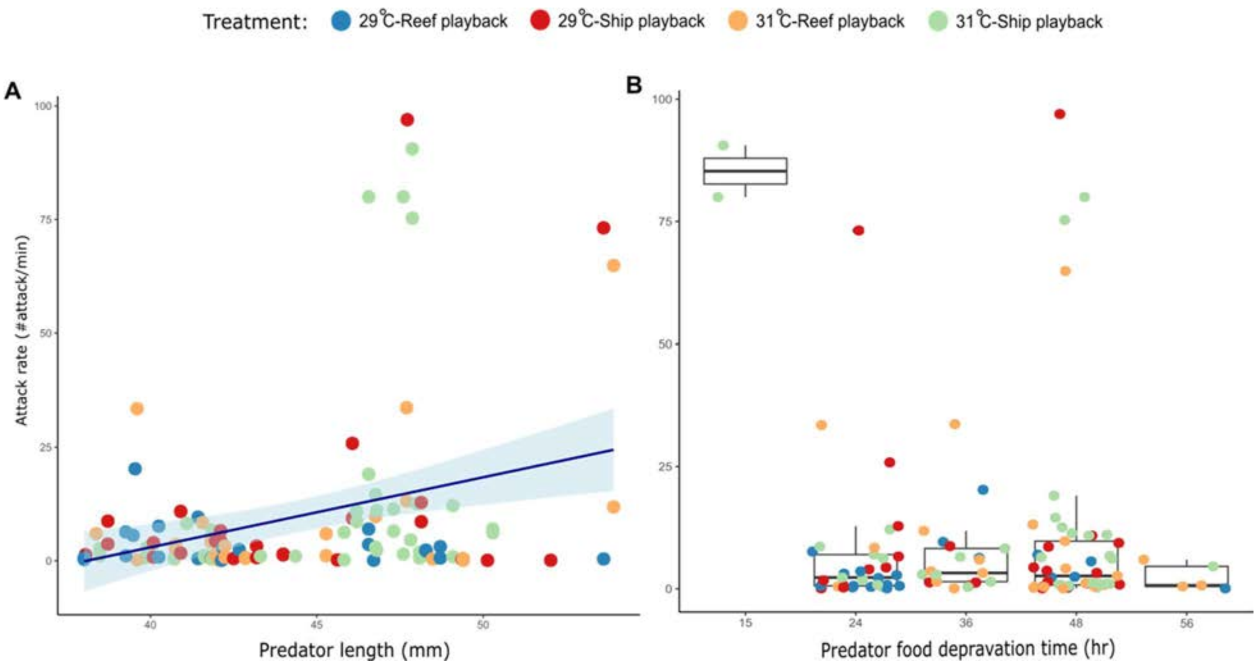

Figure D5. Relationship between attack rate and (A) Predator length and (B) Predator food depravation time (mean ± s.e.). Blue line in panel (A) indicates the nature relationship between both variables.

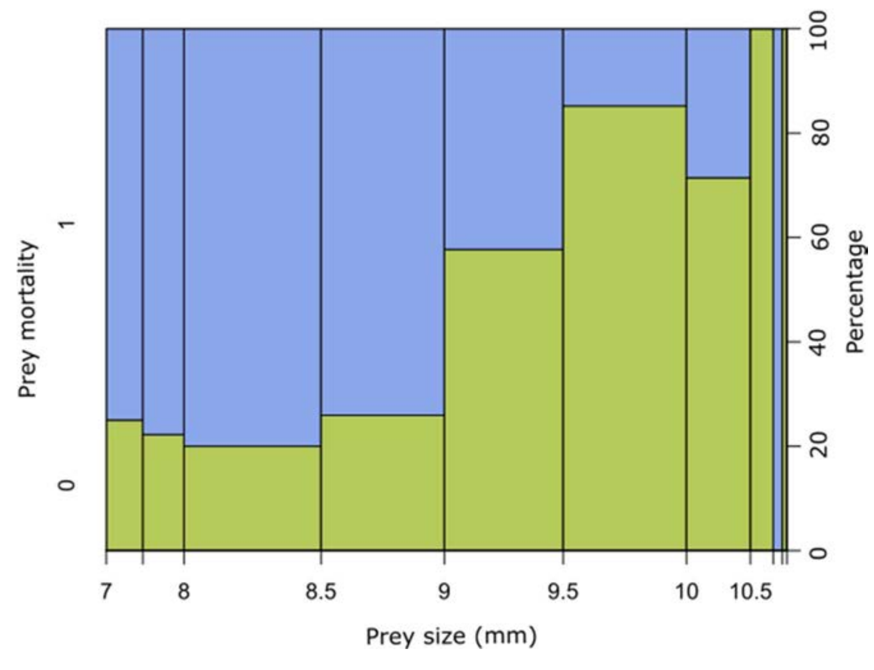

Figure D6. Relation between prey mortality and prey size. Prey mortality 1 = prey consumed, 0 = prey not consumed. Bars width indicate the proportion of data points within each prey size category.

