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The effect of motorboat noise on early life stages of coral reef fishes

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Statement on the Contribution of Others

This work was done in collaboration with my supervisors Prof. Mark McCormick, A/Prof. Jodie Rummer and Dr. Stephen Simpson as well as my field assistant Eric Fakan. All of these collaborators participated in the development of research ideas, and editorial processes. Eric Fakan also was involved in carrying out experiments. Sophie Nedelec, Harry Harding and Tim Gordon assisted with sound recordings. Wayne Morris contributed technical assistance. Funding was provided by the ARC Centre of Excellence for Coral Reef Studies, the College of Science and Engineering at James Cook University (McCormick and Rummer) and a James Cook University International Postgraduate Research Scholarship (Jain-Schlaepfer).

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Ethics

All work complied with JCU Animal Ethics Committee regulations (permit: #A2089 and #A4208).

General Abstract

Underwater anthropogenic noise is now recognized as an environmental pollutant. Vessel noise is the most common source of underwater anthropogenic noise. With vessel noise increasingly being propagating through the world's waters, it has become of urgent concern to understand the effects of boat noise on aquatic organisms and reduce negative impacts. I focused on fish, which have received less research attention than mammals in terms of anthropogenic noise impacts. In particular, I examined early life stages, which are important to fish population persistence, but are understudied with respect to the impacts of anthropogenic noise. In order to gain insight into the animal's perception of anthropogenic noise and their associated mechanistic responses, I examine indicators of physiological stress during exposure to boat noise in my study species. The aims of my studies were to increase empirical evidence of the effects of boat noise on early life stages of coral reef fishes so that noise mitigation programs can be developed and implemented more effectively.

Chapter 2 investigated whether fish embryos were affected by boat noise and whether the effects differed with engine type (e.g., two vs. four stroke engines). Embryos of the damselfish *Amblyglyphidodon curacao* were used in a novel recording setup to examine changes in heart rate, which can indicate stress in response to real boat noise. Findings showed an increase in embryo heart rate when boats were passing. The magnitude of increase was greater upon exposure to boats with two-stroke engines compared to the response to four-stroke outboard engines. These are the first field data demonstrating that fish embryos can be affected by boat noise. I also analyzed the differences between two-stroke and four-stroke sound spectra and concluded that a new metric – total sound energy produced above the

sound detection threshold and within the sound detection range of the animal (relSEL) – may be useful in indicating differences between biological effects of different noises.

Chapter 3 examined the energetic cost of boat noise induced stress in settlement stage coral reef fishes, using respirometry to measure oxygen uptake rates and approximate metabolic costs associated with the secondary stress response initiated by boat noise**.** The energetic cost of boat noise induced stress is relevant because a high energetic cost would be associated with depleted energy available for other fitness promoting processes and activities. I examined oxygen uptake rates in settlement-stage *Pomacentrus amboinensis,* and employed a new design for intermittent flow respirometry that does not require pumps and therefore allows quiet acoustic conditions during respirometry trials. Findings showed no effect of boat noise playback on oxygen uptake rates. These differing results in stress response indicators between the two species and life stages highlights the complexity of responses of fish to anthropogenic noise. Both of these studies provide a significant advancement in technology with which to monitor anthropogenic impacts during early life stages, which I hope will be used in future studies on this topic. Overall, these findings contribute to our understanding of how boat noise can affect fish and increases our understanding of how noise pollution can be managed and/or mitigated.

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Chapter 1: General Introduction

1.1 Anthropogenic Noise

Underwater anthropogenic noise is now recognized as an environmental pollutant (Boyd et al. 2011, Williams et al. 2015). Rapidly increasing sources of anthropogenic sound worldwide have become of concern, as sound is a vitally important cue to many aquatic organisms. Travelling further and about five times faster in water than in air, sound is often more reliable than visual cues that can be obscured by suspended sediment and attenuation with depth (Slabbekoorn et al. 2010). The recognition that anthropogenic activities can effect aquatic organisms through noise generation is a fairly recent development, first proposed in the context of whale communication in 1971 (Payne and Webb 1971), and has only become a field of study in the last few decades (Williams et al. 2015). To date, most of the research has focused on marine mammals (Simmonds et al. 2003), but detrimental effects of anthropogenically-produced noise are increasingly being recognized as widespread across taxa (Popper and Hastings 2009; Morley et al. 2014).

1.2 Vessel noise

While there are many anthropogenic sources of underwater noise, including sonar, pile driving, seismic shooting, and renewable energy devices, motorized vessels are the most pervasive anthropogenic source of underwater noise (Andrew et al. 2002, Popper 2003). Increasing global populations, economic growth, and industrialization means that large vessel numbers and prevalence will also grow worldwide. For example, with industrialization, cargo shipping has doubled approximately every 20 years (Hawkins and Popper 2014), and there has been an approximate 3.3 dB increase in open ocean noise per decade (Frisk 2012). Small boats, such as recreational powerboats, are also widespread and growing in popularity. For example, in 2014, approximately 15.8 million recreational motorboats were registered in the U.S. (NMMA 2015). On a more local scale, also in 2014, 250,231 recreational vessels were registered in Queensland, Australia – home to the Great Barrier Reef – which was a 10% increase from the previous year (*Recreational Vessel Census June 2014* 2014).

Motorized vessels create both broadband noise, where energy is continuous over a range of frequencies, and tonal sound, which occurs at specific frequencies. Vessel components that generate noise are largely from the propulsion mechanism and associated parts (e.g., propellers, rotating shafts, gear teeth, reciprocating parts, fluid flow turbulence and mechanical friction; Ross 1976). Cavitation accounts for much of the power of noise generated by vessels (up to about 80%) and is caused by static pressure decreases as the propeller moves faster. When the pressure reaches vapor pressure, it generates bubbles that create loud noises when they collapse, and can cause a high intensity noise that peaks at 50-150 Hz and can reach 10 000 Hz (Ross 1976). Propeller singing, with a peak tonal range of 100 to 1000 Hz, involves the water motion contributing to resonant vibrational frequencies of a propeller and occurs especially when propellers are damaged or when multiple propellers are spinning asynchronously (Richardson et al. 1995). Other sources that can contribute to a vessel's noise signature include hydrodynamic noise from water flowing over the hull of the vessel and machinery being used on the vessels (Jong 2009).

The sound spectra produced depends on vessel type. Large ships, such as commercial carriers and supertankers with greater power, slower turning engines, and large drafts generally create high intensity, low frequency noise, with sound power being greatest below about 50 Hz and caused primarily by propeller blades and secondarily by shaft rotation and engine cylinder firing (Richardson et al. 1995). Broadband source levels of ships range from about 170 dB re 1µ Pa @ 1 m to 190 dB re 1 µPa @ 1 m, with larger ships generally producing more noise (Richardson et al. 1995, Hatch et al. 2008, McKenna et al. 2012). As low frequency sounds propagate further through the water than higher frequency sounds, ship noise can have an effect on aquatic ecosystems over long distances. Much less work has been done to characterize the sound profiles coming from small vessels, such as recreational vessels, tugs, and fishing boats. Small vessels are quite diverse in the noise they produce but generally, compared to large, slow ships, small vessels have broader frequency content (at times > than 10 kHz) and higher peak tonal frequencies (100 Hz to 6 kHz) (Simmonds et al. 2003). Source levels are typically lower than large ships but can vary considerably (McKenna et al. 2012). Very few studies have characterized the particle motion of vessel noise, making it difficult to know at this time to what degree the particle motion sound signature differs between vessels. Nedelec et al. (2014; 2016) characterized motorboat sound and found that motorboats with a 25 hp engine, driving between 50-100 meters from the recording device, produced an average particle motion power spectral density of 30-60 dB re 1 ($\mu m/s^2)^2$/Hz) between 1 and 3000 Hz. Power spectral densities differed considerably between sound pressure and particle motion, though peaks appear to be within similar frequency ranges. Clearly, both large vessels and small motorboats have the potential to impact aquatic life through the noise they generate. In this study, I focused on noise from small motorboats rather than large vessels, as motorboats tend to be more prevalent outside of shipping lanes and ports, such as in coastal environments, lakes, and waterways where impacts to numbers of species and ranges of life stages could be more pronounced.

1.3 Effects of boat noise on fishes

Fishes may be particularly vulnerable to the impacts of motorboat noise because they have frequency sound detection ranges that overlap with the sound spectra of motorboats (Slabbekoorn et al. 2010). Over their evolutionary history, the fishes have evolved a diverse variety of structures used for sound detection, and their sound detection sensitivities depend on those structures. Sound detection ranges vary across species and with ontogeny (Wright et al. 2010), but in general, fishes generally hear from 50 Hz to ranges spanning 500-1500 Hz depending on species (Popper and Hastings, 2009). The intensity at which sound is audible (i.e., the sound detection threshold) varies with frequency as well. There are two components of sound that can be detected by fish: particle motion and sound pressure (reviewed by Nedelec et al. 2016). These two components of sound do not typically have a direct relationship in coastal environments; in other words, particle motion cannot be predicted from sound pressure and visa versa. Thus, it is crucial to measure both to understand the nature of the sound detected by fishes. Particle motion is the oscillatory motion transmitted through neighboring particles, which is how sound propagates, while sound pressure is the difference in pressure caused by the oscillatory particle motion. Virtually all fish species can detect particle motion using otolith organs that vibrate in relation to receptor sensory hair cells (Popper and Fay 2011). However, the particle motion sound detection mechanism is limited in sensitivity. Thus, some fish species use gas-filled swim-bladders or other gas bubbles to detect sound pressure, which can expand their sound detection ranges, though ranges over which fish detect either component of sound are quite variable (Popper and Fay 2011) and may affect the extent to which different species are affected by motorboat noise.

Motor motorboat noise can affect many important ecological processes in adult fishes. Small motorboat noise can mask important vocalizations used for courtship and territory defense (Codarin et al. 2009) and/or alter outcomes of interactions (Sebastianutto et al. 2011). Some soniferous fishes have been found to decrease calling rates during and following motorboat passage (Luczkovich and Sprague 2008), which may decrease courting success. Other species have been found to increase vocalization (Picciulin et al. 2012), perhaps to compensate for conspecifics having a harder time sound detection. Other aspects of reproduction have also been found to be affected by boat noise including nest maintenance (Picciulin et al. 2010), nest defense (Bruintjes and Radford 2013), and offspring survival (Nedelec et al. 2017). Boat noise can also trigger avoidance responses (Ona and Godø 1990, Sarà et al. 2007), which can increase energy usage and disrupt fitness-promoting activities, such as feeding (Bracciali et al. 2012).

Motorboat noise can also affect physiological processes in adult fishes. Increases in ventilation rates, blood cortisol concentrations, metabolic rates, and heart rates, which are all indicators of stress, have been observed in the presence of boat noise (Wysocki et al. 2006, Graham and Cooke 2008, Purser et al. 2016). Boat noise is a perceived threat and generally not a direct, lethal threat. However, the energy required to mount the stress response upon perceiving the boat noise as a potential threat can be detrimental to the organism, as it takes away from the energy that can be allocated to fitness promoting physiological processes (e.g., immune response, growth, reproduction) and behaviors (e.g., feeding, escaping predators).

While most studies have been on adult fishes, boat noise has also been found to detrimentally affect fishes during the larval and juvenile life stages. Several studies have examined effects of boat noise on behaviors affecting recruitment of larval and juvenile fishes. Holles et al. (2013) found that the navigation decisions of the cardinalfish, *Apogon doryssa*, were affected when larvae were exposed to continuous motorboat noise. Less coral reef fish larvae settled in the vicinity of motorboat noise playback compared to ambient reef-sound playback (Simpson et al. 2016). In the Baltic sea, fish species that depend on vegetation for habitat recruited less frequently to areas where there was high motorboat activity (Sandström et al. 2005). However, not all fish species are negatively affected by boat noise in terms of larval recruitment; Jung and Swearer (2011) found that intermittent motorboat noise had little effect on temperate reef fish larvae settlement patterns. Another important process found to be affected in juveniles is startle and predator escape responses, which can impact juvenile fish survival (Simpson et al. 2016b). A few studies have found boat noise to induce a stress response in juvenile fish (Spiga et al. 2012a, Nichols 2014), but juvenile fish may habituate or recover quickly (Bruintjes et al. 2016; Nedelec et al. 2016). Many coral reef fishes undergo a population bottleneck during the juvenile settlement stage. In a meta-analysis by Almany and Webster (2006), it was determined that approximately 56% of reef fishes die in first two days of settlement, thus stressors that alter condition or fitness promoting behaviors during the settlement stage or the time leading up to this life stage (embryo and larval stage) have potential to drastically alter chances of survival, population structure, and ecosystem dynamics.

In fish embryos, very little is known about the effects of boat noise. To date, only one study has been published, and the authors found no effect of boat noise on hatchings success, post-hatching larval growth, or larval survival (Bruintjes and Radford 2014). There is evidence that coral reef fish embryos start sound detection through the latter part of their development, *in ovo* (Simpson et al. 2005) and therefore may also be affected physiologically by anthropogenic noise. Additionally, some of the sound spectra produced by boat noise falls within their sound detection range. Because of their small size and rapid development, embryos are particularly sensitive to environmental perturbations (e.g., temperature, pollutants). Environmental perturbations at the embryo life stage can have carryover effects for neural, sensory, muscular and morphological development (Roussel 2007, McCormick and Gagliano 2010), that may affect growth and survival upon hatching (Gagliano et al. 2007).

Given the ability of early life stages of fish to detect sound, and that healthy early life stages are critical to healthy adult populations, it is important to understand the effects of boat noise on early life stages. Indeed, the effects of boat noise on early life stages of fishes has been included as a research priority by the European Commission in the Marine Strategy Framework Directive (Borsani et al. 2015), and many other countries will hopefully soon follow.

1.4 Stress and non-lethal impacts of boat noise

Stress is the adaptive response of the body to deal with real or perceived stressors in order to maintain homeostasis (Barton 2002). In vertebrates, real or perceived stressors stimulate the hypothalamic–pituitary–adrenal axis (HPA; or the hypothalamic–pituitary–interrenal (HPI) axis in fish) and chromaffin cells, resulting in the release of glucocorticoid hormones and catecholamines into the blood stream (Sapolsky et al. 2000, Barton 2002). The release of glucocorticoid and catecholamine hormones stimulates the secondary stress response consisting of physiological changes related to metabolism, hydromineral balance, respiration, immune and cardiovascular function that help the animal cope with the stressor. In general, these changes reallocate energy and resources from long term growth, survival, and reproduction toward immediate survival by mobilizing energy, increasing oxygen uptake, and generally suppressing immune function (Bonga 1997). Stress also modifies behavioral patterns (e.g. foraging, vigilance, predator escape) from those conducive to long-term fitness to those that aid in immediate survival. Examining indicators of stress response can be used to assay an animals' perceptions, which can be especially useful when there are no behavioral metrics that can be used (for example in fish embryos). Additionally, physiological responses are mechanistic and can often be more repeatable than behavioral measures. Indicators of the stress response can also indicate when the allostatic overload is reached, in other words, the point at which an animal begins to alter their physiology and behavior to cope with the real or perceived stressor (Wikelski and Cooke 2006). For these reasons, examining indicators of an animal's stress response can be useful to understanding the impacts of anthropogenic stressors. In some cases, an animal's stress response can be maladaptive. When a perceived threat is not actually an immediate threat to an animal's survival (i.e., boat noise), then the reallocation of energy and resources from long term fitness to short term survival could affect the animals' ability to properly grow, maintain health, and reproduce, especially if the stressor is often present, forcing the animal to continually mount a response. In the case of boat noise, the noise itself is unlikely to be lethal or cause physical injury, thus the stress response it induces may be maladaptive and, over time, have negative consequences for the animal. For all the above reasons, I used indicators of stress response as a tool to examine the effects of boat noise.

1.5 Boat noise regulation

Noise from motorboat activities propagates in the air, water, and onboard. Regulatory frameworks for onboard crew conditions have been in place for a few decades (Badino et al. 2012). Underwater noise regulations are rare, and, to my knowledge no regulations based on how different noises affect aquatic ecosystems exist, despite underwater boat noise being considered a threat to marine environments by many international organizations (e.g. International Union for the Conservation of Nature (IUCN), the International Whaling Commission (IWC), the International Council for the Exploration of the Sea (ICES) and the International Maritime Organization (IMO); Badino et al. 2012). It is thus of urgent importance to understand the effects of boat noise on aquatic life and research approaches to reducing harmful effects so that evidence-based regulations of boat noise can be put in place.

As boat noise becomes more prevalent, successful mitigation likely depends on altering boat noise production through boat engine engineering designs that are less noisy rather than decreasing boat prevalence. Thus, successful mitigation of boat noise requires understanding what aspects of boat noise need to be reduced to minimize the impacts on animals of interest and the engineering designs that are required to do this. Very few studies have compared the noise produced by different engineering designs of boats. Even fewer studies have examined the consequences of different types of noise produced by boats on fish, but a summary of those that do is presented here. Kuznetsov et al. (2012) found the distance at which fish reacted to a boat depended on the vessel, but they do not relate this to the sound characteristics produced by different vessels. Sarà et al. (2007) found captive tuna changed their schooling patterns with differing characteristics depending on the type of vessel that approached: ferry, hydrofoil, or an outboard motorboat. Graham and Cooke (2008) found that fish increased their heart rates after 60 seconds exposure to the noise associated with a canoe paddle, an electric troll motor, or a motorboat combustion engine. The most dramatic effect was following exposure to the combustion engine, and fish took the longest to recover (40 minutes). The troll motor had the second greatest effect and a 25 min recovery time. The canoe paddle had the least effect and a 15 minute recovery time. In summary, current research demonstrates that the effect of vessel noise does differ with vessel; however, the characteristics of the noise responsible for this difference have rarely been examined.

Whether a fish responds to vessel noise depends on whether the noise exceeds a reaction threshold somewhere above the sound detection threshold, within the frequency range audible to the fish; for example cod avoidance reaction levels are about 30 dB above the sound detection threshold of about 76 dB (bellow 1000 Hz) (Wood 2011). Quite a lot of work has been done on sound detection thresholds, but less on reaction thresholds and there is no clear relationship between the two (Kastelein et al. 2008); however sounds of higher intensities within the sound detection ranges of fish are more likely to exceed reaction thresholds and cause a response (Knudsen et al. 1992). Stress response has been found to increase with sound intensity. For example, during playbacks to juvenile giant kelpfish, stress response increased linearly with decreasing distance of recorded boat, (Nichols et al. 2015). Thus, I might hypothesise that boat engineering designs with the highest noise intensity within the sound detection range of the fish of interest may have the most effect.

The most common difference in engine engineering design in small motorboats is four-stroke versus two-stroke engine. In this study I examine the difference in effect on fish caused by these two engine types. I hypothesize that two-stroke engines have a greater effect, as the intensity of noise they generate is generally higher. One other study has examined differences in these two engine types with respect to their effects on risk assessment in juvenile coral reef fishes, and found two-stroke engine noise to have a considerable effect on risk assessment behaviours, and four-stroke engine noise to have minor effects on behaviours (McCormick et al. 2018).

