

SOUND BITES: THE IMPACT OF BOAT NOISE ON FRESHWATER FISH

A Thesis Submitted to the
College of Graduate Studies and Postdoctoral Studies
In Partial Fulfillment of the Requirements
for the Degree of Master of Science
In the Department of Biology
University of Saskatchewan

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Abstract

Noise pollution is rapidly becoming more prevalent on a global scale, yet it is one of the least studied anthropogenic disturbances. Sound has low attenuation in water where it travels five times further than it does in air. Such effects, coupled with the wide spatial and temporal distribution of anthropogenic noise, makes noise pollution a major concern for aquatic species that may lack refuge from this modern-day stressor.

This thesis explores how boat noise impacts freshwater fish behaviour and is divided between a lab and field experiment. The lab experiment investigated how fathead minnow and brook char's anti-predator responses to a looming stimulus was influenced by play-back tracks. Tracks were either recordings of boat noise from different distances (100, 250, 500 and 1000 m) or ambient lake recordings for our control. The field experiment looked at how yellow perch's oxygen consumption, or stress level, was influenced by exposure to boat noise at different distances away from the running motor (same distances as lab experiment) compared to ambient lake noises.

The results from our field experiment showed generally that the closer the boat was to the yellow perch, the more stressed the individual was. Furthermore, the majority of the fish exposed to boat noise at any distance used significantly more DO (dissolved oxygen) compared to individuals that were only exposed to ambient lake noises. In contrast, the lab experiment showed no evidence that boat noise influenced fathead minnow or brook char anti-predator responses; however, use of pre-recorded tracks in the laboratory may minimize effects. Future research needs to continue to understand how to use play-back tracks in a lab setting to create more ecologically relevant conditions. This research adds to the limited literature on how anthropogenic noise is influencing freshwater species.

Acknowledgements

Thank you to my supervisor Dr. Maud Ferrari who has been a phenomenal mentor, teacher and role model throughout my master's. Additionally, thank you to Dr. Mike Pollock and Dr. Doug Chivers who supported me and offered a lot of guidance and knowledge. I was tremendously fortunate to have had these three amazing mentors who encouraged and allowed me to grow as a person and researcher.

I also would like to thank all my fellow lab mates from the APEC and Chivers lab who helped and offered support when I needed it most. The level of teamwork and comradery I have experienced throughout my masters has not only helped me complete my experiments but also to become a more well-rounded person. Specifically, I would like to thank Reid Bryshun for the constant support, encouragements and help even when our field of studies did not overlap. Furthermore, Dr. Adam Crane has been a major help, supporter, mentor and role model for me. Finally, Alex Preagola, Gabriel Achtymichuk, Stephen Pecylack and Hunter Stevens who all volunteered to help me with my field and lab experiments. Thank you does not seem to express the gratitude I feel towards my lab mates who truly helped me achieve my experiments and research.

I would also like to thank the University of Saskatchewan Biology Department who funded me through a Biology Graduate Scholarship.

Finally, I would like to thank my friends and family who supported me through this journey. Specifically, Daniel Sanche who helped and taught me how to convert my videos into photos. My father, Mike Fedoroff, and uncle, Ted Hus, who helped me build my looming apparatus. I wish I could list everyone's name who aided me throughout my masters, and I appreciate all the support and help I was able to receive throughout this experience.

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Chapter 1: Introduction

1.1 Noise Pollution in Aquatic Environments

1.1.1 Sources of noise pollution

Noise pollution, especially within aquatic environments, is a rapidly growing environmental issue; however, it is one of the least studied anthropogenic disturbances and could be considered a new kind of pollutant (Putland et al. 2019). Since the industrial revolution, noise pollution has been quickly increasing and has become a hazard of major concern (Hawkins et al. 2014; Holmes et al. 2017). There is a variety of human activities that are contributing to the increasing noise in aquatic environments such as construction, oil and gas operations, shipping, military activities, fishing, and recreational boating (Hawkins et al. 2014). As human activities continue to spread and advance, noise sources polluting terrestrial and aquatic environments has also become more diverse and widespread (Hawkins et al. 2014).

Initially, research into sound pollution focused on marine environments and species, particularly the impact of high-intensity and acute sources of sound pollution such as sonar, pile driving and seismic air-guns (Pearson et al. 1992; Codarin et al. 2009; Slabekoorn et al. 2010; Mickle and Higgs 2018). The impact of lower-intensity and more chronic noise pollution sources on aquatic species and ecosystems has only recently started to be investigated. However, the majority of this low-intensity data has, once again, been studied in marine settings and focused on marine species (Andersson et al. 2012; Hawkins et al. 2014; Mickle and Higgs 2018). Although some of this data is assumed to be parallel and similar to what we would find within freshwater systems, it is difficult, and dangerous to make those assumptions with limited empirical data within freshwater environments (Popper and Hastings, 2009; Hawkins et al. 2014). For example, studying anthropogenic noise in a freshwater system may be different than the marine environment due to the topography of both systems. Measuring sound transmission in the open ocean compared to shallow, freshwater environments has contrasting methodologies and results in different noise profiles. Overall, it is more difficult to measure noise properties in shallow systems (Mickle and Higgs 2018). Although shallow marine environments near the coast may be considered equally complicated in measuring noise profiles, there is an increase in

sounds speed as depth, temperature and salinity increases (Mickle and Higgs 2018; Putland et al. 2019).

Pleasure crafts, such as motorboats, are increasing in popularity around the world and are a growing source of low-intensity and chronic noise pollutants (Simpson et al. 2016; Mickle and Higgs 2018). Within Canada, the boating industry is very lucrative, bringing in over \$4.4 billion in revenue every year, and from a study conducted in 2012, discovered that over 35% of Canadians participate in boating and that approximately 4.3 million Canadians owned and are operating pleasure crafts (National Marine Manufacturers Association Canada 2012). In conclusion, within North America, the growing interest and ownership in motorboats, raises concerns as to how this environmental stressor might impact marine and freshwater organisms (National Marine Manufacturers Association Canada 2019; Simpson et al. 2016; National Marine Manufacturers Association Canada 2012). This thesis will focus on noise pollution caused by motorboats within freshwater systems.

1.1.2 The physics behind underwater acoustics

Noise can be transmitted more efficiently through water, in contrast to a similarly widespread pollutant such as light. Sound travels five times farther in water than it does in air and has lower attenuation in water—it can therefore be heard several kilometers away from the source (Scholik and Yan 2001; Voellmy et al. 2014; Ferrari et al. 2018). Because of the unique relationship between water and sound, as well as the wide distribution of anthropogenic noise, many aquatic species may lack the shelter or ability to escape from this disrupting pollutant (Ferrari et al. 2018). Although recreational motorboats do not have the amplitude or the low frequency produced by larger, commercial shipping carriers, the concern with these smaller pleasure crafts is that they are usually found in shallower systems and therefore, closer in proximity to aquatic organisms (Holmes et al. 2017). For some fish species, such as adult yellow perch, the possibility to move to deeper water is an option, however, for other species such as salmon, suckers and darters, the requirement for shallower bodies of water is necessary (Lyons 1987; Schilt 2006). Furthermore, many fishes take refuge in shallower areas of a lake to avoid predation from larger fish. It is also hypothesized that shallower systems can provide niche habitats and is a rich source of food (Lyons 1987).

When sound moves through water, it gains kinetic energy and travels as a propagated longitudinal elastic wave (Hawkins and Popper 2018). Detection of sound by fish is often described in terms of sound pressure and particle motion (Coffin et al. 2014). Sound pressure is a scalar value and can be described by the intensity of a sound using temporal and frequency characteristics (Hawkins and Popper 2018). This is a well understood component in acoustics and similarly can be easily measured (Gray et al. 2016). Sound pressure can give a fish insight to the general intensity of the sounds; however, because it is scalar, it does not give any information as to the direction in which the noise is coming from (Coffins et al. 2014; Hawkins and Popper 2018). Although not all fish can detect sound pressure or use it to the extent of others, it is believed that majority of fish can detect particle motion as the primary basis for their hearing (Popper and Fay 2011; Gray et al. 2016). Particle motion is a vector quantity in contrast to sound pressure being a scalar unit. Whereas sound pressure can only describe the magnitude of a noise, particle motion can describe both magnitude and direction (Coffin et al. 2014).

1.1.3 The diversity of fish hearing

In order to understand how noise pollution impacts fish, it is important to understand how they hear. There is major variation among fish species in terms of their internal hearing structures (Popper and Fay 2011). Although fish hearing is highly variable among species, there is considered to be a “basic” ear structure that includes semicircular canals, their respective sensory cristae and the three otolith organs that are the saccule, lagena and utricle. Sensory epithelia, which contains sensory hair cells, can be found to be in close proximity of both the cristae and all three otolith organs (Figure 1.1) (Popper and Fay 2011). Depending on the size of the fish there can be hundreds upon thousands of these sensory hair cells. This is the make-up of the most basic fish hearing system (Popper and Fay 2011). However, throughout evolutionary history, the basic structure has changed and adapted, leading up to a massive expansion of diversity among fishes. One of the more recent evolutions in fish hearing is essentially a type of hearing aid that allows fish to not only sense particle motion, but also to detect sound pressure. What allows fish to detect sound pressure is an intimate connection between the swim bladder and the inner ear allowing fish in the super-order ostariophysii to hear across a wider auditory range, and because of this, they are often deemed “hearing specialists” (Scholik and Yan 2001; Popper and Fay 2011).

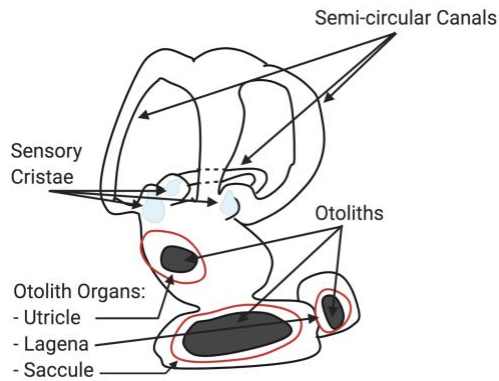


Figure 1.1. Illustration depicting the basic ear structure found in fish (created with biorender.com).