Table D1. Summary output of final models fitted

Response variable	Parameter	X^2	P value	Variance	Standard deviation
Prey speed Linear mixed-effects model (LMER)	Temperature	14.33	**<0.001**		
	Noise	0.187	0.665		
	Temperature * noise	0.723	0.395		
	Prey length	2.44	0.117		
	Predator length	4.79	**0.028**		
	Food depravation	13.01	**0.011**		
	Predator identity (random effect)			3696	60.79
Prey escape distance Linear mixed-effects model (LMER)	Temperature	0.339	0.560		
	Noise	0.257	0.611		
	Temperature * noise	0.235	0.627		
	Prey length	0.02	0.886		
	Predator length	0.02	0.865		
	Food depravation	3.86	0.425		
	Predator identity (random effect)			0	0
Prey reaction distance Linear mixed-effects model (LMER)	Temperature	1.07	0.3		
	Noise	0.004	0.946		
	Temperature * noise	2.11	0.145		
	Prey length	2.10	0.146		
	Predator length	2.00	0.156		
	Food depravation	4.15	0.385		
	Predator identity (random effect)			0	0
Predator attack distance Linear mixed-effects model (LMER)	Temperature	0.80	0.368		
	Noise	0.30	0.578		
	Temperature * noise	0.02	0.869		
	Prey length	0.27	0.602		
	Predator length	0.64	0.421		
	Food depravation	4.45	0.347		
	Predator identity (random effect)			0	0
Maximum attack speed Linear mixed-effects model (LMER)	Temperature	0.751	0.385		
	Noise	1.12	0.289		
	Temperature * noise	0.29	0.585		
	Prey length	2.08	0.148		
	Predator length	0.11	0.732		
	Food depravation	8.28	0.081		
	Predator identity (random effect)			60722	246.4
Attack rate	Temperature	0.02	0.886		
	Noise	0.554	0.456		

Response variable	Parameter	X^2	P value	Variance	Standard deviation
Linear mixed-effects model (LMER)	Temperature * noise	0.972	0.324		
	Prey length	1.68	0.194		
	Predator length	11.07	**<0.001**		
	Food depravation	32.24	**<0.001**		
	Predator identity (random effect)			0	0
Capture success Logistic regression (GLMER)	Temperature	0.93	0.334		
	Noise	1.31	0.250		
	Temperature * noise	0.31	0.576		
	Prey length	23.33	**<0.001**		
	Predator length	3.11	0.077		
	Food depravation	1.23	0.872		
	Predator identity (random effect)			2.62	1.61
Trials with no predator attack Logistic regression (GLMER)	Temperature	0.86	0.94		
	Noise	0.09	0.17		
	Temperature * noise	0.74	0.74		
	Predator identity (random effect)			0.13	0.37

Appendix E: Supporting Information for Chapter 5

Figure E1. ***Acanthochromis polyacanthus*** **with brood at Lizard Island, northern GBR** (photo credit: M, McCormick).

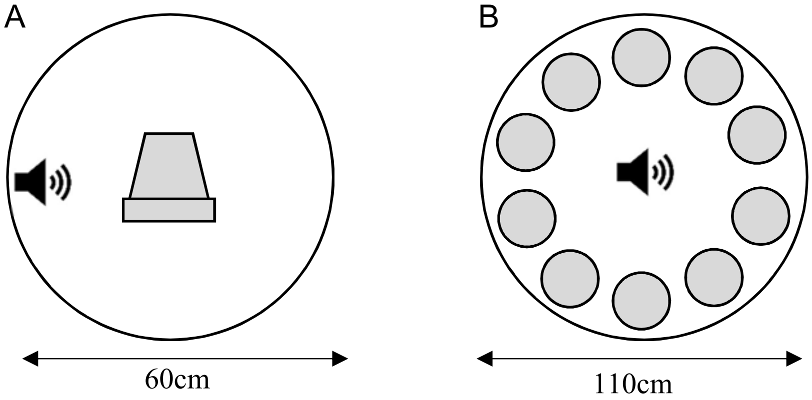

Figure E2. Schematic diagram of laboratory set-up. (A) Aerial view of adult's tank, nest positioned in centre, audio symbol indicates position of speaker. **(B)** Aerial view of juvenile tank, speaker was placed in the centre of the tank facing upwards, and juveniles were in the buckets positioned around the speaker.

