THE BEHAVIOURAL EFFECTS OF ANTHROPGENIC NOISE ON RAINBOW TROUT IN A HOLDING FACILITY

By

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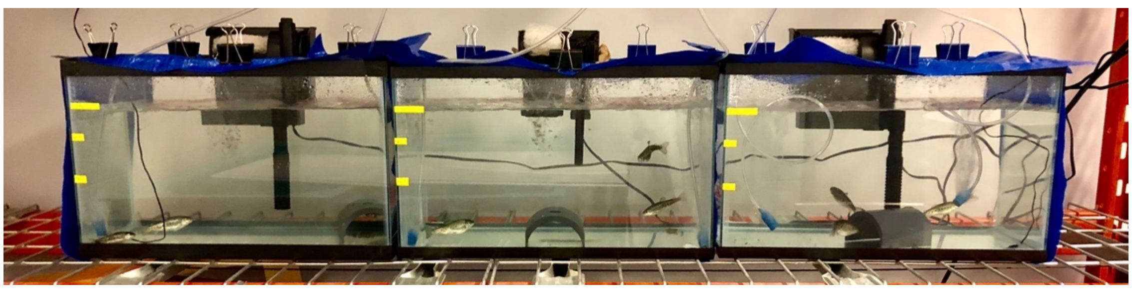

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THE BEHAVIOURAL EFFECTS OF ANTHROPGENIC NOISE ON RAINBOW TROUT IN A HOLDING FACILITY

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Major Advisor

Second Reader

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ABSTRACT

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Keywords: pile driving, anthropogenic noise, rainbow trout, Biology Aquatics Facility

There is a growing concern within the scientific community about the effects of anthropogenic noise on fish. Pile driving creates large amounts of noise, and especially in the creation of bridges it has the ability to affect fish. Additionally, fish are now being used in research studies within laboratories and can be exposed to a wide variety of noises. Chronic exposure to extreme noises can lead to an increase in stress, reduced growth, and ultimately mortality. The purpose of this thesis is to explore what behavioural effects sounds from pile driving and closing doors have on the behaviour of Rainbow Trout (*Oncorhychus mykiss*). To determine their effects, parameters measured were the amount of time spent hiding, in groups and in the upper portion of the tank, on a reference day, while the trout were exposed to recorded sounds, and during breaks with sounds that simulated pile driving and closing doors.

The effects to the test sounds were limited to the amount of time spent in groups, which was higher during the reference day when no noise was playing, suggesting fish were dispersing as a result of the sounds. No differences in the amount of time spent hiding or in the upper portion of the tank were found as they were approximately the same during the reference and test days. Although effects were limited to group behaviours, the effects of our tests sounds may have had an impact cortisol levels, which was not tested.

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ACKNOWLEDGMENTS

I would first like to thank my supervisor, Dr. Brian McLaren, for his continuous patience and support throughout the production of this thesis. Dr. McLaren's knowledge and understanding on the topic were an invaluable resource. Dr. McLaren went continuously over the top to help me in any way I needed, and I am truly thankful for this. I would also like to thank Dr. Patricia Alderson for her continuous help in guiding me throughout the Animal Use Protocol application process as well as welcoming me into the Biology Aquatics Facility to conduct my research. Dr. Patricia Alderson was also kind enough to be my second reader, with her veterinarian background I cannot think of anyone else who was more suited for this topic area. I would also like to acknowledge the support I received from registered veterinary technician Trista King who raised and cared for the Rainbow Trout used in this research project, without her, this study surely would have not been possible. Trista assisted me with a wide range of issues and questions I came up with and she truly made working with the Rainbow Trout a great experience I will not forget. Lastly, I want to thank the entire Animal Care Committee (ACC) for taking the time to review and approve my project. The ACC ensured I complied with all the ethical standards put forth by the Canadian Council on Animal Care. Thank you.

INTRODUCTION

Anthropogenic noise has long been a concern with respect to its effects on fish in their natural habitat. With the introduction of new technologies to develop and manage the natural environment, such as pile driving equipment, noise sources have been increasing in number; do these noises affect the behaviours of fish? Studies have shown that fish use sound in determining the location of their prey, as well as in communication for defending territories (Amoser 2005). Humans for the longest time were limited in the places where they could live; however, with advancements in technology, we now see humans living in more remote areas, resulting in increased land development. Sound affects fish differently depending on the sound's duration, the fish's specific hearing ability, and the particular fish species (Wysocki 2007).

Over the past decades, there has been a substantial increase in anthropogenic noise from development around water bodies with the creation of bridges and buildings. The noises are able to penetrate water, polluting the underwater environment with noise. Noise pollution affects the natural noises fish hear regularly such as water currents, prey and moving sediments. Exposure to different types of noise such as those from pure tones and broadband white noise ranging from 142 to 170 dB have been known to cause temporary hearing loss in fish (Popper and Clarke 1976).

The increased stress levels created from noise could have effects on growth, reproduction, disease susceptibility and the overall survival of the species (Wysocki 2007). To understand this topic further, it is crucial a controlled environment is used to

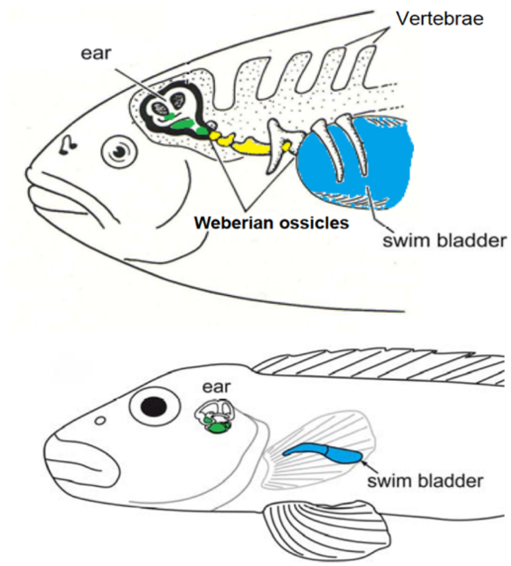

Figure 1 Different hearing structures of fish. What is considered to be a hearing specialist is on top, a hearing generalist on bottom (Popper et al. 2006)

ensure factors such as water quality and temperature are standardized. This study was conducted over the span of ten days in Lakehead University's Biology Aquatics Facility. Using different external sounds, such as those created from pile driving and the loud sound created from a closing door, captive Rainbow Trout (*Oncorhychus mykiss*) were watched using a camera for changes in their swimming patterns and behaviour as result of being exposed to the noise. The recorded noise was played every three minutes to replicate actual events the fish could encounter in the wild (repeated pile driving), as well as in the lab (closing doors). Three tanks housed three fish each. Each tank had dividers on either side to ensure the reactions were separate among tanks and test subjects. All three tanks were placed in the same room to ensure environment standardization. All fish were placed at the same distance from the audio source. Factors such as water quality, temperature, light and any other disturbances were controlled to ensure accurate test results between individual test subjects.

STATEMENT OF PURPOSE

The intent was to obtain a better understanding of how development around water bodies should take into consideration the noise they are producing and how it may be affecting local populations of fish.

The aim of this thesis was to study sound-induced behavioral changes in fish using captive Rainbow Trout as a model species. I explored short term behavioural parameters, which are indicators of sound related stress, disturbance and deterrence. Here, I examined the effects of two different sounds ranging in frequency and loudness to provide insights that may benefit future studies of indoor and outdoor sound impact studies. Furthermore, my findings may also raise awareness for sound levels around construction projects and to show construction companies and other researchers the importance of taking fish hearing into consideration.