What allows ostariophysan fish to detect sound pressure is the unique connection between their swim bladder and ear, more commonly referred to as a Weberian ossicle. When the pressure from the sound reaches the fish, it sets into motion the swim bladder that is filled with gasses which are at different densities and uniformity than water. This movement from the swim bladder is translated by the Weberian ossicle connection that re-emits a particle motion that can produce oscillations of the sensory epithelium on the otoliths, therefore, providing that pressure detection (Figure 1.2) (Popper and Fay 2011). It should be noted that ostariophysans can get increased particle motion from the swim bladder as well. However, this auditory information is believed to not give additional information for directionality as it comes from one direction only (from the swim bladder). Therefore, it is believed that the Weberian ossicle may just increase noise sensitivity (Hawkins and Popper 2018; Hawkins and Johnstone 1978; Yan et al. 2000). However, “hearing generalists”, or non-ostariophysan fish do not have this unique connection and therefore, will have narrower auditory ranges compared to ostariophysans (Scholik and Yan 2001; Popper and Fay 2011).

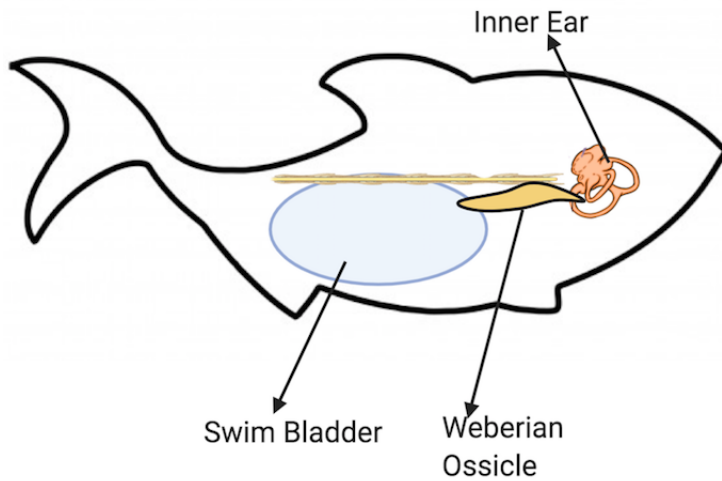


Figure 1.2. Illustration of the Weberian ossicle connection between the swim bladder and inner ear (created with biorender.com).

When a fish is exposed to a sound wave, the tissues of the fish, including the inner ear, move with the particle motion. This phenomenon occurs because the fish is of similar density to the surrounding water. However, the otoliths within the inner ear have a higher density and therefore create a type of shearing force (Dijkgraaf 1960; de Vries 1950; Hawkins and Popper 2018). This occurs because while part of the fish is moving in one direction, the otoliths are moving differently due to their higher density. This force is also applied to the hair cells of the sensory epithelium and ultimately results in the ability to detect directionality. In this example the otoliths were stimulated directly. However, ostariophysan fish, who have Weberian ossicles stimulate the otoliths indirectly through the unique connection between the inner ear and swim bladder. As the fish is surrounded by the sound wave, the swim bladder is also stimulated at a different motion compared to the rest of the fish, resulting in the otoliths indirectly responding to the pressure component of the sound wave. This Weberian ossicle connection has allowed ostariophysan otoliths to become stimulated at a wider range of frequencies compared to non-ostariophysans (Hawkins and Popper 2018). Although hearing in fish has been studied for decades there are still gaps in the literature. Two main points that require further research are, first, how hearing capabilities vary among species and second, what behavioural responses fishes exhibit to sound (Hawkins and Popper 2018).

1.1.4 Methodological approaches to noise pollution

With noise pollution studies it can be difficult to determine end-points because it is very rare to observe death or injury for chronic, less intense noise exposure studies such as boat noise. However, this chronic pollutant can alter behavioral changes that may lead to decreased fitness. Therefore, throughout the literature, it is generally agreed that although harder to measure, behavioural changes are more critical than physiological changes when determining how anthropogenic noise is impacting fish (Ferrari et al. 2018; Hawkins et al. 2014).

By using behavioural methods to measure how anthropogenic noise impacts aquatic species, the accumulated scientific understanding of how noise affects fish has grown significantly. Some of the behaviours that have been observed to be altered by anthropogenic noise are shoaling behaviours, communication, foraging, mutualism, and avoidance and detection of predators (Herbert-Read et al. 2017; Mickle and Higgs 2018; Magnhagen et al. 2017; Nedelec et al. 2017; Simpson et al. 2016; Hasan et al. 2018). Ultimately, understanding how aquatic organisms are influenced by anthropogenic noise can help advance our understanding of the bigger picture as to how ecosystems are impacted.

1.2 Escape Behaviours

1.2.1 History of Looming Stimulus

How a prey responds to a predator is one of the most critical behaviours for survival and fitness, and therefore, is a valuable response to study (Herberholz and Marquart 2012). Measuring and understanding startle responses have been used for decades on a variety of animal species (Peek and Card 2016). Specifically, using a looming object to mimic a predator strike so as to understand the circuits and physiology behind escape movements by prey has been a popular method across phyla such as primates (i.e. humans, monkeys), birds (i.e. pigeons), amphibians and reptiles (i.e. frogs, turtles), arthropods (i.e. crabs, crayfish, flies, locusts) and of course a variety of fish species (Ball and Tronick 1971; Schiff et al. 1962; Wang and Frost 1992; Hayes and Saiff 1967; Ingle and Hoff 1990; Hemmi 2005; Wine and Krasne 1972; Holmqvist and Srinivasan 1991; Fotowat and Gabbiani 2007; Simpson et al. 2015; Anderson et al. 2019; Peek and Card 2016). Reacting to a potential predator strike involves several physiological components. For example, prey need to be able to take visual information and translate that to

muscular and motor stimulation. In other words, prey must be able to use sensory-motor integration to respond to a predator (Fotowat and Gabbiani 2011; Herberholz and Marquart 2012). However, when a prey reacts to a predator there are many puzzle pieces at play that need to be considered. Often times, prey interact with predators while accomplishing other important behaviours such as foraging, mating, and habitat choice. Therefore, prey must consider a trade-off between potentially finding a mate or food and being eaten (Lima and Dill 1990; Herberholz and Marquart 2012).

As previously mentioned, testing startle responses using a looming object has been used on multiple species and phyla (Fotowat and Gabbiani 2011; Peek and Card 2016). Every animal that has spatial vision has evolved neural circuits that are specialized to execute this critical behaviour (Fotowat and Gabbiani 2011). The majority of animals respond to a looming stimulus similarly which involves motor responses. First the animal moves away from the potential threat, followed by either a fast startle movement, or protecting vulnerable parts of their body (Fotowat and Gabbiani 2011).

1.2.2 Visual cues for fish and the C-start response

Teleost fishes' startle response to a looming stimulus has been studied extensively to understand the mechanisms behind the movement (Zottoli and Faber 1979; Preuss et al. 2006; Bhattacharyya et al. 2017). Mauthner cells (M-cells) are behind the quick, rapid burst (~0.1ms) movements in startle responses for teleost fish (McCormick et al. 2019). The M-cell is a reticulospinal neuron that projects from the hindbrain and collects sensory information. Motor neurons are connected to the M-cells which allow it to produce a startle response (Preuss et al. 2006; Bhattacharyya et al. 2017). Past research has shown that visual, auditory and physical stimuli can elicit this behaviour (McCormick et al. 2019; Zottoli and Faber 1979; Eaton and Emberley 1991).

The startle response produced by the activation of the M-cells creates a movement in fish commonly known as the C-start response, which has two to three stages (Weihs 1973; Eaton and Emberley 1991). The first stage of the response begins with a musculature contraction on one side of the fish's body. If viewing the fish from above, the contraction often looks like the fish is making a "C" with its body as the head and tail is angled towards one side, most likely directed away from the threatening stimuli. The fish, now in the familiar C shape, enters stage two, which

allows the fish to have a burst of speed to start an escape from the stimulus. Finally, at the end of stage two or beginning of stage three, the fish returns to a normal swimming pattern (Weihs 1973; Eaton and Emberley 1991).

As with the majority of behaviours, there are costs and benefits that the organism needs to consider. The C-start response can deplete a lot of energy that could be used for foraging or mating. Therefore, using a C-start response when a stimulus is not threatening can result in failure to perform other critical behaviours. An individual only wants to respond to actual life-threatening stimuli and avoid using a C-start response to non-threatening stimuli (Hemmi 2005; Herberholz and Marquart 2012).

Studying an organisms startle response to threatening stimuli have also been used to understand how a variety of pollutants are impacting escape behaviours (Anderson et al. 2019). Research is now investigating how specific pollutants impact the time for individual fish to respond to a threatening stimulus. Latency is often used as a measurement for how quickly an organism responds once a looming stimulus is released (Voellmy et al. 2014). Therefore, the question many researchers have started to explore is how certain pollutants can impact the latency to escape a stimulus (McCormick et al. 2019; Anderson et al. 2019).

1.3 Research Objectives

The main objective of this thesis is to explore how boat noise impacts the physiology and behaviour of freshwater fish. I used two experiments to answer different questions related to the main objective. The first experiment explores how boat noise impacts yellow perch (*Perca flavescens*) metabolic rate. Furthermore, it examines how being exposed to boat noise at different distances impacts a fish's oxygen consumption. Previous research has shown that exposure to boat noise can increase metabolic rate and cortisol levels, indicating a fish has increased stress levels in the presence of boats (Wysocki et al. 2006; Simpson et al. 2016). However, to our knowledge, there is little to no data on how exposure to boat noise at varying distances impacts a fish's stress levels. Therefore, this experiment aims to understand how being exposed to boat noise at varying distances can impact yellow perch's metabolic rate. This experiment took place within a field environment using similar methodologies employed by Simpson et al. (2016) within a marine setting. We hypothesize that fish exposed to boat noise will have greater oxygen consumption compared to those that are exposed to ambient lake conditions. Furthermore,

yellow perch that are exposed to boat noise at a closer distance will have greater oxygen consumption compared to those that are exposed to boat noise at greater distances.