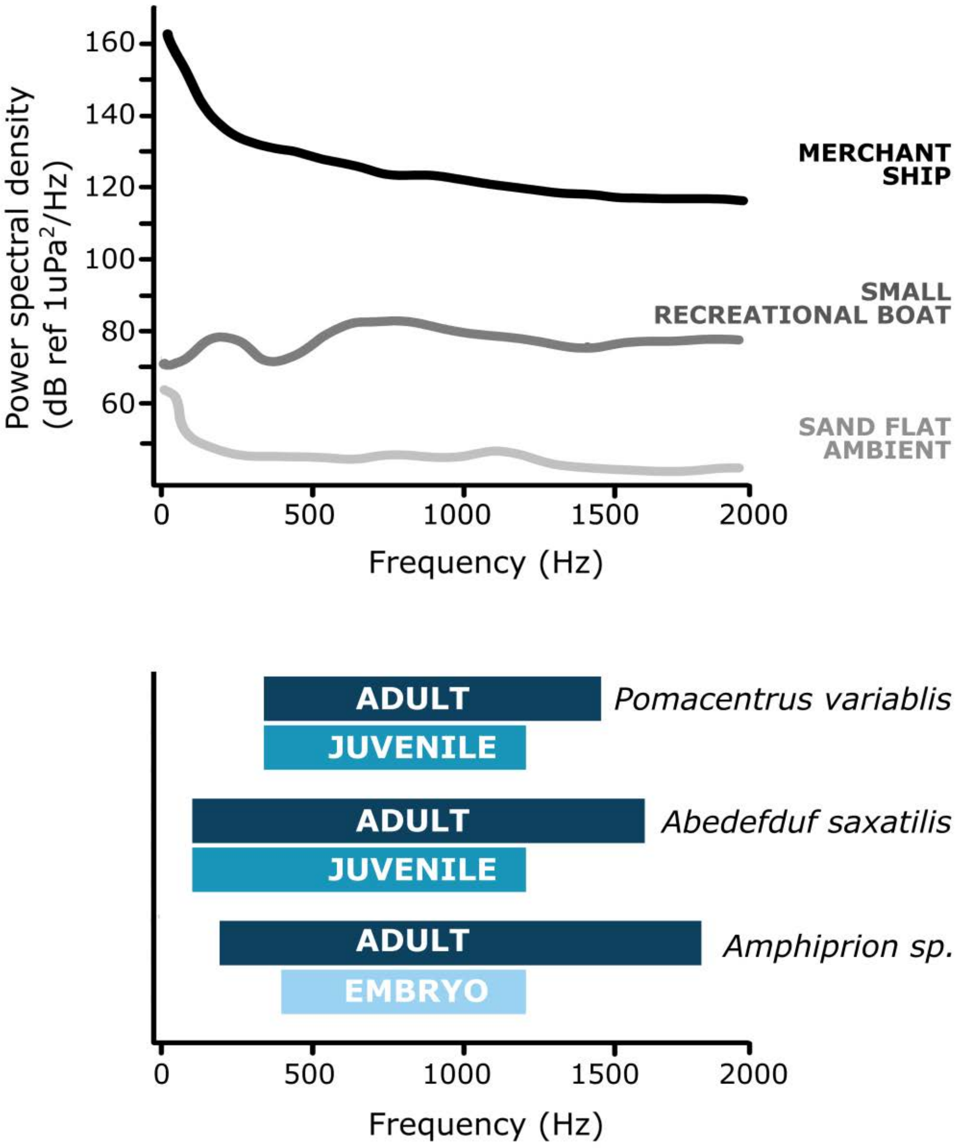

Figure 1. 1: Approximate power spectral density of a merchant ship (Wales and Heitmeyer 2002), small recreational boat with a two-stroke powered engine (Simpson et al. 2016b) and natural ambient noise on a sand flat (Simpson et al. 2016b). Sound detection ranges of Pomacentrid species: *Pomacentrus variablis* (Kenyon 1996), *Abedefduf saxatilis* (Egner and Mann 2005) and *Amphiprion sp.* (Simpson et al. 2005, Parmentier et al. 2009).

1.6 Thesis outline

While many studies are finding impacts of motor vessel noise on fishes there is still relatively little known about the impacts on fish during their early life stages of fish and few evidence-based recommendations on how to mitigate the negative impacts of vessel noise. In order to begin to address these knowledge gaps the over-arching goals of my thesis were: 1) to increase empirical evidence for effects of small motorboat noise on early life stages of coral reef fish, and 2) to investigate a potential mitigation strategy of using quieter engine types. My study focused on early life stages of coral reef fish, both because few studies examine early life stages despite evidence suggesting they develop sound detection capabilities as an embryo (Figure 1.1) and because it is a stage at which stressors are likely to have population consequences. This thesis is set up as two self-contained manuscripts, one of which is already published at the time of submission (Ch 2).

In these studies, I took a conservation physiology approach. Examining the physiological responses of animals to stressors is increasingly used to solve conservation problems, because physiological responses can be the only available assay of the animals perception (e.g. in the case of fish embryo), and because they can indicate when the allostatic overload is reached at which an animal begins to alter physiology and behavior to cope with the stressor problems (Wikelski and Cooke 2006).

In **Chapter 2**, I examine whether boat noise affects embryonic coral reef fishes, and whether the effect differs with engine type. I examine the effect of boat noise on wild-collected embryos, in the field, using real boats. This is an important advancement because acoustic stimulus can have different qualities during playbacks, due to the environment through which the sound propagates, properties of the speaker, and any sound frequency clipping or unusual background sounds that occurs during recordings (Rogers et al. 2016). Additionally, while many studies have found detrimental effects of boat noise few have examined how these effects can be reasonably mitigated. Thus, informing management was also a goal in the chapter and I accomplish this by examining differences in effects between recreational boat engine types (two-stroke versus four-stroke engines).

I examine heart rate, which is related to the secretion of catecholamine stress hormone beginning during embryonic development (Bagatto 2005). The implications of increased heart rate are: 1) the energetic cost of increased heart rate may lead to decreased energy available for fitness promoting processes such as growth and predator escape once hatched, and 2) increased heart rate indicates a secondary stress response which may be associated with other detrimental physiological changes.

In **Chapter 3**, I examine a proxy for the energetic cost of boat noise induced stress in settlement stage coral reef fishes by using respirometry to approximate metabolic costs associated with the secondary stress response initiated by boat noise. Closed respirometry has been previously used to address these questions; however, closed respirometry is limited in that it can only take a single measurement of oxygen consumption per fish (Armstrong-Smith, 2016; Simpson et al., 2016). Intermittent flow-through respirometry provides a more accurate and precise method of determining oxygen consumption rates, as continuous measurements can be taken on the same individual, measurements can be taken pre- and post-boat noise exposure and recovery times can be determined. Additionally, oxygen concentrations in the chambers can be maintained well above oxygen concentration that could induce a hypoxia response, and fish can be acclimated in the chambers. To date, advanced methods of respirometry have not been used on coral reef fishes to assess the effects of boat noise, one of the problems being that these methods involve pumps that expose the fish to loud noise that is not part of the stimulus of interest. I therefore fill this knowledge gap by creating a gravitational intermittent-flow system that does not use pumps.

In **Chapter 4**, I discuss the implications of these studies and how it adds to current knowledge on the topic of the effects of boat noise on fishes. I also suggest direction for future study.

Chapter 2: Impact of motorboats on fish embryos depends on engine type

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Nature and extent of the intellectual input of each author: Authors co-developed research questions. SJS developed research methods with assistance from other authors. SJS carried out data collection with assistance from EF. SJS carried out all data analyses and made all figures and tables. SJS wrote the first draft and received editorial input from JLR, SDS and MIM.

2.1 Abstract

Human generated noise is changing the natural underwater soundscapes worldwide. The most pervasive sources of underwater anthropogenic noise are motorboats, which have been found to negatively affect several aspects of fish biology. However, few studies have examined the effects of noise on early life stages, especially the embryonic stage, despite embryo health being critical to larval survival and recruitment. Here, I used a novel setup to monitor heart rates of embryos from the staghorn damselfish (*Amblyglyphidodon curacao*) in shallow reef conditions, allowing us to examine the effects of *in situ* boat noise in context with real-world exposure. I found that the heart rate of embryos increased in the presence of boat noise, which can be associated with the stress response. Additionally, I found two-stroke outboard-powered boats had more than twice the effect on embryo heart rates than did four-stroke powered boats, showing an increase in mean individual heart rate of 4.6% and 1.9%, respectively. To my knowledge, this is the first evidence suggesting fish embryos can be stressed by boat noise and highlights the need to explore the ecological ramifications of boat noise stress during the embryo stage. Also, knowing the response of marine organisms caused by the sound emissions of particular engine types provides an important tool for reef managers to mitigate noise pollution.

2.2 Introduction

Human generated noise is changing natural soundscapes worldwide. Boat noise is the most prevalent source of underwater anthropogenic noise and is becoming recognized in international legislation as a prevalent anthropogenic pollutant that is increasing (Slabbekoorn et al. 2010, International Maritime Organization 2011, Badino et al. 2012, Borsani et al. 2015). While boat noise has been found to have a variety of biological impacts on a broad range of taxa (Nedelec *et al.*, 2014; Rolland *et al.*, 2012; Simpson *et al.*, 2016), data are insufficient to provide the evidence needed to inform policy geared toward mitigating biological and environmental impacts. Current boat noise regulations are developed based on assessments of airborne emissions affecting comfort of onboard living conditions or that of inhabitants near ports, but not the impacts of noise on aquatic life (Badino et al. 2012). Successful mitigation likely depends on altering boat noise production rather than decreasing boat prevalence, because boat numbers continue to increase. Yet, to my knowledge, no studies have examined the responses of aquatic organisms to noise from different types of boat engines.

The early life stages of marine organisms can be particularly susceptible to environmental perturbations, especially at key development stages when sensitivities are high (e.g., Mager et al. 2017). While most research that documents the importance of the early life history to population dynamics focuses on the larval phase (Peck et al. 2012), it is of course preceded in most species by an egg phase whose sole purpose is development driven and fueled by maternally provisioned endogenous yolk reserves. Because of the small size and rapid development, embryos are particularly sensitive to disruption by environmental perturbations (e.g., temperature shock, pollutants) with carryover effects for neural, sensory, muscular and morphological development (Roussel 2007, McCormick and Gagliano 2010), that may flow on to effect growth and survival (Gagliano et al. 2007).

Here I investigate a coral reef fish species during the vulnerable embryonic life-stage; a life-stage identified as a research priority in relation to anthropogenic noise effects by the European Commission in the Marine Strategy Framework Directive (Borsani et al. 2015). Boat noise has been shown to affect many biological processes in fish including parental care

(Nedelec et al. 2017), navigation (Holles et al. 2013), foraging (Voellmy et al. 2014), and survival under a predator threat (Simpson *et al.*, 2016). However, to my knowledge only a single study has examined effects of noise on fish at the embryonic life-stage (Bruintjes and Radford 2014), despite evidence suggesting that fishes begin to respond to sound during embryonic development (Simpson et al. 2005) and that embryo health is important to larval growth and cohort survival (Bailey and Houde, 1989; McCormick and Nechaev, 2002; Simpson *et al.*, 2005). The present study represents a significant advancement on Bruintjes and Radford's study by manipulating the embryos' acoustic environment in the field using real boats and by considering both the pressure and particle motion conditions during experimental exposures. Additionally, I compare effects of two-stroke outboard engines to quieter four-stroke engines.

I use heart rate as an indicator of the stress response in fish embryos. Heart rate is a reliable indicator of stress and has been frequently employed as an indicator in other studies (Nimon et al. 1996, Bunt et al. 2004, Borell et al. 2007, Graham and Cooke 2008, Atherton and McCormick 2015). Heart rate increases (β-adrenoreceptor-mediated) directly in response to stressors caused by the stimulation of the hypothalmic-sympathetic-chromaffin-cell axis and the production of catecholamines (Barton 2002, Bagatto 2005). Therefore, heart rate provides a logistically feasible indicator of stress response, suggesting increased energy mobilization and use in fish embryo.

2.3 Methods

Study species and collection

The staghorn damselfish (*Amblyglyphidodon curacao*) is an omnivorous damselfish that forms pairs during the breeding season when males make nests on vertical projections of dead substrate (Goulet 1995). Eggs are laid in a monolayer and are defended from predators, principally by the male. At Lizard Island on the northern Great Barrier Reef, Australia (14°41’S, 145°27’E), during summer sea temperatures of approximately 28ºC, embryos hatch 5 days post fertilization. The sagittal otoliths that form the basis of the acousticolateralis system form during embryogenesis, and it is likely that these embryos have a functioning acoustic system prior to hatching (Simpson et al. 2005). As embryos do not have a gas filled swim bladder, sound detection is likely driven by particle motion auditorily and via neuromast cells (Sarrazin et al. 2010).

Four-day-old *A. curacao* embryos were collected from the reefs around Lizard Island from 12 clutches and 9 different nesting sites/fathers between 21 and 29 October 2016. In order to collect and age the embryos, sheets of clear plastic were wrapped around dead coral branches at breeding sites and monitored daily for egg deposition. Plastic sheets were collected 4 days after egg deposition and placed into a seawater filled 9 L plastic bag, which was then placed into a seawater filled polystyrene box (to reduce noise disruption and temperature change) and driven slowly by boat (with a quiet four-stroke engine) to a nearby beach (See supplementary material Figure A2.1 for an analysis of acoustic exposure during transport). Eggs were then kept in the plastic bags within the polystyrene box in the shade on the beach, isolated from any further boat noise, until their experimental treatment (less than 4 hours later). Seawater was replaced in the plastic bags and box every 30 mins, and water temperature was kept within 1˚C of local sea temperature.

Acoustic stimuli

Three different acoustic stimuli were used in experimental treatments: ambient conditions (with background biophonic noise produced by fishes and invertebrates resident on patch reefs within the bay, but without any boats operating in the area), two-stroke powered boats, and four-stroke powered boats. Boat stimuli consisted of boats driven at 0 to 35 km/h at 10–200 m from the experimental setup. Seven boats were used in total; four aluminium-hulled 5m long boats with 30 hp Suzuki two-stroke outboard engines (model DT30) and three boats of the same design but with 30 hp four-stroke outboard engines (model DF30A).

In order to characterize the differences in acoustic conditions in the experiment, three recordings of acoustic pressure and particle motion conditions were made for each of the treatments, where a different boat was used in each of the boat noise recordings. Recordings were made at the location of the experimental trials, 1 m above the ocean bottom, from a kayak in 2–5 m water. Acoustic-pressure recordings were taken using an omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1V/µPa; frequency range 0.02–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport MS). Particle motion recordings were taken simultaneously using a triaxial accelerometer (M20L; sensitivity following a curve over the frequency range 0–2 kHz; calibrated by manufacturers; Geospectrum Technologies, Dartmouth, Canada). Both the accelerometer and hydrophone were connected to a digital 8-track recorder (F8 field recorder, sampling rate 48 kHz, Zoom Corporation, Tokyo, Japan). Using the same recording equipment, a recording was made in the polystyrene container of seawater on a boat to quantify the acoustic conditions to which embryos were exposed during transport (Figure A2.2). Calibration parameters for the recording levels used were determined by recording a pure sine wave signal from a function generator, with the voltage measured using an in-line oscilloscope. Sound files were cropped in Audacity 2.1.2 (http://www.audacityteam.org), and acoustic analyses were calculated using PaPAM 0.872 (Nedelec et al. 2016b) in Matlab Compiler Runtime 8.3 (https://au.mathworks.com).

The root mean square of the power spectral density (PSD) was used to characterise the acoustic treatments. The PSD describes the acoustic power across frequencies, while the root-mean-square (RMS) of the PSD provides an average across frequencies (Merchant et al. 2015). The RMS PSD of each treatment (ambient, two-stroke, and four-stroke) was calculated for 1 min tracks, where three passes of different boats of the same treatment were spliced together (to get an average between soundscape replicates), or three ambient tracks were spliced together in the case of the ambient treatment. Using the same tracks, the sound exposure level (SEL) within the estimated sound detection range of the embryos (400–1200 Hz) was calculated. SEL describes the cumulative sound energy over time (Merchant et al. 2015). Consistency analysis, which indicates the percentage of time that the amplitude of sound is greater than a given threshold (Nedelec et al. 2016b), was also calculated for these at a threshold of 110 dB re $\mu Pa^2 Hz^{-1}$ (at 400–1200 Hz). This sound pressure sound detection threshold is the best estimate of the embryos sound detection thresholds based on previous studies with pomacentrid fishes (Table 2.1). Consistency was then multiplied by SELs to give an estimate of the cumulative sound energy that embryos were exposed to for each treatment (relSEL, Figure 2.1).

Table 2. 1: Approximate sound detection thresholds found in other studies on pomacentrid fishes.

Reference	**Species**	**Life stage**	**Sound detection range (Hz)**	**Pressure threshold range (dB re 1 µPa)**
(Wysocki et al. 2009)	*Chromis chromis*	Adult	100-500	100-110
(Parmentier et al. 2009)	*Amphiprion ocellaris, frenatus and clarkii*	Adult	100-1800	100-150
(Kenyon, 1999)	*Pomacentrus variablis*	Adult	300-1200	80-140
(Kenyon 1996)	*Pomacentrus variablis*	Post settlement juvenile	300-1100	At 13mm: 120-140 At 20mm: 110-130
(Egner 2004)	*Abudefduf saxatilis*	Post-settlement juveniles	100-1200	110-150
(Wright et al. 2011)	*Pomacentrus nagasakiensis, Pomacentrus amboinensis*	Settlement stage larvae	100-2000	90-110
(Simpson et al. 2005)	*Amphiprion ephippium*	Embryo	At 3 days: 400-700 At 9 days: 400-1200	At day 3: 140-150 At day 9: 100-140

Experimental design

Heart rate was measured using a recording apparatus located on a shallow (2–5 m) sandy bottom site, adjacent to a reef (25 meters), in front of Lizard Island Research Station (14˚ 40’ S, 145˚ 28’ E), Great Barrier Reef, Australia. The apparatus consisted of an Olympus Stylus T-4 camera with an i-Das UCL-02 lens (125mm/ +8 macro lens), a Perspex stage in front of the lens, and a dive torch. To film the embryos, a strip of plastic sheet onto which embryos had been laid was attached to the stage and illuminated from behind by the torch (see supplementary material Figure A2.2 for photograph). Before each trial, a strip of the plastic sheet containing the egg clutch was cut off and taken by a snorkeler to the video apparatus. The camera was then focused on one to four individual embryos with visible heartbeats. Following 15 min of habituation in ambient conditions (natural ambient sound in the absence of boats), heartbeats were recorded using the camera for a further 2 min under pre-treatment, ambient conditions, followed by 1 min of one of three acoustic stimuli treatments (ambient, two-stroke, four-stroke; Figure 2.1). The three treatments were alternated in a different order each day. There were 21 different individuals exposed to each treatment.

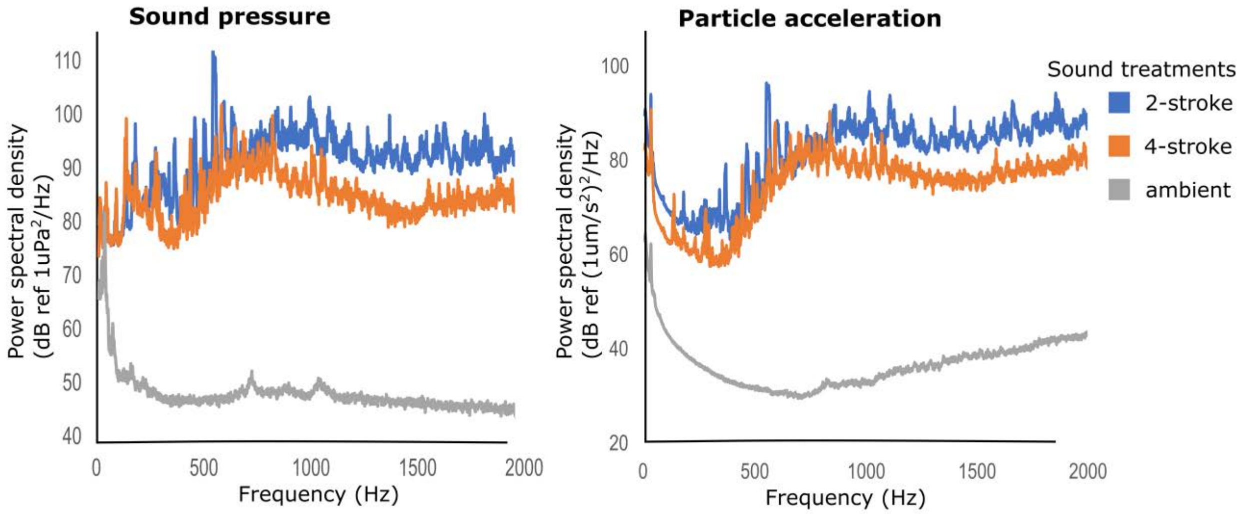

Figure 2. 1: Power spectral density of the sound treatments to which *Amblyglyphidodon curacao* embryos were exposed: two-stroke powered boat noise, four-stroke powered boat noise, and natural ambient conditions. Spectral content is shown in sound pressure (left) and particle acceleration (right). Analyses were conducted in paPAM using one minute tracks that combined three separate recordings of each treatment to give the average sound profile of the three recordings. For boat tracks, each of the three recordings used in a track were from a different boat to account for variability between boats with the same engine type.