RESEARCH QUESTIONS AND HYPOTHESES

1. Is there a difference between the amount of time spent cover seeking during the audio playback of a door closing and pile driving and during periods when there is no sound being played? Hypothesis: There will be more time spent cover seeking during audio playback.
2. Is there a difference between the amount of time spent in groups during audio playback of a door closing and pile driving and during periods when there is no

sound being played? Hypothesis: There will be more time spent in groups during audio playback.

3. Is there a difference between the amount of time spent in the upper portion of the tank during audio playback of a door closing and pile driving and during periods when there is no sound being played? Hypothesis: Fish spend a greater amount of time in the upper portion of the tank when no noise is being played.

LITERATURE REVIEW

Until recently, the effects of sound on fish have not been studied extensively. Additionally, how sound affects their behaviour and overall general health was somewhat a mystery. Many people argued that sound does not affect the behaviour and health of fish and therefore is not worth investigating. Prior to sound studies conducted in the early 1800's by Swiss physicists like Jean-Daniel Colladon, it was presumed that the underwater environment was more or less silent. This was largely due to our lack of knowledge of how sound travelled and because our air-adapted human ear is not adapted at picking up underwater sounds. However, with advancements in what we know about a fish's dependence on hearing, there is a growing global concern about the effects of human generated sound on fish and other aquatic organisms.

The natural environment is exposed to a wide variety of noises on both land and in water. These range from noises created by wind and water currents to those created from anthropogenic sources such pile driving and seismic surveys. The main effect of these noises on fish is to pollute the underwater environment, ultimately to affect the hearing of species that depend on it for hunting prey (Popper 2008). In general, anthropogenic noise caused by humans has the ability to mask biological sounds and negatively impact the behaviour of fish that are acoustically dependent. The purpose of this literature review is to examine what studies have been conducted to date on the effects of anthropogenic noise on fish and what is known already about the potential impacts of noise on fish species that occur in close proximity to human activity.

In a paper published by Slabbekoorn (2012), the effects of anthropogenic noise on Zebrafish (*Danio rerio*) was examined thoroughly in a lab setting using two tanks connected via a tunnel. Both tanks contained an underwater speaker that would alternately play different noises at varying frequencies; the fish would then be watched for changes in their behavior. After being exposed to sounds, the fish would be monitored to see whether or not they would avoid the sound by swimming through a tunnel to the other (quiet) tank. The fish confirmed that they were hearing the noise by attempting to move escape/move away from it. Fish exposed to noise, such as the ones used in Slabbekoorn's study, demonstrated an increased heart rate and elevated cortisol levels as a reaction to the noise (Wysocki et al. 2006). Other effects that were witnessed included startled responses and erratic swimming movements, sudden changes in swimming speed, strengthened group cohesion, and a delayed first entry to the upper half of the tank.

SOUND FROM PILE DRIVING

Pile driving is the process in which poles are driven into the soil during construction; it later provides the foundation for buildings and other structures. The process requires the use of large machinery to lift large weights to a predetermined height, from which they are then released and allowed to collide with the pole below

(California Department of Transportation 2015). Impact sounds result from a rapid release of energy that occurs when two objects hit one another. The impact of the weight colliding with the pole creates a substantial noise that can be heard from kilometers away. When a pile-driving hammer strikes a pile, the impact sound propagates in the air and a pulse propagates down the length of the pile. There is also a significant vibration generated from pile driving that can be felt in the surrounding buildings and a large surrounding area depending on the soil type. The severity of vibrations depends on the size and nature of the pile driver chosen. The different pile drivers available in construction projects include vibratory, impact hammer and push.

The impact of pile driving also includes flexural stress waves in the wall of the pile that couple with surrounding fluids (air and water) to radiate sound. The effects of pile driving on fish can depend heavily on the frequency of the sound; continuous sounds may also produce different results than a sequence that has a “recovery” time between pile drives (California Department of Transportation 2015). In most cases, sounds of pile driving under water are characterized by multiple rapid increases and decreases in sound pressure over time. Thus, it is possible that certain locations could receive higher levels of sound farther away from the pile than at locations closer.

EFFECTS OF PILE DRIVING ON FISH HEARING AND BEHAVIOUR

A paper published by Blenkle (2010) reported that the damage incurred from being exposed to noise from pile driving depends on its duration and severity. Temporary hearing loss (also known as temporary threshold shift) can occur when fish are exposed to higher levels of noise for shorter periods of time. Exposure is affected by factors such as the repetition rate, frequency, duration and life history of the fish. The study, conducted in Northern Scotland, examined the effects of pile driving sounds on two species of fish (cod and sole) in the marine environment, using two large net pens placed offshore and a specialized underwater loudspeaker that was capable of playing back sounds up to 170 dB (Blenkle 2010). The net was fastened to the sea floor on a relatively flat surface in the proximity of the speaker. The researchers were successfully able to replicate the sound of a pile driver that was located far away and also examined the particle motion created, which is considered of great importance for species that are sensitive to particle motion. Almost 45% of the cod and 32% of the sole showed signs of movement or spatial response to the sounds of the researcher's pile driving.

In a similar *in situ* study, researchers examined the behavioral effects of vessel noise on Bluefin tuna (*Thunnus thynnus*) housed in a cage on the Mediterranean Sea floor. They witnessed changes in swimming direction, an unconcentrated swimming structure and uncoordinated swimming behaviour (Sara et al. 2007). This study ultimately showed that noise pollution caused behavioral deviations within tuna. Another study was conducted on the behavioral reactions of Shiner Perch, Chinook Salmon and Northern Anchovy to the noise created from pile driving (Abbott and Marty 2004). Fish were placed in cages 25 feet below water and the pile driving equipment was approximately 32 feet away from the fish. Researchers reported no behavioral changes in the fish after examining them for one minute.

EFFECTS OF PILE DRIVING ON FISH ANATOMY AND PHYSIOLOGY

How fish experience stress is much harder to identify than the case for mammals; quantifying this measure is even more difficult. Previous studies have shown that increased background noise or exposure to sudden large sound waves affects human physiology in a negative way, so it would be safe to assume they would affect the physiology of fish (Hastings et al. 2005). These effects do not always result in damage or sudden death; a more common assumption for fish is a subsequent decline in health over time resulting in higher susceptibility to predation. For example, vibration of an aquarium's walls was shown to create a slow decline in health of Rainbow Trout with effects visible one to five days later (Gilham et al. 1985). The decline was determined by examining serum cortisol levels.

Studies have examined the effects of pile driving on a fish's eyes, swim bladder and overall health. Caltrans (2001) examined the effects of pile driving on fish located at varying distances to the pile driving. Fish located 50 m from the sound source suffered high rates of mortality with bleeding and damage to the swim bladder. The amount of damage and mortality was greater for fish that were located closer to the source. Similarly, Abbott and Bing-Sawyer (2002) investigated the effects of pile driving on health and mortality in Sacramento Blackfish (*Orthodon microleidotus*). Fish were housed in cages under water at varying depths and then were exposed to 40 pile drives that would reach levels of approximately 193 dB per strike. Upon completion of the study, fish were examined for both behavioral changes and damage to organs. After examining the fish for 5 h, the researchers reported no noticeable changes to the fish's behavior. Autopsy revealed significant damage to the swim bladder of fish located closer to the pile driving source than those farther away. In another study, Shiner perch and Rainbow Trout were exposed to pile driving sounds at varying distances ranging from 23 to 314 m from a pile driving operation (Caltrans et al. 2004). Fish were then observed for 48 h for any changes to their behaviour after exposure to the sound, before being frozen for necropsy. As with previous studies, results showed no changes to behaviour but varying levels of trauma to organs among both fish species, more so in *Cymatogaster aggregata*. However, fish that were housed behind a bubble curtain to reduce the impact of the sound experienced less trauma then those that were not shielded.