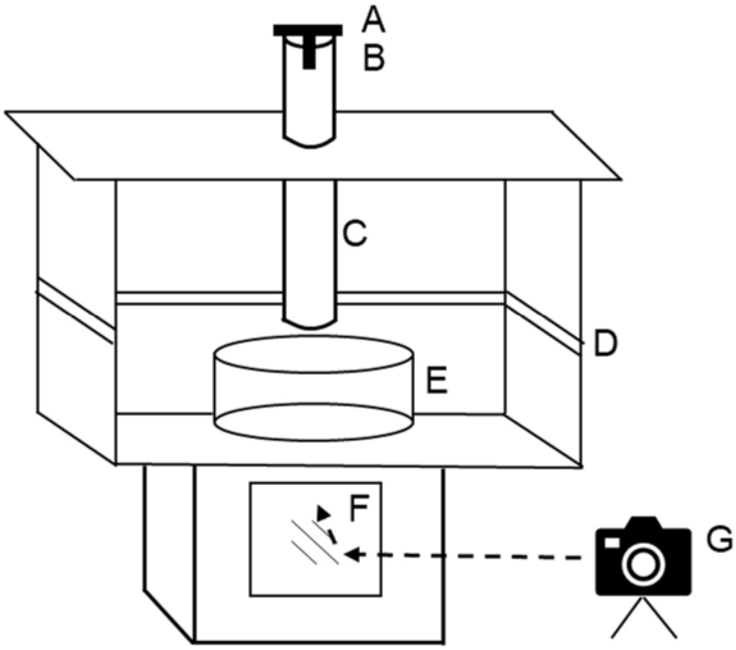

Figure E3. **Schematic of the experimental set up.** Experimental arena for the escape response trials of *Acanthochromis polyacanthus*. Electromagnet **(A)**, tapered weight **(B)**, pipe **(C)**, LED lights **(D)**, experimental arena **(E)**, mirror at a 45° angle **(F)** and camera **(G)**.

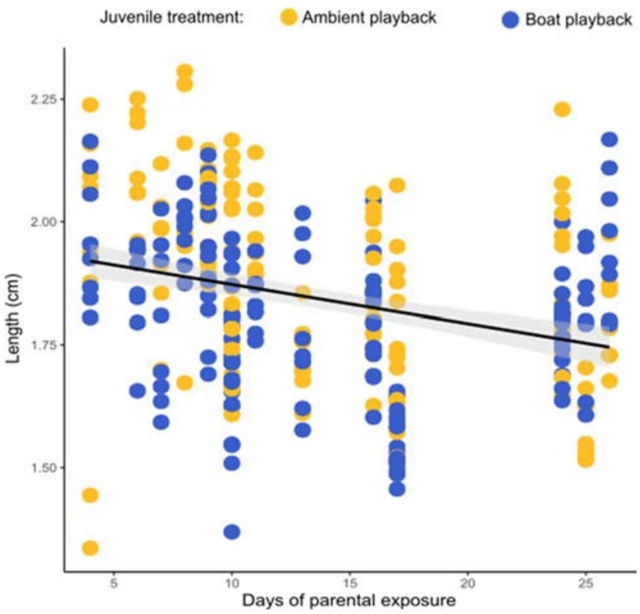

Figure E4. Relationship between standard length and days of parental exposure of *Acanthochromis polyacanthus* juveniles exposed to boat or ambient reef noise after hatching. Black line indicates the nature of the relationship between both variables.