Analyses

Heartbeats were counted in 20 s intervals, blind to treatment. To determine the time at which embryos were affected by boat noise, heart rate was initially plotted over time for embryos exposed to ambient conditions and those exposed to two-stroke boat noise during treatment (Figure 2.2). These results suggested that it takes 140 s for embryos' hearts to reach their full response to boat noise. Therefore, heart rate measurements taken in the 2 min following the first 140 s of boat noise were averaged within individuals to represent heart rate during treatments, and the heart rate measurement taken during the 2 min of pre-treatment, ambient conditions were averaged within individuals to represent heart rate during pre-treatment.

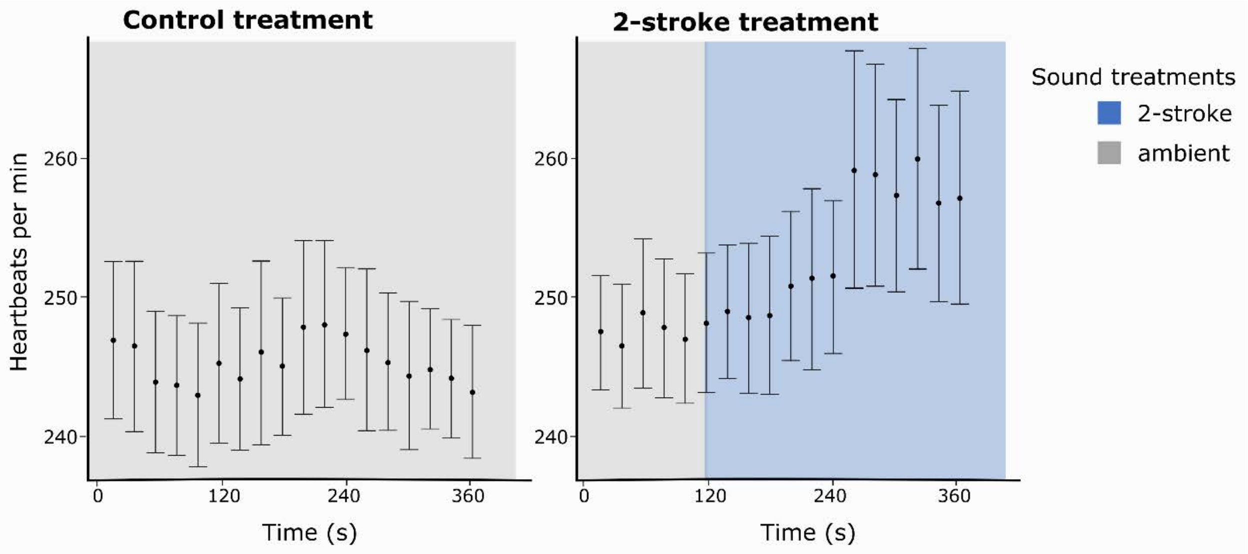

Figure 2. 2: Heart rate of 4 day old *Amblyglyphidodon curacao* embryos, following 15 mins of habituation in the recording apparatus. Embryos were exposed to either ambient conditions for 360 seconds (left; sample size 13) or 120 seconds in ambient conditions followed by 240 seconds of two-stroke powered boat noise driving at 0–35 km/h at 10–200 m from embryos (right; sample size 18). The full heart rate response to boat noise appears to occur 140s after initiation of exposure to boat noise.

A linear mixed effects split-plot model was fitted to the data using maximum likelihood and implemented using the “lmer” function in the “lme4” R package (Bates et al. 2015). Treatment was included as a between individuals fixed effect, and time (pre-treatment/treatment) was included as a within individuals fixed effect, thereby incorporating the repeated measures element of the design into the analysis. A fixed treatment–time interaction was included to determine whether changes in heart rates within individuals differed with treatment. Individual and clutch were added as random factors without interactions. The assumption of normality was met, and the response variable (heart rate) was square root transformed to meet the assumption of homogeneity of variance. Within-group correlation structure did not improve the model and thus was not incorporated (Logan 2010). The “lsmeans” function in the “lsmeans” package was used post-hoc to identify where differences among means occurred (Lenth 2016).

2.4 Results

Acoustic analysis

In general, boats with two-stroke engines generated more noise than boats four-stroke engines. The RMS acoustic pressure (PSD) generated by boats within the estimated sound detection range of *A. curacao* embryos (400–2000 Hz, Table 2.1) was 125 dB re $\mu Pa^2 Hz^{-1}$ for four-stroke engines and 132 dB ref $\mu Pa^2 Hz^{-1}$ for two-stroke engines (Figure 2.3). Within this same frequency range the average particle acceleration was 77 dB re $\mu m s^{-2} Hz^{-1}$ for four-stroke engines and 84 dB re $\mu m s^{-2} Hz^{-1}$ for two-stroke engines. The cumulative sound energy in sound pressure over a min (SEL) above an estimated sound detection threshold for *A. curacao* embryos of 110 dB re $\mu Pa^2 Hz^{-1}$ and within their estimated sound detection range (400–2000 Hz) was 0 dB re $\mu Pa^2 s^{-2} Hz^{-1}$ (ambient conditions), 13 dB re $\mu Pa^2 s^{-2} Hz^{-1}$ (four-stroke engines), and 35 dB re $\mu Pa^2 s^{-2} Hz^{-1}$ (two-stroke engines; Figure 2.3).

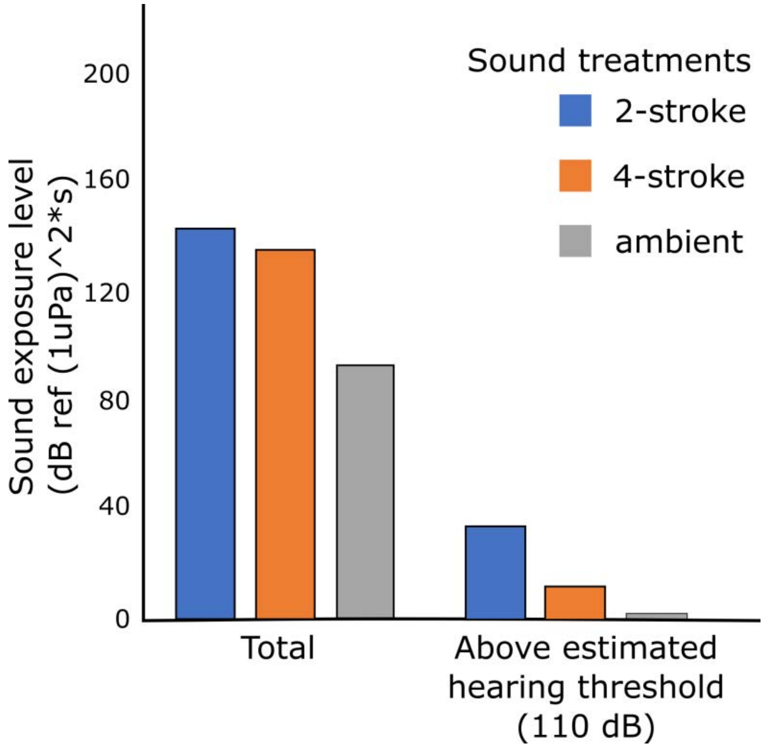

Figure 2. 3: Sound exposure levels (SELs) are the cumulative sound energy at 400–1200 Hz (the estimated sound detection range of *Amblyglyphidodon curacao* embryo) over 1 min. The SELs are shown in sound pressure for each of three sound treatments: a two-stroke powered boat, a four-stroke powered boat noise, and natural ambient conditions. Total SELs as well as the SELs above the estimated sound detection thresholds of *A. curacao* embryos are shown (relSEL, calculated by SEL* % consistency above 110 dB re $\mu Pa^2 Hz^{-1}$).

Effect of boat noise

When compared to embryos under ambient conditions, heart rate of A. curacao embryos significantly increased during exposure to boat noise, as demonstrated by a significant treatment-time (time = pre-treatment or treatment) interaction ($F_{2,20} = 21.0$, $p < 0.001$; Figure 2.4). Embryos that were exposed only to ambient conditions did not show a significant change in heart rate between pre-treatment and treatment periods (t66 = 0.5, p = 0.6). Embryos exposed to two-stroke engine noise showed a statistically significant mean increase in heart rate of 4.6 % ± 3.5 above that under ambient conditions (t66 = -8.6, p < 0.001). The effect of four-stroke engine noise was less than half of the effect of two-stroke engine noise, showing a mean increase in heart rate of 1.9 % ± 1.7 from ambient conditions (t66 = -3.7, p < 0.001). When clutch was included in the model as a fixed effect instead of a random effect, there was a significant effect of clutch on heart rate ($F_{11,46}$=6.3, p<0.001), but no treatment-time-clutch interaction indicating that the effect of boat noise on individual heart rate did not differ with clutch ($F_{9,46}$=1.9, p=0.07).

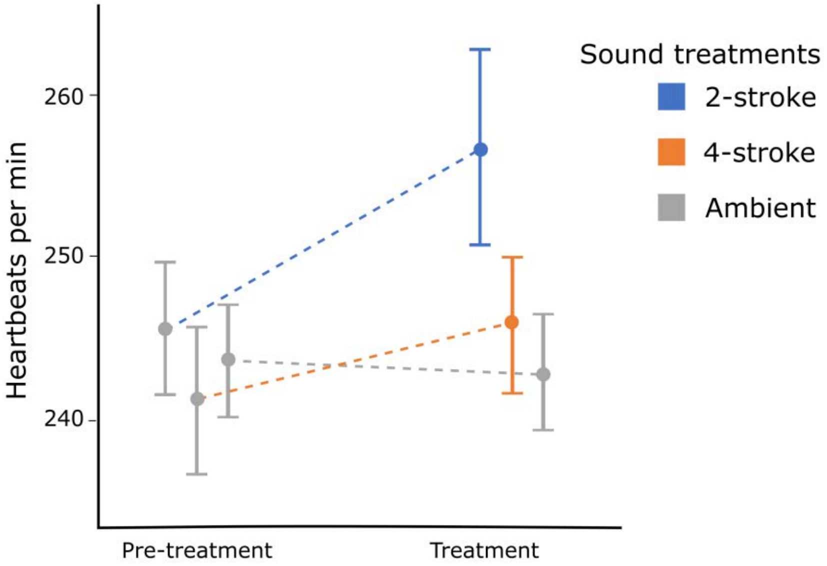

Figure 2. 4: Change in heart rate of *Amblyglyphidodon curacao* embryos from pre- treatment conditions (ambient) to treatment conditions (ambient, two-stroke engine, or four-stroke engine), where heart rate was negated for the first 140 s of the treatment conditions to allow the response to be reached. Both two-stroke and four-stroke treatments involved a boat

driving at 0–35 km/h at 10–200 m from embryos. The graph displays inter-individual means, and bars represent 95 % confidence intervals.

2.5 Discussion

I found increased heart rate in *A. curacao* embryos in response to boat passage,. Increased heart rate indicates the initiation of an adrenergic stress response, which is ultimately responsible for activating metabolic pathways and the mobilization of energy substrates to deal with perceived challenges (Armstrong 1986, Lucas 1994, Barton 2002). In the case of boat noise, the perceived challenge is not a threat, so the energy expenditure associated with the stress could be detrimental to the embryos by depleting energy that could have otherwise been allocated to fitness promoting processes. However, from the data collected in this study, I cannot say whether the energetic cost of the stress induced by boat noise is sufficiently large to have impacts on body condition and fitness (Frid and Dill 2002). If boat noise induced stress significantly depletes embryonic energy reserves, it may affect subsequent recruitment to coral reefs. Growth until feeding in the post-yolk sac larval stage is dependent on the available yolk sac energy reserves (McCormick and Nechaev, 2002). Thus, depletion of the endogenous embryonic energy reserves can reduce larval growth. Studies have found that larger and faster growing larvae have higher survival, which is related to larger larvae having increased ability to compete for food, resist starvation, and avoid predation (e.g., Bailey and Houde, 1989; Jenkins and King, 2006; Peck *et al.*, 2012; Sogard, 1997). Furthermore, in many populations, a strong link has been found between larval abundance and recruitment (Cushing 1990, Leggett and Deblois 1994, Karjalainen et al. 2000). Thus, the depletion of energy reserves associated with boat noise induced stress may affect young-of-year recruitment by reducing growth. Further experiments are required to quantify the magnitude of these energetic costs associated with boat noise induced stress and determine whether these costs affect recruitment by affecting embryo survival or causing carryover effects to later life stages (e.g. McCormick and Gagliano, 2010).

The embryos heart rates increased by 1.9 % and 4.9 % on average with the passage of two-stroke and four-stroke powered boats, respectively. Any additional stress caused by the experimental procedure likely make these estimates more conservative, as they would decrease the ability of the embryos to respond to other stressors. An increase in heart rate of 4.9 % with the passage of two-stroke powered boats may indicate a considerably severe stress response when compared to increases in heart rate associated with conspecific alarm odours found in other fish species. A study on another pomacendrid (*Amphiprion melanopus*) found that embryos responded to conspecific alarm odours, arguably the most stressful cues that could be perceived, with an average increase in heart rate of 6.6 % and 12.2 % on days 6 and 7 of development over an 8 day development period (Atherton and McCormick 2015). On day 4 of development, *Melanotaenia duboulayi* showed a 8.9 % increase in heart rate in response to conspecific alarm odour (Oulton et al. 2013). In a detailed experimental study of the affect of cortisol on developmental rhythms during embryogenesis, McCormick & Nechaev (2002) found that the experimental elevation of cortisol resulted in a 4 to 14 % increase in heart rate, and that magnitude of increase was dependent upon developmental stage. Overall these changes were enough to alter the size of larvae at hatching such that larvae with higher heart rates were smaller in size. These findings were further supported by a study that looked at the interrelationships between egg, embryo and larval characteristics at the individual level (Gagliano and McCormick 2009), suggesting that perturbations within the embryonic stage can have strong carryover effects into future life stages. Therefore, it is possible that the increases in heart rate observed in the study in the presence of boat noise may indicate a stress response that could have carryover effects to future life stages.

Our finding that *A. curacao* embryos exhibit a stress response when exposed to motorboat noise contributes to the growing body of evidence that vessel noise can have detrimental effects on fishes. At juvenile and adult life stages, several other studies have found boat noise to instigate a physiological stress response (Nichols, 2014; Simpson *et al.*, 2016; Spiga *et al.*, 2012) and behavioural changes (Holles et al. 2013, Voellmy et al. 2014) in fishes. At the embryonic life-stage, the few studies related to boat noise show variability in the sensitivity of embryos to boat noise. The playback of chronic boat noise was not found to effect growth and survival of embryonic cichlids (*Neolamprologus pulcher*) in the laboratory (Bruintjes and Radford 2014). In the marine mollusc *Stylocheilus striatus*, chronic boat noise playback decreased embryonic survival by 21% and by a further 22% upon hatching (Nedelec et al. 2014). Differences among species in their tolerance and reaction to anthropogenic noise may be expected from differences in the development of sound detection systems and their sensitivities (Wright et al. 2011); a topic that remains unexplored for most species of fishes and invertebrates.

Our study is the first to assess the effects of *in situ* boat noise on embryonic fish. Using real boat noise in a field setting is an important advancement because sound is altered through its replication by speakers and by resonance, reflection, and differential absorption within a tank environment (see Rogers *et al.*, 2016 for a discussion of tank acoustics and drawbacks). Additionally, while particle motion and sound pressure components of sound have a direct relationship in the far field (fish would often experience sound in the far field in their natural environment), they do not when the sound source is in close proximity (such as a speaker in a tank). Many fishes hear both particle motion and sound pressure components of sound, and when a fish responds to sound, it is often uncertain to which component the fish is responding. Thus, it is difficult to adjust sound exposure levels in a tank experiment to the levels fish would experience in their natural environment, and therefore, it is more informative to conduct aquatic noise pollution studies in the field. It is an important advancement to find evidence that embryonic fish can display a stress response to boat noise, suggesting that it is important for future studies to examine the consequences of this stress response and whether effects carry-over to later life stages. An examination of the capacity to habituate to chronic exposure would be another research direction and for future studies. However, there are potentially many confounding factors in a long-term field experiment (e.g., effects on parental care, nest predators etc.); so, the current finding that embryos can be acutely stressed by boat noise forms an important foundation for future work.

Another important advancement of this study is that I found the effect of boat noise on embryos differed with source of the acoustic disturbance (i.e., engine type). I found the effect of two-stroke powered boats on embryo heart rates to be more than twice that of four-stroke powered boats. When comparing the acoustic signatures of the two engine types, only a small difference was found in PSD, the most common metric used in noise pollution studies. However, I found a marked difference between two-stroke and four-stroke engines in the SEL produced above the estimated sound detection threshold and within the sound detection range of *A. curacao* embryos (relSEL), which may account for the differences in heart rate responses found between the two engine types (Figure 2.3). There are currently not enough studies on particle motion sound detection thresholds to estimate the SEL above the particle motion sound detection threshold or fish embryo. Additionally, there is no standard unit of particle motion sound making it difficult to compare between studies (Nedelec et al. 2016a). It is thus important to develop a standard and conduct more research on particle motion sound detection thresholds. I suggest that measuring the relSEL of the species and life-stage may be a more appropriate metric for determining effects of noise pollution, as it is more indicative of what the organism may actually experience.

It is currently unclear whether the relatively small but significant changes in heart rate caused by boat noise are ecologically relevant and have repercussions for subsequent early life history dynamics. This study lays a strong methodological foundation for further studies that will examine the potential for habituation to boat noise by embryos and the relative importance of carryover effects to later life stages. It is only by examining how noise perturbations affect all major life stages that the importance of windows of developmental sensitivity (Fawcett and Frankenhuis, 2015) and carryover effects can be integrated into our understanding of how environmental perturbations such as noise affect the dynamics of marine organisms. Knowing whether different types of engines produce different magnitudes of disturbance is important as it gives aquatic resource managers an effective tool with which to mitigate the impacts of noise through restrictions on maximum sound outputs.

Chapter 3: Boat noise does not alter metabolic performance of a newly-settled coral reef fish

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This chapter will be submitted to PLoS ONE.

Nature and extent of the intellectual input of each author: Authors co-developed research questions. SJS developed research methods with assistance from other authors. SJS carried out data collection with assistance from EF. SJS carried out all data analyses and made all figures and tables. SJS wrote the first draft and received editorial input from JLR, SDS and MIM.

3.1 Abstract

Boat noise is increasing dramatically worldwide and has been found to have detrimental effects on fishes. It is therefore important to develop tools to measure the circumstances under which fishes are affected by boat noise. Measuring whole organism oxygen uptake rates as a proxy for metabolic rates can be a useful way of quantifying stress and its associated energetic costs. I demonstrate that a single small submersible pump often used in respirometry systems can expose fish to noise levels comparable to boat noise. Therefore, I designed and used a pumpless, noiseless aquatic intermittent-flow respirometry system to monitor changes in oxygen uptake rates of juvenile damselfish exposed to either the noise from an outboard-power boat or natural ambient conditions. No significant change in oxygen uptake rates were detected in newly settled *Pomacentrus amboinensis* in response to the playback of boat noise. This suggests there is either no stress response occurring or no immediate energetic cost to the fish in response to the boat noise playback. The respirometry system designed in this study is valuable to future respirometry studies as it eliminates pump noise as a secondary unintentional stressor.