EFFECTS OF DIFFERENT SOUNDS ON FISH

There have been several studies on the effects of different sounds on different species of fish. Enger (1981) examined what occurred when Atlantic cod were exposed to a high-intensity sound for an extended duration of time. The high-intensity pure tones resulted in damage to sensory hair cells. Auditory hair cells are critical to a fishes hearing ability, and the damage was determined by using an electron microscope able to see sensory hair cells. Hastings et al. (1996) examined the effects when freshwater fish (specifically velvet cichlid) were exposed to an hour-long continuous 180 dB sound, which similarly caused damage to the fish's sensory hair cells. The sound from an air gun at a 1-m distance, which reached a level of over 222.6 dB, caused considerable damage to the ciliary bundles of the sensory hair cells of the saccular sensory epithelium in *Pagrus auratus* (McCauley et al. 2003). Sound recorded from road traffic was played back underwater in the 90-100 dB range to the Otophysan Fish (*Cyrinella venusta*) and the sound was causing significant rises to cortisol levels in the test fish (Crovo et al. 2015). In addition, hair cell damage was observed and is thought to have resulted in a shift of hearing sensitivity. These results demonstrate that even when a fish is exposed to high pitch sound, damage may only be seen days later, raising the question of how many fish are being affected undetected when a loud sound is produced in the environment.

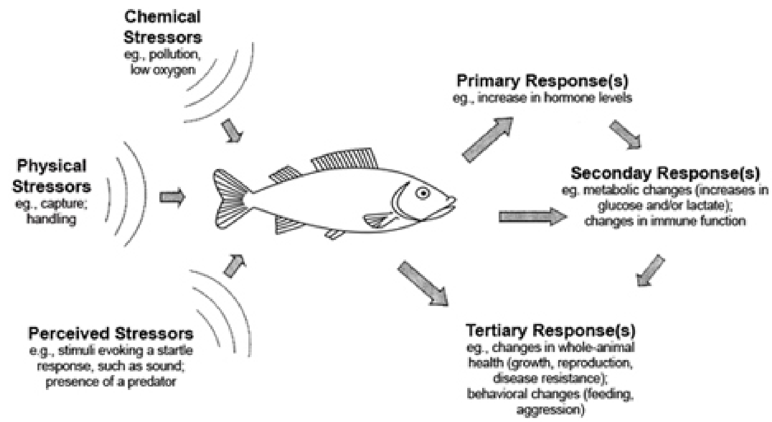

Figure 2. Different stressors fish can experience; they include physical, chemical and other perceived stressors, which can cause primary, secondary and/or whole-body responses (Barton 2002).

FISH ADAPTING

Animals have been known to adapt to unusual noises in their environment by a means of switching modality (Radford 2014). Animals have multiple ways to communicate with one another, and if one modality fails another will be used, as demonstrated in Three-spined Sticklebacks (*Gasterosteus aculeatus*). Another example includes the Rock-Dwelling Cichlids (Teleostei: Cichildae) of Lake Malawi, which normally depens on the use of acoustic, chemical and visual cues in the mating process, as water at the depths they occupy offers little light. Given that they have three different ways of relaying messages, it would be expected that if one of these cues became muffled or distorted from an anthropogenic noise like a boat motor, a greater dependence will occur in the other cues.

FISH HEARING IN AQUACULTURE FACILITIES

There have been several studies that have examined the effects of long-term noise exposure on fish in aquaculture facilities. Fish in aquaculture production facilities are exposed to various noises created from equipment such as filtration systems, water pumps, aerators and blowers that are capable of increasing ambient noise levels within their tanks. Several fish species, including Rainbow Trout, are artificially bred in captivity and are used extensively in research studies that have varying levels of sound. For hearing specialists (fish with specialized hearing structures) the noise can cause hearing loss, whereas with fish considered to be hearing generalists may experience no effect on hearing. Rainbow trout are considered to be hearing generalists whereas species like catfish are considered to be hearing specialists. Smith et al. (2004) examined the effects of being exposed to 170 dB of white noise over the span of 20 d in Goldfish and Nile Tilapia. Significant hearing loss occurred in Goldfish, which are considered hearing specialists, whereas Nile Tilapia, a hearing generalist, experienced no effect. Davidson et al. (2008) reported no changes to Rainbow Trout growth or survival when exposed to intensive white noise played at 150 dB. The fish did, however, exhibit a stress response when exposed to bursts of 149 dB sound but seemed to acclimate over time. Acclimation of loud noises is crucial for normal function in Rainbow Trout, as they are found in noisy natural environments, such as rivers with fast moving water and coarse sediments. In contrast, fish such as Catfish and Carp have more sensitive hearing and could be impacted by the noises created in aquaculture facilities as they are considered a hearing specialist with a boney connection between their swim bladder and inner ear (Wysocki et al. 2007). Not all sounds in aquaculture facilities are detrimental for the fish; for example, the sound created from automatic feeding systems results in a positive reaction associated with feeding, a positive occurrence (Crovo et al. 2015). Novel sounds, however, may induce behavioral changes due to anxiety or curiosity (Neo et al. 2015).

CASCADING EFFECTS OF NOISE ON OTHER SPECIES

Anthropogenic noise created by humans may have cascading effects that go beyond single species. Anthropogenic noise, such as the noise created from pile driving, may cause shifts in relative species densities in a given area, thus effecting prey and other species (Saeed et al. 2016). Different noises may result in declines of certain species and increases in others. Disturbance can increase the amount of time a fish hides or retreats leading to shifts in foraging efficiency, which can have effects on the availability of food to predators (Purser et al. 2011). Furthermore, noise induced stress could also reduce the speed at which a fish reacts to approaching predators, increasing its chance of mortality. Effects of water pollutants other than noise have already shown not only to affect fish, but also other species which rely on them as a food source. Surface feeding fish use the sounds surface waves in order to locate prey such as insects that have fallen into the water (Hoin-Radkovsky et al. 1984). Such predators that use noise to hunt may be at a disadvantage when found in a noisy environment. Purser and Radford (2011) examined how foraging performance was affected by brief and prolonged periods of noise and concluded that the playback of anthropogenic noise affected swimming behavior, resulting in a decreased prey attack efficiency and increased food handling errors. Herring were found to be able to detect ultrasound produced by whales and avoid them (Doksaeter et al. 2009).