The second experiment was conducted within a lab setting using play-back tracks and examined how boat noise impacts freshwater fishes' anti-predator behaviour to a looming stimulus. Two freshwater species, brook char (*Salvelinus fontinalis*) and fathead minnows (*Pimephales promelas*), were used to understand how anti-predator behaviour is impacted by boat noise. Similar to the first experiment, I continued to explore how exposure to boat noise recordings at varying distances impacted the fishes' ability to respond to a looming threat. Previous research has shown trends that aquatic organisms have latent responses to a looming stimulus when exposed to anthropogenic noise (Simpson et al. 2016; McCormick et al. 2018; McCormick et al. 2019). Therefore, we hypothesize that fish exposed to boat noise will result in an increased latent response to the looming stimulus compared to fish exposed to ambient lake tracks. It is further hypothesized that fish exposed to boat noise recorded at a closer distance will have increased latency to the looming stimulus compared to fish exposed to boat noise tracks recorded at a greater distance.

As previously mentioned, there is limited data explaining how anthropogenic noise impacts freshwater species and ecosystems (Hawkins et al. 2014). This thesis provides greater insight into the potential impacts of boat noise on freshwater fishes and gives more empirical data required for conservation work and policy makers (Reid et al. 2019).

1.4 Ethical Statement

The following experiments were approved by the University of Saskatchewan's University Animal Care Committee (UACC) and Animal Research Ethics Board (AREB) under the protocol #20160058. Fish that were euthanized at the end of the trials were given tricaine methanesulfonate (MS-222) and a blow to the head.

Chapter 2: The effect of boat noise on brook char and fathead minnow anti-predator responses

2.1 Introduction

There are over 32,000 known fishes; however, we have studied only a small fraction of these fish and how anthropogenic noise impacts them. In order to get a clear understanding of how man-made noise impacts a species' behaviour, it is vital to investigate how different species respond (Hawkins et al. 2014). To identify which species are more sensitive to anthropogenic noise, we need to continue to test and research ways of classifying the species that appear to be more susceptible to noise (Hawkins et al. 2014). While there are limited studies that have considered the difference between how species react to noise pollution, the aforementioned experiments do suggest interspecific differences (Voellmey et al. 2014; Magnhagen et al. 2017).

When exposing fish to anthropogenic noise there are generally three hypotheses as to how the pollutant can influence behaviour. An increase in anthropogenic noise could cause distraction, stress or mask important cues for the individual; however, these hypotheses are not necessarily mutually exclusive (Simpson et al. 2015; Wright et al. 2007). Distraction, stress and masking are common themes for researchers to use to explain how important behaviours are influenced by anthropogenic noise (Simpson et al. 2015). Within this chapter I focus on how anti-predator behaviours, specifically escape responses, are influenced by boat noise.

There are several examples from the past decade of researchers observing prey responding to a predatory event inappropriately when exposed to anthropogenic noise (Voellmy et al. 2014; Simpson et al. 2015; Simpson et al. 2016; Ferrari et al. 2018; Hasan et al. 2018). Although there are some cases in which fish respond more quickly to a threatening stimulus, the majority of research points to prey having a latent response. In 2016, Simpson et al. explored how Ambon damselfish (*Pomacentrus amboinensis*) reacted to a looming threat under boat noise and ambient conditions. When damselfish were exposed to boat noise there was a significant latency in response compared to fish that were exposed to ambient conditions. It was proposed that the latent response was a side effect from noise-induced stress (Simpson et al. 2016). A similar study conducted in the field looked at how juvenile damselfish (*Pomacentrus wardi*) responded to a looming stimulus when exposed to ambient or boat noise from a 2-stroke or 4-stroke motor (McCormick et al. 2017). Similar to Simpson et al. (2016), the damselfish had almost a 40%

slower response time when exposed to a 2-stroke motor compared to individuals exposed to ambient or 4-stroke engines (McCormick et al. 2017). Both these studies supported the hypothesis that stress could be a major influence for the inappropriate response to a looming threat. However, it is difficult to test how masking and/or distraction could be playing a role as well (Simpson et al. 2016; McCormick et al. 2017)

The main objective of this chapter is to explore how the antipredator behaviour of two fish species, the brook char (*Salvelinus fontinalis*) and fathead minnow (*Pimephales promelas*) are impacted when exposed to boat noise. Experiments were conducted on each species to test whether exposure to boat noise impacted their startle response to a looming stimulus. Furthermore, we tested how playback tracks taken from varying distances from the boat would influence their response time. We hypothesized that fish exposed to playback tracks taken closer to the boat would respond with increased latency compared to fish exposed to ambient lake noise or tracks taken from a greater distance.

2.2 Methodology

2.2.1 Study Species Collection and Care

Fathead minnows were collected by seine net from Blackstrap Lake, SK in the summer of 2019. They were transported to the Aquatic Predation and Environmental Change (APEC) laboratory at the University of Saskatchewan (U of S). Here they were housed in groups of ~10 fish per 40-L tanks. They were maintained on a strict 16:8 hr light:dark schedule, were fed finely grounded flake food once a day and underwent a 10% water change every second day.

Temperature was maintained around 18°C, and no secondary sex characteristics were observed.

Brook char were supplied by Wild West Steelhead fisheries/hatchery on Diefenbaker Lake, SK and transported to the R.J.F. Smith Centre for Aquatic Ecology. Here they were divided and held in two 1700-L tanks for approximately two months before being transferred to the APEC lab facility where they were kept in stream tanks. Within APEC, they experienced a 16:8 hr light:dark schedule, were fed commercial pellets once a day and underwent a 10% water change every day. Temperature was maintained around 16°C to reduce mortality and maintain water quality.

2.2.2 Audio Collection and Track Development

Audio for this experiment was recorded in Diefenbaker Lake, SK, using an omnidirectional hydrophone (CR1 hydrophone, calibration sensitivity -198.960 SPL (dB re 1 μ PA) rms; sensitive to frequency ranges between 0.001 kHz to 48 kHz; using a SpectraDaq-200 precision data acquisition sound card; Cetacean Research Technology, Seattle, WA, USA). The hydrophone was positioned at around a 2-m depth and supported by a small, inflatable, anchored Zodiac boat. Two 5-m V-aluminium hulled motorboats equipped with identical 4-stroke 40 hp Mercury engines were used for the boat noise recording. I refer to them as white boat and black boat for the rest of the experiment.

We recorded both boats at travelling speeds of ~10-30 km/h at 100, 250, 500 and 1000 m away from the hydrophone location. We also recorded ambient (i.e., no boat) tracks at four different locations. This was done in case there was a sound anomaly in a specific location that we could not detect but our fish could and, therefore, giving us more unique ambient tracks to work with. Finally, we randomized the order in which the tracks were recorded (i.e. ambient, white boat or black boat at 100, 250, 500 or 1000 m). Again, this was to prevent a sound anomaly only showing up on one of the distances recordings. In the end, we had a total of twelve 15-min long recordings: white boat at all four distances, black boat at all four distances and four ambient recordings. When we detected another boat that would disrupt a recording, we were able to remove these parts during sound editing.

Using Audacity 2.3.0 and our field recordings, we created fifty unique playback tracks for the experiment; five 2-min test tracks per boat per distance, five 2-min test tracks for ambient and another five 15-min tracks for ambient that were used during the acclimation period.

2.2.3 Noise Analysis

Boat and ambient noise recorded in the field was analyzed in SpectraPLUS (Pioneer Hill Software) using the overlay tool (size of FFT 8192; 1042 points) producing sound pressure spectrums (Figure 2.1). This data revealed the characteristics of boat and ambient noise in the field; however, it was important to re-analyze the sound profiles in the lab setting where testing occurred. To understand how the sound characteristics changed in a lab setting, we played tracks for each sound treatment in a test tank using an underwater speaker (ECOXGEAR). While a

track was playing, we re-recorded using the omnidirectional hydrophone. The recording was similarly analyzed using the overlay tool on SpectraPLUS (Pioneer Hill Software; size of FFT 8192; 1042 points), producing unique sound pressure spectrums. Finally, noise profiles from the field and lab were compared by plotting line graphs illustrating the sound pressure differences (Figure 2.2). This is an important step to ensure that fish were experiencing similar sound pressures and frequency as recorded in the field.

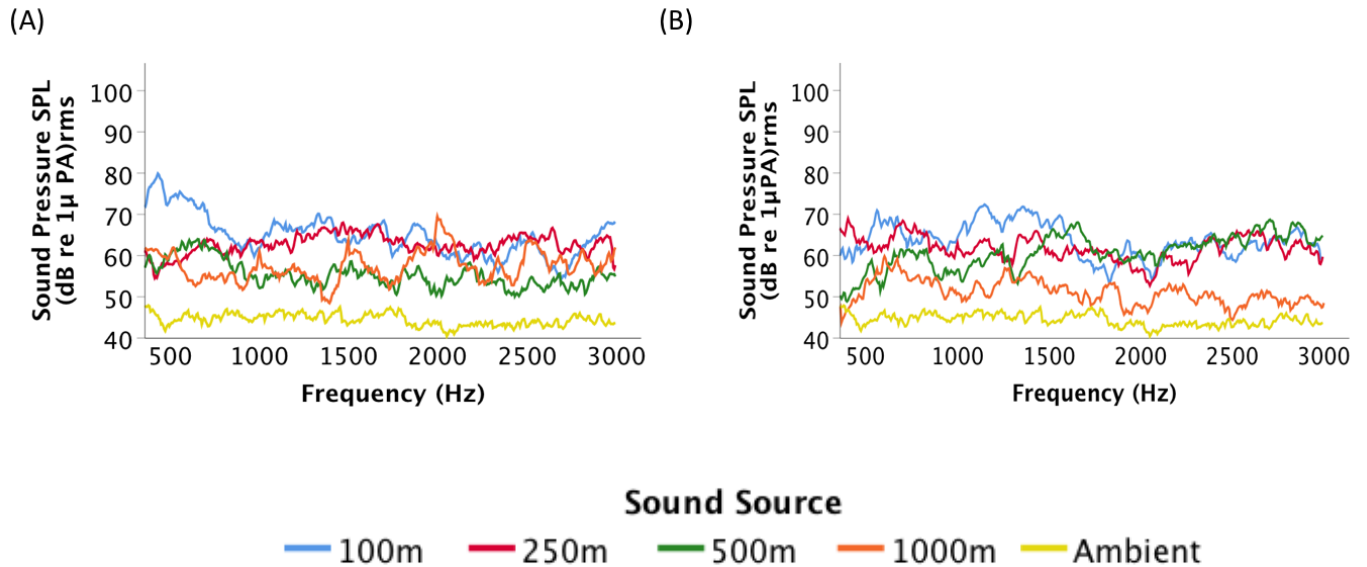


Figure 2.1. Sound pressure spectrums from boat noise and ambient lake conditions in the field. (A) represents recordings from the white boat and (B) represents recordings from the black boat. Blue lines represent recordings taken at 100 m, red lines were from 250 m, green lines were from 500 m, orange lines were from 1000 m and yellow represents ambient controls.