3.2 Introduction

Underwater anthropogenic noise is recognized as a global pollutant that is negatively affecting aquatic life (Boyd et al. 2011, Williams et al. 2015). Boat noise is the most prevalent form of underwater anthropogenic noise (Andrew et al. 2002, Popper 2003) and is increasing markedly with increases in the global population, economy, and industry. For example, with industrialization, cargo shipping has doubled approximately every 20 years (Hawkins and Popper 2014), and there has been an approximate 3.3 dB re 1 μPa increase in open ocean noise per decade (Frisk 2012). Smaller boats, such as recreational powerboats, are also widespread and growing in popularity. In 2014, there were 250,231 recreational vessels registered in the state of Queensland, Australia – the location of the Great Barrier Marine Park – which was a 10 % increase from the previous year (*Recreational Vessel Census June 2014* 2014). That same year there were approximately 15.8 million recreational motorboats registered in the USA (NMMA 2015). This high prevalence of boats has the potential to affect a wide array of aquatic taxa.

Fishes are a globally important resource that have been shown to be vulnerable to the effects of boat noise. Evidence has shown boat noise to effect several aspects of fish biology including parental care (Bruintjes and Radford 2013), navigation (Holles et al. 2013), foraging (Bracciali et al. 2012, Voellmy et al. 2014), habitat selection (Simpson et al. 2016a) and survival (Simpson et al. 2016b). An increasing number of studies have also demonstrated that fishes exhibit a stress response when exposed to boat noise. For example, exposure to boat noise has been associated with an increase in circulating cortisol levels (Spiga et al. 2012a, Nichols et al. 2015), increased cardiac output (Graham and Cooke 2008), increased oxygen uptake rates (Simpson et al. 2016b), and increased ventilation rates (Bruintjes et al. 2016, Nedelec et al.

2016c). The stress response in fish involves the activation of brain centers causing the release of catecholamine and corticosteroid hormones (Barton 2002). These hormones mediate the physical changes to cope with the stressor; such as mobilization of energy through metabolic pathways, increased oxygen uptake for metabolism and the circulation of energy through increased cardiac output (Bonga 1997). In general, stress responses involve the reallocation of energy from long term outcomes such as growth and reproduction to immediate survival and tasks necessary for coping with the stressor (Elsasser et al. 2000). Therefore, when a fish exhibits a stress response to a stimulus that is not directly harmful, it can be indirectly detrimental to the fish, as stress responses are energetically expensive. The energetic cost associated with various stressors is often estimated using respirometry, the measure of an organisms oxygen uptake (Beyers et al. 1999, Lankford et al. 2005, Clark et al. 2013, McLeod et al. 2013). Most energy (ATP) produced in fishes occurs through aerobic metabolism, which uses oxygen, thus oxygen uptake measurements are an estimate of a fishes' energy use (Chabot et al. 2016). In this study, I use respirometry to estimate the energetic cost of boat noise on fish.

To date, in the context of boat noise, short term experiments (minutes) using a closed respirometry system that determined a single slope (rate of oxygen uptake), has been the only protocol used to determine oxygen uptake rates of fishes. This is because such a system avoids the use of noisy pumps and allows for exposure of fish to real noise sources in open water conditions (Simpson et al. 2016b). However, intermittent flow respirometry is considered to be a more accurate system to measure oxygen uptake rates (Snyder et al. 2016, Svendsen et al. 2016a). Intermittent flow respirometry involves the chamber being intermittently flushed with clean oxygenated water, which avoids nitrogenous waste buildup, the creation of hypoxic conditions through oxygen uptake, and the decrease in pH associated with carbon dioxide production (Steffensen 1989, Svendsen et al. 2016a). Intermittent flow also better controls for oxygen diffusion into the chamber from the surrounding water bath (Snyder et al. 2016). Continuous oxygen measurements are often taken in intermittent flow respirometry, improving accuracy and precision of calculations of oxygen uptake rates and allowing for changes to be monitored within individuals over time. Continuous measurement also allows for fish to be habituated to chambers prior to determining oxygen uptake changes in response to a stressor. It is also possible to examine whether individuals respond to the introduction of a stressor or habituate to stressors over time when repeated measures are carried out on an individual. Unfortunately, the pumps used in traditional intermittent flow respirometry produce additional noise that could mask or alter sound treatments, making this design inadequate for noise studies, and raising a more general question about whether pump noise could act as an additional stressor in other studies using intermittent flow respirometry.

In this study, I aimed to estimate the energetic cost of the stress response induced by the playback of boat noise in a common coral reef fish, the Ambon damselfish, *Pomacentrus amboinensis*. To achieve this, oxygen uptake rates were measured using a quiet pumpless intermittent-flow respirometry system. Given that previous studies using closed respirometry systems have found a marked increase in oxygen uptake rates associated with real boat noise and playback (e.g.,Simpson et al. 2016b), it was expected that this more sensitive respirometer would detect with more precision a marked increase in oxygen uptake in fish exposed to the playback of boat noise versus ambient conditions.

3.3 Methods

Study species and collection

P. amboinensis are a demersal coral reef fish species common to the Indo-Pacific. They have a pelagic planktonic larval phase that lasts 15–23 days, before the fish settle back onto reefs as juveniles (Kerrigan 1996). Settlement-stage larvae were collected in November and December 2016 using light traps (Meekan J. H. Carleton, A. D. McKinnon, K. Flynn and M. Furnas 2003) set offshore from Lizard Island Research Station (14˚ 40’ S, 145˚ 28’ E), Great Barrier Reef, Australia. All collection and experimental protocols complied with James Cook University Animal Ethics Committee regulations (permit: #A2089 and #A4208).

Fish were collected from traps in the morning and transported in 60 L containers of aerated seawater by boat (See Figure A3.1 for sound spectra) to the research station where they were sorted by species. Fish destined for use in experiments were maintained in aquaria with a constant, quiet supply of clean seawater and natural lighting. Sound exposure in aquaria was minimized by placing sound insulating materials below aquaria and adjusting waterflow to avoid bubbles (See Appendix 1 for sound spectra). Fish were fed hatched *Artemia* sp. twice daily *ad libitum* and maintained for 2–11 days.

Respirometry Setup

An intermittent flow respirometry chamber was designed such that electric submersible pumps would not be needed, to minimize noise exposure other than that coming specifically from noise treatments (Figure 3.1). Four 12 ml glass chambers were gravity-fed clean seawater via header tanks, which also avoided air bubble accumulation in the chambers. Temperatures in the four chambers were maintained at 31 ± 0.5 ˚C using a water bath. This is slightly higher than field temperature which were found to be 28 ± 0.7 ˚C and 29 ± 0.6 ˚C for December and

November respectively at 2m; however, temperatures as high as 32 ˚C were recorded in the field in January and February (McCormick 2016). A ball valve controlled flush cycles in the chambers and was closed and opened using a timed actuator. This device created no detectible sound in the chambers (Figure 3.4). Cycles consisted of a 10 min measurement period (valve closed) and 5 min flushing (valve open). Oxygen concentrations were measured in the chambers using oxygen-sensitive REDFLASH dye on contactless spots on the inside of the chambers linked through fiber optic cables to a Firesting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany). Measurements were temperature and barometric pressure compensated through the Firesting software using an inbuilt barometer and a Firesting temperature cable. Sensors were 100% calibrated using air saturated seawater before each trial and 0% calibrated as needed using saturated sodium sulphite solution. Chambers and associated tubing were cleaned with 10% bleach solution and freshwater between each trial to avoid microbial growth that could contribute to background respiration. All water containers were placed on foam insulation to minimize sound vibration transfer from the ground. Foam was also placed around the water tubing where it touched other equipment and around the bolt securing the actuator to the valve to prevent vibrations from being transferred from the actuator to the water. A vented drainage system was used to minimize drainage noise in the water bath. While this system does not actively mix the water within the chamber during the measurement periods (valve closed), mixing was observed using food coloring during a pilot, likely because of the constant fin movements in juvenile *P. amboinensis*.

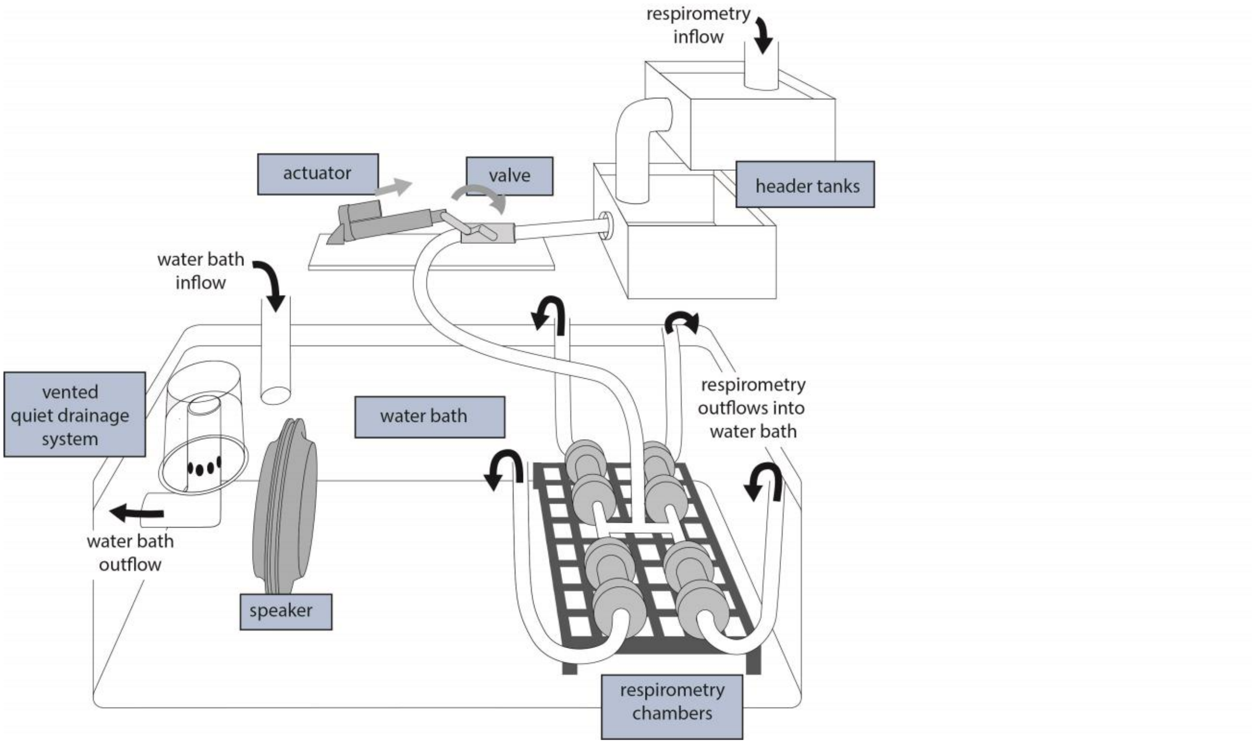

Figure 3. 1: Schematic diagram of the quiet respirometry system used in this experiment. A timed actuator and valve were used rather than pumps to reduce noise in the system. Additionally, a vented drainage system was used for quiet outflow, and submerged pipes were used for quiet inflow. Two header tanks were used to prevent bubbles from moving through the system. Insulation was placed between the actuator and the valve anywhere the pipes touched an external surface and below every water tank and the actuator to reduce vibration transfer from the environment to the water.

Acoustic Stimuli

Two different types of acoustic stimuli and three replicates of each stimuli type were recorded and used in playback during the experiment. The two types of acoustic stimuli used were daytime ambient open water conditions (control treatment) and the sounds from 5 m-long aluminium boats with 30 hp two-stroke engines (Suzuki DT30) driving at 0 to 35 km/h 10–

200 m from the recording device. For each replicate of the boat noise stimuli, a different boat of similar design was operated. Each recording was approximately 5 min. Each recording was repeated and spliced together without abrupt sound changes to form both a 30 min and 24 h track using Audacity 2.1.2 (http://www.audacityteam.org).

Sound treatments were played using a speaker system positioned 50 cm from the chambers, suspended halfway through the water column. The speaker was centered over the chambers by placing the chambers on a gridded plastic rack. The sound system as a whole consisted of a battery (12v 7.2 Ah sealed lead-acid), an MP3 player (SanDisk ClipJam), an amplifier (M033N, 18W, frequency response 0.04–20 kHz; Kemo Electronic GmbH, Germany), and a speaker (University Sound UW-30; maximal output 156 dB re 1 µPa at 1m, frequency response 0.1–10 kHz; Lubell Labs, Columbus, OH). Playback sound levels were adjusted so that pressure and particle motion-based power spectral density (PSD) levels of recordings of playback most closely resembled PSD levels of field boat noise and ambient recordings.

Sound pressure and particle motion recordings of acoustic stimuli were made 1 m above the ocean bottom from a kayak in 2–5 m water. Sound pressure recordings were taken using a suspended omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1V/µPa; frequency range 0.02–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport MS). Particle motion recordings were simultaneously taken using a triaxial accelerometer (M20L; sensitivity following a curve over the frequency range 0–2 kHz; calibrated by manufacturers; Geospectrum Technologies, Dartmouth, Canada). Both the accelerometer and hydrophone were connected to a digital 8-

track recorder (F8 field recorder, sampling rate 48kHz, Zoom Corporation, Tokyo, Japan) or the hydrophone was connected to a separate digital recorder (PCM-M10, 48 kHz sampling rate, Sony Corporation, Tokyo, Japan). Using the same recording equipment, a recording was also made of the transport process (i.e., from the light trap soundscape, via 60L container), and the respirometry soundscape with and without movement of the actuator. To demonstrate the importance of not using pumps in the respirometry setup, recordings were made of a submersible EHEIM pump (type: 1102.360; series: 14072; 240V; Hmax: 3.0; l/Std: 2500-5000) commonly used in respirometry, inside the water bath and a pump in an external bucket pumping water into the water bath through a one meter long, two cm wide silicon tube (Figure 3.5). Additionally, recordings of boat and ambient playback were made inside a glass barrier (a 1L glass beaker filled with seawater and partially submerged; Figure 3.6), to validate that sound reaching fish within glass chambers is comparable to the absence of a glass barrier.

Calibration parameters were determined by measuring a voltage on an oscilloscope, where the voltage produced was a pure sine wave signal from a function generator. Sound analyses were calculated using PaPAM 0.872 (Nedelec et al. 2016b) in Matlab Compiler Runtime 8.3 (https://au.mathworks.com).

Experimental Procedure

Fish were fasted for 9h prior to any experimentation to ensure a post-absorptive state (Niimi & Beamish, 1974) and were then habituated to the respirometry chambers overnight with playback of one of the three replicates of ambient conditions. The following day, between 10am and 1pm, the playback track was changed systematically in a block design to one of six, 30-minute tracks of one of two treatments (one of the three ambient replicates, or one of the three boat noise replicates) followed by at least three hours of the original ambient sound track. Background (e.g., microbial) oxygen uptake rates were measured before and after every trial. Following trials, fish were blotted dry and immediately weighed using a Mettler Toledo balance before being placed in a recovery tank. Subsequently all fish were released back onto the reef.

Analysis

All oxygen uptake data were analysed using LabChart (ADInstruments, Dunedin, New Zealand). The average slope ($R^2 \geq 0.95$) was calculated every min during 6 min of the closed phase of the respirometry cycle. A mean was then taken on these six calculations to give an estimate of oxygen uptake rates during each cycle. This process was repeated for four respirometry cycles prior to soundtrack change, the two cycles following the first sound track change and the two cycles following the second sound track change. Slopes of oxygen over time (S) were converted to oxygen uptake rates.

$\dot{M}O_2$ (Rummer et al. 2016):

$$\dot{\mathrm{M}}\mathrm{O}_2 = \mathrm{SVM}^{-1}$$

Where V is the volume of the respirometer minus that of the fish (calculated as density of water times M) and M is the blot dry mass of the fish. Fish oxygen uptake rates were then determined by subtracting the proportional background oxygen uptake rates from the total oxygen uptake rates.

Four ambient and two boat respirometry profile outliers were removed. These data points had negative oxygen uptake rates and variation from individual means that were greater than 50%. These may have arisen due to the fiber optic cables becoming misaligned with the oxygen-sensitive REDFLASH dye on contactless spots on the inside of the chambers. The final sample size used in analyses was 13 fish per treatment.

A linear mixed effects model was fitted to the data using maximum likelihood and implemented using the "aov" function in the "stats" R package. Treatment (boat noise or ambient) and respirometry cycle (4 cycles before the treatment track, 2 cycles during the treatment track and 2 cycles following the treatment track) were included as fixed main effect, and a treatment-time interaction was also included. Fish individual and temperature were added as random factors without interactions. The assumption of normality and homogeneity of variance were examined graphically and found to be satisfied.

3.4 Results

Oxygen consumption rates showed expected increase with temperature and expected variation throughout a day; showing higher oxygen consumption during the day (Figure 3.2). The average oxygen uptake found across individuals during the day-time 30 min treatment period was 1215 ± 317 mg O_2 Kg^{-1}. Playback boat noise and ambient conditions are comparable to *in situ* boat noise and ambient conditions (Figure 3.3). Boat noise playback was slightly less intense than *in situ* in terms of sound pressure and slightly more intense in terms of particle motion. Ambient playback conditions were more intense that *in situ* across the sound detection range of fishes in terms of sound pressure. In terms of particle motion ambient playback was slightly less intense than *in situ* ambient until about 1400 Hz and then slightly more until 2000 Hz. The actuator creating the flush cycles in the respirometry system produced no detectible sound in the system (Figure 3.4). In contrast, when a submersible EHEIM pump was included in the water bath containing the respirometry chambers, sound levels reached PSD levels comparable to boat noise, demonstrating the importance of designing a pumpless respirometry system (Figure 3.5). Even when the pumps were external to the water tank, a considerable amount of sound travelled through the 2cm wide 1 meter long silicon tubing. Playback PSD sound levels through a glass barrier were comparable to in the absence of a glass barrier, suggesting that sound measurement taken outside of the fish chambers (as chambers are too small for sound measurement instruments) are comparable to the sound reaching fish in chambers (Figure 3.6).

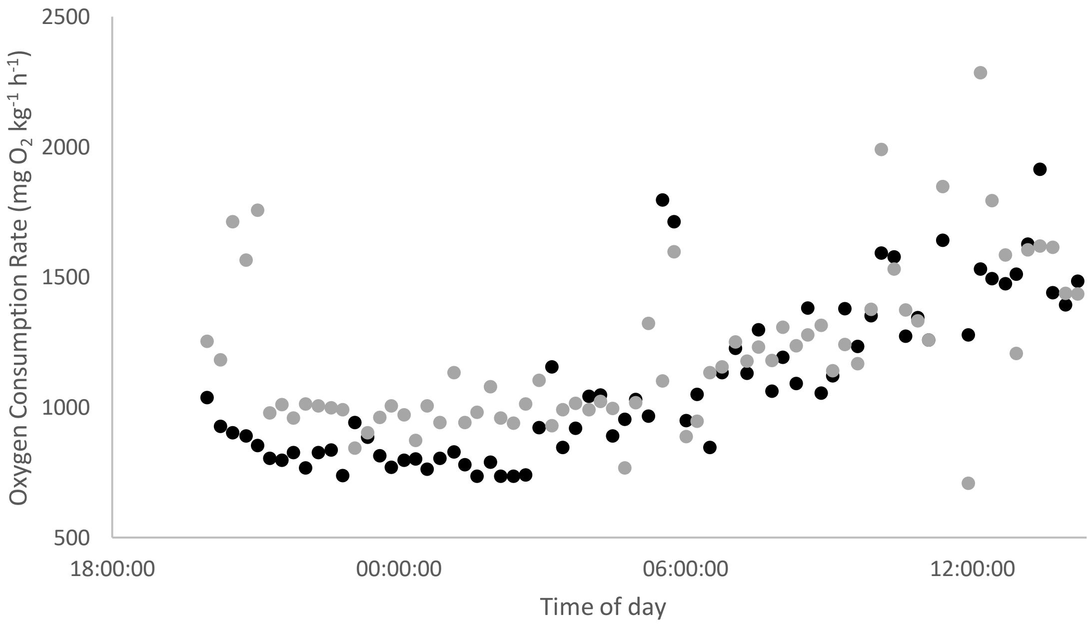

Figure 3. 2: Representative oxygen consumption rate of two individual *Pomacentrus amboinensis* from being put into respirometry chambers simultaneously at 19:00 pm to prior to being removed from chambers at 1:00 pm the next day.