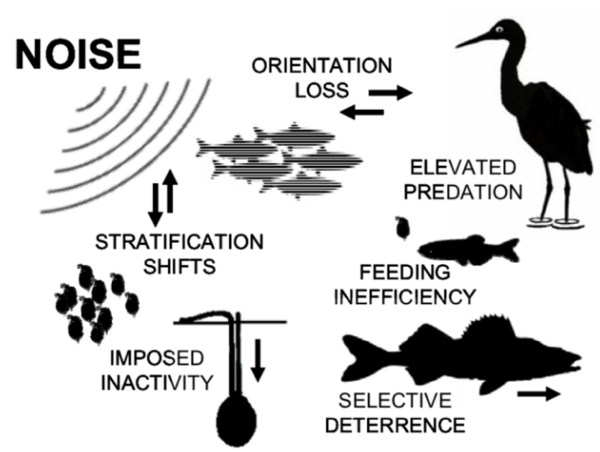

Figure 3 Examples of how anthropogenic noise can cause shifts in relative species densities in horizontal and vertical directions. These effects may go far beyond single species effects. (Popper et al. 2016)

THE PROCESS OF SOUND TRAVELLING THROUGH WATER

Hearing is the detection of propagated vibratory energy by the ear (Gans 1992). Throughout history, there have been many theories proposed as to how exactly sound travels through water and is subsequently heard by other creatures. Our understanding of how sound travels through air, and most importantly through water, has evolved and changed. Greater advancements in both technology and knowledge would ultimately allow us to prove and disprove theories. Sound is actually capable of travelling at far greater speeds underwater than it is above water because particles are located closer together (Chavez and Sosa 1984). Particles under water are better capable of transmitting vibration to one another.

All hearing is based on the mechanosensory hair cells transducing vibrations into electrical signals (Nedelec et al. 2016). The vast majority of mammals use the sound pressure element of sound whereas fish and many invertebrates use the particle motion component. Sound is propagated vibratory energy, and so sound waves propagate because particles next to a vibrating source are moved back and forth in an oscillatory motion (Gans 1992). Further, these particles then move the particles next to them and so on which results in the creation of vibratory energy. Particle oscillations can either be detected directly by hair cells that protrude into the medium (air and water) or by the relative motion between the body and a solid structure in the ear to which the hair cells are attached (Nedelec et al. 2016). With the bodies of fish being composed of mainly water, they are coupled directly to the medium, in this case the water (Banner 1968). With this in mind, the whole body of the fish vibrates as a sound wave passes through water, the reason the concept of particle motion in water is very important.

METHODS

To assess the effects of varying sounds on fish, a species that could represent a wide range of cold water habitats found in Canada was selected, *Oncorhynchus mykiss*. This study was conducted in Lakehead University's Biology Aquatics Facility over a duration of 10 days during the month of January 2018.

ANIMAL MAINTENANCE AND HOUSING

Three tanks were set up in a separate room in the facility (BAF Room CB0026L), which was found to be substantially quieter than the rest of the facility (Figure 8). The fish were housed in three long stock tanks (10 x 20 x 11 inches) with each tank having its own filtration and aeration systems. Separating the study fish from the rest of the facility behind a closed door created a situation in which other noises did

Figure 4. Tanks inside the Aquatic facility housing numerous fish species. Filtration systems can be seen towards the bottom left and bottom right (Rugo 2018).

not interfere. The tanks were placed beside one another with a blue privacy divider as to not allow the fish to see the other tanks and possibly react to another fish's response to

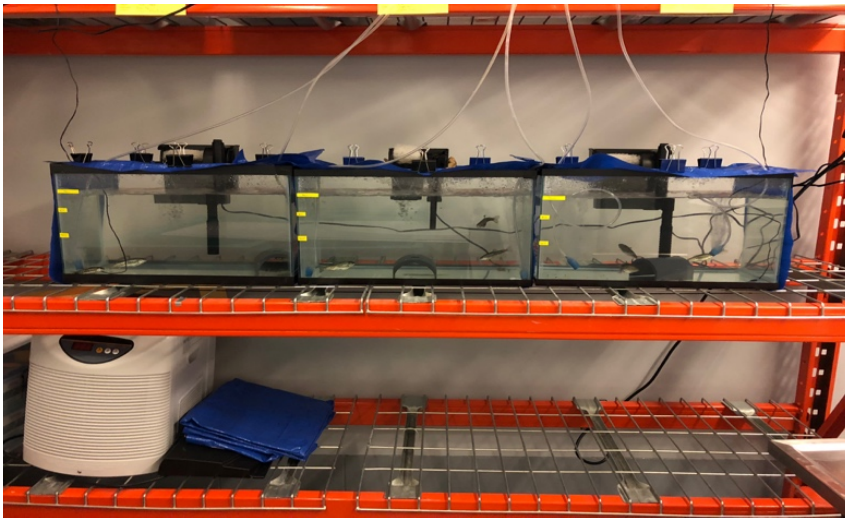

Figure 5. Three tanks used to study the effects of noise on Rainbow Trout. Each tank was separated with a blue divider. There were three fish per tank and all conditions such as water quality and temperature were standardized (Rugo 2018)

the study sounds. Daily water changes of approximately 50% were provided for all three tanks during the study, and water conditions were kept in all tanks as similar as possible.

Background noise was measured and maintained between 60 and 65 dB, much quieter than the rest of the BAF. Routinely daily care such as feeding, water quality testing, and maintaining temperature, lighting and pH levels was conducted and monitored by a Registered Veterinarian Technician within the facility. The fish were kept on a 14/10 light/dark cycle (light switched on from 6:00 am - 7:30 pm) and were fed dry food once a day using commercial trout chow. For this study, nine Rainbow Trout were used, divided into the three static tanks, and given an acclimation period of ten days to their new environment.

ARTIFICIAL NOISE STIMULUS PREPARATION

Once the acclimation period was over, the fish were exposed to the different test noises, which included a recorded pile driving noise and door closing noise. Two sound treatments were used with varying temporal patterns: a continuous sound represented by a pile driving noise and an intermittent irregular sound represented by a closing door. The sound of pile driving would be played for three continuous minutes and during that time the fish would be watched for different reactions to the test sound. The continuous sound represented by pile driving ran at a fast pulse rate (1-1; Figure 6). After the pile driving noise was played, a rest period of 10 min was provided to allow the fish to relax and recuperate before the next sound was played. During the break time, the decibel reading in the room ranged between 64-65 dB. After the break, the intermittent irregular sound represented by the recorded door closing sound was then played every 30 s for 3 min before again giving the fish another break period. As each sound was played, the fish would be observed for changes in behavior. The overall elapsed time to play each set of noises with rest periods taken into consideration was approximately 52-53 min, which was repeated every day for 10 d. A reference day was selected during the study period where no noise would be played, fish would be watched for their natural behaviour in a quiet environment. The reference day would later be used to determine the effects the test sound were having on the fish during audio playback.

ETHICAL APPROVAL

In regard to the ethical treatment of the study fish, testing done in the Aquatic facility required special permission from the animal care committee (ACC). This project would have not gone ahead without first being reviewed and approved for its ethical practices by the ACC. The animal use protocol (AUP) took approximately two months to plan and gather the necessary information before being submitted for review. After submission, Dr. McLaren and myself (Cameron Rugo) attended the November 21st meeting held by ACC members to review the project proposal and make any necessary changes. At first, break periods were set to 7 min, but the committee recommended they be raised to 10 min to ensure the fish had enough time to take a break between test noises.

TRAINING

In order to be allowed to conduct research within the aquatic facility, various training modules needed to be completed before beginning. These training modules included WHIMIS training, Worker Health and Safety Awareness Training, completion of CCAC Training Modules and an in-person training session inside the actual facility facilitated by a registered veterinarian technician. This training was required to minimize the chance of injury during the study and to ensure animals were being treated ethically and humanely.

