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**Behavioural response of Sea Lamprey (*Petromyzon marinus*) to acoustic stimuli in a
laboratory and field setting**

By

Victoria L.S. Heath

A Thesis
Submitted to the Faculty of Graduate Studies
through Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

2021

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**Behavioural response of Sea Lamprey (*Petromyzon marinus*) to acoustic stimuli in a
laboratory and field setting**

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January 14th, 2021

DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

Chapter 2 of the thesis was co-authored with Scott Miehl, Nicholas Johnson and Dr. Dennis Higgs under the supervision of Dr. Dennis Higgs. Chapter 3 of the thesis was co-authored with Dr. Dennis Higgs under the supervision of Dr. Dennis Higgs. In all cases, the key ideas, primary contributions, experimental designs, data analysis, interpretation, and writing were performed by the author, and the contribution of co-authors was primarily through the provision of data collection, statistical analysis, and editing of the manuscript. Scott Miehl and Nick Johnson contributed to the collection of data and provided feedback on refinement of ideas and editing of the manuscript; Dennis Higgs contributed to the statistical analysis and provided feedback on refinement of ideas and editing of the manuscript.

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<i>Chapter 2</i>	<i>Heath, V.L.S., Miehls, S., Johnson, N., Higgs, D. M. (In Press). Behavioural response of Sea Lamprey (Petromyzon marinus) to acoustic stimuli in a small stream</i>	<i>Accepted for Publication</i>

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ABSTRACT

Sea lamprey are invasive in the Laurentian Great Lakes and parasitically feed on valued fishes. Migration barriers and selective pesticides are used to control sea lamprey, but there is a desire to develop additional control tools such as traps with deterrents. Sound has been used as a deterrent for other invasive species but its potential for manipulating sea lamprey behavior in natural stream conditions remains untested. The behavioural threshold for sea lamprey nor a behavioural comparison of life stages has also not been established. Here, behavioral responses of upstream migrating adult sea lamprey in response to low frequency sounds of 70 or 90 Hz was tracked in a small stream using passive integrated transponder (PIT) telemetry. The low frequency sounds shifted sea lamprey distribution with up to 30% more sea lamprey detected on PIT antennas without sound compared to PIT antennas with sound playing. The same frequency tones were used for behavioral responses of adult and juvenile sea lamprey and were tracked in a lab setup. The low frequency sounds changed the sea lampreys behaviour with juvenile and adult sea lamprey showing similar swimming behavioural thresholds and twitch (startle) behavioural thresholds for both frequencies. Future studies could continue testing low frequency sounds in natural setting for use as a natural deterrent at sea lamprey barriers to push sea lamprey toward traps at different life stages and continued studies in a lab setting could be useful for knowledge of the behaviour of sea lamprey to apply to traps for population control.

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CHAPTER 1

CONTROLLING THE INVASIVE SEA LAMPREY: PAST, PRESENT AND FUTURE

The Invasive Sea Lamprey

Invasive species are considered to be the leading cause of animal extinctions worldwide (Clavero and Garcia-Berthou, 2005). The growth and extension of invasive species poses a strong threat to native species with the rate of introduction of non-native freshwater fish worldwide increasing (Gozlan et al. 2010; Hermoso et al. 2011). It is important to consider potential unintended outcomes of implementing invasive species management programs such as increases in non-target invasive species that could be avoided with more studies (Prior et al. 2018). To efficiently manage invasive species predictive tools are needed for vulnerable areas and where their impacts will be most severe (Kulhanek et al. 2011). To improve pest management, we must first understand the behaviour of the individual and how it can be manipulated. The behavioural ecology, that is, its relation to its environment, is also an important factor.

Sea lamprey (*Petromyzon marinus*), which parasitize economically important fishes fish during the juvenile stage, colonized the Laurentian Great Lakes during the late 1900s and caused severe losses for commercial fishing (Chapman and Bolen, 2015). In the Great Lakes, sea lamprey are much larger and more abundant than smaller native lamprey and the fish that become prey are not equipped to tolerate a parasite of this size (Siefkes, 2017). Consequently, in the Great Lakes, a larger proportion of the preyed upon fish die from sea lamprey attacks, where sea lamprey function more as a predator than a parasite (Swink, 1990, 2003; Madenjian, 2008). Sea lamprey use suction cup mouths to attach to the side of prey and with pointy teeth and rasping tongues feed on their blood and body fluids while selecting for larger hosts (Farmer and Beamish 1973; Swink, 2003; Siefkes,

2017). After invading the Great Lakes by the early to mid-1900s, sea lamprey caused a significant decline of many native fish species including lake trout (*Salvelinus namaycush*), which were driven to low abundance in the various Great Lakes (Smith and Tibbles, 1980; Hansen, 1999). To attempt to combat and reverse the devastating impacts of the sea lamprey, the federal governments of Canada and the United States established the Great Lakes Fishery Commission by treaty in 1955 to coordinate fisheries management, implement a research program to promote the rehabilitation of Great Lakes fisheries, and develop and implement a sea lamprey control program (Siefkes, 2017). Individual control methods are not entirely effective, which is a problem when controlling the population. If even a few lamprey survive or get past barriers, they can produce a large number of eggs and their expansions continue. Sea lamprey produce 60,000 eggs on average with up to 90% survival (Manion and Hanson, 1980). The Great Lakes Fishery Commission (GLFC) is looking to diversify the strategies and limit costs for controlling sea lamprey in the Great Lakes (Great Lakes Fishery Commission 2011). A combination of multiple methods is the best way to get the maximum effect with new techniques always being studied and implemented.

The vulnerability of sea lamprey depends on its' life cycle. The life cycle of the sea lamprey is quite complex with multiple terms used to describe the stages of the life cycle. The life stages of the sea lamprey start at ammocoetes (or larvae) which grow to transformers (or metamorphosing lampreys) to juveniles (metamorphosed but sexually-immature lampreys, including downstream migrants, and feeding-phase "adults") to upstream migrants to sexually-mature adults (Docker et al. 2015). Adult lampreys are concentrated, and thus vulnerable to predation, during their upstream migration and

spawning. (Docker et al. 2015). While ammocoetes spend most of the time motionless (Quintella et al. 2005), the downstream migration of newly metamorphosed lampreys to feeding grounds can be fast and mainly at night (Dawson et al. 2015). The environmental factors encountered during spawning migration vary widely among species and populations, demanding both physiological and behavioral plasticity (Moser et al. 2015). Sea lamprey in the Great Lakes appear to exhibit behaviors similar to those of the marine form which has access to larger rivers and migrate for longer times and greater distances (Clemens et al. 2010; Vrieze et al. 2011; Moser et al. 2015).

Current Control Methods for Sea Lamprey

Current control methods range from barriers; including mechanical and electrical; selective toxicants, sterilization of males and other strategies used with traps such as pheromone use (Smith and Tibbles, 1980). Lampricides are currently the most effective and most commonly used for sea lamprey control by itself. Sterilization of males has shown promise but has not been supported with data and has a focus on areas with fewer sea lamprey. Finally, barrier and traps show promise for increased efficiency by their ability to work in conjunction with other strategies. While all possible methods are used, they all come a different set of benefits and issues.

Barriers can be the easiest to make or find since they can be naturally present in an area or put in a narrow waterway to block the progression of lamprey to new areas. Electrical barriers are used to stun spawning lamprey but can affect a variety of other organisms that appear near the electrical field (Lavis et al. 2003). Mechanical barriers can be man-made or natural from the landscape that impedes the path of lamprey, but also stops

native fish from swimming up streams (Lavis et al. 2003). After barriers are removed, sea lamprey will recolonize habitats or move into new territory (Gardner et al. 2012).

Lampricides are used to kill larval lamprey before they grow and become parasitic in open waters. Lampricide treatment, a selective toxicant, was responsible for about a 70% reduction observed in the 1990s (Brege et al. 2003). Chemical treatments may affect the biology of the waterbody and information on the effects on surrounding habitats are not complete (Hubert, 2003). Pesticides also leave room for other problems such as a growing population of resistant lamprey to the chemical or effects on non-target species (Sawyer, 1980). With continued use of the same lampricide, resistance behaviours or internal response may lead to a generation of lamprey that are resistant leading to higher concentrations needed or new approaches (Dunlop et al. 2017).