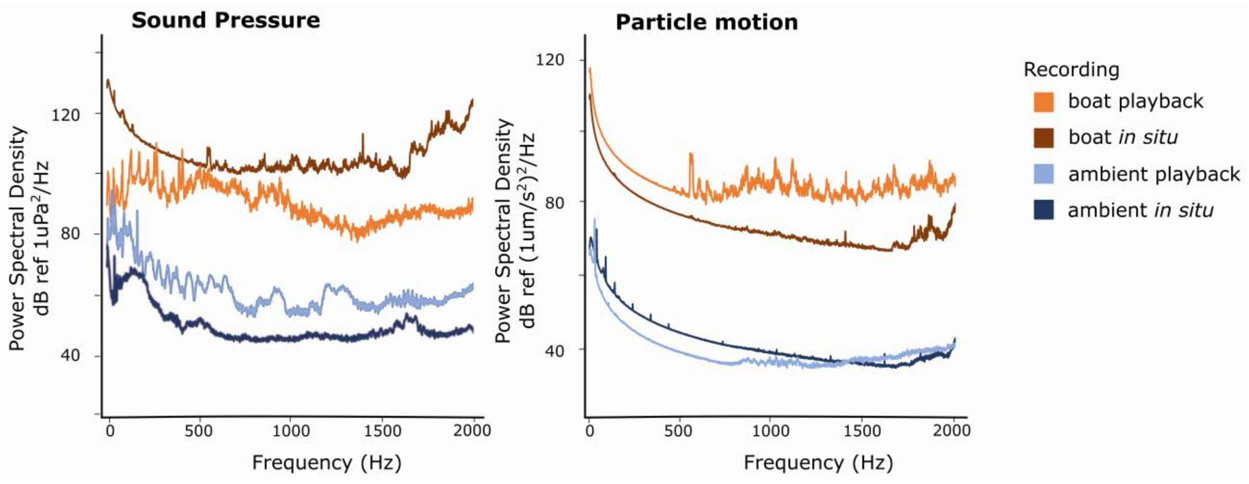

Figure 3. 3: Power spectral density of recordings of ambient and boat noise (30 hp two-stroke engines driving at 0–35 km/h 10–200 m from the recording devices) and playback in the tank in the location in which the respirometer chambers were set up. Spectral analyses show sound pressure (left) and particle acceleration (right) in the audible range for *Pomacentrus amboinensis*. Analyses were conducted in paPAM using one minute tracks that combined three separate recordings of each treatment to give the average sound profile of the three recordings. For boat tracks, each of the three recordings used in a track were from a different boat to account for variability between boats with the same engine type.

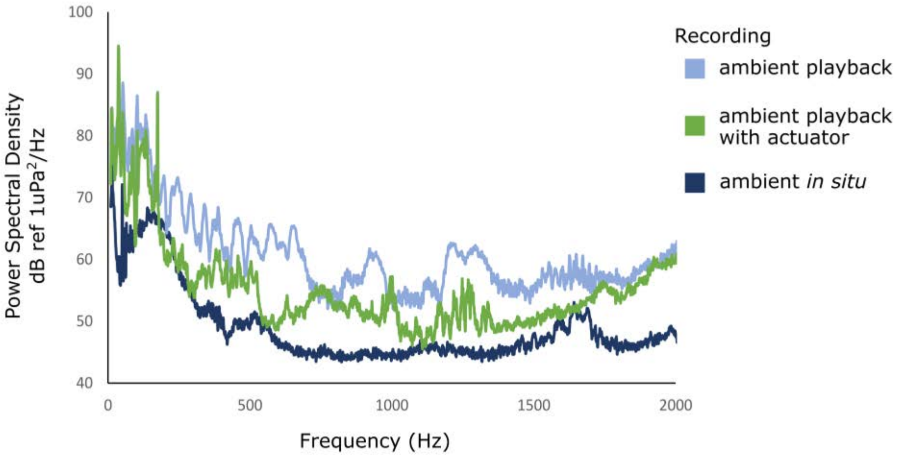

Figure 3. 4: Sound pressure power spectral density taken at the location of respirometry chambers during ambient playback when the actuator was in motion and when it was not in motion, compared to *in situ* ambient.

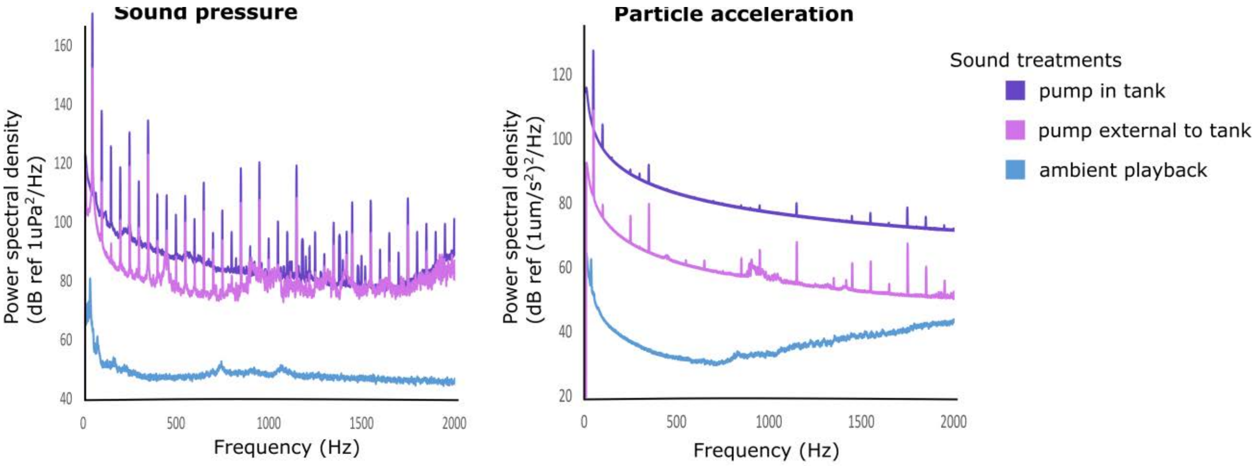

Figure 3. 5: Power spectral density in sound pressure and particle acceleration taken at the location of the chambers within the water bath used during respirometry trials. The figure compares the ambient playback used during respirometry trials to the sound produced by a EHEIM pump (type: 1102.360; series: 14072; 240V; Hmax: 3.0; I/Std: 2500-5000) typical of respirometry setups, both in the tank at the location of the speaker and external to the tank pumping water into the tank through a 1m long plastic tube.

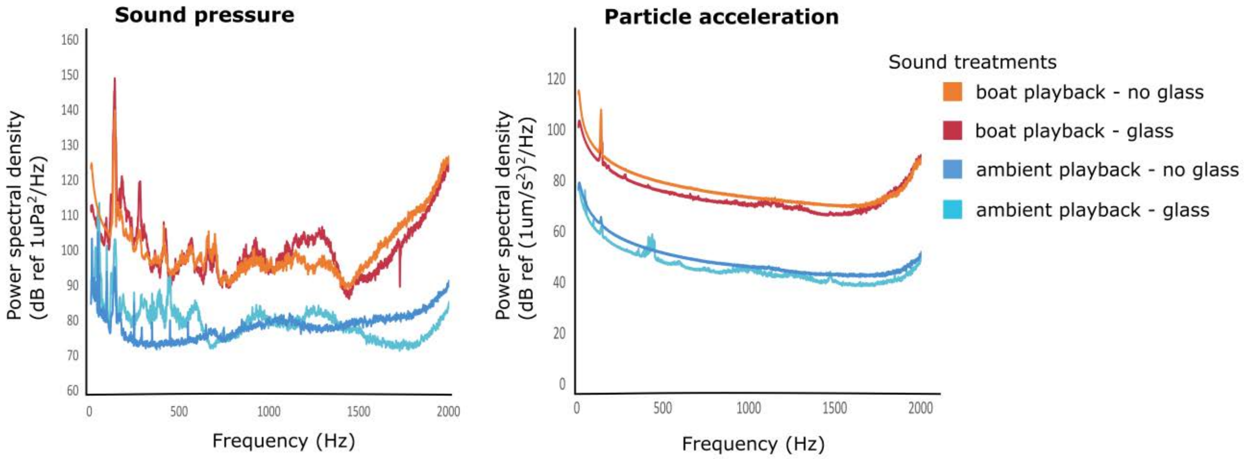

Figure 3. 6: Comparison of ambient and boat playback in the respirometry water bath when there is a glass barrier and when there is no glass barrier present. Recordings were taken at the location of the respirometry chamber. To measure the playback through a glass barrier the recorder was placed inside a seawater-filled glass beaker within the water bath, where the water bath water level was slightly below the rim of the beaker.

Boat noise was found to have no effect on oxygen uptake rates of *P. amboinensis* juveniles (Figure 3.7). No significant effect of time ($F_{7,168}$= 0.6, p=0.7), treatment ($F_{1,24}$= 1.9, p=0.2) or time-treatment interaction ($F_{7,168}$= 0.4, p=0.9) was found when controlling for individual and temperature. Therefore, no difference was found in oxygen uptake when individuals went from being exposed to ambient conditions over four 15 min intervals to being exposed to boat noise for two 15 min intervals. Nor were there changes in the two 15 min intervals of ambient conditions exposure following the boat noise exposure. Individuals exposed to eight 15 min intervals of ambient conditions also showed no significant change between time periods. Temperature ($F_{8,199}$= 10.7, p<0.001) and individual ($F_{25,182}$= 20.9, p<0.001) both had significant effect when tested as main effects. Temperature ranged from 30.0˚C to 32.4˚C.

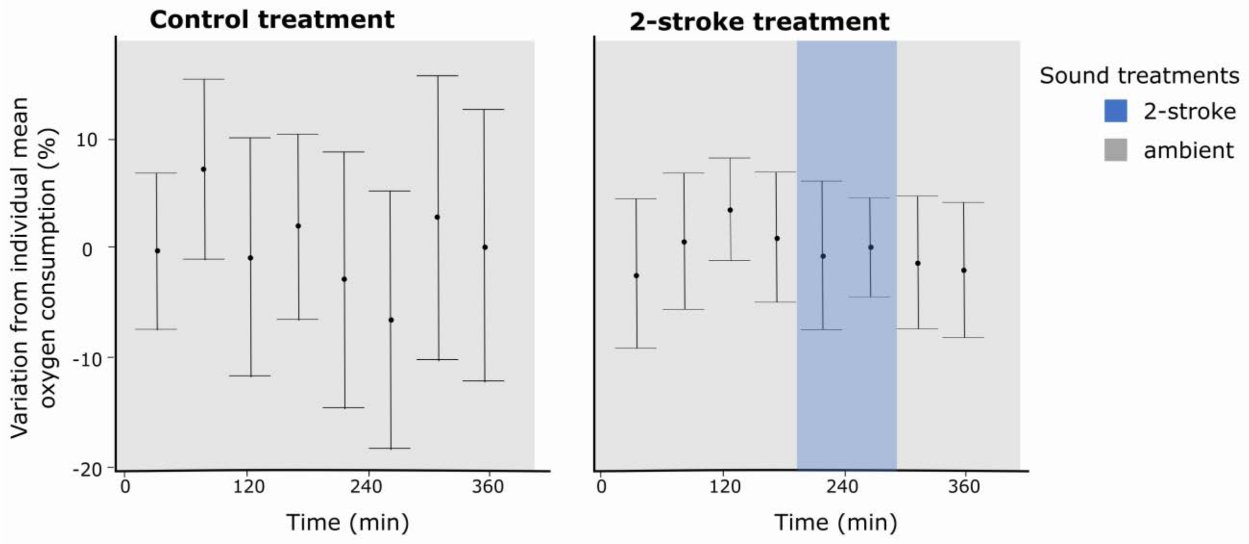

Figure 3. 7: Percent variation from individual mean oxygen uptake rates in *P. amboinensis* juveniles exposed to playback of ambient coral reef sound, or playback of a boat with a 30hp two stroke engine driving at 10-200 m. Bars show 95% confidence limits.

3.5 Discussion

In this study, I created a pumpless, quiet intermittent flow respirometry system to test the effects of noise on fish oxygen uptake rates. I found no influence of playback of recordings of noise from small boats powered by two-stroke outboard engines on the oxygen uptake rates in settlement-stage *P. amboinensis*. This suggests that either the playback of boat noise was not stressful for the fish, or that there was no immediate (within 4 hours) energetic cost to the physiological changes associated with this stress response to a single boat noise exposure, or that I was unable to detect the stress response using this approach.

Since I found no evidence to suggest that boat noise causes a stress response in settlement stage *P. amboinensis* it suggests that the mechanism by which boat noise affects settlement-stage fish behavior [e.g., settlement (Simpson et al. 2016a), and predator escape behaviors (Simpson et al. 2016b, McCormick et al. 2018)] may not be via the stress response but rather may involve distraction or may be masking important sound cues. However, care needs to be taken in interpreting these results of no stress effects of boat noise, as it may be that the response is just not detected by measuring oxygen uptake rates. It is possible that fish were compensating for the extra energy associated with the stress or that part of their response involved preparation for increased oxygen and energy demand without actually using more oxygen. For example, while fish are expected to react to predatory threats with stress, whether fish respond to a predatory threat with increased oxygen uptake has been found to depend on the locomotory behaviour induced by anti-predatory behaviour (Huuskonen and Karjalainen 1997). Similarly, the finding that oxygen uptake rates did not change upon exposure to boat noise may indicate that *P. amboinensis* does not increase activity in response to boat noise but may still show a stress response. The fish may be preparing for increased oxygen demand by increasing cardiac output and ventilation rates and preparing for increased energy demand by mobilizing energy (e.g., glycogen may be broken down into glucose and circulated through the blood, which is an oxygen independent process). In other fishes, boat noise has been found to affect several such indicators of stress including cardiac output (Graham and Cooke 2008), cortisol secretion (Spiga et al. 2012a) and ventilation rates (Simpson et al. 2015). If a stress response was occurring in the experimental fish, then data suggests that there was no immediate energetic cost to this response. It is possible however that there are delayed costs. For example, stress in fish has been shown to decrease appetite (Bernier 2006), thereby affecting subsequent acquisition of energy. Additionally, I acknowledge that playback of noise in tanks is not identical to noise propagated through the organisms' natural environment and it is therefore possible that differing results would be found in the field; however, the recordings of playback conditions in the tank share many characteristics with the original *in situ* sound conditions (Figure 3.3).

My results are unexpected, as most previous studies examining oxygen uptake rates in marine organisms using less precise methods have found an increase in oxygen uptake rates upon exposure to boat noise. An increase in oxygen uptake rates was observed using closed respirometry in shore crabs (*Carcinus maenas*) in the presence of ship noise playback (Wale et al. 2013) and in shrimp (*Crangon cragon)* exposed to elevated ambient noise levels (Regnault and Lagardere 1983). Also using closed respirometry, Simpson et al. (Simpson et al. 2016b) found that settlement stage *P. amboinensis*, the same species as used in the experiment, showed a 20 and 33% greater decrease in oxygen concentrations over 30 min when exposed to tank boat noise playback and real boat noise in the field, respectively, when compared to those exposed to ambient conditions. Similar to my findings, oxygen uptake rates were not found to increase with boat noise playback in giant Australian cuttlefish (*Sepia apama*) using a pump-based intermittent flow system (Woodcock et al. 2014).

There are several possible reasons for why Simpson et al. (2016b) found an increase in oxygen uptake rates using closed respirometry, while I did not find an effect of boat noise despite using the same species and life-stage. Intermittent flow respirometry allowed us to habituate fish in their chambers overnight; whereas, the closed respirometry experiment of

(Simpson et al. 2016b) did not incorporate a habituation period. Thus, in the closed respirometry experiment, fish may have been responding to multiple stressors, and were thus already stressed fish upon exposure to the boat noise, meaning that the boat noise trials were effectively a secondary stress test. The alternative is also possible, that the overnight habituation period could add the stress of prolonged confinement in a small chamber, meaning that fish in both treatments were chronically stressed and so any additional stressor was undetected due to a ceiling effect in the stress response, though this seems unlikely. Another possible explanation for different results found between techniques is the size of the chamber. Clark et al. (Clark et al. 2013) recommends a 20:1 to 100:1 ratio of water volume to fish mass when building respirometry systems, which not only allows for enough resolution to detect changes in oxygen concentration over a reasonable period of time but also prevents the organism from moving around too much, thus altering measurements from what would be deemed resting to those more representative of active or routine. The intermittent flow respirometry chambers were each 22 ml in volume in this study (approximately 100:1 ratio of water to fish mass), while 120 ml (approximate 500:1 ratio of water to fish mass) chambers were used in the closed respirometry system, which would allow fish more room to move and thus potentially expend energy (i.e., use more oxygen). Potentially, when the closed respirometry approach was used fish were displaying greater swimming behaviour or flight responses in response to boat noise but were able to display in to a greater extent, and thus showed a significant increase in oxygen uptake rates that was not seen when fish were confined in the intermitted flow chambers.

The respirometry system designed in this study may be of great value to other experiments. To my knowledge this is the first intermittent flow respirometry system for aquatic organisms that has a flush cycle mechanism that does not produce noise that can be detected in the chambers (Figure 3.4). Data produced expected relationships with temperature, the expected decrease in oxygen uptake rates following being caught and placed in the respirometer and expected diel/diurnal fluctuations in oxygen uptake rates (higher during the day), validating the performance of the respirometry system (Figure 3.2). The average oxygen uptake found across individuals in the experiement (1215 ± 317 mg O_2 Kg^{-1}@ an average of 31˚C) is comparable to oxygen uptake rates found in another coral reef Pomacentrid (*Pomacentrus moluccensis*; 1100 mg@ an average of 31.5˚C). Sound recordings demonstrated that having a single small submersible pump exposes fish to noise comparable to a motorboat going past at 10-200 meters (Figure 3.5). Intermittent-flow respirometry systems generally have at least one pump creating flush cycles but many also have a second recirculating pump (Clark et al. 2013, Rummer et al. 2016). Additionally, many experiments using respirometry systems run multiple respirometry chambers at once that each have a flush and recirculating pump (Roche et al. 2013, Killen et al. 2014). This means in a four-chamber system, 8 pumps could be running at once exposing fish to very high intensities of noise. Even if pumps are placed in external water tanks in an attempt to minimize noise exposure (e.g. Woodcock et al. 2014) a considerable amount of sound appears to travel through connective piping or tubing (Figure 3.5). While in this experiment we did not find evidence that boat noise altered oxygen uptake rates, the sound produced by multiple pumps in a respirometry system may have an effect on oxygen uptake rates and it remains unknown how other species and life-stages respond to noise in terms of oxygen uptake rates. Thus, the respirometry system design in this study is ideal for other experiments looking at noise pollution effects on aquatic organisms and is likely beneficial to respirometry experiment examining effects of other stressors as it eliminates pump noise as a secondary unintentional stressor.

My data suggest there are no immediate energetic costs to *P. amboinensis* juveniles when exposed to boat noise playback. I recommend, however, that further investigation into the nature of the stress responses to boat noise in fish be done. I also suggest the energetic costs of chronic boat noise exposure, and individual fitness and population-level consequences merits more research.