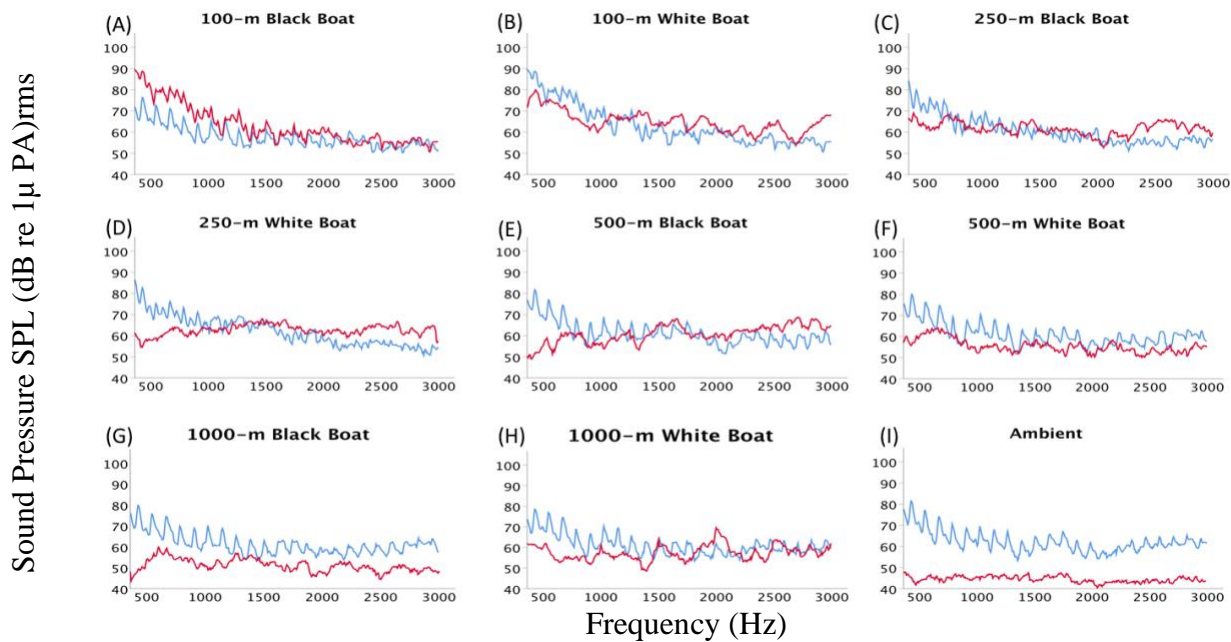


Figure 2.2. Sound pressure spectra illustrating the difference between the different treatments recorded in the field (red line) versus in the lab (blue line).

2.2.4 Experimental Set Up

Three testing chambers were built to test the fish. Testing chambers consisted of 6.8 L glass aquaria that were divided by white perforated plastic. The two areas in the tank included a testing area (closer to the looming device), and a sound chamber that enclosed the speaker and thermometer (Figure 2.3). To reduce unintentional visual cues, we modified the sides of the aquarium. The side furthest from the observer was wrapped in black, opaque plastic; however, the side facing the observer was wrapped in a grey tint that had a 5% visual light transmission. The tint allowed us to observe the test fish with minimal visual disturbance to the fish. In order to ensure that the fish behaviour could be recorded and that the looming device was visible, we illuminated the testing tanks with one light on the side, and one light directly above. A high-speed camera (Casio EX-ZR100) was positioned above each test tank and trials were video recorded at 480 fps (frames per second).

The looming apparatus, illustrated in figure 2.3, was composed of a metal frame positioned above the testing tank and had a 75 cm wooden dowel attached to it. At the end of the dowel was a 10-cm diameter white circle with a 4.5 cm diameter black circle positioned in the

middle. Once released, the looming stimulus therefore appeared as an enlarging black circle to the test fish (Anderson et al. 2019; Simpson et al. 2016). The dowel was pulled back and attached to an electromagnet that had a push button allowing us to release the looming device with minimal disturbance. Once released, the looming stimulus swung freely towards the tank but a tether attached to the metal frame stopped it 3 cm from the tank, preventing it from striking the glass (Figure 2.3). Throughout the experiment we would conduct 50% water changes after every test fish to ensure that temperature and dissolved oxygen remained consistent.

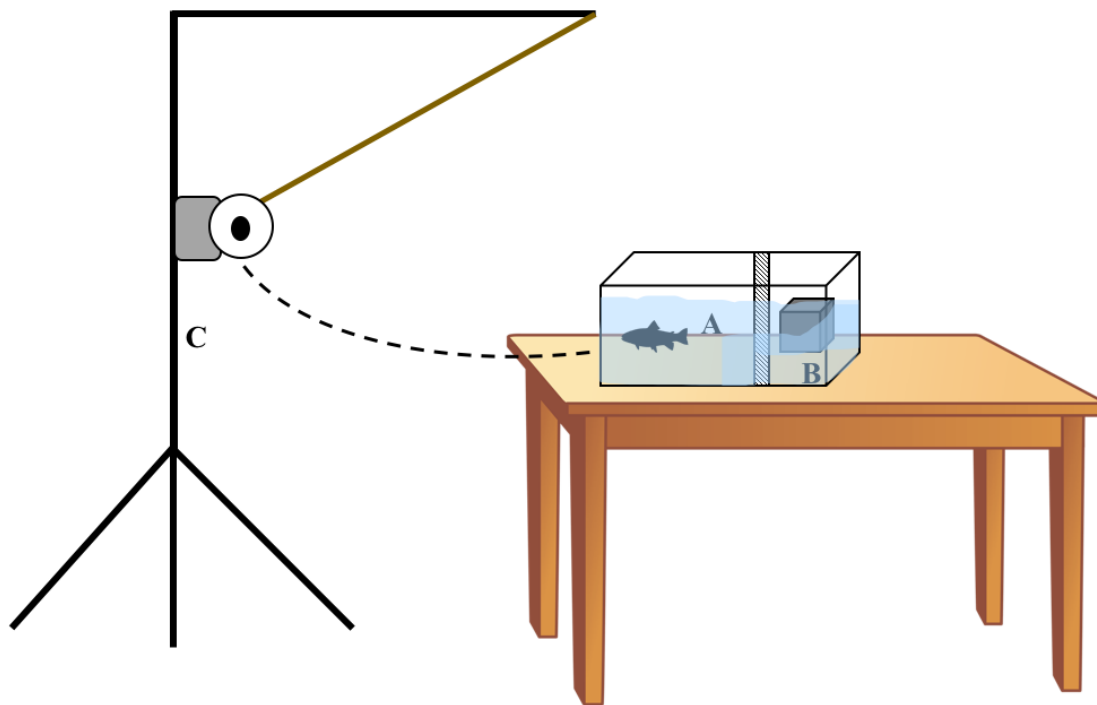


Figure 2.3. Experimental set up for the looming device and testing tanks. Section A represents the testing chamber, section B represents the sound chamber and section C represents the looming device.

Once a test fish was placed into a testing tank, it was given 15 min to acclimate while being exposed to one of five ambient soundtracks. After the acclimation period, a 2 min test track (ambient, or boat noise at one of the four distances) was randomly assigned. The fish was given 1 min exposure time to the test track without triggering the stimulus. Once the second minute began, the camera was turned on, and the looming stimulus was fired when the fish was in an area we defined as the “strike zone” (Figure 2.4). If the fish did not enter the strike zone

during this time frame, we released the looming stimulus at the 2 min mark, therefore limiting the amount of exposure difference between test fish. If a fish was not in the appropriate angle we removed them from analysis. Finally, after each trial the fish were euthanized using an overdose of MS-222 and a blow to the head. This methodology for euthanasia was in accordance with our animal use protocol. Once euthanized, we recorded the fish's weight and body length. Brook char trials were conducted first, followed by the fathead minnow trials.

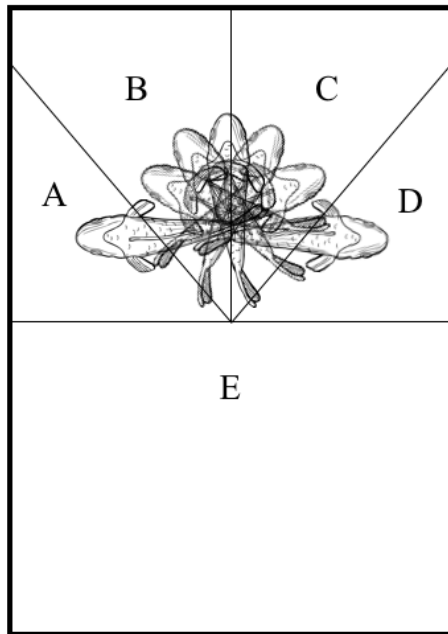


Figure 2.4. Top view of testing tank illustrating the acceptable positions for a fish to be considered in the “strike zone”. The “strike zone” parameters required the fishes head to be in either sections A, B, C or D and the tail directed towards section E or the sides of the tank. This provided a 180° angle the fish could be oriented in.

2.2.5 Video Analysis

Individual image frames were extracted as Portable Network Graphics (PNG) images using FFMPEG V4.2, with each individual frame representing 2.0833ms of video. Response time was calculated by determining the frame in which the looming device was triggered and the frame in which a fast-start response was apparent. The difference in frames between these events was then divided by 480fps to determine latency response time.