The sterilization of males are also used in spawning waters. This is where male lamprey are caught, sterilized and then released back into spawning streams so that the successful sterilized males will breed with females producing eggs with a lower viability (Bergstedt et al. 2003; Hanson, and Manion, 1980). Reproductive potential of the females is greatly reduced and can be used in a number of areas without affecting the environment or behaviour of the lamprey but has a limited area of effect with only a few spawning couples in a stream (Bergstedt et al. 2003; Hanson, and Manion, 1980).

Traps are another form of control with tremendous amount of potential, if used as part of an integrated program. Physical removal of a species is more socially acceptable, but requires information on the spatial distribution of the lamprey. Traps catch many non-target species unless specially made and designed sea lamprey traps are used, which are

more complex (McLaughlin et al. 2007). Trap efficiency and capture rate have been shown to be disappointingly low in St. Mary's River in the Great Lakes, which could be due to low encounter rate, that is, the amount of lamprey that interact with the trap (Holbrook et al. 2016). Low efficiency traps have great potential to raise their catch rate by deploying more traps, moving the traps to a new area, or using attractants or repellents with the traps (Bravener and McLaughlin 2013; Holbrook et al. 2016). Traps should be set in areas where large abundances of lamprey pass by as they spawn thus increasing the encounter rate (Bravener and McLaughlin 2013). Understanding or controlling the behaviour of lamprey can lead to crucial increases in the success of control methods, especially traps, where information about how close and when lamprey enter could be very helpful (Bravener and McLaughlin 2013). Traps can be used with other catalysts to increase their trapping efficiency such as pheromones, which are used to control lamprey behaviour.

Synthesized male pheromones can be used for attracting female lamprey into traps (Johnson et al. 2009). Alarm cues from adult lamprey are used to deter or ensure an avoidance behaviour from lamprey away from protected streams (Imre et al. 2010). Chemosensory alarm cues elicit strong behavioural responses night or day and during spawning season. Adult lamprey can be more active during the day when water temperatures are high (Rocco et al. 2014; Wagner et al. 2011). Some chemosensory cues are less effective when the lamprey are resting or hiding (Rocco et al. 2014). Using traps and barriers in association with pheromones can increase the effectiveness of both of these control techniques (Hume et al. 2015). A potential problem for chemosensory cues is how well the lamprey smell, if there are other chemicals in the river that affect the

sense of smell, the lamprey may no longer respond (Imre et al. 2010). Improving the efficiency of traps and barriers would help with sea lamprey control by removing the adult lamprey before they have a chance to reproduce (McLaughlin et al. 2007).

Even though ongoing physiological research continues to sharpen the effectiveness and selectivity of previous techniques, there is a strong desire to develop new, innovative techniques that could provide levels of effectiveness and selectivity yet to be seen and prevent catastrophic population increases if one of the current techniques is rendered ineffective (e.g. development of lampricide resistance; Siefkes, 2017). Barriers, like sound, are a new control measure that obstruct the movement of lamprey without affecting the environment; this includes the use of sensory modalities (Noatch and Suski 2012). Sensory modalities can be exploited to increase trap success by attracting lamprey to stimuli they are naturally responsive to (Johnson et al. 2009). These non-lethal and non-invasive measures to deter lamprey away from vulnerable areas provide a new insight on how to control and limit lamprey. Techniques that can trap animals in streams without barriers are of interest since they may have reduced negative effects on other species (Bergstedt, and Twohey 2007).

Traps are used for a number of species and the efficiency of the traps can be manipulated by looking at attraction/pushing towards use, trap design and trap positioning. The encounter rate, the entering rate, and exiting rate of traps is what determines the effectiveness of a trap, by increasing one or all of them you can increase the effectiveness of traps overall. Individual differences in the species can affect these rates, by linking or predicting the behaviour of the species one can design a trap that works better to control a population. Aquatic invasive organisms have environmental conditions that are harder to

not change but other factors can be, such as adding pheromone as bait. For traps to work, lamprey need to encounter the trap, enter the trap and stay in the trap until the trap is checked. Sea lamprey behavior affects both the duration within each state as well as the transitions between states (Bravener and McLaughlin 2013).

Sound Application

Detection of sound by fishes typically involves two primary sensory systems – the ear, and the lateral line. An important difference between these systems is the distance from the fish over which they function. The lateral line system detects signals that originate relatively close to a fish whereas the ear system detects signals at further distances from the fish (Popper and Carlson 1998). Consequently, when developing effective behavioral guidance technologies for fish using underwater sound, fish must be able to ‘hear’ the projected sounds. Specifically, the sound frequencies used must be within the detection range of the fish species of interest, signal compositions must be of a type that are repellent to fish and the sound level used must be high enough to elicit an appropriate behavioral response (Popper et al. 2019). Higgs and Radford (2013) found that sound responses in fish are not only driven by the ear but also by the lateral line system, which usually detects vibrations, and while it is difficult to separate both systems of detection, it is important to realize the contribution of both. Sea lamprey are most responsive to sounds below 100 Hz (Mickle et al. 2019) but this could be a response to either the vibrations detected by the lateral line or sound detected in the ear. For practical or applied use of sound, the process used to detect the sound is not very important as long as the response to sound is useful for control. The question of which system is used is important for evolutionary knowledge and comparison to other fish species. By knowing which

system is used by sea lamprey for responding to sound, we can determine the evolutionary significance of lateral line or inner ear system and whether one system is compensating for another. The process of one system being used and another being ignored is what drives evolution of these species, and knowing how the species has evolved to use one or both systems can help with determining how those systems started out and their connection on the evolutionary tree to other similar species (i.e. convergence and divergence).

Sound has been used to control behaviour in other fish and marine animals and therefore there is potential effectiveness on sea lamprey. Previous studies on Silver Carp (*Hypophthalmichthys molitrix*), another invasive species, shows they are deterred by broad-band sounds and the round goby (*Neogobius melanostomus*), also invasive, was attracted to reproductive calls, suggesting sound has potential for use as a control measure (Vetter et al. 2015; Isabella-Valenzi, and Higgs, 2016). Previous research has also shown acoustic stimuli can guide teleost fishes around power intakes (Ross et al. 1993, Maes et al. 2004, Sonny et al. 2006) and can generally direct fish movement (Knudsen et al. 1994, Sand et al. 2000, Pegg and Chick 2004). Different sound frequencies have been used in studies such as low frequencies and harmonics used to reduce whale collisions with fishing gear in Canada (Lien et al., 1989) while artificially-generated sounds have been used to induce an avoidance response in fish (Van Derwalker, 1967). Freshwater fish can have a multitude of effects from exposure to sound including behavioural and physiological responses such as an increase in activity and avoidance, which could be a sign of stress (Mickle and Higgs 2018). Many other fish have shown avoidance or erratic behaviour from boat noise, which produce low

frequency noise constantly (Enger et al. 1993; Pieniasek et al. 2020). The behavioural response thresholds of other underwater organisms has been examined by exposing them to differing intensity levels, showing the lowest threshold with the highest sensitivity (Stanley et al. 2011) or using sound behavioural thresholds to use low intensity sound as an acoustic barrier (Qin et al. 2020). Sea lamprey's auditory system and responses are poorly studied but previous studies have shown they do respond to acoustic stimuli in the lab and can hear at low frequencies of sound (Mickle et al. 2019). The specific behavioural thresholds of sea lamprey are unknown and the application of sound in a field setting also remains untested although behavioural reactions of some marine species to loud sounds (100-175 re 1 μ Pa) range from directional changes, movement and avoidance (Roberts et al. 2016; Deleau et al. 2020).

Sound is not always the perfect option for management applications. Sound can affect non-target species by interrupting or masking the communication of other underwater species. Masking or interruptions in communication can have negative impacts on breeding, feeding or predation of freshwater fish species (Mickle and Higgs 2018). Many fish have shown changes in behaviour, for example, avoidance or swimming away, from low frequency sounds like boat noise (Enger et al. 1993). Sound also cannot be used in certain landscapes such as loud areas with lots of background noise such as waterfall areas, experimental sound would not be loud enough for sea lamprey to hear it and respond or would only cover a small area. Sound does not work in all settings, low frequency sounds are less effective in shallow and hard-bottomed areas since they propagate poorly (Rogers and Cox 1988). The constrictions on sound application, either on the negative impacts on other species or the restriction of usable sites, means that

while sound is a viable option, research must be done before sound can be used in a practical setting.