Chapter 4: General discussion

Motor vessel noise is increasing dramatically in many ecosystems (Hawkins and Popper 2014). Given the common exposure of fishes to small motorboat noise, the goal of this study was to increase knowledge on the effects of boat noise on fishes. I was particularly interested in early life stages as they are important to population maintenance and considered a research priority (Borsani 2015). This study also aimed to provide evidence useful to informing mitigation strategies for the impact of boat noise on fishes.

4.1 Effects of boat noise on early life stages

To expand our understanding of the effect of boat noise on early life stages of fish, I examined indicators of the stress response in two early life stages of coral reef fishes. In *A. curacao* embryos, I found an increase in heart rate in the presence of boat passage, and in settlement stage *P. amboinensis*, I found no change in oxygen uptake rates in the presence of boat noise playback. Both increases in cardiac output (heart rate and stroke volume) and in oxygen uptake (e.g., oxygen uptake at the gills, blood oxygen transport capacity, branchial oxygen diffusion capacity) are associated with the stress response in fish and are β-adrenoreceptor mediated via catecholamine release (Barton 2002). It was surprising that I found an effect at the embryo life stage but not the settlement stage, because settlement stage fish have better sound detection than embryos (Simpson et al. 2005) and a more developed endocrine and nervous system with which to respond to stressors. There were, however, a number of differences between the experiments on the different life stages that may account for these findings: I used different metrics that were more suited to each life stage, and different species of pomacentrid. Additionally, I was able to use real boat noise for the embryo experiment, but not for the settlement stage experiment, because of the logistics of respirometry. It is also possible that by the juvenile life stage, the fish have already been exposed to boat noise enough to have habituated to it.

Simpson et al. (2016) previously found that the passage of two-stroke-powered motorboats caused an increase in oxygen uptake in juvenile fish. However, they used a closed respirometry system, but used both boat noise playback and real boat noise. My study used the same species that were collected using the same methods and at the same age. The only things that differed were the respirometry system and the year. It is possible that the young-of-year were of different health or sound detection ability. For example, Gagliano's work shows differences in settlement stage *P. amboinensis* sound detection ability between the light traps in which they were caught (Gagliano et al. 2008). Perhaps it is more likely that the difference in response was due to the difference in respirometry apparatus. The intermittent flow respirometry apparatus used in the current study is an advancement in both accuracy and precision for measuring oxygen uptake rates compared to closed respirometry, as it allows a habituation period prior to trials and several measurements per individual (Snyder et al. 2016, Svendsen et al. 2016b). The current study provides no evidence to suggest that boats initiate a primary stress response in settlement stage fish. This suggests that the mechanism by which boat noise affects settlement-stage fish behavior [e.g., settlement (Simpson et al. 2016a), activity, flight response, and predator escape behaviors (Simpson et al. 2016b, McCormick et al. 2018)] may not be via the stress response but rather may involve distraction or may be masking important sound cues. The closed respirometry system used by Simpson and colleagues (Simpson et al. 2016b) had no true control because habituation was not possible, but had the benefit of the possibility of measuring oxygen uptake rates in the presence of real boat noise. The ideal system would be an intermittent flow system that could be used *in situ*, in the field, an area certainly warranting future research. Future work should also examine the possibility that settlement-stage fish are, indeed, stressed but compensating in some other way.

In fish embryos, the finding that boat noise can affect heart rate is novel. It is remarkable that, after only 4 days of development, embryos are already initiating a stress response upon exposure to certain acoustic cues. During embryogenesis, otolith formation occurs, but the swim bladder and lateral line, which also form a fishes acousticolateralis system, do not generally form prior to hatching (Simpson et al. 2005). With respect to the primary stress response, embryos develop the ability to release catecholamines but generally are not be capable of secreting corticosteroids until after hatching (Bagatto 2005, Szisch et al. 2005, Applebaum et al. 2010). To my knowledge, there are only two studies examining boat noise impacts on fish embryos (Bruintjes and Radford, 2014; Fakan et al., unpublished), and both use playback recordings of boat noise rather than real boat noise. It is an important advancement to conduct the study in the field with real boat noise, as soundscapes can be altered using playback conditions, as soundscapes are dependant on speaker quality, and can also depend on the propagation environment (Rogers et al. 2016). Bruintjes and Radford (2014) found that hatching success in cichlid embryos was not affected by long-term playback of boat noise. Fakan *et al.* (unpublished) found heart rate to increase by approximately 10% in the presence of boat noise in two species of reef fishes, *Amphiprion melanopus* and *Acanthochromis polyacanthus*. Additionally, in one of the two species (*Ac. polyacanthus*), yolk sac reserves were depleted more quickly when exposed to boat noise treatments, but survival through to hatching was not affected. In the marine invertebrate, *Stylocheilus striatus*, chronic boat noise playback was found to decrease embryo survival by 21 % and by a further 22 % once embryos hatched (Nedelec et al. 2014). My study adds to this knowledge base by validating that boat noise also has an effect on another species of Pomacentrid, in the embryonic stage and additionally in the animal's natural environment using real boat noise. To further our understanding, future research should focus on the cost of stress and the survival implications of boat noise induced stress in fish embryos. This could be accomplished by correlating heart rates and oxygen uptake rates to estimate the metabolic cost of boat noise induced stress and doing long-term studies on how chronic boat noise affects development.

The embryos heart rates increased by 1.9 % and 4.9 % on average upon exposure to the passage of four-stroke and two-stroke powered boats, respectively. An increase in heart rate by 4.9 % upon exposure to two-stroke powered boat passage may be comparable, based on other studies on other species, to the stress induced by a predator response and enough to alter development. For example, conspecific alarm cues resulted in a 6.6 % to 12.2 % increase in heart rate in *Amphiprion melanopus* (day 6 and 7 of development; Atherton and McCormick, 2015), and a 8.9 % increase in heart rate in *Melanotaenia duboulayi* (day 4 of development; Oulton et al. 2013). McCormick & Nechaev (2002) found that experimentally elevated cortisol resulted in a 4 to 14 % increase in heart rate. These changes were enough to alter the size of larvae at hatching such that larvae with higher heart rates were smaller in size and these alterations can have strong carryover effects to future life stages (Gagliano and McCormick 2009).

Conclusions from both Chapters 2 and 3 provide a significant advancement in technology with which to monitor anthropogenic impacts at early life stages in fishes. In Chapter 2, the field heart rate measuring system is the first system capable of recording heart rates in fish embryos in the field. In Chapter 3, the quiet respirometry system is the first intermittent flow system with a soundscape comparable to natural ambient conditions. The field heart rate measuring system and quiet intermittent flow respirometry system designs could be used as tools to solve other conservation questions. For example, these physiological tools could be used to measure whether early life stages of fishes are affected by other anthropogenic sources of noise such as large ships, pile driving, wind turbines, seismic shooting etc.

4.2 Mitigating effects of boat noise

There are very few restrictions regarding boat usage in coastal regions; yet, the number of boats is increasing exponentially. For example, in the Great Barrier Reef Marine Park, only 20% of the marine park is restricted to shipping passage, and less than 1% is restricted to recreational vessels (GBRMPA, 2011). As boat noise has been found to have many adverse effects on fishes, it is important to provide a biological foundation and evidence base to communicate to managers facing management decisions. Unfortunately, there is still a paucity of information on which to base recommendations that could ameliorate the effects of boat noise on populations and communities. Several international conservation bodies recognize underwater anthropogenic noise as a problem; however, none provide quantitative recommendations and guidelines for mitigation (Badino et al. 2012). Thus, it was my goal to produce evidence to inform mitigation, by comparing the effect of different engine types on fish.

Boats differ in acoustic signatures, prevalence, and area of coverage. Aspects of a boat's acoustic signature that can differ include intensity or power over different frequencies and the bandwidth over which the greatest intensity of noise is generated (Richardson et al. 1995). Additionally, noise varies with boat design, state of maintenance, as well as the environment through which the sound propagates (Ross 1976). Sound can be detected differently, depending on proximity to the boat. For example, noise is generally most powerful in the bow-ward direction of the boat (Ross 1976). Not surprisingly, determining how different engineering designs in boats alter the boat noise produced and the effect of the noise on wildlife, can be very complex.

To begin to relate engineering designs of boats to biological impacts, I compared effects of two-stroke and four-stroke powered motorboats on *A. curacao* embryos and found that four-stroke engines had less of an impact than two-stroke engines. McCormick et al. (2018) also found striking differences in how four-stroke and two-stroke engines affected juvenile coral reef fishes. They found that, in the presence of two-stroke engine noise, juvenile *P. amboinensis* did not respond to alarm cues and a looming stimulus, but they responded 'normally' in the presence of four-stroke engine noise. It is an encouraging finding that four-stroke powered motorboats do not have as much of an effect on reef fishes when compared to two-stroke powered motorboats. This finding suggests that noise is an anthropogenic pollutant that could be mitigated through engineering designs that reduce and/or change the noise that is produced.

In Chapter 2, I suggest a new sound metric that I think better represents an animal's perception of sound than what has been commonly used – power spectral density (PSD), which takes the average sound power into consideration. The new metric (relSEL) is the SEL relative to the auditory capability of the organism or, in other words, the sound energy above the sound detection threshold and within the sound detection range of the study species (Figure 4.1). This metric is calculated by the percent consistency of the noise pollution above the sound detection threshold and within the sound detection range of the study species times the SEL of the noise pollutant. The relSEL metric allows the amount of sound likely to be perceived by an organism to be visualized. Thus, relSEL may be useful in predicting by how much different noise pollutants can affect an organism with only the organism's sound detection threshold across their sound detection range (i.e., using an audiogram) and a sound recording of the noise pollutant. For example, in Chapter 2, I found that embryos heart rates increased by 2.6 times more in the presence of a two-stroke powered motorboat than in the presence of a four-stroke powered motorboat. Similarly, the relSEL for the embryos in the presence of a two-stroke powered motorboat is 2.7 times that of a four-stroke powered motorboat. One complication to using the relSEL metric is that fish hear in both particle motion and sound pressure, but there has been little work done on the particle motion sound detection thresholds of fishes. Also, there is no standard unit of particle motion, making comparisons between studies difficult (Nedelec et al. 2016a). Future research should focus on particle motion sound detection thresholds of fishes, which would allow us to determine the relSEL in both particle motion and sound pressure. Additionally, relSEL is based on physiological thresholds, which can differ from thresholds of effect on behaviours. Behavioural thresholds have received much less work than physiological thresholds of sound detection and also warrant future study. More studies correlating relSEL to effects on organisms would be useful in validating the use of relSEL as a metric.

The relSEL metric may also give insight into how the effects of boat noise may change with ontogeny. In Figure 4.1, sound detection thresholds and ranges of different life stages are estimated (see Table 2.1 for references on which estimates are based) and used to calculate relSEL for the presence of two-stroke and four-stroke powered boats. Resultant relSEL values suggest that, as sound detection develops with ontogeny, fishes are exposed to greater amounts of sound stimuli, but the difference between two-stroke and four-stroke sound exposure diminishes. We may therefore expect a greater effect of boat noise on adults, unless habituation is a factor. Also, four-stroke engines appear to always be better for coral reef fishes, as they have less of an effect on than two-stroke engines, but make the biggest difference at early life stages.

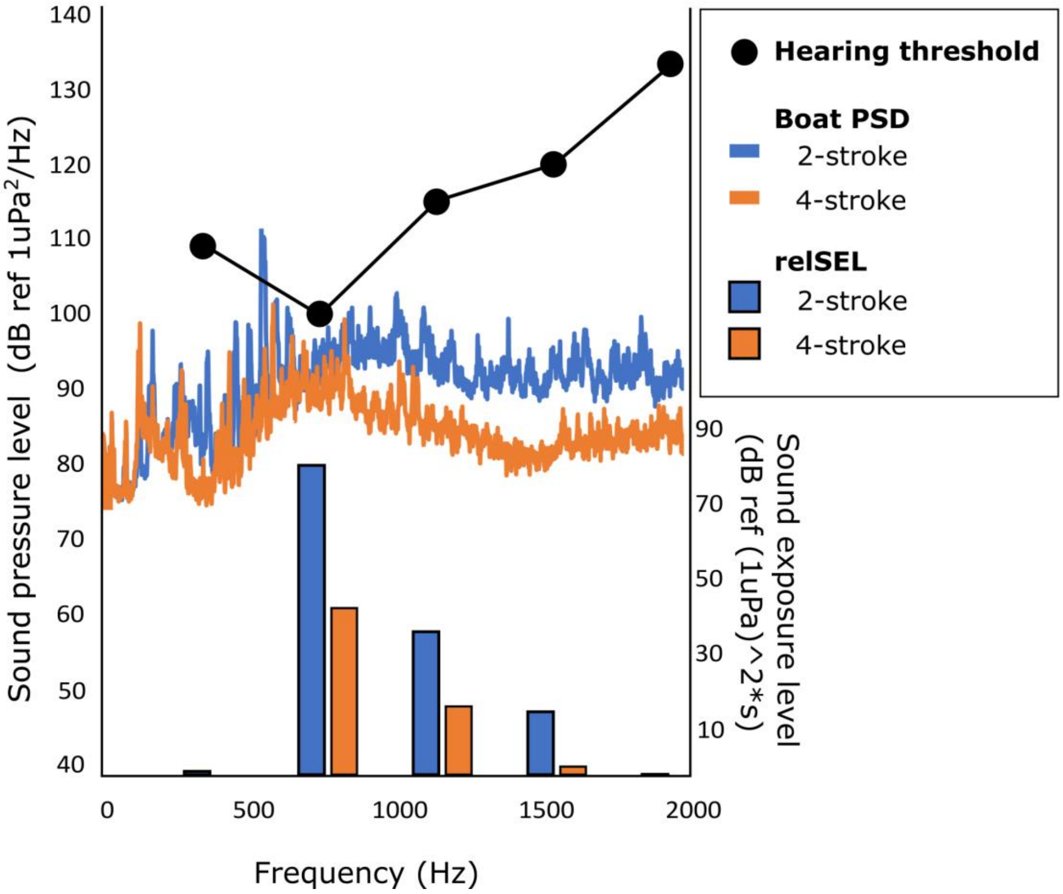

Figure 4. 1: Illustration of various sound metrics related to noise pollution effects on organisms, using damselfish *Pomacentrus variablis* as an example. Metrics displayed are: a) sound detection threshold of organism (left axis), b) power spectral density (PSD) of noise the pollutant (left axis), and c) a new metric proposed in this study (relSEL; right axis) which is the sound exposure level (SEL) of the noise pollutant relative to the auditory capability of the organism. In this case the sound detection threshold is of 20mm juvenile *P. variablis* (Kenyon 1996) and the PSD of the noise pollutant is of a 5m-long boats with either a two-stroke or four-stroke outboard engine. The relSEL is calculated from the SEL of each boat engine type, multiplied by the percent consistency above the sound detection threshold of *P. variablis* (right axis).

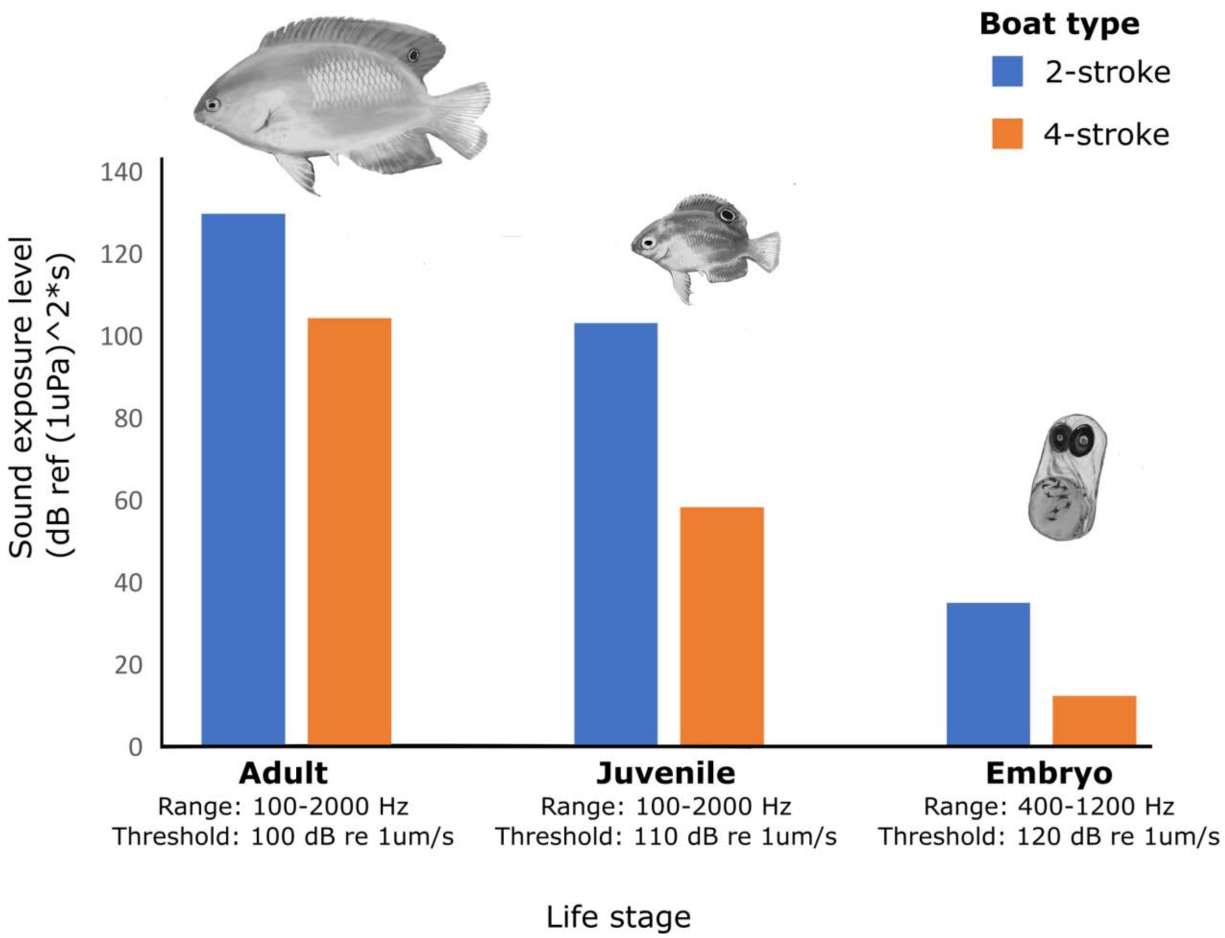

Figure 4. 2: Sound exposure level of the noise pollutant relative to the auditory capability (relSEL) of coral reef fish embryos, juveniles, and adults. The relSEL is calculated by the SEL of each boat type multiplied by the percent consistency above the approximate sound detection threshold of each life stage within the sound detection range of each life stage (specified below each life-stage heading; Table 2.1). Boats were 5 m long with either a two-stroke or four-stroke engine, driving at 10-200 meters.

The difference in sound produced by two-stroke and four-stroke engines may be explained by the difference in the timing of their engine processes. There are four main processes that occur in an engine: intake, compression, combustion, and exhaust. Intake involves the intake of fuel and air into the combustion chamber. Compression involves compressing the air to raise the energy potential. Combustion involves the fuel igniting and exploding, which pushes the piston creating useable energy for the car. Exhaust involves the used air/fuel combination being pushed out from the combustion chamber. The explosion generated during combustion creates a loud noise, as does the exhaust of hot air under pressure when it meets the outside air that is not under pressure (Muscari and Mascio 2013, Leclere et al. 2015). In four-stroke engines, a separate piston stroke occurs for intake, compression, combustion, and exhaust phases (Basshuysen et al. 2004). In two-stroke engines, the intake and compression phase occur during one stroke and the combustion and exhaust phase occur during the second piston stroke (Blair 1996, Basshuysen et al. 2004). Because the two-stroke engine does not have a designated exhaust phase, there is a shorter amount of time available to clear the cylinder, thus the exhaust has to be free flowing and so fewer noise reducing features can be used. Because the exhaust process occurs while the air is expanding in two-stroke engines, rather than after air expansion has completed in four-stroke engines, the engine air reaching the outside air is at higher pressure in two-stroke engines and therefore may create a louder exhaust noise as a result. Additionally, combustion and exhaust, which both generate noise, occur simultaneously in two-stroke engines but not in four-stroke engines, which means that maximum sound levels would be higher in two-stroke engines even though average sound power would be similar to that of four-stroke engines.