DATA COLLECTION

Data was collected with an iPhone 8 plus to record reactions of all fish during the play back of our test sounds. Footage was recorded at resolution of 1080 pixels and 60 frames per second to make playback viewing easier and clearer. The camera was placed 1.3 me away from the tank and was not moved for the duration of the study to ensure all footage was the same. After all the footage was recorded during the ten-day study period, it was edited using Final Cut Pro to make shorter video clips, for easier analysis. The videos were split according to the test sounds: pile driving, door closing and break.

STATISTICAL ANALYSIS

I compiled the data in SPSS and used a repeated measures ANOVA for the ten-day dataset comprising of separate tests for total time (during the 3-min interval) spent hiding, total time spent as part of a pair or group of three (defined as a maximum distance from a neighboring fish of 3-4 cm), and total time spent in the upper portion of the tank (swimming up). Swimming in the upper portion of the tank was considered to be whenever a fish swam above the yellow 50% fill marker located on every tank. Bonferroni-adjusted, pairwise comparisons followed each ANOVA done to assess specific differences between noise conditions, with a significance level equivalent to 0.05. A fourth three-way ANOVA was done for the three variables recorded during the reference day, this would be later used to determine what effects the sound had on the fish.

HUMAINE EUTHANASIA

In order to end the study, the test fish were humanely euthanized following strict animal use protocols under the supervision of a registered veterinarian technician. As the fish were raised in an aquaculture facility, they cannot be released due to the strict government regulations put forth as they are considered to be study fish.

RESULTS

Playback of the pile driving noise reached a peak of approximately 90 dB every six milliseconds, the peak noise signaling the driver hitting the head, the loudest part of pile driving(Figure 6). Background noise of the pile driving sound track never fell below 70 dB with the exception of once at the 121-millisecond mark.

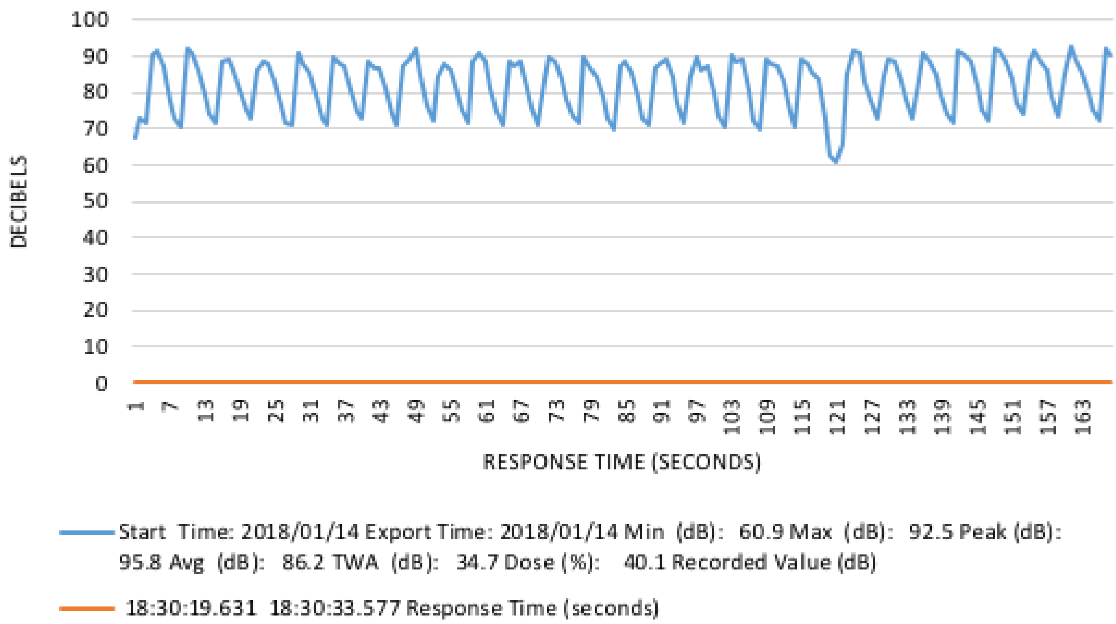

Figure 6. Recorded decibels in study room during playback of pile driving noise (Rugo 2018).

The sound used to represent a loud door closing within the facility reached approximately 95 dB at its highest point (Figure 8). During the break, sound levels in the

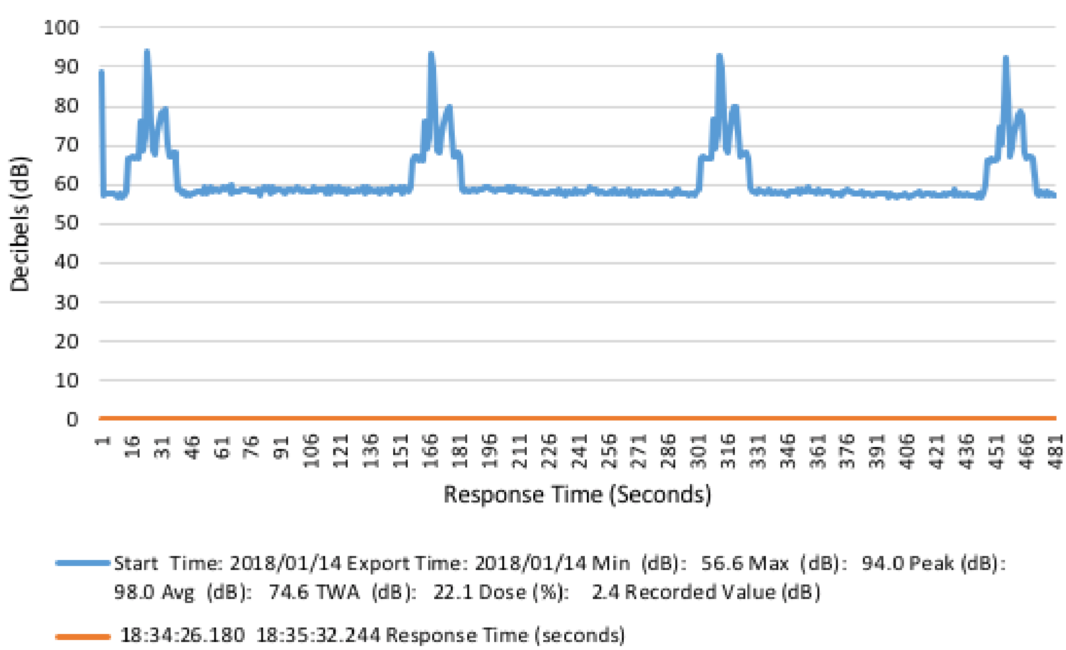

Figure 7. Recorded decibels in study room during playback of a door closing (Rugo 2018).

room would decrease to approximately 60 dB, which represented noise created from the filters and aerators in the room.