Conservation

Conservation can be a complex topic and for a viable conservation method the integration of multiple disciplines is needed, like behavioural knowledge, offering a new perspective or insight to a solution (Madliger, 2012). Recently, the intersection of sensory ecology and conservation biology has become of interest for successfully applying sensory-based organismal principles to conservation practice (Madliger et al. 2016). Sensory ecology is the study of how organisms collect information and respond to triggers in their environment and can include cues from the organism's environment interacting with the organism (Dusenbery, 1992; Bowdan and Wyse, 1996). Understanding the underlying mechanisms, the sensory triggers or processes can aid the interpretation of the behavioural responses of the organism. Both sensory ecologists and conservation biologists are interested in the interaction of an animal and its environment but sensory ecologists are interested in the individual while the conservationist focuses on a healthy and stable population and ecosystem (Madliger, 2012). However, we need to know the individual specifics to help the ecosystem as a whole. For example, knowing why some organisms are more impacted by certain stimuli than others, like environmental changes, or why some respond well to anthropogenic sounds while others do not (Mickle and Higgs, 2018). Knowing the degree and effectiveness of the organism's flexibility will let us know how well they can cope and lead to multi-scale implementation opportunities (Mickle and Higgs, 2018). Sensory systems play a central role in guiding animal behaviour and can be manipulated to alter behavioural outcomes to limit negative

interactions between humans and animals. Systematic studies on a pest's behaviour will have important implications for future control technologies.

Observing and understanding behavioural patterns is an important step. The relationship between the fish, the environment and a new technology is an important one to consider leading to possible significant improvements by leading to improved fish capture, fewer costs, less by-catch, and overall less impact on the environment (Madliger, 2012).

Sensory-based approaches can be used to take advantage of the link between specific sensory cues, their interpretation, and the behavioural decisions associated with them.

Population control, in terms of invasive species, can also benefit from research focused on sensory traps. For example, other research is identifying promising control mechanisms for other invasive species using chemical signalling including: crayfish (*Pacifasticus leniusculus*) (Stebbing et al. 2004), and cane toads (*Rhinella marina*) (Crossland et al. 2012), or using environmental controls in the round gobies (*Neogobius melanostomus*) (Isabella-Valenzi, and Higgs, 2016). Sensory traps can be highly targeted, less labour intensive, and less likely to affect non-target species or cause environmental degradation (Madliger, 2012). To optimize sensory-related approaches, research will have to focus on individual species and the effects on each and look at variation in signaling or reception across different life history stages to allow for effective targeting (Sorensen and Stacey 2004). Many animals exhibit and respond to complex signals that rely on more than one sensory modality (Hebets and Papaj 2005), it may also be beneficial to consider multiple sensory modalities for a single species, for example, the use pheromone signals with acoustic or light attractants (Sorensen and Stacey 2004). In conclusion, to produce success stories for any pest control program, one would need to

gain an intimate understanding of the biology and ecology of the target organism (Siefkes, 2017).

Thesis Objectives

The purpose of my first data chapter is to monitor the distribution of upstream migrating sea lamprey to understand if low frequency sounds that produced behavioral responses previously in a laboratory setting would also produce a response in the field and if that response could be used to manipulate sea lamprey movement. My specific objectives were to determine if distribution patterns of sea lamprey migrating upstream could be altered by application of both 70 and 90 Hz acoustic stimuli. By exposing migrating adult sea lamprey to low frequency tones (70 and 90 Hz) in a stream setting, we are able to quantify any behavioural or swimming change.

The purpose for my second data chapter is to quantify the behavioural threshold of sea lamprey to low frequency tones known to cause a response at higher intensities (Mickle et al. 2019; Heath et al. In Press). Specifically, I am answering the question: what is the minimum sound level needed for sea lamprey to detect and change their behaviour. I also explore and test the possibility of a difference in hearing threshold between juveniles and adults and what this may mean for their behavioural responses. These goals are accomplished by exposing sea lamprey to two tones (70 and 90 Hz) in a range of sound intensities and quantifying behavioural responses to both the onset and offset of these tones.

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CHAPTER 2

BEHAVIOURAL RESPONSE OF SEA LAMPREY (*PETROMYZON MARINUS*) TO ACOUSTIC STIMULI IN A SMALL STREAM

Introduction

Sea lamprey (*Petromyzon marinus*) are highly invasive in the Laurentian Great Lakes and require a multinational control effort to suppress their population and allow native species recovery (Christie and Goddard 2003). Sea lamprey lower the abundance of native fish (Smith, and Tibbles 1980) and change the size structure of populations by specifically targeting larger fish, leaving the smallest of the species to survive (Farmer and Beamish 1973). Sea lamprey populations are present in all the Laurentian Great Lakes and are now decreasing towards, or at, management target numbers but still over half of the Great Lakes are above target with Lake Superior and Lake Huron over 100,000 adult sea lamprey (Great Lakes Fishery Commission <http://www.sealamprey.org/status.php>). Spawning female sea lamprey can produce around 45,000–79,000 eggs with up to 90% survival (Manion and Hanson 1980; Docker et al. 2019) so maintaining populations below management targets is a significant challenge. For over 60 years, the sea lamprey control program has been entirely dependent on chemical pesticides and physical barriers to migration to suppress sea lamprey populations to target levels (Miehls et al. 2020). Because sea lamprey are highly damaging to the Great Lakes fishery and because current control is entirely dependent on the combination of physical barriers and pesticides, managers are increasingly calling for additional control measures in an integrated approach (McLaughlin et al. 2007).

The adult migratory phase, when sea lamprey leave the open water environment and return to streams to spawn, may present an opportunity for additional control. In the

Laurentian Great Lakes, adult sea lamprey typically migrate upstream in May and June when water temperatures range from 8-16° C and spawn during June and July when temperatures range from 16-24° C (Johnson et al. 2015). Capture and removal of migratory adults may hold promise as an additional control measure (Velez-Espino et al. 2008; Miehl et al. 2020), however capture rate has been disappointingly low at many trap locations, perhaps due to low encounter rates (Bravener and McLaughlin 2013). Catalysts such as pheromone (Johnson et al. 2013) or alarm cues (Hume et al. 2015) have been tested along with non-physical cues such as electric leads (Johnson et al. 2016) to increase trap efficacy but each possess drawbacks. Traps and barriers for adult sea lamprey could be more effective if an adverse sensory cue could be integrated to push sea lamprey away from barriers and toward traps. Acoustic stimulation is one potential stimulus that deserves further investigation.

Sound has been used to control behaviour in other fishes (Knudsen et al. 1994, Sand et al. 2000, Pegg and Chick 2004). Silver Carp (*Hypophthalmichthys molitrix*), another invading species may be deterred by broadband sounds (Vetter et al. 2015) while fish in the families Clupeidae (Ross et al. 1993; Maes et al. 2004), and Cyprinidae (Sonny et al. 2006) can be guided away from power plant intakes using low frequency sounds. Though the sea lamprey's auditory system and responses are poorly studied (Lowenstein et al. 1968; Hagelin 1974; Popper and Hoxter 1987; Johnson et al. 2015), they do respond to acoustic stimuli in the lab and can hear at low frequencies of sound, showing increased activity levels with exposure to 70 Hz and 90 Hz specifically (Mickle et al. 2019). The use of sound to guide sea lamprey into traps, block sea lamprey from vulnerable streams, or deter sea lamprey entrance in fish passage scenarios has great potential for sea lamprey

control in the Great Lakes. However, whether and how sea lamprey behaviorally respond to low frequency sounds in natural streams has yet to be studied.

Here we monitored the distribution of upstream migrating sea lamprey to understand if low frequency sounds that produced behavioral responses previously in a laboratory setting would also produce a response in the field and if that response could be used to manipulate sea lamprey movement. Our specific objectives were to determine if distribution patterns of sea lamprey migrating upstream could be altered by application of both 70 and 90 Hz acoustic stimuli.