Two-stroke engines are quite common. In 2007, 90% of small motorboats in Australia were powered by two-stroke engines, and 69% of sales were two-stroke engines ("Comparative Assessment of the Environmental Performance of Small Engines: Marine Outboards and Personal Watercraft" 2007). In Europe, two-stroke engines made up 30% of outboard motor sales, 15% of sales in the USA. New Australian emission regulations (*Australia Government: Department of Environment and Energy. Outdoor power equipment and marine engines.* 2017) will effectively prohibit the sale of new two-stroke engines that do not comply with the new exhaust standards. There are two-stroke engines on the market that do. My research suggests that it may be important to also create noise standards. Using relSEL may be a useful approach to determine noise limits based on the sound detection capabilities of species targeted for conservation, or a broad range of species.

This research highlights that, in order to mitigate the effects of boat noise on marine life, it is necessary that we understand the effects of sound on organisms, how these effects interact with the characteristics of the sound produced, and how this is affected by the engine engineering design. This thesis also highlights the complexity of the responses fish have upon exposure to boat noise and provides new methodological tools for analyzing the magnitude of effects on early-life stages of fishes.

References

Andrew, R.K., Howe, B.M., Mercer, J.A., and Dzieciuch, M.A. 2002. Ocean ambient sound: Comparing the 1960s with the 1990s for a receiver off the California coast. Acoust. Res. Lett. Online **3** (2): 65. doi:10.1121/1.1461915.

Applebaum, S., Wilson, C.A., Holt, G.J., and Nunez, B.S. 2010. The onset of cortisol synthesis and the stress response is independent of changes in CYP11B or CYP21 mRNA levels in larval red drum (*Sciaenops ocellatus*). Gen. Comp. Endocrinol. **165**: 269–276. doi:10.1016/j.ygcen.2009.07.003.

Armstrong-Smith, E.F., and Sciences, B. 2016. Damselfish in Distress : an exploration of context-dependent stress responses of coral reef fish exposed to anthropogenic noise. Open Research Exeter.

Armstrong, J.D. 1986. Heart rate as an indicator of activity, metabolic rate, food intake and digestion in pike, *Esox lucius*. J. Fish Biol. **29**: 207–221.

Atherton, J.A., and McCormick, M.I. 2015. Active in the sac: Damselfish embryos use innate recognition of odours to learn predation risk before hatching. Anim. Behav. **103**: 1–6. Elsevier Ltd. doi:10.1016/j.anbehav.2015.01.033.

Australia Government: Department of Environment and Energy. Outdoor power equipment and marine engines. (2017). Available from http://www.environment.gov.au/protection/air-quality/emissions-standards/outdoor-power-equipment-and-marine-engines [accessed 13 March 2018].

Badino, A., Borelli, D., Gaggero, T., Rizzuto, E., and Schenone, C. 2012. Normative framework for ship noise: Present and situation and future trends. Noise Control Eng. J. **60**(6): 740–762. doi:10.3397/1.3701045.

Bagatto, B. 2005. Ontogeny of cardiovascular control in zebrafish (*Danio rerio*): Effects of developmental environment. **141**: 391–400. doi:10.1016/j.cbpb.2005.07.002.

Bailey, K.M., and Houde, E.D. 1989. Predation on Eggs and Larvae of Marine Fishes and the Recruitment Problem. Adv. Mar. Biol. **25**(C): 1–83. doi:10.1016/S0065-2881(08)60187-X.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. **42**(3): 517–525. doi:10.1093/icb/42.3.517.

Basshuysen, V., Schäfer, R., and Schäfer, F. 2004. Internal combustion engine handbook-basics, components, systems and perspectives.

Bates, D., Machler, M., Bolker, B., and Walker, S. 2015. Fitting Linear Mixed-Effects Models Using "lme4." J. Stat. Softw. **67**: 1–48. doi:10.18637/jss.v067.i01.

Bernier, N.J. 2006. The corticotropin-releasing factor system as a mediator of the appetite-suppressing effects of stress in fish. Gen. Comp. Endocrinol. **146**: 45–55. doi:10.1016/j.ygcen.2005.11.016.

Beyers, D.W., Rice, J.A., Clements, W.H., and Henry, C.J. 1999. Estimating physiological cost of chemical exposure: integrating energetics and stress to quantify toxic effects in fish 1. **822**: 814–822.

Blair, G.P. 1996. Design and simulation of two-stroke engines. Society of Automotive Engineers, Warrendale, PA.

Bonga, S.E.W. 1997. The Stress Response in Fish. Physiol. Rev. **77** (3): 591–625.

Borell, E. Von, Langbein, J., Després, G., Hansen, S., Leterrier, C., Marchant-forde, J., Marchant-forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D., and Veissier, I. 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals — A review. **92**: 293–316. doi:10.1016/j.physbeh.2007.01.007.

Borsani, J., Faulk, R., and Merchant, N.D. 2015. Impacts of noise and use of propagation models to predict. Cefas Contract Rep. C6082 (July). doi:10.13140/RG.2.1.1512.5841.

Boyd, I., Frisk, G., Urban, E., Tyack, P., Ausubel, J., Seeyave, S., Cato, D., Southall, B., Weise, M., Andrew, R., Akamatsu, T., Dekeling, R., Erbe, C., Farmer, D., Gentry, R., Gross, T., Hawkins, A., Li, F., Metcalf, K., Miller, J.H., Moretti, D., Rodrigo, C., and Shinke, T. 2011. An International Quiet Ocean Experiment. Oceanography **24**(2): 174–181. doi:10.5670/oceanog.2009.80.COPYRIGHT.

Bracciali, C., Campobello, D., Giacoma, C., and Sarà, G. 2012. Effects of nautical traffic and noise on foraging patterns of Mediterranean Damselfish (*Chromis chromis*). PLoS One **7**(7). doi:10.1371/journal.pone.0040582.

Bruintjes, R., Purser, J., Everley, K.A., Mangan, S., Simpson, S.D., and Radford, A.N. 2016. Rapid recovery following short-term acoustic disturbance in two fish species. R. Soc. open Sci. **3**: 150686. doi:10.1098/rsos.150686.

Bruintjes, R., and Radford, A.N. 2013. Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. Anim. Behav. **85** (6): 1343–1349. Elsevier Ltd. doi:10.1016/j.anbehav.2013.03.025.

Bruintjes, R., and Radford, A.N. 2014. Chronic playback of boat noise does not impact hatching success or post-hatching larval growth and survival in a cichlid fish. PeerJ. **2**: e594. doi:10.7717/peerj.594.

Bunt, C.M., Cooke, S.J., Schreer, J.F., and Philipp, D.P. 2004. Effects of incremental increases in silt load on the cardiovascular performance of riverine and lacustrine rock bass, *Ambloplites rupestris*. Environ. Pollut. **128**(3): 437–444. doi:10.1016/j.envpol.2003.09.015.

Chabot, D., McKenzie, D.J., and Craig, J.F. 2016. Metabolic rate in fishes: Definitions, methods and significance for conservation physiology. J. Fish Biol. **88**(1): 1–9. doi:10.1111/jfb.12873.

Clark, T.D., Sandblom, E., and Jutfelt, F. 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. **216**: 2771–2782. doi:10.1242/jeb.084251.

Codarin, A., Wysocki, L.E., Ladich, F., and Picciulin, M. 2009. Effects of ambient and boat noise on sound detection and communication in three fish species living in a marine protected area (Miramare, Italy). Mar. Pollut. Bull. **58**(12): 1880–1887. Elsevier Ltd. doi:10.1016/j.marpolbul.2009.07.011.

Comparative Assessment of the Environmental Performance of Small Engines: Marine Outboards and Personal Watercraft. 2007. Environment Link and Vehicle Design and Research P/L, prepared for Department of the Environment and Water Resources.

Cushing, D.H. 1990. Plankton Production and Year-class Strength in Fish Populations: An Update of the Match/Mismatch Hypothesis. *In* Advances in Marine Biology. doi:10.1016/S0065-2881(08)60202-3.

Egner, S.A. 2004. Auditory sensitivity of the sergeant majors (*Abudefduf saxatilis*) from post-settlement juvenile to adult.

Egner, S.A., and Mann, D.A. 2005. Auditory Sensitivity of Sergeant Major Damselfish *Abudefduf Saxatilis* from Post-Settlement Juvenile to Adult. Mar. Ecol. Prog. Ser. **285**: 213–222.

Elsasser, T.H., Klasing, K.C., Filipov, N., and Thompson, F. 2000. The Metabolic Consequences of Stress: Targets for Stress and Priorities of Nutrient Use. *In* The biology of animal stress: basic principles and implications for animal welfare. *Edited by* G. Moberg and J.A. Mench. CABI Publishing. pp. 78–110.

Fawcett, T.W., and Frankenhuis, W.E. 2015. Adaptive explanations for sensitive windows in development. Front. Zool. **12**(1): 1–14. doi:10.1186/1742-9994-12-S1-S3.

Frid, A., and Dill, L.M. 2002. Human-caused disturbance stimuli as a form of predation risk. Conserv. Ecol. **6**(1): 11.

Frisk, G. V. 2012. Noiseonomics: The relationship between ambient noise levels in the sea and global economic trends. Sci. Rep. **2**(1): 2–5. doi:10.1038/srep00437.

Gagliano, M., Depczynski, M., Simpson, S.D., and Moore, J.A. 2008. Dispersal without errors: symmetrical ears tune into the right frequency for survival. Proc. R. Soc. B Biol. Sci. **275** (1634): 527–534. doi:10.1098/rspb.2007.1388.

Gagliano, M., and McCormick, M.I. 2009. Hormonally mediated maternal effects shape offspring survival potential in stressful environments. Oecologia **160**: 657–665.

Gagliano, M., McCormick, M.I., and Meekan, M.G. 2007. Survival against the odds: ontogenetic changes in selective pressure mediate growth-mortality tradeoffs. Proc. R. Soc. London, Ser. B Biol. Sci. **274**(1618): 1575–1582. doi:10.1098/rspb.2007.0242.

Goulet, D. 1995. Temporal patterns of reproduction in the Red Sea damselfish *Amblyglyphidon leucogaster*. Bull. Mar. Sci. **57**(3): 582–595.

Graham, A.L., and Cooke, S.J. 2008. The effects of noise disturbance from various recreational boating activities common to inland waters on the cardiac physiology of a freshwater fish, the largemouth bass (*Micropterus salmoides*). Aquat. Conserv. Freshw. Ecosyst. **18**: 1315–1324. doi:10.1002/aqc.

Hatch, L., Clark, C., Merrick, R., Van Parijs, S., Ponirakis, D., Schwehr, K., Thompson, M., and Wiley, D. 2008. Characterizing the relative contributions of large vessels to total ocean noise fields: A case study using the Gerry E. Studds Stellwagen Bank national marine sanctuary. Environ. Manage. **42**(5): 735–752. doi:10.1007/s00267-008-9169-4.

Hawkins, A.D., and Popper, A.N. 2014. Assessing the impacts of underwater sounds on fishes and other forms of marine life. Acoust. Today **10**(2): 30–41.

Holles, S., Simpson, S.D., Radford, A.N., Berten, L., and Lecchini, D. 2013. Boat noise disrupts orientation behaviour in a coral reef fish. Mar. Ecol. Prog. Ser. **485**: 295–300.

Huuskonen, H., and Karjalainen, J. 1997. Predator-induced respiratory responses in juveniles of vendace *Coregonus albula*, whitefish *C. lavaretus*, perch *Perca fluviatilis* and roach *Rutilus rutilus*. Environ. Biol. fishes **49**: 265–269.

International Maritime Organization. 2011. Noise from Commercial Shipping and its Adverse Impacts on Marine Life. Germany.

Jenkins, G.P., and King, D. 2006. Variation in larval growth can predict the recruitment of a temperate, seagrass-associated fish. Oecologia **147**(4): 641–649. doi:10.1007/s00442-005-0336-5.

Jong, C. a F. De. 2009. Characterization of ships as sources of underwater noise Surface ship underwater noise. NAG/DAGA Int. Conf. Acoust.

Jung, C.A., and Swearer, S.E. 2011. Reactions of temperate reef fish larvae to boat sound. Aquat. Conserv. Mar. Freshw. Ecosyst. **21**(4): 389–396. doi:10.1002/aqc.1190.

Karjalainen, J., Auvinen, H., Helminen, H., Marjomaki, T.J., Niva, T., Sarvala, J., and Viljanen, M. 2000. Unpredictability of fish recruitment: interannual variation in young-of-the-year abundance. J. Fish Biol. **56**(4): 837–857. doi:10.1006/jfbi.1999.1206.

Kastelein, R.A., Heul, S. van der, Verboom, W.C., Jennings, N., Veen, J. van der, and Haan, D. de. 2008. Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Mar. Environ. Res. **65**(5): 369–377. doi:10.1016/j.marenvres.2008.01.001.

Kenyon, T.N. 1996. Ontogenetic changes in the auditory sensitivity of damselfishes (pomacentridae). J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol. **179**(4): 553–561. doi:10.1007/BF00192321.

Kerrigan, B.A. 1996. Temporal patterns in size and condition at settlement in two tropical reef fishes (Pomacentridae: *Pomacentrus amboinensis* and *P. nagasakiensis*). Mar. Ecol. Prog. Ser. **135** (1–3): 27–41. doi:10.3354/meps135027.

Killen, S.S., Mitchell, M.D., Rummer, J.L., Chivers, D.P., Ferrari, M.C.O., Meekan, M.G., and Mccormick, M.I. 2014. Aerobic scope predicts dominance during early life in a tropical damselfish. Funct. Ecol. **28**(6): 1367–1376. doi:10.1111/1365-2435.12296.

Knudsen, F.R., Enger, P.S., and Sand, O. 1992. Awareness reactions and avoidance responses to sound in juvenile Atlantic salmon, *Salmo salar L*. J. Fish Biol. **40**(4): 523–534. doi:DOI 10.1111/j.1095-8649.1992.tb02602.x.

Kuznetsov, Y.A., Bocharov, L.N., Akulin, V.N., and Kuznetsov, M.Y. 2012. Marine Bioacoustics and the Regulation of Fisheries. Adv. Exp. Med. Biol. **730**(2007): 575–577. doi:10.1007/978-1-4419-7311-5.

Lankford, A.S.E., Adams, T.E., Miller, R.A., and Cech Jr., J.J. 2005. The Cost of Chronic Stress: Impacts of a Nonhabituating Stress Response on Metabolic Variables and Swimming Performance in Sturgeon. Physiol. Biochem. Zool. Ecol. Evol. Approaches **78**: 599–609.

Leclere, Q., Drouet, J., Parizet, E., Leclere, Q., Drouet, J., Extraction, E.P., En-, A.O.F.D., Leclere, Q., Drouet, J., and Parizet, E. 2015. Extraction and analysis of diesel engine combustion noise faculty of mechanics and technology.

Leggett, W.C., and Deblois, E. 1994. Recruitment in marine fishes: Is it regulated by starvation and predation in the egg and larval stages? Netherlands J. Sea Res. **32**(2): 119–134. doi:10.1016/0077-7579(94)90036-1.

Lenth, R. V. 2016. Least-Squares Means: The R Package lsmeans. J. Stat. Softw. **69**(1): 1–33. doi:10.18637/jss.v069.i01.

Logan, M. 2010. Biostatistical Design and Analysis Using R: A Practical Guide. Willey-Blackwell.

Lucas, M.C. 1994. Heart rate as an indicator of metabolic rate and activity in adult Atlantic salmon, *Salmo salar*. J. Fish Biol. **44**: 889–903. doi:10.1111/j.1095-8649.1994.tb01262.x.

Luczkovich, J.J., and Sprague, M.W. 2008. Does Boat Noise Affect Spawning Sound Production of Soniferous Fish in Shallow Estuarine Systems? Bioacoustics **17**(1–3): 203–205. doi:10.1080/09524622.2008.9753817.

McCormick, M.I. 2016. Temperature data from Sunbird Reef (2m). Sunbird Reef, Lizard Island Research Station.

McCormick, M.I., Allan, B.J.M., Harding, H., and Simpson, S.D. 2018. Boat noise impacts risk assessment in a coral reef fish but effects depend on engine type. Sci. Rep. **8**(1): 1–11. Springer US. doi:10.1038/s41598-018-22104-3.

McCormick, M.I., and Gagliano, M. 2010. Carry-over effects: the importance of a good start. 11th Int. Coral Reef Symp. (10): 305–310. Ft. Lauderdale, Florida, 7-11 July 2008. Available from file:///C:/Mark's files/Electronic articles/Mine/McCormick & Gagliano ICRS08.pdf.

Mccormick, M.I., and Nechaev, I. V. 2002. Influence of cortisol on developmental rhythms during embryogenesis in a tropical damselfish. J. Exp. Zool. **293**(5): 456–466. doi:10.1002/jez.10138.

McKenna, M.F., Ross, D., Wiggins, S.M., and Hildebrand, J.A. 2012. Underwater radiated noise from modern commercial ships. J. Acoust. Soc. Am. **131**(1): 92–103. doi:10.1121/1.3664100.

McLeod, I.M., Rummer, J.L., Clark, T.D.C., Jones, G.P., McCormick, M.I., Wenger, A.S., Munday, P.L., McCormick, M.I., and Munday, P.L. 2013. Climate change and the performance of larval coral reef fishes: the interaction between temperature and food availability. Conserv. Physiol. **1** (1): cot024. doi:10.1093/conphys/cot024.

Meekan J. H. Carleton, A. D. McKinnon, K. Flynn and M. Furnas, M.G. 2003. What determines the growth of tropical reef fish larvae in the plankton: food or temperature? Mar. Ecol. Prog. Ser. **256**: 193–204. doi:10.3354/meps256193.

Merchant, N.D., Fristrup, K.M., Johnson, M.P., Tyack, P.L., Matthew, J., Blondel, P., and Parks, S.E. 2015. Measuring acoustic habitats. Meth. Ecol. Evol. doi:10.1111/2041-210X.12330.

Morley, E.L., Jones, G., and Radford, A.N. 2014. The importance of invertebrates when considering the impacts of anthropogenic noise. Proc. R. Soc. B Biol. Sci. **281**(1776): 20132683. doi:10.1098/rspb.2013.2683.

Muscari, S.I.R., and Mascio, A. Di. 2013. Ship underwater noise assessment by the acoustic analogy. Part I: nonlinear analysis of a marine propeller in a uniform flow. **18**(4): 547–570. doi:10.1007/s00773-013-0227-0.

Nedelec, S.L., Campbell, J., Radford, A.N., Simpson, S.D., and Merchant, N.D. 2016a. Particle motion: The missing link in underwater acoustic ecology. Methods Ecol. Evol. **7**. doi:10.1111/2041-210X.12544.

Nedelec, S.L., Campbell, J., Radford, A.N., Simpson, S.D., and Merchant, N.D. 2016b. paPAM User Manual for version 0.872.

Nedelec, S.L., Mills, S.C., Lecchini, D., Nedelec, B., Simpson, S.D., and Radford, A.N. 2016c. Repeated exposure to noise increases tolerance in a coral reef fish. Environ. Pollut. **216** (May): 428–436. Elsevier Ltd. doi:10.1016/j.envpol.2016.05.058.