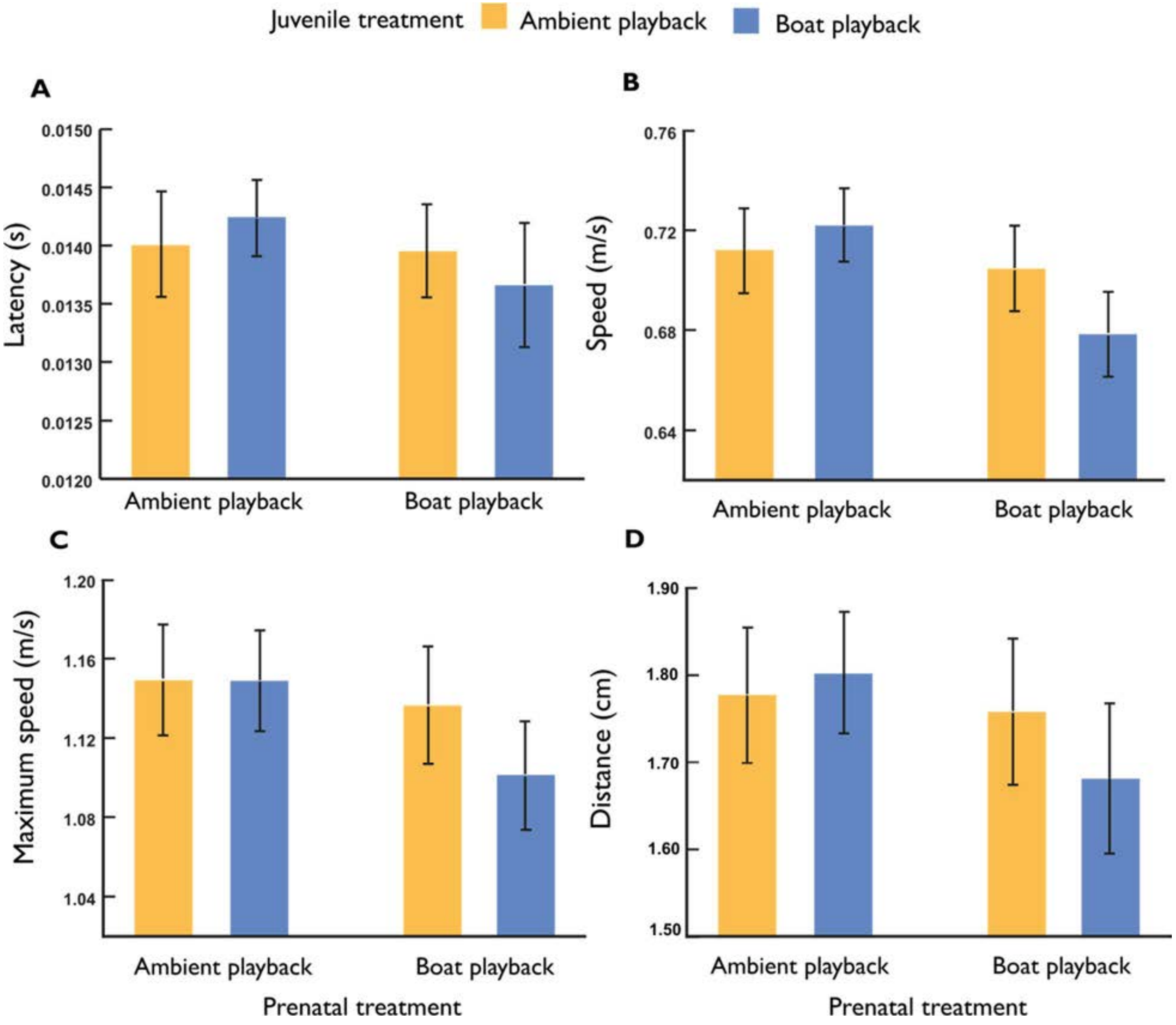

Figure E5. Locomotor and non-locomotor variables of escape response of *Acanthochromis polyacanthus* juvenile prenatally exposed to boat or ambient reef noise playback and then exposed to boat or ambient reef noise after hatching. (A) Latency (mean ± s.e.), **(B)** Speed (mean ± s.e.), **(C)** Maximum speed (mean ± s.e.) and **(D)** Distance (mean ± s.e.).

Table E1. Final number of breeding pairs and offspring tested in the present study. Breeding pairs and clutches of offspring were reared in two different treatments: control (ambient reef playback) and boat noise.

Noise treatment	Number of parent pairs	Number of pairs producing offspring	Number of juveniles tested for escape response		
			Total	Treatment	Individual
Control (ambient reef playback)	14	13	284	Control (ambient reef playback)	92
				Boat noise	96
Boat noise	11	9	173	Control (ambient reef playback)	54
				Boat noise	57

Table E2. Effects of boat noise on non-locomotor and locomotor escape response variables of *Acanthochromis polyacanthus* juveniles. V = variance, s.d. = standard deviation

Dependent variable	Fixed effects						Random effects	
	Embryo treatment	Juvenile treatment	Embryo treatment * juvenile treatment	Days of parental exposure	Length	Distance to stimulus	Clutch	Juvenile tank
Response latency	X^2=0.19, p=0.682	X^2=0.06, p=0.794	X^2=0.33, p=0.565	X^2=3.57, p=0.058	X^2=5.17, p=0.022	X^2=44.25, p>0.001	v=1.058^{e-06}, s.d=0.001	v=0, s.d=0
Distance	X^2=1.05, p=0.303	X^2=0.01, p=0.888	X^2=1.37, p=0.240	X^2=4.17, p=0.040	X^2=12.63, p>0.001	X^2=7.38, p=0.006	v=9.004^{e-}10, s.d=3.001^{e-}05	v=4.604^{e-}10, s.d=2.146^{e-}05
Speed	X^2=0.83, p=0.362	X^2=3^{e-}04, p=0.986	X^2=1.01, p=0.313	X^2=4.12, p=0.042	X^2=13.58, p>0.001	X^2=6.86, p=0.008	v = 0.002, s.d = 0.046	v = 0.001, s.d = 0.034
Maximum speed	X^2=0.07, p=0.780	X^2=0.06, p=0.803	X^2=0.08, p=0.773	X^2=7.37, p=0.006	X^2=7.3, p=0.006	X^2=1.48, p=0.223	v=0, s.d=0	v=0.004, s.d=0.064