Methodology

Adult sea lamprey used for experimentation were captured from the Ocqueoc and Cheboygan Rivers, Michigan, in May and June of 2018 and 2019. Sea lamprey were maintained prior to experimentation in 1000 L indoor, covered tanks at US Geological Survey, Hammond Bay Biological Station. Tanks were supplied with water from Lake Huron at ambient temperatures, which typically ranged from 4 – 10⁰ C. The animals in this experiment were cared for in accordance with the Guide to Care and Use of Experimental Animals at University of Windsor (AUPP #07-11). Experimental protocols involving the handling of fishes were carried out in accordance with United States federal guidelines for care and use of animals and were approved by the American Fisheries Society through the “Use of Fishes in Research Committee, 2014” (Jenkins et al. 2014). Permits were obtained, after coordination with local landowners, from the Michigan Department of Natural Resources, and the U.S. Fish and Wildlife Service.

Experiments were conducted in Schmidt Creek near Rogers City, MI, USA (45°29'23.3"N 83°55'42.0"W). The experimental stream reach was 7.2 m wide and

averaged 0.66 m in depth in 2018 and 0.88 m in 2019 at its deepest point (Table 1), with an average of 0.45-0.65 m depth across the channel. The experimental stream section was 55-60 m from the outflow to Lake Huron with an average velocity of 0.19m/sec with a higher velocity of 0.25 m/s in the center of the stream and a slower velocity of 0.11 m/s near the banks. Stream substrate was sand. The average temperature of the stream was 16.2⁰ C in 2018 and 16.8⁰ C in 2019 (Table 1). Animals were transported by truck with a large water tank and air bubbler to help with the short trip from the field station to the experiment stream. Sea lamprey were then placed directly into cages in the stream to start acclimating and were transported with plastic bins from container to cage. In all cases, transport took less than 30 minutes. Schmidt Creek is a known sea lamprey stream, with sea lamprey migrating up the stream annually for spawning. Schmidt Creek is ideally situated for our experiments as it provides a good width for our equipment, the water is slow moving, and has the right depth to avoid missing any sea lamprey as they pass. The animals that are not recaptured in traps set up farther in the stream, can be recorded in other years by their PIT tag. Sea lamprey that are recapture and were used in this experiment can be used in other experiments unrelated to sound.

To document the location and timing of sea lamprey movement through a transect of Schmidt Creek, four Passive Integrated Transponder (PIT) detection antenna (Oregon RFID) were set up across the entire stream width. The order of antennas facing upstream and looking left to right were A1, A2, A3, A4 (Fig 2.1). Each antenna spanned 1.8 m across the stream. Each antenna could detect a PIT tag from about 0.5 m in front and behind the antenna, leading to sea lamprey being detected before, after and right under the antenna.

In-stream experiments were conducted in May through July in both years. Fifteen to 20 sea lamprey were released each night depending on availability (20 sea lamprey, half male and half female in 2018 and 15 sea lamprey, all female in 2019) with a total of 320 sea lamprey used in 2018 and 270 in 2019 with each experimental day counting as one data point (n=1). Adult sea lamprey were measured for length and weight then tagged with a PIT tag before being organized into groups for experiments. PIT tags were surgically implanted by inserting the tag through a 3 mm incision made with a scalpel approximately 3 cm back from the last gill pore on the ventral side of the sea lamprey. A 32 mm HDX PIT tag was inserted and the incision was sealed with Vetbond™ surgical adhesive. Sea lamprey were not anesthetized prior to PIT tagging with the PIT tagging procedure taking less than 10 seconds and no deaths were recorded due to the tagging process. Sea lamprey were given 24 hours to recover. Anesthetizing compounds (MS222) are likely to impact olfactory sense in sea lamprey and chemosensation is an important sensory modality in this species. The day of a trial, sea lamprey were placed in an acclimation cage (1 m³) approximately 50 m downstream from PIT antennas, from noon to 5 pm and sea lamprey were given at least an hour to acclimate before cages were opened. The cage either opened automatically at 9 pm or was opened manually after 1 pm the day of the experiment. Because sea lamprey are nocturnal (Wigley 1959), the majority of sea lamprey did not leave the cage until dark even when the cage was opened during the day. The traps could not be opened in the dark due to safety for the experimenter; therefore, the cage was opened before sunset. Sea lamprey were recorded by the antenna array as soon as the cage was opened but the majority of detections were after or during sunset (Fig 2.2). The PIT tag arrays and datalogger were checked each

morning after the experiment and data from the previous night were collected on a laptop computer.

Two underwater speakers (Clark Synthesis Diluvio AQ339; Lubell Labs) were placed on cement plates in the stream, in the antenna array. The speakers were connected to an amplifier (Scosche SA300), a 12-Volt car battery for power and a MP3 player to control acoustic stimuli. In 2018, both speakers were placed by Antenna 4, near the right side of the stream near the bank to try and impact all sea lamprey moving up the right side of the stream and compare to left side of the stream with and without sound (Figs 2.1 A and B). In the second year, 2019, the speakers were both placed in front of Antenna 3 (Figs 2.1 C and D). Speakers were moved in 2019 because Antenna 3 was in the deeper part of the stream, where sea lamprey were more likely to encounter the speaker based on detection data from 2018. In 2018, one sound frequency treatment (control, 70 Hz, 90 Hz) was tested each night and treatment was randomized across the study period and in 2019, treatments were played sequentially in sets of three (control, then 70 Hz, then 90 Hz). Tones with frequencies of 70 or 90 Hz were played at the loudest amplitude level the equipment could produce (160 dB re 1 μ Pa for 90 Hz and 150 dB re 1 μ Pa for 70 Hz 1 m in front of the speakers). Sound levels of the experimental tones and background noise within the study area were mapped during both years using a hydrophone (Inter Ocean System Inc. – Acoustic 127 Calibration and System Model 902). Background noise levels averaged 122 dB re 1 μ Pa when no sound was playing. The sound fields for both years were similar, with sound by the speaker for 70 Hz being around 165 dB and for 90 Hz being above 170 dB for both years and showing similar decaying patterns with distance (Fig 2.1. A, B, C, D). The sound pressure levels further than 5 m from the speaker were

indistinguishable from background levels measured. Sound level is presented in pressure units, rather than particle motion because while sea lamprey should only be able to detect the particle motion component of the sound field (Mickle and Higgs 2018) it was not possible to accurately measure particle motion of a sound source in a flowing stream with available equipment. Pressure units are also more likely to be useful in an applied context if incorporating speakers into trapping protocols as pressure units are easier to measure in the field compared to particle acceleration. Sound was played up to 12 hours each night starting at 2100 hrs and finishing at 0900 hrs or until the system ran out of power, which was usually around sunrise (0600 hrs). In 2019, any detections that were outside of the time that sound would have stopped (due to equipment running out of battery) were left out of analysis. The same assumptions were used for 2018, where we did not have the equipment to monitor the sounds but had the same experimental setup. In 2019, a hydrophone (Loggerhead Instruments) was used to record sounds playing to ensure the equipment continued functioning the entire night and to fully characterize the sound actually emitted by the speakers for each frequency used (Fig 2.3).

Statistical analysis

Only detection data for sea lamprey on the night they were released were used in the analysis to control for prior exposure and experiences in the stream (Hume et al. 2015). For all trials, we are sure that all sea lamprey that passed the antenna were exposed to sound. In all cases that we can verify, we had full sound power being emitted while sea lamprey were passing the array, only detections during active sound presentation were used for analysis. If a tagged sea lamprey swam near the junction of two antennas, the read record would show detections on both nearly simultaneously of each other. These

readings were not included since they could not be classified as either antenna. There were fewer joint detections than detections on individual antennas for both years (2018: 18% joint detections, 2019: 28% joint detections). If more than one antenna was triggered, that was not next to one another (*e.g.* A1 and A3) within 2 seconds of each other, the assumption was of a mistake made by the system and both detections were removed assuming sea lamprey could not realistically swim between non-adjacent antennas in that time. Antennas were 1.8 m apart and average swim speed of sea lamprey is 0.3-0.4 m/sec so it would take them > 7 seconds to reach the other antenna swimming normally (Vrieze et al. 2011). Sea lamprey can burst swim at nearly 4 m/sec so they could move between antennas in less than 0.5 seconds, but that is unlikely.