Nedelec, S.L., Radford, A.N., Pearl, L., Nedelec, B., Mccormick, M.I., Meekan, M.G., and Simpson, S.D. 2017. Motorboat noise impacts parental behaviour and offspring survival in a reef fish. Methods Ecol. Evol. doi:10.1111/2041-210X.12544

Nedelec, S.L., Radford, A.N., Simpson, S.D., Nedelec, B., Lecchini, D., and Mills, S.C. 2014. Anthropogenic noise playback impairs embryonic development and increases mortality in a marine invertebrate. Sci. Rep. **4**(Figure 1): 5891. doi:10.1038/srep05891.

Nichols, T.A. 2014. Boat engine noise induces physiological stress and reduces predation risk in coastal marine fish. A thesis presented to the Faculty of San Diego State University.

Nichols, T.A., Anderson, T.W., and Širović, A. 2015. Intermittent noise induces physiological stress in a coastal marine fish. PLoS One **10**(9): 1–13. doi:10.1371/journal.pone.0139157.

Nimon, A.J., Schroter, R.C., and Oxenham, R.C. 1996. Artificial Eggs : Measuring Heart Rate and Effects of Disturbance in Nesting Penguins. Physiol. Behav. **60**(3): 1019–1022.

NMMA. 2015. 2014 Recreational boating statistical abstract. Natl. Mar. Manuf. Assoc.

Ona, E., and Godø, O.R. 1990. Fish reaction to trawling noise: the significance for trawl sampling. Rapp. procès-verbaux des réunions / Cons. Perm. Int. pour l'exploration la mer **189**: 159–166.

Oulton, L.J., Haviland, V., and Brown, C. 2013. Predator recognition in rainbowfish, *Melanotaenia duboulayi*, embryos. PLoS One **8**(10): 8–11. doi:10.1371/journal.pone.0076061.

Parmentier, E., Colleye, O., and Mann, D. 2009. Hearing ability in three clownfish species. J. Exp. Biol. **212**(13): 2023–2026. doi:10.1242/jeb.030270.

Payne, R., and Webb, D. 1971. Orientation b means of long range acoustic signaling in baleen whales. Ann. New York Acad. Sci. **188**: 110–141. Available from doi:10.1111/j.1749-6632.1971.tb13093.x.

Peck, M.A., Huebert, K.B., and Llopiz, J.K. 2012. Intrinsic and Extrinsic Factors Driving Match-Mismatch Dynamics During the Early Life History of Marine Fishes. *In* Advances in Ecological Research, 1st edition. Elsevier Ltd. doi:10.1016/B978-0-12-398315-2.00003-X.

Picciulin, M., Sebastianutto, L., Codarin, A., Calcagno, G., and Ferrero, E.A. 2012. Brown meagre vocalization rate increases during repetitive boat noise exposures: A possible case of vocal compensation. J. Acoust. Soc. Am. **132**(5): 3118–3124. doi:10.1121/1.4756928.

Picciulin, M., Sebastianutto, L., Codarin, A., Farina, A., and Ferrero, E.A. 2010. In situ behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a Marine Protected Area. J. Exp. Mar. Bio. Ecol. **386**(1–2): 125–132. Elsevier B.V. doi:10.1016/j.jembe.2010.02.012.

Popper, A.N. 2003. Effects of anthropogenic sounds on fishes. Fisheries **28**(10): 24–31. doi:10.1577/1548-8446(2003)28.

Popper, A.N., and Fay, R.R. 2011. Rethinking sound detection by fishes. Hear. Res. **273**(1–2): 25–36. doi:10.1016/j.heares.2009.12.023.

Popper, A.N., and Hastings, M.C. 2009a. The effects of anthropogenic sources of sound on fishes. J. Fish Biol. **75**(3): 455–489. doi:10.1111/j.1095-8649.2009.02319.x.

Popper, A.N., and Hastings, M.C. 2009b. The effects of human-generated sound on fish. Integr. Zool. **4**(1): 43–52. doi:10.1111/j.1749-4877.2008.00134.x.

Purser, J., Bruintjes, R., Simpson, S.D., and Radford, A.N. 2016. Condition-dependent physiological and behavioural responses to anthropogenic noise. Physiol. Behav. **155**: 157–161. Elsevier Inc. doi:10.1016/j.physbeh.2015.12.010.

Recreational Vessel Census June 2014. 2014. Queensland.

Regnault, M., and Lagardere, J. 1983. Effects of ambient noise on the metabolic level of *Crangon cragon* (Decapoda, Natantia). **11**: 71–78.

Richardson, W.J., Greene, J.C.R., I, M.C., and Thomson, D.H. 1995. Marine mammals and noise. Academic Press, San Diego, California.

Roche, D.G., Binning, S. a, Bosiger, Y., Johansen, J.L., and Rummer, J.L. 2013. Finding the best estimates of metabolic rates in a coral reef fish. J. Exp. Biol. **216**(Pt 11): 2103–10. doi:10.1242/jeb.082925.

Rogers, P.H., Hawkins, A.D., Popper, A.N., Fay, R.R., and Gray, M.D. 2016. Parvulescu revisited: small tank acoustics for bioacousticians. *In* The effects of noise on aquatic life II. Springer, New York. pp. 933–941.

Rolland, R.M., Parks, S.E., Hunt, K.E., Castellote, M., Corkeron, P.J., Nowacek, D.P., Wasser, S.K., and Kraus, S.D. 2012. Evidence that ship noise increases stress in right whales. Proc. Biol. Sci. **279**(1737): 2363–8. doi:10.1098/rspb.2011.2429.

Ross, D. 1976. Mechanics of Underwater Noise. Pergamon Press, Pergamon, New York.

Roussel, J.-M. 2007. Carry-over effects in brown trout (*Salmo trutta*): hypoxia on embryos impairs predator avoidance by alevins in experimental channels. Can. J. Fish. Aquat. Sci. **64** (5): 786–792. doi:10.1139/f07-055.

Rummer, J.L., Binning, S.A., Roche, D.G., and Johansen, J.L. 2016. Methods matter: considering locomotory mode and respirometry technique when estimating metabolic rates of fishes. Conserv. Physiol. **4**(February): 1–13. doi:10.1093/conphys/cow008.

Sandström, A., Eriksson, B.K., Karås, P., Isaeus, M., and Schreiber, H. 2005. Boating and navigation activities influence the recruitment of fish in a Baltic Sea archipelago area. J. Hum. Environ. **34**(2): 125–130. doi:10.1579/0044-7447-34.2.125.

Sapolsky, R.M., Romero, L.M., and Munck, a. U. 2000. How do glucocorticoids influence stress responses? Endocr. Rev. **21**(April): 55–89. doi:10.1210/er.21.1.55.

Sarà, G., Dean, J.M., D’Amato, D., Buscaino, G., Oliveri, A., Genovese, S., Ferro, S., Buffa, G., Lo Martire, M., and Mazzola, S. 2007. Effect of boat noise on the behaviour of bluefin tuna *Thunnus thynnus* in the Mediterranean Sea. Mar. Ecol. Prog. Ser. **331**: 243–253. doi:10.3354/meps331243.

Sarrazin, A.F., Nunez, V.A., Sapede, D., Tassin, V., Dambly-Chaudiere, C., and Ghysen, A. 2010. Origin and Early Development of the Posterior Lateral Line System of Zebrafish. J. Neurosci. **30** (24): 8234–8244. doi:10.1523/JNEUROSCI.5137-09.2010.

Sebastianutto, L., Picciulin, M., Costantini, M., and Ferrero, E.A. 2011. How boat noise affects an ecologically crucial behaviour: The case of territoriality in *Gobius cruentatus* (Gobiidae). Environ. Biol. Fishes **92**(2): 207–215. doi:10.1007/s10641-011-9834-y.

Simmonds, M., Simmonds, M., Dolman, S., Dolman, S., Weilgart, L., and Weilgart, L. 2003. Oceans of noise: a WDCS Science Report. Wdcs: 784–788. doi:10.1109/OCEANS.1997.624092.

Simpson, S.D., Purser, J., and Radford, A.N. 2015. Anthropogenic noise compromises antipredator behaviour in European eels. Glob. Chang. Biol. **21**: 586–593. doi:10.1111/gcb.12685.

Simpson, S.D., Radford, A.N., Holles, S., Ferarri, M.C.O., Chivers, D.P., McCormick, M.I., and Meekan, M.G. 2016a. Small boat noise impacts natural settlement behavior of coral reef fish larvae. Adv. Exp. Med. Biol. **875**: 1041–1048. Springer Science+Business Media, New York.

Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, M.C.O., Chivers, D.P., McCormick, M.I., and Meekan, M.G. 2016b. Anthropogenic noise increases fish mortality by predation. Nat. Commun. **7**: 10544. doi:10.1038/ncomms10544.

Simpson, S.D., Yan, H.Y., Wittenrich, M.L., and Meekan, M.G. 2005. Response of embryonic coral reef fishes (Pomacentridae: *Amphiprion spp*.) to noise. Mar. Ecol. Prog. Ser. **287**: 201–208. doi:10.3354/meps287201.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., and Popper, A.N. 2010. A noisy spring: The impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. **25** (7): 419–427. Elsevier Ltd. doi:10.1016/j.tree.2010.04.005.

Snyder, S., Nadler, L.E., Bayley, J.S., Svendsen, M.B.S., Johansen, J.L., Domenici, P., and Steffensen, J.F. 2016. Effect of closed v. intermittent-flow respirometry on hypoxia tolerance in the shiner perch *Cymatogaster aggregata*. J. Fish Biol. **88**(1): 252–264. doi:10.1111/jfb.12837.

Sogard, S.M. 1997. Size selective mortality in the juvenile stages of teleost fishes: A review. Bull. Mar. Sci. **60**(3): 1129–1157.

Spiga, I., Fox, J., and Benson, R. 2012a. Effects of short- and long-term exposure to boat noise on cortisol levels in juvenile fish. *In* The effects of noise on aquatic life. New York. doi:10.1007/978-1-4419-7311-5.

Spiga, I., Fox, J., and Benson, R. 2012b. Potential effects of long-term exposure to boat noise on the growth, survival and nutrient retention in juvenile fish. *In* The effects of noise on aquatic life. New York. pp. 255–257. doi:10.1007/978-1-4419-7311-5.

Steffensen, J.F. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiol. Biochem. **6**(1): 49–59.

Svendsen, M.B.S., Bushnell, P.G., and Steffensen, J.F. 2016a. Design and setup of intermittent-flow respirometry system for aquatic organisms. J. Fish Biol. **88**(1): 26–50. doi:10.1111/jfb.12797.

Svendsen, M.B.S., Bushnell, P.G., and Steffensen, J.F. 2016b. Design and setup of intermittent-flow respirometry system. **88**(1): 26–50. doi:10.1111/jfb.12797.

Szisch, V., Papandroulakis, N., Fanouraki, E., and Pavlidis, M. 2005. Ontogeny of the thyroid hormones and cortisol in the gilthead sea bream, *Sparus aurata*. Gen. Comp. Endocrinol. **142** (1–2): 186–192. doi:10.1016/j.ygcen.2004.12.013.

Voellmy, I.K., Purse, J., Flynn, D., Kennedy, P., Simpson, S.D., and Radford, A.N. 2014. Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim. Behav. **89**: 191–198.

Wale, M.A., Simpson, S.D., Radford, A.N., and Radford, A.N. 2013. Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. doi:10.5061/dryad.36f65.

Wales, S.C., and Heitmeyer, R.M. 2002. An ensemble source spectra model for merchant ship-radiated noise. J. Acoust. Soc. Am.**111**(3): 1211–1231. doi:10.1121/1.1427355.

Wikelski, M., and Cooke, S.J. 2006. Conservation physiology. Trends Ecol. Evol. **21** (2) : 38-46. doi:10.1016/j.tree.2005.10.018.

Williams, R., Wright, A.J., Ashe, E., Blight, L.K., Bruintjes, R., Canessa, R., Clark, C.W., Cullis-Suzuki, S., Dakin, D.T., Erbe, C., Hammond, P.S., Merchant, N.D., O'Hara, P.D., Purser, J., Radford, A.N., Simpson, S.D., Thomas, L., and Wale, M.A. 2015. Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. **115**: 17–24. Elsevier Ltd. doi:10.1016/j.ocecoaman.2015.05.021.

Williams, S.E., Bolitho, E.E., and Fox, S. 2003. Climate change in Australian tropical rainforests: an impending environmental catastrophe. Proc. R. Soc. London B. **270**(August): 1887–1892. doi:10.1098/rspb.2003.2464.

Wood, B.R. 2011. Fish reaction to tonal vessel noise - Comparison with ICES CRR 209 methodology. Appl. Acoust. **72**(7): 399–411. Elsevier Ltd. doi:10.1016/j.apacoust.2010.12.002.

Woodcock, S.H., Johansen, J.L., Steer, M.A., and Gaylard, S.G. 2014. Regional Sustainability Planning in the Upper Spencer Gulf Investigating potential impacts of shipping on giant Australian cuttlefish Final Report – April 2014. (April): 1–54.

Wright, K.J., Higgs, D.M., Cato, D.H., and Leis, J.M. 2010. Auditory sensitivity in settlement-stage larvae of coral reef fishes. Coral Reefs **29**(1): 235–243. doi:10.1007/s00338-009-0572-y.

Wright, K.J., Higgs, D.M., and Leis, J.M. 2011. Ontogenetic and interspecific variation in sound detection ability in marine fish larvae. Mar. Ecol. Prog. Ser. **424**: 1–13. doi:10.3354/meps09004.

Wysocki, L.., Codarin, A., Ladich, F., and Picciulin, M. 2009. Sound pressure and particle acceleration audiograms in three marine fish species from the Adriatic Sea. J Acoust Soc Am **126** (May): 4. doi:10.1121/1.3203562.

Wysocki, L.E., Dittami, J.P., and Ladich, F. 2006. Ship noise and cortisol secretion in European freshwater fishe. Biol. Conserv. **128**: 501–508. doi:10.1016/j.biocon.2005.10.020.

Appendix

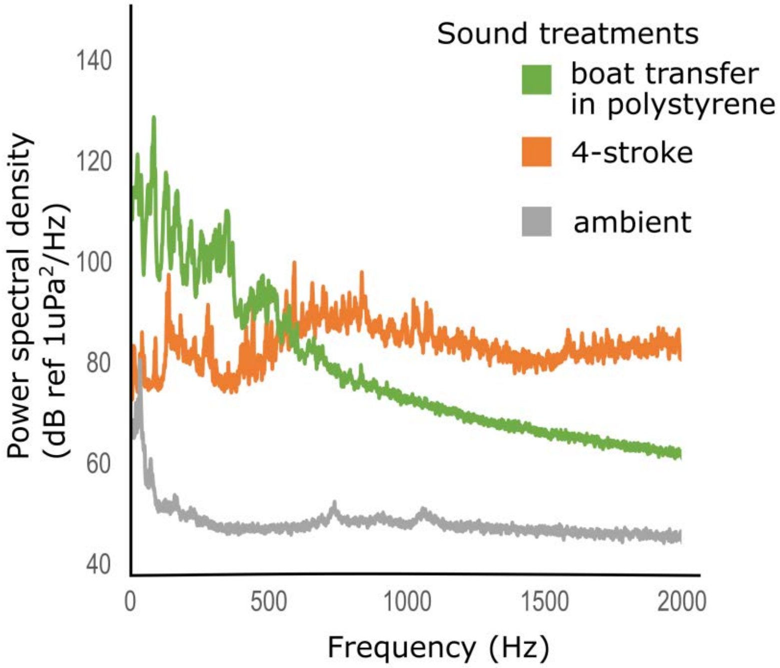

Figure A2. 1: Sound pressure mean (RMS) power spectral density that *Amblyglyphidodon curacao* embryos were exposed to during transport in a polystyrene container on a four-stroke powered boat to the experiment location, compared to four-stroke powered boat noise from underwater and underwater ambient noise. The boat was travelling at 20 km/h during transport and 0–35 km/h, 10–200 m from the sound recording device during the underwater recording. Analysis was conducted in paPAM.

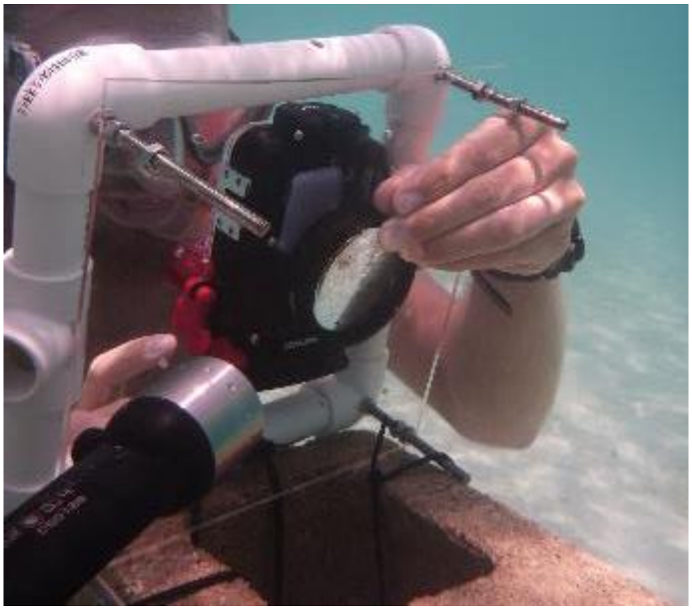

Figure A2. 2: Photograph of heart rate video recording setup used to examine heart rate responses of *A. curacao* embryos to real boat noise.

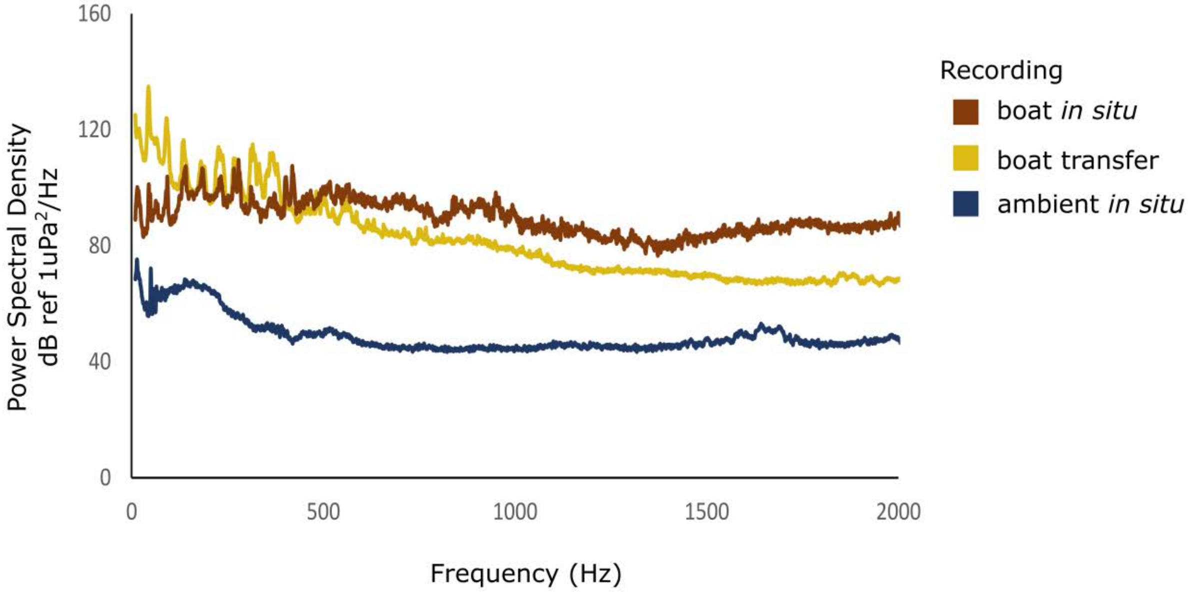

Figure A3. 1: Sound pressure power spectral density that *Pomacentrus amboinensis* juvenile were exposed to during transport in a Nally bin on a two-stroke powered boat to the experiment location, compared to two-stroke powered boat noise from underwater and underwater ambient noise. The boat was travelling at 20 km/h during transport and 0–35 km/h, 10–200 m from the sound recording device during the underwater recording.