A behavioral reaction to the test sounds was limited to grouping when the ten test days were compared to the reference day ($F_{3,8} = 3.96$, $p = 0.02$; Figure 9). Time spent in

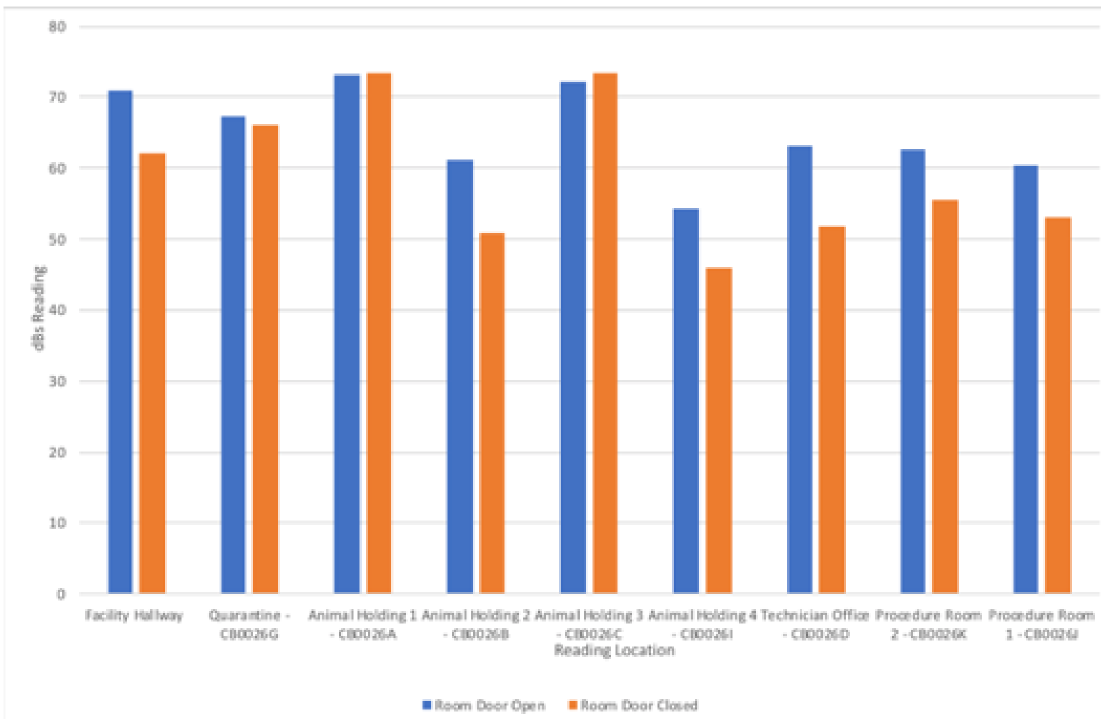

Figure 7. Sound readings inside the BAF with doors open and doors closed (Trista King 2017).

groups was higher during the reference day when there was no noise playing (comparing door closing, $p = 0.04$, and pile driving, $p = 0.04$) and it was significantly lower during audio playbacks, and nearly significantly different between breaks and during audio playbacks ($p = 0.07$), suggesting fish were dispersing as a result of the noise. There were no differences in hiding ($F_{3,8} = 0.17$, $p = 0.92$) and swimming up ($F_{3,8} = 0.04$, $p = 0.99$). Fish were hiding for approximately the same amount of time during the reference and test days.

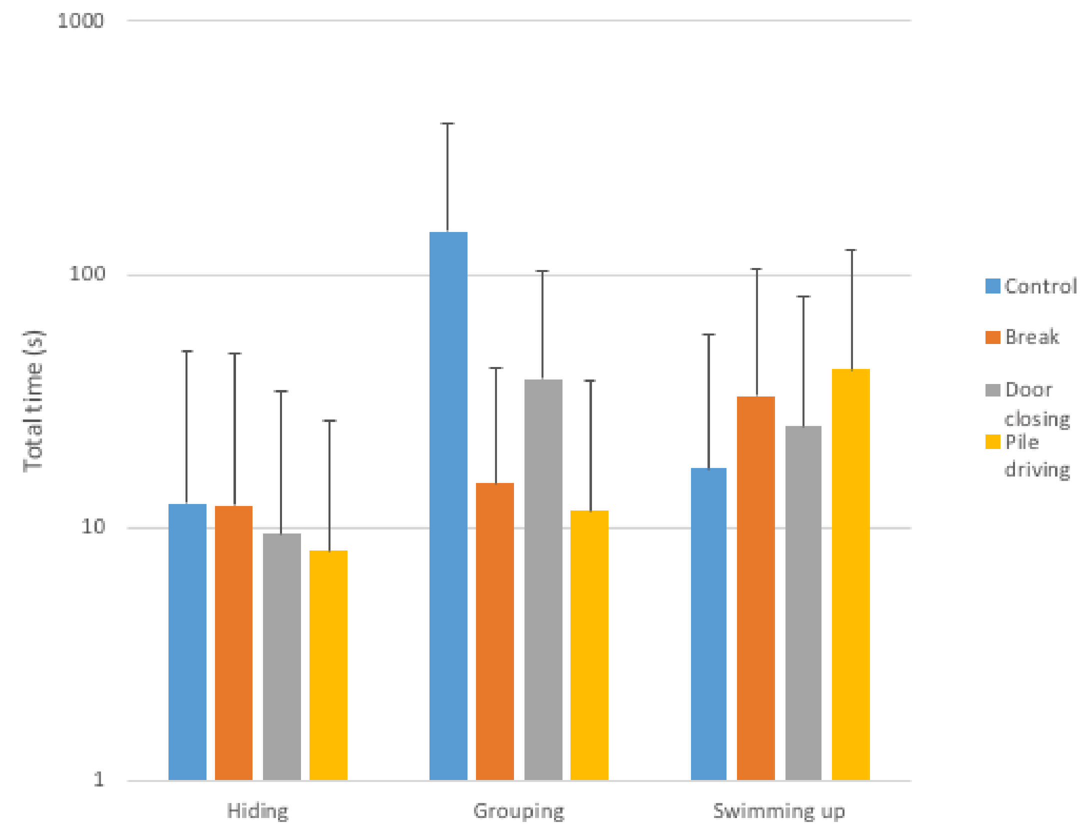

Figure 8. Amount of time recorded hiding, grouping and swimming up when one test day is compared to the reference day; the error bars show 95% upper confidence limits in estimates.

There were, on the other hand, no significance differences in hiding ($F_{3,8} = 0.82$, $p = 0.48$), grouping ($F_{3,8} = 3.86$, $p = 0.07$), or swimming up ($F_{3,8} = 1.18$, $p = 0.36$) over the ten repeated-measure days (Figure 10).

DISCUSSION

TIME SPENT IN GROUPS

Opposite to what was hypothesized, there was a smaller amount of time spent in groups during a day of audio playback when compared to the reference day, suggesting fish were dispersing as a result of the noise. Possibly, Rainbow Trout are just not as inclined as other fish species to swim in groups. Rainbow Trout enjoy swimming against

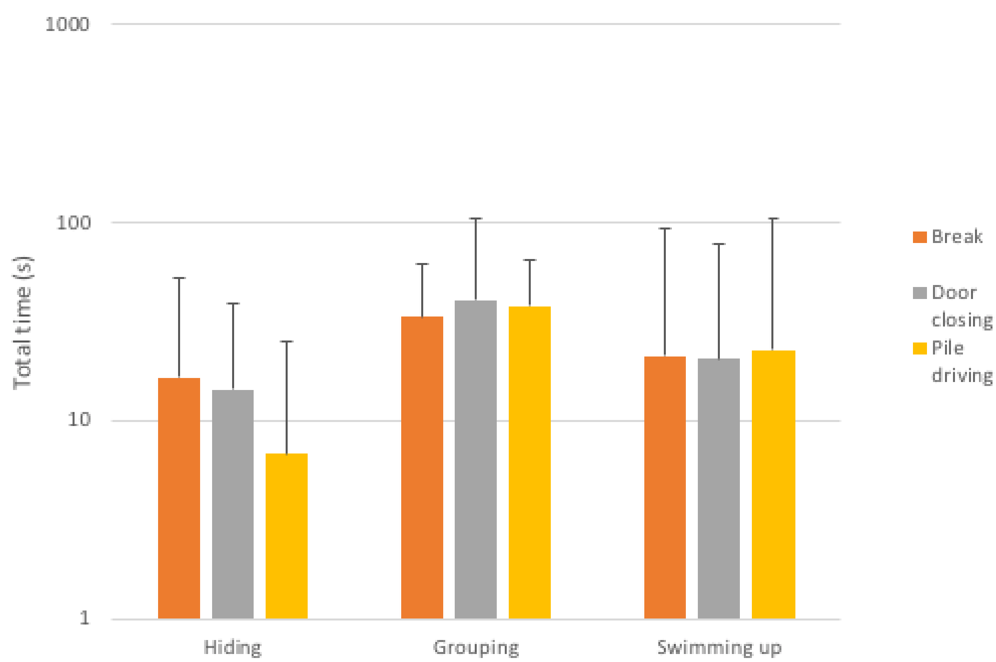

Figure 9. Amount of time recorded hiding, grouping and swimming up over ten repeated-measure days; the error bars show 95% upper confidence limits in estimates.