Detection data from 2018 and 2019 were analyzed separately because the location of the sound source differed between the years (*i.e.* different position of the speaker, different river conditions (Table 1.)) leading to a significant year effect (Antenna X Year interaction for first detection, $p=0.03$). To determine if sound influenced the distribution of sea lamprey in the stream, two behavioral metrics were investigated: (1) What antenna an individual sea lamprey was first detected on (First Detection, *i.e.* the first antenna recorded for that sea lamprey's PIT tag number), (2) which antennas an individual sea lamprey was detected on throughout the night (Proportion Overall Detections). We calculated the proportion detections by dividing the number of sea lamprey detected on each antenna for one night (first detections or proportion detections) over the total number of sea lamprey detected the same night. Next, we calculated the differences in proportion during 70 Hz and 90 Hz nights from corresponding control proportion values. We compared the differential proportions of detections per night among antennas as a

function of treatment (70 Hz, 90 Hz) using Generalized Linear Model (with gaussian distribution) with a Tukey HSD post hoc analysis and R was used (R Core Team, 2013), this was used since the normal distribution tests showed abnormal data. We first analyzed the effect of sound on the mean difference in proportion of sea lamprey first detected on each antenna comparing 70 Hz, 90 Hz, and control. We then analyzed the effect of sound on the mean proportion of sea lamprey detected out of all the sea lamprey that were detected that night on each antenna comparing different frequencies (multiple detections at the same antenna are only counted once, a sea lamprey can have a maximum number of four detections throughout the night, one on A1, A2, A3 and A4).

Results

In total, 590 adult sea lamprey were used over 15 experiment nights in 2018 and 18 experiment nights in 2019. Out of 320 sea lamprey used in 2018 and 270 in 2019, 204 sea lamprey (64%) were detected in 2018 and 148 (72%) were detected in 2019 the night of release.

1.1 First Detection Difference for 2018

Comparing all antennas, there was a significant effect of antenna where sea lamprey were first detected when sounds were played relative to control trials (DF= 3, 35 Residual Deviance=1.3611 $p<0.01$; Fig 2.4A). There was no significant difference between the 70 and 90 Hz Treatments (DF= 1, 38 Residual Deviance=1.9080 $p=0.355$; Fig 2.4A) and there was no significant Antenna X Treatment interaction (DF= 3, 32 Residual Deviance=1.2459 $p=0.398$; Fig 2.4A). Subsequent post hoc testing showed Antenna 3 was significantly different from Antenna 2 and 4 for 70 Hz suggesting that the difference

in detections on the antennas was mainly due to playing 70 Hz, leading to lower detection rates on Antennas 2 and 4 and higher rates on Antenna 3.

1.2 First Detection Difference for 2019

Comparing all antenna, there was a significant effect of antenna at which sea lamprey were first detected when sounds were played (DF= 3, 43 Residual Deviance=1.8274 $p<0.01$; Fig 2.4B). There was no significant difference between the 70 and 90 Hz Treatments (DF= 1, 46 Residual Deviance=2.3801 $p=0.817$; Fig 2.4B) and there was no Antenna X Treatment interaction (DF= 3, 40 Residual Deviance=1.8016 $p=0.902$; Fig 2.4B). Subsequent post hoc testing showed no significant difference ($p<0.01$) between any antenna for either 70 or 90 Hz although a difference of $p<0.1$ was found between Antenna 2 and 3 for both 70 and 90 Hz with higher rates of detection on Antenna 2 and lower rates on Antenna 3.

1.3 Lamprey Proportion Overall Difference for 2018

1.3 Lamprey Proportion Overall Difference for 2018

Comparing all antennas, there was no significant effect of antenna at which sea lamprey were detected overall when sounds were played relative to control trials (DF= 3, 35 Residual Deviance=2.1715 $p=0.339$; Fig 2.5A) and no significant difference between the 70 and 90 Hz Treatments (DF= 1, 38 Residual Deviance=2.3892 $p=0.264$; Fig 2.5A) and there was no Antenna X Treatment interaction (DF= 3, 32 Residual Deviance=2.0723 $p=0.675$; Fig 2.5A).

1.4 Lamprey Proportion Overall Difference for 2019

Comparing all antenna, there was no significant effect of antenna at which sea lamprey were detected overall when sounds were played relative to control trials (DF= 3, 43

Residual Deviance=2.7190 $p=0.130$; Fig 2.5B) and no significant difference between the 70 and 90 Hz Treatments (DF= 1, 46 Residual Deviance=3.0937 $p=0.692$; Fig 2.5B) and there was no Antenna X Treatment interaction (DF= 3, 40 Residual Deviance=2.653 $p=0.803$; Fig 2.5B).

Discussion

Sea lamprey moved away from low frequency sound sources in both years when first moving upstream and these results are consistent with the hearing ability of sea lamprey and previous behavioral results in laboratory microcosms (Mickle et al. 2019). Sea lamprey were being pushed away from the antennas with speakers when sound was playing and moving across the river to the next closest place, the adjacent antenna, leading to an increase in proportion of first detections compared to control trials. Since sea lamprey have been shown to hear low frequency noise (Mickle et al. 2019), it is likely that our results could be due to the behavioural response of hearing and keeping clear of the sound. The sea lamprey's response to sound is similar to the multitude of effects sound has on freshwater fish including behavioural and physiological responses (Mickle and Higgs 2018). Previous research on using sound as an aversive stimulus on sea lamprey has shown limited success but did show an increase in activity where an increase in activity and avoidance due to sound could be a sign of stress (Mickle and Higgs 2018). Sound has been used as a deterrent or behavioural guide in many other studies (Ross et al. 1993; Sand et al. 2000; Sonny et al. 2006; Vetter et al. 2015) and in the current study has been shown as a useful deterrent for adult sea lamprey.

In 2018, there was a reduction in first detection, and a trend toward a reduction in overall detections of sea lamprey, on antenna 2 during 70 Hz treatments. This distributional shift

may have been due to sea lamprey association preferences. A lower proportion of sea lamprey detected on antenna 2, 3.6 m away from the speaker, could be due to sea lamprey at that spot in the stream choosing to associate with the increased number of sea lamprey being pushed to antenna 3. This distributional shift, however, is unlikely the result of association preference as sea lamprey have demonstrated little association or schooling behavior with other migratory sea lamprey in the past (Siefkes et al. 2005). Sea lamprey were possibly attracted toward the faint sound emitted from the speaker at antenna 4 but then repulsed by the increasing sound level closer to the source. While this pattern is interesting, we could not address this hypothesis within the constraints of the current study. Detailed behavioral response data (such as video) would need to be collected and analyzed to determine if avoidance and attraction responses varied with sound levels.

Though not statistically significant, a pattern emerged with mean proportion of detections during both years, especially for 70 Hz, showing a lower mean average of sea lamprey detections on antennas with sound compared to control trials, and a higher mean average of sea lamprey detections on adjacent antennas with no sound, compared to control trials. With different analyses showing the same pattern, there does seem to be an underlying implication that sound is affecting sea lamprey behaviour during nights with sound even with repeated interactions. While there is some evidence that sea lamprey habituate to olfactory cues (Imre et al. 2016) it is unknown if the same can be said for acoustic cues. Studies have shown low frequency sounds as a deterrent for other freshwater species with no habituation even after long time exposure (Knudsen et al. 1994; Sonny et al. 2006). Sea lamprey found in streams and rivers are likely to be detected multiple times at one

location due to circling behaviour, our results indicate that sound may affect sea lamprey behaviour even with multiple interactions from sea lamprey returning to the same spot (Vrieze et al. 2011).

Sound was able to affect the early, first detection, behaviour of sea lamprey with sea lamprey moving around the antennas with sound while migrating upstream. Sea lamprey seemed to prefer taking a path with less low-frequency sound interference, at least 1.8 m (one antenna) away from the sound. Low frequency noises in water areas are related to anthropogenic noise, usually boat noise, which sea lamprey may be encouraged to avoid (Mickle et al. 2019). Many other fish have shown avoidance or swimming away from boat noise which produce low frequency noise constantly (Enger et al. 1993). In the current study, however, low frequency tones were used which has shown to be attractive to predatory fishes (Richard 1968) and sea lamprey have been found attached to boats meaning that they may not have the same behavioural reaction to boat noise (Howe et al. 2006). Adult sea lamprey showed increased activity and surface breaches in the lab for 70 Hz (Mickle et al. 2019) which could be linked to a sudden loud sound field to startle the sea lamprey and introduce a sudden new obstacle they were not expecting. Sea lamprey may be inclined to stop or turn around when faced with sudden, loud, low frequency tones, similar to reactions seen in other species (e.g. Blaxter and Hoss 1981; Kastelein et al. 2008; Mickle et al. 2020) and sea lamprey have been shown to go through bursts of speed and times of attaching to substrate and move in circular patterns in rivers and streams (Vrieze et al. 2011).