2.2.6 Statistical Analysis

Data was analyzed for outliers using a boxplot; however, other than checking for data entry errors, no outliers were removed. Weight and fork length of both brook char and fathead minnows were examined to determine if there was a pattern with size and response using a comparison of regression lines (Type III SS, model: treatment + length/weight + treatment*length/weight + error); however, there was nothing of significance (brook char comparison of regression lines; $F_{1,4}=1.973$, $p=0.098$) (fathead minnow comparison of regression lines; $F_{1,4}=1.071$, $p=0.372$). Chi-squares were run to ensure that there was no bias on responses depending on test apparatus. Brook char showed no significant difference between tanks; however, the tank used affected the responses of fathead minnows to the looming stimulus (brook char chi-square; $\chi^2_{0.05,2}=5.679$, $p=0.058$) (fathead minnow chi-square; $\chi^2_{0.05,2}=18.272$, $p<0.001$). Furthermore, we explored whether time exposed to test track impacted response using a comparison of regression line (Type III SS, model: distance + exposure + distance*exposure + error), and found no significant pattern (brook char comparison of regression lines; $F_{1,74}=1.014$, $p=0.459$) (fathead minnow comparison of regression lines; $F_{1,50}=0.896$, $p=0.669$). Data did not meet assumptions for normality (KS test, $0.132<Z<0.217$, $p<0.001$); however, the data was homoscedastic based on residual plots.

After analyzing all potential patterns in the data, we ran final models for both fathead minnows and brook char; however, both species models looked different. For brook char in order to test how exposure to the sound treatments influenced response time we ran a one-way ANOVA (response latency as the response variable, noise treatment as the factor (ambient, 100 m, 250 m, 500 m and 1000 m)). Similarly, for fathead minnows we ran a one-way ANOVA for response time (response latency as the response variable, noise treatment as the factor (ambient, 100 m, 250 m, 500 m and 1000 m)); however, we also had boat and tank as random factors. Finally, we analyzed count data of fish that did not respond versus those that did respond to the looming stimulus by using a chi-square across treatment.

2.3 Results

For fathead minnows, the result of the one-way ANOVA (which included tank and boat as random factors) revealed no significant difference for how the minnows responded to the

looming stimulus depending on the noise they were exposed to (one-way ANOVA, $F_{1,3}=0.010$, $p=0.998$) (Figure 2.5). Similarly, for brook char, the result of the one-way (no random factors were included in this model) revealed no significant difference for how brook char responded to the looming stimulus depending on the noise they were exposed to (one-way ANOVA, $F_{1,4}=0.695$, $p=0.596$) (Figure 2.6). Finally, there was no significant difference in whether or not fish responded to the looming stimulus based on the noise treatment they were exposed to (fathead minnow; Pearson Chi-square, $\chi^2_{0.05,4}=4.966$, $p=0.291$; brook char; Pearson Chi-square, $\chi^2_{0.05,4}=1.239$, $p=0.872$) (Figure 2.7 and 2.8).

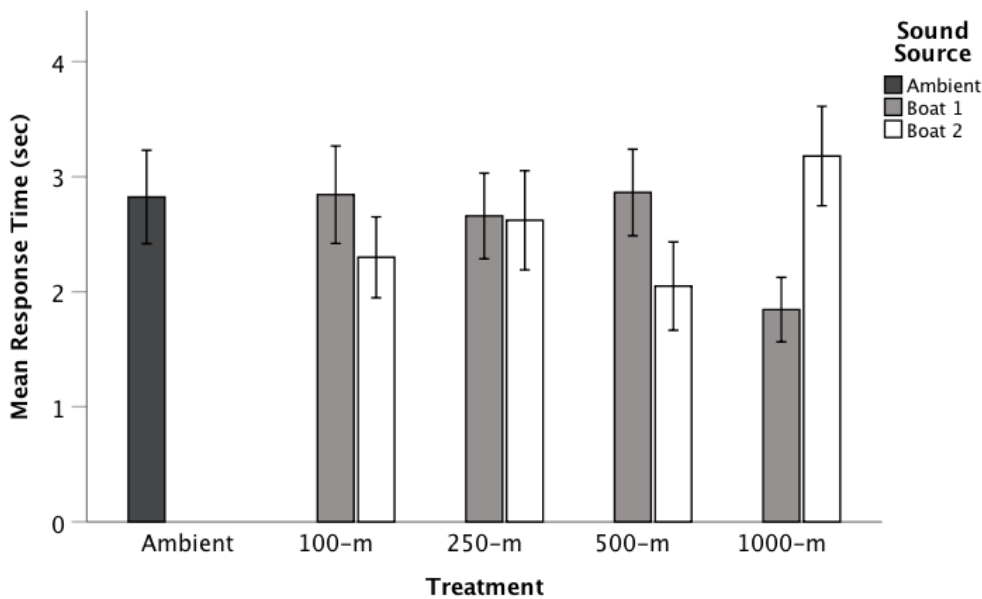


Figure 2.5. Bar graph (SE \pm 1) illustrating mean response time (sec) in fathead minnows for each distance treatment.

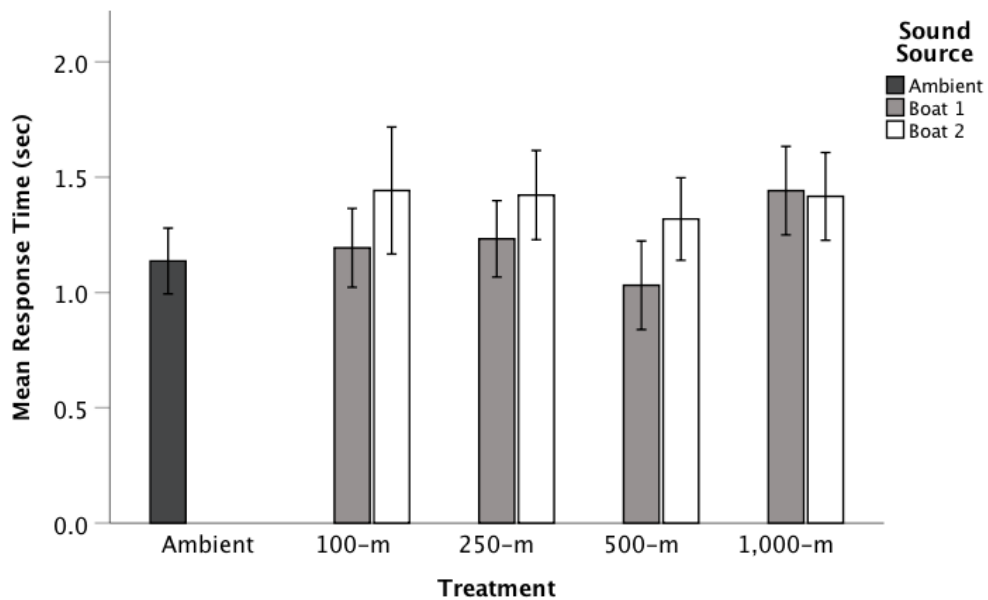


Figure 2.6. Bar graph (SE \pm 1) illustrating mean response time (sec) in brook char for each distance treatment.

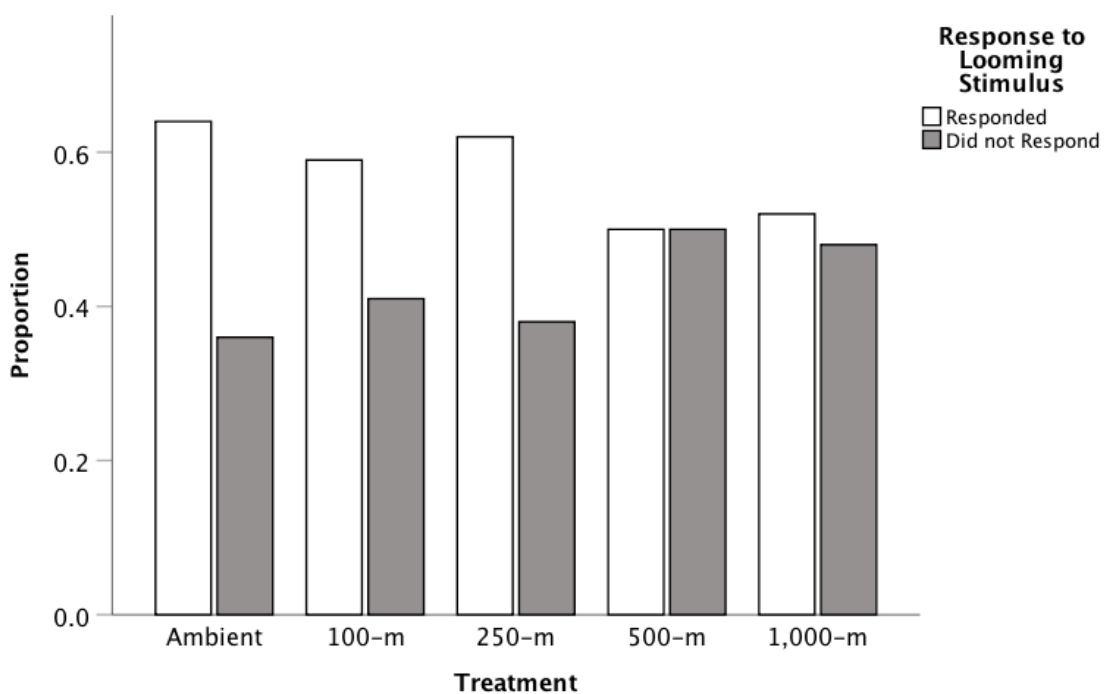


Figure 2.7. Bar graph illustrating the mean proportion of responses for fathead minnows.