strong currents as they do in nature. Further, dispersal may be an evolutionary defense mechanism which would allow for survival of the fittest when being chased by prey.

The tanks where the fish were housed are considered to be small when compared to open water, they do not allow for the fish to escape like they normally would if found in another scenario. Furthermore, when comparing the tank environment to that of a lake or river, elements such as rocks, vegetation and sediment help better reflect sound waves and an *in situ* study may have different outcomes (Glushkov et al. 2013).

TIME SPENT IN THE UPPER PORTION OF THE TANK

Time spent in the upper portion of the tank did not show any major differences during the playback of our audio and break periods or when comparing the test day to the reference day. Some fish demonstrated a repetitive behaviour wherein they swam behind the filter intake pipe, which was producing a strong current. Rainbow Trout in lakes and rivers are accustomed to swimming in strong currents and is most likely why this was witnessed. Other times recorded as swimming in the upper portion of the tank included when a territorial fish chased another fish from its hiding location. Both these behaviors were witnessed regardless of noise being played and suggests the test noises did not impact the fish's placement within the tank.

TIME SPENT HIDING

The amount of time spent hiding during the playback of our test noises did not change with either of the sounds. More often than not, certain fish would demonstrate a repetitive behaviour in regard to hiding. Other fish that did not regularly hide would rarely seek cover during audio playback for fear of other territorial fish. These non-hiding fish would remain in their particular position within the tank and rarely venture into the hiding place provided. Hiding was considered to be whenever a fish would swim under the hiding place (cut in half piping) provided, and I did not interpret staying in a corner or swimming behind the filter cartridge to be considered hiding even though this could most certainly be interpreted to be hiding.

GROWTH RATES, HEALTH AND MORTALITY

Over the course of this study, no mortality was encountered. The sounds played during trials were purposely set to levels that do not cause the fish damage. One effect that was observed was dominant fish, such as fish 1, were better able to intercept food giving it a significant advantage in growth rate (Table 1). Although I did not compare lengths and weights of fish exposed to noise against others that were not, it is worth mentioning that an increasingly amount of food was found in the bottom of the tank as the trials progressed. The increase of food found in the bottom of the tank may suggest a decrease in feeding rates due to noise stimulus.

Table 1. Length and weight measurements at the time of euthanasia per fish.

Fish	Length (cm)	Weight (g)
1	11.4	19.25
2	10.5	16.51
3	10.6	19.37
4	10.5	15.71
5	10.5	14.88
6	9.01	9.62
7	9	8.6
8	9.2	8.88
9	9.1	10.27
Average	9.98	13.68

Another important aspect to the effects of anthropogenic noise exposure on fish is elevated cortisol levels. Cortisol is one of the most commonly measured indicators of stress on fish as it can be measured easily and accurately using various methods

(Mommsen et al. 1999). In this particular study, cortisol levels were not measured. Previous studies conducted on Rainbow Trout exposed to high levels of sound ranging from 115-150 dB for eight months did not result in differences in stress levels among fish (Wysocki et al. 2006). Alternatively, the fish may have inhibited increased cortisol levels at the beginning but over time became used to the sound and resulted in their stress levels disappearing.

SHIFTING HEARING THRESHOLDS

The hearing thresholds of our fish may have been altered due to the noisy environment located within the facility, specifically in room CB0026C where the fish were initially raised. Sound levels fish experience in this room are approximately 70 dB due to various equipment located in the room such as filtration and aeration systems. Studies on the hearing thresholds of Rainbow Trout raised in aquaculture facilities reported that over time their sensitivity to sound decreased (Wysocki et al. 2007). Constant elevated background noise is thought to increase the threshold at which they react to sound. The ability for Rainbow Trout to adapt to a noisy environment is important as they live in noisy natural habitats such as those found in creeks and torrents (Lugli and Fine 2003). It is therefore assumed that the fish in this study may have become accustomed to the test noises over time given both to prior and current exposure to sound levels.

In an intriguing part of this study, the background noise associated with the filtration and aeration systems was shut off briefly during a period of resetting the filtration systems. This change to an almost silent environment within the tanks and led the fish to panic, rapidly swimming around the tank quickening their gape.

HEARING IN TROUT

Further reasons for the lack of significant results in this study include the actual hearing ability of Rainbow Trout. They do not possess specialized accessory hearing structures when compared to species like gold fish (Wysocki et al. 2007). Consequently, they have a limited hearing bandwidth and sensitivity, and are known as hearing generalists. Previous studies have confirmed that fish species with different overall hearing sensitivity are affected differently by exposure to noise of a given level. These effects range from substantial noise-induced hearing loss to no effect at all. With this said, although the rainbow trout in our study were not significantly affected by our test noises, other species with more sensitive hearing like catfish and carp could be affected by the noises created from pile driving and a closing door (Wysocki et al. 2007).

LIMITATIONS OF THE STUDY

The vast majority of studies of fish hearing incorporated the use of speakers placed under water. The speaker in this study was placed 1.3 m outside the tank and the distance could have affected how the sound was penetrating the water and being reflected. Furthermore, the sound created from a closing door within the facility (specifically the main door separating the facility to the entrance) was considerably lower than the sound tested, which exceeded sound levels of 90 dB. Therefore, the sound created from a closing door was unlikely to cause sound-induced stress in Rainbow Trout in this particular facility.

LACK OF STIMULUS

Overall, the fish used in this study did not show major changes in their behaviour as a result of our test noises. After examining almost ten hours of recorded footage, I found that numerous fish developed repetitive behaviors, which could have been interpreted to be a reaction of the test sound but were most likely a learned repetitive behavior. One fish demonstrated a repetitive territorial behaviour with a favorite hiding spot in the tank (Figure 11).

Figure 10. Example of a fish using the provided hiding location (cut in half piping) and demonstrating a territorial behaviour (Rugo 2018)

This fish would remain hiding for an extended amount of time only to come out to chase other fish in the tank away as they approached too closely its hiding spot. This behaviour was witnessed regardless of sound being present during playback. Most fish in this study chose to remain in certain areas of the tank. This behaviour could have been a result of poor stimulus within the tanks. This information could be useful to other researchers who further pursue similar studies on the effects of sound on fish. Some recommendations that could be drawn from this study include to have two tanks set up connected via a tunnel that allows the fish to escape the sound being produced. Data collected over a longer period of time could also show developing behaviors that were not seen during the 10-d period.