The sound system in this experiment did not completely repel all sea lamprey or push them completely to the other side of the river and the results, especially for multiple

detections, varied suggesting limitations. A possibility for the variability in sea lamprey detection is their natural swimming behaviour. Sea lamprey can swim around 1.2-1.5 km/hr with faster speeds in open waters and slightly slower in spawning areas (Vrieze et al. 2011). Lampreys go through bursts of speed and times of rest where they are attached to substrate when there is high velocity water conditions (Quintella et al. 2004; Quintella et al. 2009). In our experiment, the water flow was quite slow and had a sandy terrain, which would make it harder for sea lamprey to attach, this would lead sea lamprey to be moving more often and quicker leading to less time to respond or detect the sound stimuli. Another possible limitation is lampreys do respond to weak, low frequency electrical fields but the differences in lampreys behavioural response range from decreased activity levels in adults but an increase in activity in juveniles so more studies are needed to know the extent of the effect on behaviour (Bodznick and Northcutt 1981; Chung-Davidson et al. 2004, Chung-Davidson et al. 2008). Traps set with electric leads have shown to guide lampreys as they try to avoid the electric signal, which could be part of the reason for sounds use as a deterrent (Johnson et al. 2016). Electrical fields are present when sound stimuli are played from a speaker and sea lamprey may be avoiding the electrical signals from the speaker rather than the acoustic signal but more studies are needed. The final possible limitation discussed here is that temperature also has a large effect on lampreys activity with increasing temperatures leading to lampreys being more active during the day when sound was not playing (Binder and McDonald 2008a). Binder and McDonald (2007) found that lampreys activity was highest two hours after dark, which declined to low activity at around 4 AM. Similar to our results of a median first arrival time of 21:30-23:00 both years (Fig 2.2), around 50% of total lampreys activity

was found between 21:00 and 00:00 in previous research (Binder and McDonald 2007). Sea lamprey are primarily nocturnal at the beginning of their migration and usually hide throughout the day (Wigley 1959), but as water temperature increases, so does their diel activity (Wigley 1959; Binder and McDonald 2008a). During spawning, lampreys are not fully nocturnal anymore with more activity during the day (Binder and McDonald 2008b). Longer daytime activity levels, when sea lamprey are not being recorded, could lead to less or some abnormal activity by sea lamprey during the night especially later in the season when the temperature increases (Table 1). The possibility of using sound needs to be expanded to include the shortcomings and the potential use of sound in other situations but can still provide a substantial advantage for behaviour control.

The low frequency tones did not repel all sea lamprey or push them to the other side of the river, but a more powerful speaker or additional speakers may produce stronger avoidance. Adult sea lamprey have been shown to have an auditory threshold level of 144-150 dB re 1 μ Pa (Mickle et al. 2019) and sound in the experiment area attenuates quite fast leading to levels below 140 as close as 2 m away from the source. With low frequency sounds less effective in shallow and hard-bottomed areas since they propagate poorly, due to interaction with surface and substrate leading to rapid attenuation (Rogers and Cox 1988), a louder sound system would be detectable further downstream and affect the movement earlier. Another option is to move the speakers to an open, soft-bottomed area, where low frequency sounds will travel much farther (Popper and Carlson 1998). Taken together, our results do show that sound deters sea lamprey the first time they swim past speakers and has potential as a stimulus to direct sea lamprey towards existing traps. Sound as a barrier can be used to guide sea lamprey behaviour while not impacting

the environment like many physical deterrents. One of the problems of sound as a deterrent is that it may impact other organisms that are present in the rivers or streams during the sea lamprey spawning season but if sound were only used during peak sea lamprey spawning times the impacts on native species could be lessened as the sound will have no long-lasting effects and will not propagate farther than the speaker array. Future studies could include a synergistic approach or involve multiple modality communication between sea lamprey and their environment, which is still not fully understood, to control sea lamprey with multiple senses stimulated together to elicit stronger responses (Ferrari et al. 2008; Johnson et al. 2015). For example, eyesight has been shown useful as an attractant with more traps becoming more effective with lighted entrances, which could be used with sound to guide the sea lamprey towards the traps (Purvis et al. 1985, Stamplecoskie et al. 2012).

Understanding or controlling the behaviour of sea lamprey can lead to crucial increases in success of control methods, especially traps, where information about how close and when sea lamprey enter could be very helpful (Bravener and McLaughlin 2013). Sound can be an important link between sea lamprey and their interaction with their environment that we can use to our advantage for controlling or guiding their behaviour. In an applied setting, sound systems can be used by being set up at the sides of traps to guide sea lamprey into traps where sea lamprey would be guided to the middle of two speakers where the trap is, leading to a higher encounter rate and a higher catch rate (Bravener and McLaughlin 2013). Sound can also be used as an inexpensive diversion in rivers and streams where they have a smaller radius of effect, which can be an advantage when shaping an effective range, or be used at the mouth of important spawning rivers

and streams where water is deeper and sound would attenuate less (Sand et al. 2001). Barriers, like sound, do not affect water flow and may be the only practical option in some cases (Miehls et al. 2017). Sound also provides a more flexible system that can be adjusted seasonally or daily to help protect non-target species (Johnson et al. 2016; Miehls et al. 2017). Future directions can look at the extension of sound deterrence into new areas of sea lamprey control either in conjunction with other tools or alone.

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Figures

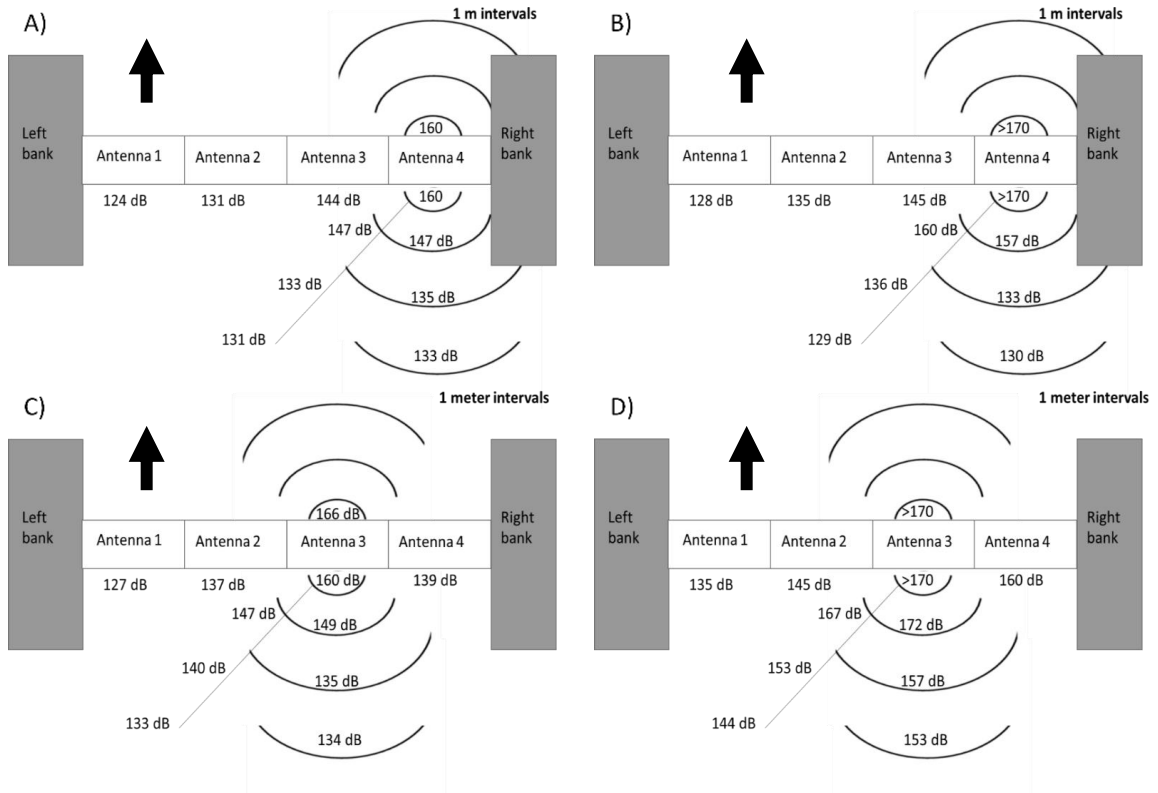


Figure 2.1. Sound field and antenna array at 70 Hz (A and C) and 90 Hz (B and D) sound presentations with sound level decay represented by contours at 1 m intervals. In 2018, (A and B) speakers were placed at antenna 4 and in 2019 (C and D) speakers were placed at antenna 3. All decibel levels are measured in dB re 1 μ Pa. Water is flowing from top to bottom in all diagrams as shown with arrows.