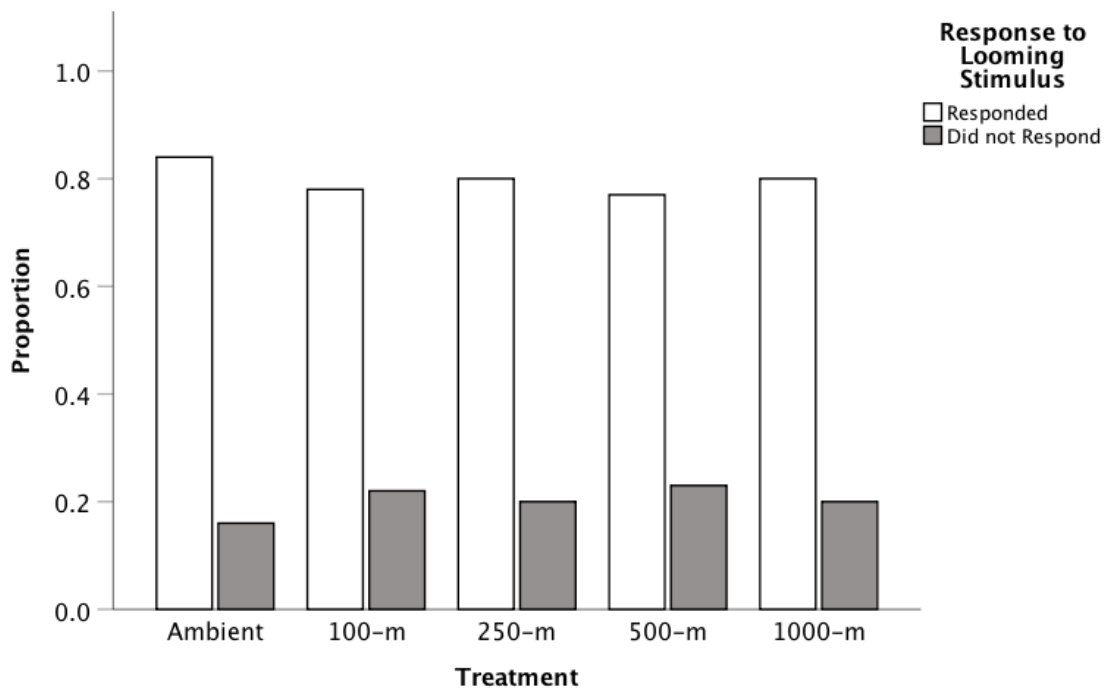


Figure 2.8. Bar graph illustrating the mean proportion of responses for brook char.

2.4 Discussion

Our study did not observe any significant effect from boat noise on the anti-predator response time in fathead minnows or brook char. There was no significant difference in how fish responded regardless of the noise treatment to which they were exposed. Therefore, we did not observe a distance effect of boat noise, nor a change in behaviour between exposure to ambient or boat noise. We cannot directly compare these two species results since the experiment took place at different times; however, brook char responded more quickly to the looming stimulus compared to fathead minnows (Figure 2.5 and 2.6). Furthermore, brook char responded at a higher frequency compared to fathead minnows (Figure 2.7 and 2.8). Finally, brook char did not show any significant changes whether they were exposed to boat 1 or boat 2. In contrast, fathead minnows responded differently to the two boats (Figure 2.5 and 2.6).

Other studies have found similar results in the sense that different species respond to anthropogenic noise in contrasting ways. A study conducted in 2018 examined the different feeding behaviours of roach (*Rutilus rutilus*) and Eurasian perch (*Perca fluviatilis*) and how motorboat noise influenced them (Magnhagen et al. 2018). The researchers observed feeding

behaviours in single and mixed species enclosures. Roach made fewer feeding attempts in both enclosures; however, perch was only hesitant in the single enclosure experiment, whereas when they were in a mixed species enclosure, they made more feeding attempts. This study also observed species-specific behavioural changes when exposed to motorboat noise in a field setting (Magnhagen et al. 2018).

A study conducted in 2017 tested how the anti-predator behaviour in two sympatric species was influenced by playback tracks of high-traffic harbours. The experiment exposed the three-spined stickleback (*Gasterosteus aculeatus*) and the European minnow (*Phoxinus phoxinus*) to a visual predator stimulus and measured their latency to respond. Contradictory to what other studies have found was that stickleback responded faster when exposed to additional anthropogenic noise compared to ambient conditions. However, minnows showed no significant difference when responding to the visual threat regardless of the sound treatment (Voellmy et al. 2017). In this sense, our experiment found similar results where we did not observe a difference in how fathead minnows or brook char responded to a looming stimulus. However, Voellmy et al. and our findings contradict what a growing number of other studies have found, which have demonstrated that added anthropogenic noise does contribute to changes in fish behaviour (Ferrari et al. 2018; Hasan et al. 2018; Magnhagen et al. 2017; Nedelec et al. 2017; Simpson et al. 2016; Sara et al. 2007). There is a large amount of empirical data on how anthropogenic noises, such as that from motorboats, influence fish behaviours; however, it should be noted that many studies have also shown that fish can return to normal behaviour if exposed to the noise repeatedly or for long periods of time (Radford et al. 2016; Holmes et al. 2017).

Overall, our study did not reveal any changes in behaviour in the presence of boat noise playback tracks. Our observations are contrasting to what we hypothesized and what several studies have found thus far. Although there are a few other studies that show non-significant results, lab experiments that deal with noise trials can be more difficult and harder to replicate wild acoustic conditions (Voellmy et al. 2014). Unfortunately, although we were able to have the majority of our tracks play back at similar acoustic levels as they were detected in the field, our ambient track was louder in a lab setting. The louder ambient track might have caused higher stress levels resulting in our test fish never acclimating to the testing conditions. In order to study how anthropogenic noise influences fish behaviour, it is important that the test fish be exhibiting normal behaviour before exposure to the pollutant. As many researchers have experienced, a

stressed test subject will not provide reliable results, and it will be more difficult to relate to other studies and to wild conditions. Furthermore, compared to several other studies, our 15 min acclimation period may have been too short with many studies using varying lengths of time to ensure their test organism had returned to normal behaviour before subjecting them to anthropogenic noise (Holt and Johnston, 2015; Magnhagen et al. 2017; Ferrari et al. 2018; Hasan et al. 2018). The combination of having a loud ambient track and too short of an acclimation period could have caused the fish to have abnormal behaviour, resulting in our unexpected observations of the fish not being impacted by motorboat noise.

Many studies that have conducted their trials in a lab setting have reported similar difficulties in getting, specifically the ambient tracks, to be similar to the original levels in the field. Studies that show the spectral analysis of their acoustic tracks display an ongoing theme of the ambient track in tanks being louder compared to ambient levels in the field (Simpson et al. 2016; Radford et al. 2016; McCormick et al. 2018; Hasan et al. 2018; McCormick et al. 2019). Even though the ambient tracks were louder, these studies did find significant behavioural changes due to anthropogenic noise.

Studies have found evidence that the impact of anthropogenic noise appears to be species specific (Voellmy et al. 2014; Magnhagen et al. 2017). Although our results were not what we expected, it does provide further evidence that the impact anthropogenic noise has on different behaviours in fish is species specific. Future studies should continue to examine how different species are impacted by anthropogenic noise, and furthermore, look at different methodologies that allow us to observe behaviour in the field to avoid alterations to the acoustical tests. Finally, having a longer acclimation period and continuing to try and get ambient playback tracks as close as possible to field levels would draw more confidence on how boat noise is influencing anti-predator behaviour in fathead minnows and brook char.

Chapter 3: The effects of boat noise on the stress levels of yellow perch (*Perca flavescens*)

3.1 Introduction

Although noise pollution is prevalent in many environments, it is a potentially greater threat within aquatic settings because of how easily it can be transmitted through water (Scholik and Yan 2002; Slabbekoorn et al. 2010; Voellmy et al. 2014). Even though motor boats pose a threat to aquatic environments, the majority of noise pollution research has focused on acute anthropogenic noise such as pile driving, seismic air guns, and sonar (Shannon et al. 2016; Mickle and Higgs 2018). However, motorboats represent a common and growing disruption to ambient noise within marine and freshwater environments (Simpson et al. 2016; Mickle et al. 2019). The majority of fishes communicate and hear within a 20-1000 Hz range, similarly, many motorboats emit a frequency that overlap within this range (Kasumyan 2005; Mann et al. 2007; Nichols et al. 2015; Mickle et al. 2019).

As previously mentioned, studies conducted in marine environments dominate the aquatic focus of noise pollution, whereas freshwater data are rare (Popper 2003; Villabos-Jiménez et al. 2017; Mickle and Higgs 2018). Although there are several examples of field experiments within a marine environment, freshwater experiments are slowly increasing (Bolgan et al. 2016). While studying noise pollution, there are certain advantages to conducting experiments within a field setting compared to that of a lab (Magnhagen et al. 2017). Lab experiments can offer controlled settings, unique to observing fish behaviours up close. However, field experiments are still considered to be the most reliable for understanding how anthropogenic noise influences organisms, because of the difficulty in accurately reproducing sound in a laboratory setting (Voellmy et al. 2014; Magnhagen et al. 2017). The primary concern with lab experiments involving boat noise is that playback tracks in a tank, or artificial environment, can have dramatically different noise profiles compared to those produced by motorboats in the field (Voellmy et al. 2014; Magnhagen et al. 2017). Although lab experiments can offer a more controlled experimental environment, they are controversial for whether their results can be ecologically relevant because of this difference in noise profiles potentially yielding a different result (Magnhagen et al. 2017). Therefore, with this experiment, we wanted to explore an ecologically-relevant field approach within a freshwater environment. We reasoned

that this would be an important step as field studies are common in marine environments but rare in the freshwater literature (Bolgan et al. 2016; Magnhagen et al. 2017).

In this experiment, we explored how being exposed to boat noise at different distances would impact yellow perch's metabolic rate, a measurement often used to gauge stress levels (Simpson et al. 2016). Fish were exposed to ambient conditions or to a boat roaming at varying distances to understand the spatial extent anthropogenic noise has on fish. As previously mentioned, noise can be easily transmitted in water and travel five times further compared to when in air (Slabbekoorn et al. 2010).

Therefore, understanding how distance can influence the stress levels of fish is an important question and one that, to our knowledge, has not been looked at in a freshwater environment. This experiment took place in a field environment and used motorboats to create the anthropogenic noise and not playback tracks. We hypothesized that boat noise would cause a significant effect on stress levels in yellow perch. Based on this hypothesis, we predicted that we would observe a greater change in dissolved oxygen (DO) consumption in yellow perch exposed to boat noise compared to ambient lake conditions. We further hypothesized the greatest change in DO consumption to be in those fish exposed to boat noise at the closest distance of 100 m. A similar experiment done within a marine environment by Simpson et al. (2016) found that fish exposed to boat noise produced a greater change in DO consumption compared to those only exposed to ambient sounds.