CONCLUSION

There is strong evidence to support that fish are negatively affected by sound at both extreme levels and constant background noise. Although results in this study did not show major differences in the behaviour of fish with sound playbacks, it should be taken into consideration that they may have experienced effects at the beginning of the study in the form of cortisol levels but eventually acclimatized to the sound being created. Furthermore, the study was specifically designed to play sounds in ranges that would not harm the fish. Future studies should take into considerations the limitations of this study and conduct a study longer than 10 d with louder noises. In addition, it may be worth studying different species as well as incorporating the measure of cortisol levels and particle motion when exposed to pure sound.

LITERATURE CITED

Andersson, M. Andersson S. Ahlsen, J. Andersson, B. Hammar, J. Persson, L. Phil, J.Sigray, P. Wikstrom, A. 2017. A framework for regulating underwater noise during pile driving. Swedish Environmental Protection Agency. 115 pp.

Abbott, R. Bing-Sawyer, E. 2002. Assessment of pile driving impacts on the Sacramento blackfish (*Orthodon microlepidotus*). Draft report prepared for Caltrans District 4. 49 pp.

Blenkle, C. McGregor, P. Gill, A. Andersson, M. Mecalfe, J. Bendall, V. Sirgray, P. Wood, D. Thomsen, F. 2010. Effects of pile-driving noise on the behaviour of marine fish. Cowrie Press. 57 pp.

Barton, B. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42: 517-525.

Banner, A. 1968. Measurements of the particle velocity and pressure of the ambient noise in a shallow bay. Journal of Acoustical Society of America. 44:6

Chavez, M. Sosa, V. 1984. Speed of Sound in Saturated Pure Water. Universidad Autónoma Metropolitana, Laboratorio de Termodinámica. 5 pp.

Caltrans, A. 2001. Pile Installation Demonstration Project, Fisheries Impact Assessment. PIDP EA 012081, Caltrans Contract 04A0148. 57 pp.

Caltrans, A. 2004. Fisheries and Hydroacoustic Monitoring Program Compliance Report for the San Francisco-Oakland Bay Bridge East Span Seismic Safety Project. Strategic Environmental Consulting, Inc. 68 pp.

Crovo, J. Mendonca, M. Holt, D. Johnston, C. 2015. stress and auditory responses of the otophysan fish, *Cyprinella venusta*, to road traffic noise. Plos One. 9 pp.

Doksaeter, L. Godo, O. Handegard, N. Kvadsheim, P. Lam, F. Donovan, C. 2009. Behvaioural responses of herring (*Clupea harengus*) to 1-2 and 6-7 kHz sonar signals and killer whale feeding sounds. Journal of the Acoustical Society of America 125: 554-64

Enger, S. 1981. Frequency Discrimination in Telosts- Central or Peripheral? Hearing and Sound Communication in Fishes. 243-255 pp.

Gans, C. 1992. An Overview of The Evolutionary Biology of Hearing. Springer-Verlag. 13 pp.

Gilham, D. Baker, B.1985. A black background facilitates the response to stress in teleosts. Journal of Endocrinology 105(1): 99-205.

Glushkov, E. Glushkova, N. Godin, O. 2013. The effect of anomalous transparency of the water-air interface for a volumetric sound source. Akusticheskii Zhurnal 59: 8-18.

Hastings, M. Popper, A. 2005. Effects of sound on Fish. California Department of Transportation. 82 pp.

Hoin-Radkovsky, I. 1984. Determination of source distance in the surface feeding fish *Pantodon buchholzi* Pantodontidae. Animal Behaviour 32: 840-851.

Lugli, M. Fine, L. 2003. Acoustic communication in two freshwater gobies: ambient noise and short-range propagation in shallow streams. Acoustical Society of America 114: 512-521.

McCauley, D. Fewtrell, J. Popper, A. 2003. High-intensity anthropogenic sound damages fish ears. Acoustical Society of America.113(1): 638-642.

Mommsen, T. Vijayan, M. Moon, T. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9(3): 211-268.

Nedelec, S. Campbell, J. Radford, A. Simpson, S. Merchant, N. 2016. Particle motion: the missing link in underwater acoustic ecology. Methods in Ecology and Evolution 7: 836-842.

No author. 2015. Assessment and Mitigation of the Hydroacoustic effects of pile driving on fish. California Department of Transportation – Department of Environmental Analysis. 532 pp.

Popper, A. Schilt, C. 2008. Hearing and acoustic behavior: basic and applied considerations. Fish Bioacoustics 21: 17-48.

Popper, A. Hawkins, A. Wysocki, E. Dittami, P. Ladich, F. 2006. Ship noise and cortisol secretion in European freshwater fishes. Biological Conservation 128: 501-508.

Purser, J. Radford, N. 2011. Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks. Plos One. 8 pp.

Radford, A. Kerridge, E. Simpson, S. 2014. Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behavioral Ecology 25: 1022-1030.

Slabbekoorn, H. 2012. Measuring behavioural changes to assess anthropogenic noise impact in Adult Zebrafish (*Danio rerio*). Proceedings of Measuring Behaviour. Pages 244-248.

Slabbekoorn, H. Bouton, N. Opzeeland, I. Coers, A. Cate, C. Popper, A. 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Cell 25: 7-9.

Sara, G. Dean, J. Amato, D. Buscaino, G. Oliveri, A. Genovese, S. Ferro, S. Buffa, G. Martire, M. Mazzola, S. 2007. Effect of boat noise on the behaviour of Bluefin tuna, *Thunnus thynnus* in the Mediterranean Sea. Marine Ecology Progress Series. 12 pp.

Wysocki, L. Davison III, J. Smith, M. Frankel, A. Ellison, W. Mazik, P. Popper, A. Bebak, J. 2007. Effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout *Oncorhynchus mykiss*. Aquaculture 288: 337-343.

APPENDICES

Table A 1 Tests of Between-Subjects Effects for hiding

Tests of Between-Subjects Effects

Dependent Variable: Day11

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.047[a]	3	.016	.171	.915
Intercept	.568	1	.568	6.179	.018
Treatment	.047	3	.016	.171	.915
Error	2.942	32	.092		
Total	3.557	36			
Corrected Total	2.989	35			

a. R Squared = .016 (Adjusted R Squared = -.077)

Table A 2 Tests of Between-Subjects Effects for grouping

Tests of Between-Subjects Effects

Dependent Variable: Grouping

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.158[a]	3	2.719	3.958	.017
Intercept	15.158	1	15.158	22.061	.000
Treatment	8.158	3	2.719	3.958	.017
Error	21.987	32	.687		
Total	45.303	36			
Corrected Total	30.145	35			

a. R Squared = .271 (Adjusted R Squared = .202)

Table A 3 Tests of Between-Subjects Effects for time spent in the upper portion of the tank

Tests of Between-Subjects Effects

Dependent Variable: Swimmingup

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.015[a]	3	.005	.036	.991
Intercept	1.501	1	1.501	10.866	.002
Treatment	.015	3	.005	.036	.991
Error	4.419	32	.138		
Total	5.935	36			
Corrected Total	4.434	35			

a. R Squared = .003 (Adjusted R Squared = -.090)

Figure A 1 Food used during the study, kept refrigerated

Figure A 2 Set up used to capture fish reactions. Included a MacBook, DSLR, speaker, sound reading device and an iPhone.

Figure A 3 Sound measuring device used to test decibel readings
during tests

Figure A 4 Set up used to measure length and weight of the study fish