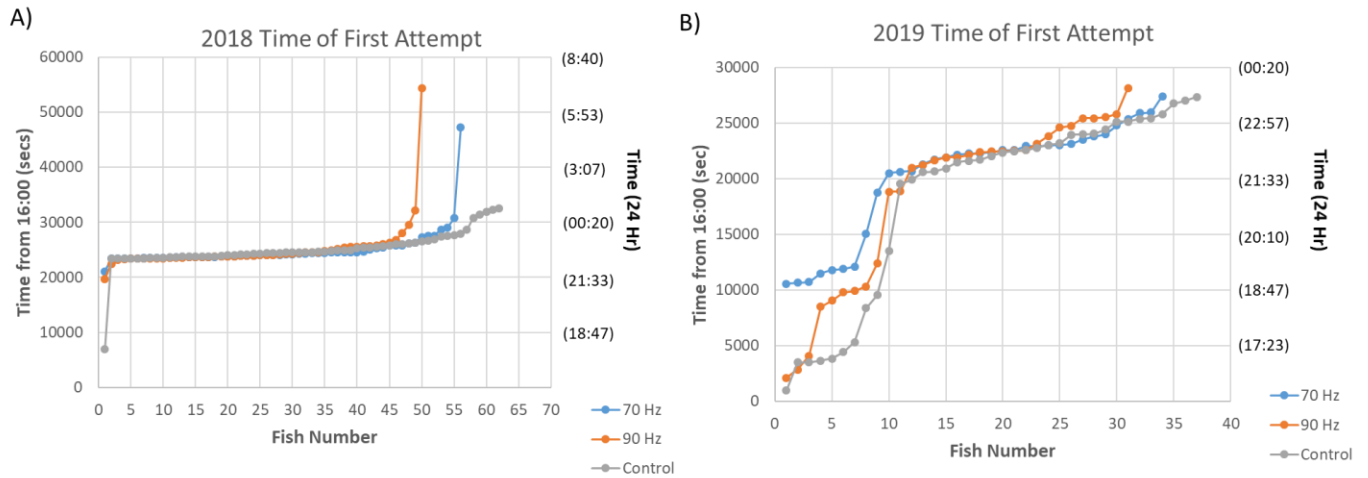


Figure 2.2. a) 2018 cumulative detection of sea lamprey by transit time in seconds (from 16:00 EST), on the left y axis, for all antenna (A1, A2, A3, A4) comparing treatment (70 Hz, 90 Hz, control shown). Each lamprey is listed on the X-axis in chronological order of arrival shown as a data point for first attempt at crossing PIT tag reader. The 24 hour clock time is shown on right y-axis. b) 2019 cumulative detection of sea lamprey by transit time in seconds (from 16:00 EST) on left y axis for all antenna (A1, A2, A3, A4) comparing treatment (70 Hz, 90 Hz, control). Each lamprey is listed on the X-axis in chronological order of arrival shown as a data point for first attempt at crossing PIT tag reader. 24 hour clock time is shown on right y-axis. Median first attempt time for both 2018 and 2019 are shown to be around 20000-25000 or 21:30-23:00 (9:30-11:00 pm EST).

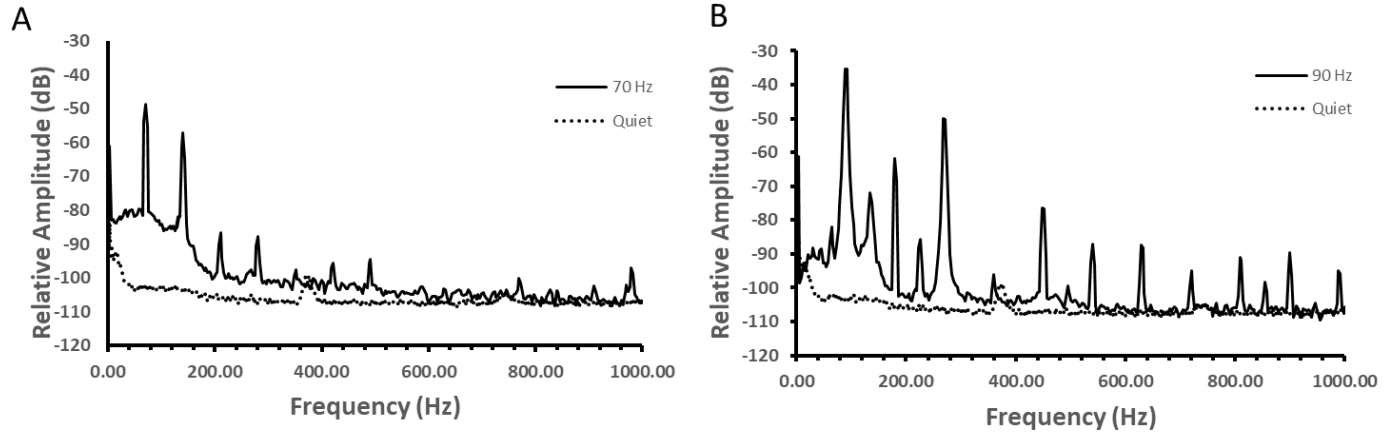


Figure 2.3. Power spectral density plots of (a) 70 Hz and (b) 90 Hz tone file presented through speakers as collected by hydrophone 1 m away from speakers in the Schmidt Creek compared to background noise (labelled as “Quiet”) where no sound was played.

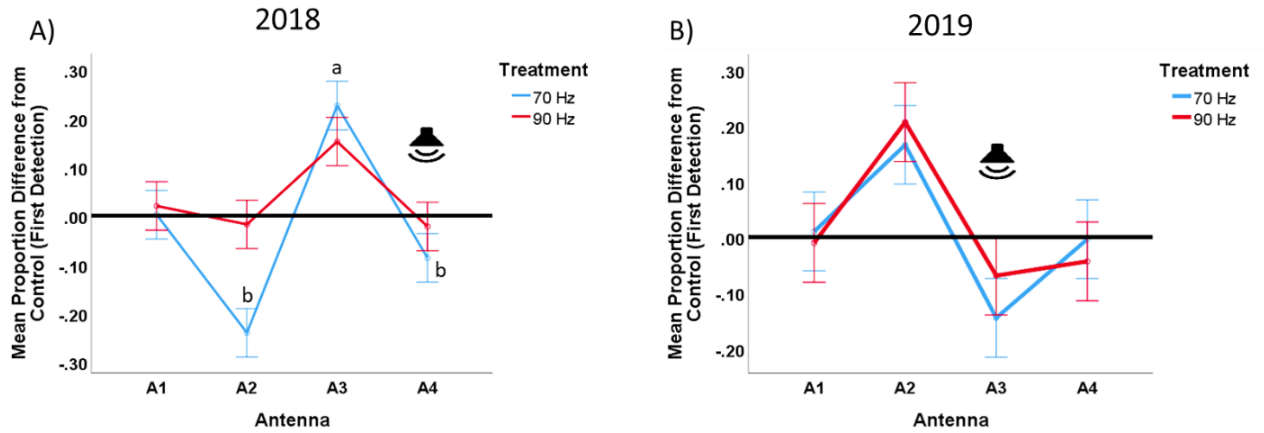


Figure 2.4. (a) First detection difference 2018, sea lamprey first detection mean proportion for all antennas (Antenna 1, 2, 3, 4) with the difference between control (no sound played) experiment nights, shown as black line at $y=0$, and 70 Hz, and 90 Hz experiment nights in 2018 (2018 $n=16$). (b) First detection difference 2019, sea lamprey first detection mean proportion for all antennas (A1, A2, A3, A4) with the difference between control (no sound played) experiment nights, shown as black line at $y=0$, and 70 Hz, and 90 Hz experiment nights in 2019. Error bars are representative of the mean (± 1 SE). The speaker symbol indicated where the speakers were placed for that year according to antenna number (2019 $n=18$). Lower case letters represent significant differences of $p < 0.01$ in 2018. In both panels positive values represent more detections from control nights and negative values represent fewer detections than control nights.