3.2 Methodology

3.2.1 Study Site, Species Collection and Care

The field experiment was conducted in Lake Diefenbaker, located in Southern Saskatchewan. With a surface area of 43,000 ha, Lake Diefenbaker is a large man-made reservoir primarily established for irrigation for the surrounding farmland (Saskatchewan Water Security Agency 2012). Because of its size it has become a popular spot for boaters and recreational activities such as fishing (Saskatchewan Water Security Agency 2012). The size of the lake allowed us to find suitable areas for our experimental setup. Furthermore, it was ecologically relevant to test the impact of boat noise on the fish in a lake that is a popular boating destination.

Juvenile yellow perch (~4 cm in length) were collected from Lake Diefenbaker using a seine net, 24 h before being used in the experiment. As mentioned previously, yellow perch are considered “hearing generalists” and, therefore, a conservative species to study for noise experiments (Roca et al. 2018). Furthermore, they were also selected for this study due to their abundance in Lake Diefenbaker, and their overall survivability. Once collected, the fish were quickly placed into 20 L buckets filled with fresh lake water, with approximately 10 fish per bucket. This stocking density helped ensure there would be no mortality overnight. The yellow perch were then left unfed and undisturbed for 24 h, except for periodic water changes performed to maintain temperature and dissolved oxygen levels. This acclimation period allowed the fish to calm down after capture and to evacuate their digestive tracks, therefore reducing inter-individual differences in metabolic rates (Simpson et al. 2016). After the trials and measurements were completed, the fish were released into Lake Diefenbaker.

3.2.2 Field experiment set up

The general objective of the experiment was to quantify the stress associated with boat noise exposure. This was accomplished by measuring the oxygen consumption of yellow perch held for 20 min under ambient (control) or boat noise conditions (boats roaming at 100, 250, 500 or 1000 m away).

Prior to the start of the experiment, we measured out a total distance of 1000 m from a starting point on the shore, referred to as point 0. Throughout this 1000 m stretch, we marked out stations located 100, 250, 500 and 1000 m away from point 0 along the shoreline. At each station, we submerged a 20 L bucket weighed down with sand, placed approximately 5 m into the lake from shore. The submerged bucket acted as the container that held our test fish during the trials (Figure 3.1). A total of two boats (n=2), both equipped with a Mercury 9.9 hp 4-stroke motor, were used to create the boat noise disturbance.

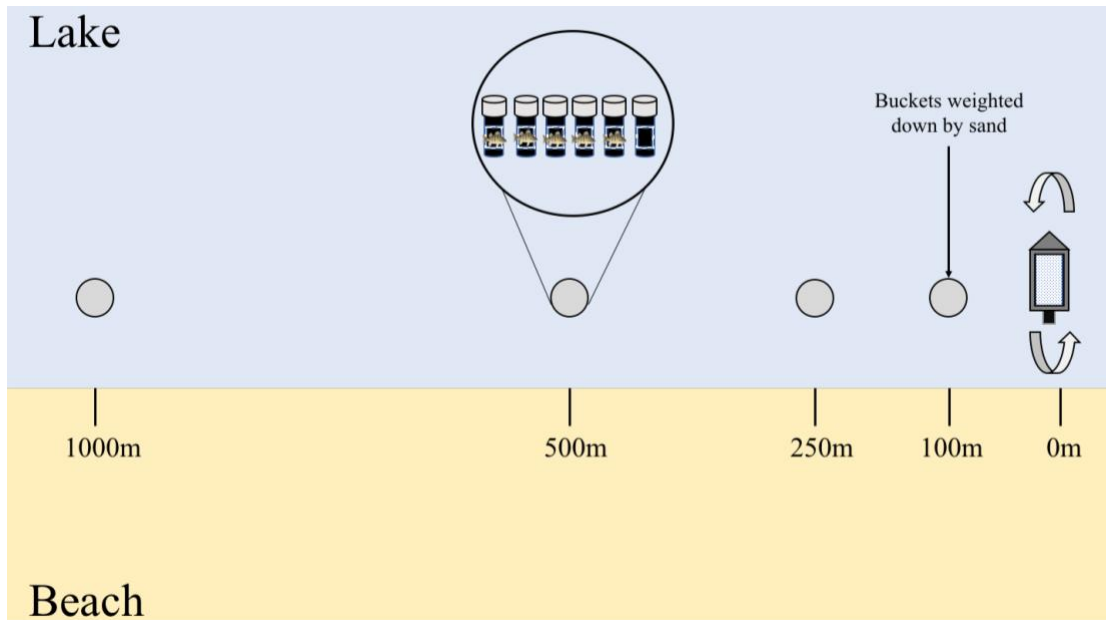


Figure 3.1. A top-view illustration depicting the experimental set up in Lake Diefenbaker.

3.2.3 Execution of experiment

The experiment started after the 24 h acclimation phase of the fish. To carry out this experiment researchers were divided into three teams. Team A was responsible for placing individual fish in jars and deploying the jars in the submerged bucket at each station. Team B was responsible for retrieving the fish at the end of the exposure phase, and then measuring the dissolved oxygen. Team C drove the boats during the exposure phase.

At the beginning of the first trial, team A started at the 100 m test point. It is important to note that we switched which end (100 m or 1000 m) team A started at for each trial to control for temporal factors. A 10 L bucket was filled with fresh lake water which was then used to fill six opaque bottles (Figure 3.1). The bottles were made of oxygen impermeable plastic and were covered in black duct tape to block light to prevent oxygen from being produced by photosynthetic microorganisms in the water. Team A placed a fish into each of the five bottles; in addition, a sixth bottle was filled similarly to the other five but no fish was placed inside. This blank bottle was used to measure changes in dissolved oxygen due to microorganisms in the water. Once a bottle was sealed, it was placed into the submerged bucket in the lake. Bottles were sealed and deployed at 1 min intervals.

Team B measured the initial dissolved oxygen of the water used to fill the bottles using a dissolved oxygen probe (ODO YSI Pro Plus). After the 20 min exposure period, this team gently retrieved the submerged bottles at 1 min intervals in the same order they were deployed. For each bottle, they waited until the 20 min mark, quickly uncapped the bottle and measured the dissolved oxygen levels in each individual bottle. Finally, before the fish were released, we weighed them using a scale (Sartorius AZ2101). Trials continued until we collected 30 measurements for each treatment (ambient, and boat exposure at 100, 250, 500 and 1000 m).

The data recorded includes the weight of the fish in grams, initial percent dissolved oxygen (*PreDO*), and post percent dissolved oxygen (*PostDO*) for both bottles with fish and the “blanks”. These values were used to determine the total change of ΔDO for the “blank” bottles and fish containing bottles. ($PreDO_{Blank} - PostDO_{Blank} = \Delta DO_{Blank}$) and ($PreDO_{Fish} - PostDO_{Fish} = \Delta DO_{Fish}$). Note that the DO values for each of the five fish containing jars were corrected by the change in DO with their respective blanks as described below.

The calculated changes in DO were then used to determine the change in oxygen saturation from just the fish. Furthermore, the weight of the fish was used to account for how the size of the fish factored into oxygen consumption. The final equation that gave us the value used in the statistics was $\frac{(\Delta DO_{Fish} - \Delta DO_{Blank})}{Weight_{Fish}} = \Delta DO\%/g_{Fish}$.

3.2.4 Statistical Analysis

Data did not meet assumptions and was non-parametric (Kolmogorov-Smirnov test on all four distances; $0.081 < Z < 0.183$, ambient $p=0.031$, 100-m $p=0.200$, 250-m $p=0.083$, 500-m $p=0.200$, and 1000-m $p=0.200$) and a Levene’s test revealed the data was heteroscedastic (Levene’s test, $F_{4,139}=2.816$, $p=0.028$). Therefore, in order to test if boat noise increased metabolic rate, or stress level, in yellow perch we ran a Kruskal-Wallis test ($\alpha=0.05$, 2-tailed), followed by a Mann-Whitney post-hoc comparisons to compare each boat treatment to the ambient control ($c=4$ comparisons). The alpha level was therefore corrected for multiple comparisons following Bonferroni correction, whereby $\alpha' = \alpha/c = \alpha/4 = 0.0125$. P-values that are compared to α' are denoted by P*. Finally, we looked at the average pre DO reading that was recorded before noise exposure, revealing that there was an average of 92.8% DO. The lowest reading was 70.7% DO and the highest reading was 106% DO.

3.3 Results

We found that fish exposed to boat noise significantly differed in oxygen consumption compared to fish exposed to ambient lake conditions (Kruskal-Wallis, $\chi^2_{0.05,4} = 14.823$, $p=0.005$, Figure 3.2). Further analysis revealed that fish exposed to boat noise at 100, 500 and 1000 m away were significantly different from ambient (Mann-Whitney U tests, $Z=-3.24$, $P^*=0.001$; $Z=-2.90$, $P^*=0.004$; $Z=-2.99$, $P^*=0.003$, for ambient vs 100, 500 and 1000 m respectively). However, fish exposed to boat noise at 250 m were not significantly different from ambient (Mann-Whitney U test, $Z=-2.33$, $P^*=0.020$).

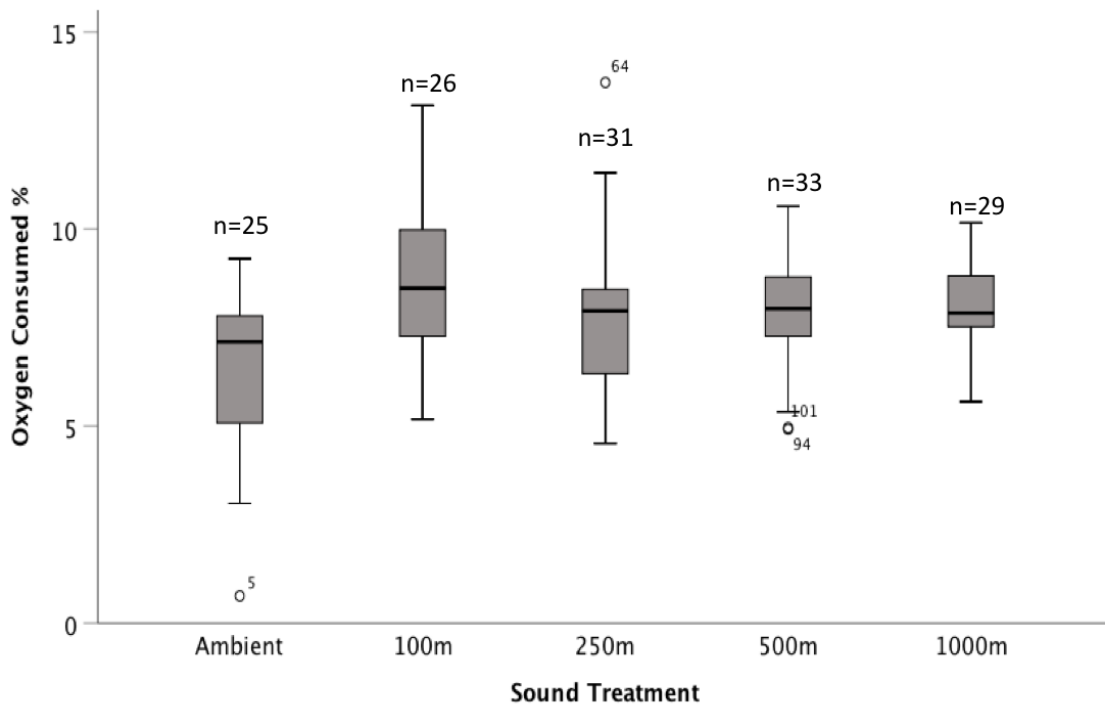


Figure 3.2. Boxplot illustrating the percent of oxygen consumed for fish exposed to ambient conditions or boat noise at 100, 250, 500 or 1000 m away. Box plot represents 1st quartile, median, 3rd quartile, minimum and maximum. Circles denote datapoints 1.5 IQR (interquartile range) away from the median.

3.4 Discussion

In this study we explored the effects of boat noise on metabolic rate in yellow perch. Furthermore, we examined how exposure to boat noise at different distances to the fish influenced stress levels. We observed that fish exposed to a boat roaming at 100, 500 and 1000 m away had a significant change in DO compared to fish exposed to ambient lake conditions. Fish exposed to boat noise at 250 m did not have a statistically significant difference compared to ambient; however, we do observe a trend of increased oxygen consumption when exposed to boat noise compared to ambient lake conditions. Based on the overall trend in our results, if we had more power within the 250 m treatment, we would expect to see a significant result at this distance as well.

This experiment reveals a trend that is similar to what other studies have found within this field; however, is the first experiment to our knowledge that looked at this effect within a freshwater lake (Simpson et al. 2015; Simpson et al. 2016). The overall trend of an increased metabolic rate and, therefore, increased stress levels is a physiological parameter that can be readily measured (Simpson et al. 2016). Although understanding how a certain pollutant can impact an organism's behaviour is an important parameter to study, this can sometimes be difficult to do within an ecologically relevant environment (Wysocki et al. 2006). By understanding physiological impacts, such as stress levels, this can give researchers insight to how a pollutant, in this case noise, is impacting an organism. Furthermore, it provides reasoning for why certain behaviours may be influenced by a pollutant (Simpson et al. 2015; Simpson et al. 2016).

Linking an increased metabolic rate and stress levels has been used to understand the physiology behind certain changes in behaviour within aquatic organisms exposed to anthropogenic noise (Simpson et al. 2016). Results of increased stress levels from noise pollutants, such as motorboats, may impact other physiological functions that can result in changes to certain behaviours (Simpson et al. 2016). Increased stress levels have been one of the potential causes for foraging and mutualism behaviour changes in fish exposed to boat noise (Magnhagen et al. 2017; Nedelec et al. 2017; Bracciali et al. 2012).

As previously mentioned, sound travels much faster and further in water compared to air, creating the potential for little shelter from this modern-day pollutant (Scholik and Yan 2001). However, few studies have looked at how exposure to boat noise at different distances may

influence stress levels or behaviour in aquatic organisms. Within this study, even a motorboat roaming at 1000 m away had a statistically significant impact on metabolic rate within yellow perch compared to when exposed to ambient lake conditions. This result highlights the potential lack of shelter aquatic organism may have from noise pollutants if they can still detect and are impacted by one motorboat at over a km away. Furthermore, it is important to note that we used a conservative motor for this experiment. A 9.9 hp motor is a smaller, less powerful motor you can purchase, with many boats being powered by much larger motors.

In summary, this study found a trend of boat noise causing increased stress levels in yellow perch compared to ambient lake conditions. It also revealed how motorboats can influence stress levels at over 1000 m away, showcasing how widespread this pollutant can be, especially in popular boating areas. With freshwater data on anthropogenic noise slowly increasing, it is important to continue to understand how new pollutants such as motorboats are influencing freshwater organisms. Little is still known on how disturbing ambient levels influences species and what consequences this poses for freshwater environments (Popper 2003; Villabos-Jiménez et al. 2017; Mickle and Higgs 2018; Currie et al. 2020).

Chapter 4: Discussion

4.1 The impact of boat noise on freshwater fishes

This study explored both a field and laboratory experiment to understand how boat noise impacts freshwater fish. Our field experiment observed that in the presence of boat noise, yellow perch had higher metabolic rates inferring a higher stress level. Furthermore, we detected a trend that the closer a fish was to the running motorboat, the more they were stressed, supporting our hypothesis that the closer a fish is to the source of the sound, the higher the stress level. The study also reinforced that even at 1 km away fish were more stressed compared to individuals exposed to only ambient lake noise. In contrast, we did not observe a change in anti-predator behaviour on brook char or fathead minnows when exposed to boat noise in our lab experiment.

In conclusion, our study did not find that boat noise impacted fathead minnows or brook char anti-predator responses. This contrasts with some publications that have observed that anthropogenic noise disrupts an organism's ability to assess risk, a behaviour that directly influences their fitness level and survival (McCormick et al. 2018). However, our field study did observe a trend of higher stress levels in yellow perch when exposed to boat noise. This study adds to the growing literature on how anthropogenic noise is impacting freshwater species. It is important not only to understand a significant impact but also to analyze experiments that do not produce a significant result. Both are equally important analysis and help to illuminate greater understanding for how boat noise is impacting our freshwater systems. Studies on anthropogenic noise is slowly advancing within freshwater environments, and therefore, it is vital to learn from different researchers' methodologies, approaches, successes, and failures. These experiments were the first to introduce distance as a factor for freshwater species and applied it in both a field and laboratory setting, providing contrasting results.

4.2 Ecological relevance

Behaviour in aquatic species has been studied for decades and are generally well understood. However, as humans continue to pollute, researchers are still learning how anthropogenic pollutants are influencing organisms (Hawkins et al. 2014). By understanding how anthropogenic noise can disrupt certain behaviours researchers can learn more on how this

pollutant impacts fitness levels and the potential impacts this will have on an ecosystem (Hawkins et al. 2014; Ferrari et al. 2018). Although research around anthropogenic noise pollution continues to centre around marine environments, freshwater populations are declining faster than marine (Reid et al. 2019). Results from marine experiments may not translate into freshwater experiments due to difference in topography, species and water chemistry (Mickle and Higgs 2018). Our research contributes to the growing body of knowledge for freshwater and supports the growing evidence for how boat noise is influencing freshwater ecosystems.

As previously mentioned, field experiment results are still considered to be more reliable for the impacts additional noise has on aquatic species. Primarily this is because of how acoustics can change within a lab setting. When using speakers to emulate a motorboat there are several limiting factors such as, sounds reflecting off of the tank can cause interference with the sound coming from the speaker, leading to frequency dips and peaks (Brown 2006). Furthermore, the reflections of the sound on the enclosure can cause further disturbances and distortions in the noise profiles that cause it to be different in comparison to noise profiles in the field (Voellmy et al. 2014).

The field experiment had an advantage of having the acoustics be authentic compared with the lab setting which relies on the use of playback tracks. Therefore, it is more difficult to extrapolate results from the lab experiment compared to field. However, as methodologies continue to develop for studying noise pollution in a lab setting, there have been promising experiments that have shown similar results between lab and field. In 2016, Simpson et al. conducted a field and lab experiment measuring metabolic rate with Ambon damselfish (*Pomacentrus amboinensis*). In the field, the fish were exposed to a running motorboat, whereas in the lab they were exposed to playback tracks. The results revealed both experiments yielded similar changes in dissolved oxygen percentage. This experiment gave more confidence to lab experiments and hope for an ability to relate it in a more ecologically sound context.

4.3 Future direction and conclusion

Although researchers are beginning to look at the physical, physiological and behavioural effects anthropogenic noise can have on aquatic animals, there still remain large gaps in the literature and our understanding of the overall effects this causes on ecosystem and community structure (Hawkins et al. 2014; Nedelec et al. 2017; Radford et al. 2016). Unfortunately, because

of the increased popularity of boating, it is predicted that rivers and lakes will see an increase in boating and fishing vessels making it important to understand how this increasing activity is going to impact fish (Hawkins et al. 2014). However, research examining how boat noise impacts freshwater fish species is rare and needs to be further explored to help mitigation efforts (Hawkins et al. 2014; Magnhagen et al. 2017; Mickle and Higgs 2018).

With noise studies starting to expand because of the increase in anthropogenic noise pollution, there is also a need to have solid methodological procedures to which researchers can adhere, allowing different research to be analyzed and compared (Hawkins et al. 2014). It is hard to say for certain whether lab studies produce realistic enough noise profiles to be reflected onto natural behaviour in the wild. It is therefore important to continue to test methodological approaches from previous studies in marine systems and species to understand if similar results will be seen in freshwater systems and species (Simpson et al. 2016).

The significance of this research is to aid conservationists and policy makers to be more informed when deciding on how to mitigate noise in freshwater systems. There are essentially two types of mitigation for noise pollution. The first is to learn and apply the results from biological research to minimize the effects of anthropogenic noise. The second is to look at the source of sound and how we can manipulate it to minimize harm to organisms. Our research on boat noise gives greater understanding to how boat noise may be impacting different freshwater fishes. Furthermore, the interest in how distance plays a role in stress on fish can help to find mitigation strategies and understand the dynamics of boat traffic on a lake, whether small or large (Hawkins et al. 2014).

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