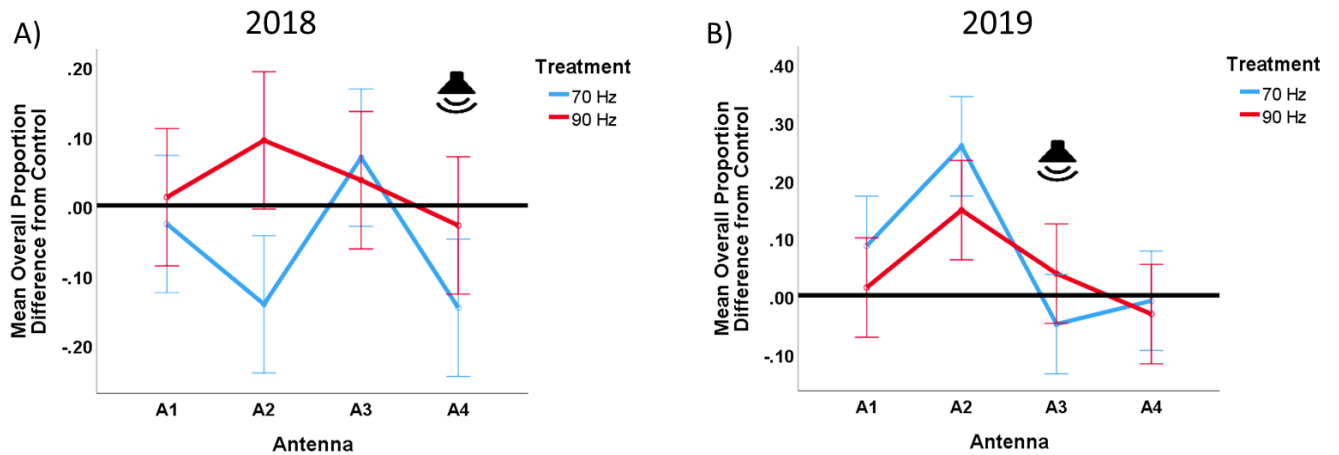


Figure 2.5. (a) Lamprey proportion overall detection 2018 represents the mean proportion of overall detections in 2018 for all antenna (A 1, A2, A3, A4) with the difference between control (no sound played) experiment nights, shown as black line at $y=0$, and 70 Hz, and 90 Hz experiment nights in 2018 (2018 $n=16$). (b) Lamprey proportion overall detection 2019 represents the mean proportion of overall detections in 2019 for all antenna with the difference between control (no sound played) experiment nights, shown as black line $y=0$, and 70 Hz, and 90 Hz experiment nights in 2019. Error bars are representative of the mean (± 1 SE). The speaker symbol indicated where the speakers were placed for that year according to antenna number (2019 $n=18$). In both panels positive values represent more detections from control nights and negative values represent fewer detections than control nights.

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CHAPTER 3
MEASURING BEHAVIOURAL THRESHOLD OF SEA LAMPREY (*PETROMYZON
MARINUS*) TO ACOUSTIC STIMULI

Introduction

The intersection of sensory ecology and conservation biology has become a recent focus of interest for better applying sensory-based organismal principles to conservation practice (Madliger, 2012; Madliger et al. 2016; Friesen et al. 2017; Swaddle et al. 2017). It is argued that if we better understand how animals interact and respond within their sensory milieu we can better increase the efficacy of our conservation and control practices to achieve desired outcomes (Madliger, 2012). Working with sensory processes, we can take advantage of the link between sensory cues and the behaviour associated with them. In cases of invasive species, sensory traps can be used to attract or deter the population to decrease their impact on the environment (Li et al. 2002; Sorensen and Vrieze 2003; Imre et al. 2010; Noatch and Suski 2012; Isabella-Valenzi and Higgs, 2016). The sea lamprey (*Petromyzon marinus*) is a highly invasive species in the Laurentian Great Lakes (hereafter LGL) and much effort has been devoted to understanding its invasion biology (McLaughlin et al. 2007; Binder et al. 2010; Miehl et al. 2020) but much remains to be known about important sensory attributes that are critical for the interaction of the sea lamprey with its environment. While the olfactory system has been well categorized (Hagelin and Johnels, 1955; Kleerekoper and Erkel, 1960; Van Denbossche et al. 1997; Zielinski et al. 2005; Buchinger et al. 2015), many other sensory systems have been understudied and this is especially the case with the auditory system. While the structure of the auditory system had been previously categorized (Lowenstein et al. 1968, Ladich and Popper 2004, Maklad et al. 2014) it was

only recently shown that sea lamprey can respond to sound (Mickle et al. 2018; Heath et al. in press). Sea lamprey are a highly invasive species in the LGL where they parasitically feed on native species and lower their population numbers (Smith, and Tibbles 1980). The sea lamprey population is becoming more manageable but still over half of the Great Lakes are above their targeted population numbers (Great Lakes Fishery Commission <http://www.sealamprey.org/status.php>). Sea lamprey are still highly dangerous to the Great Lakes ecosystem and fishery business where current control is entirely dependent on physical barrier and pesticides, scientists and managers are increasingly looking for additional control methods for an integrated approach (McLaughlin et al. 2007).

Trapping is a technique which could be used for capturing and removing animals for long-term management or eradication of small invasive populations (El-Sayed et al. 2006). Capturing sea lamprey for control or assessment purposes was an important part of the control program in the Laurentian Great Lakes and improving the trap efficiency would greatly improve the amount of sea lamprey able to be removed (McLaughlin et al. 2007). The capture and removal of sea lamprey might be an efficient control measure used with catalysts to increase the capture rate of traps, with chemicals having been studied as one possibility (Velez-Espino et al. 2008; Johnson et al. 2013; Hume et al. 2015; Miehl et al. 2020). Another possibility is acoustic stimulation used in areas where sea lamprey traps have a low rate of capture, to increase the encounter rate of sea lamprey (Bravener and McLaughlin 2013). Sound has been used to control behaviour in other fish (Knudsen et al. 1994, Sand et al. 2000, Pegg and Chick 2004, Vetter et al. 2015, Isabella-Valenzi and Higgs, 2016) and used with traps to increase the capture rate (Isabella-

Valenzi and Higgs, 2016). Sound can act as an acoustic barrier to block the movement of fish by keeping vulnerable fish away from dangerous infrastructures such as power plant intakes (Ross et al. 1993; Maes et al. 2004; Sonny et al. 2006; Qin et al. 2020). High-intensity artificially-generated sounds have also been used to induce an avoidance response in fish (Van Derwalker, 1967). Playing low frequency sounds in a lab setting has been shown to initiate a response in sea lamprey specifically with increased activity levels when exposed to 70 Hz and 90 Hz (Mickle et al. 2019). One field study (Heath et al. in press) has extended these acoustic results to a natural stream setting to show high-intensity sounds can direct sea lamprey movement but we still need more information on how sea lamprey react to lower sound levels in an effort to better understand the minimum sound levels needed to effect behavioural outcomes. Results from behavioural studies can lead to a wider knowledge of how to control lamprey's behaviour and may lead to new integrated strategies for preserving unaffected areas or help develop more effective methods of control.

The purpose of the current study is to quantify the behavioural threshold of sea lamprey to low frequency tones known to cause a response at higher intensities (Mickle et al. 2019; Heath et al. In Press). Specifically, I am answering the question: what is the minimum sound level needed for sea lamprey to detect and change their behaviour. I also explore and test the possibility of a difference in hearing threshold between juveniles and adults and what this may mean for their behavioural responses. These goals are accomplished by exposing sea lamprey to two tones (70 and 90 Hz) in a range of sound intensities and quantifying behavioural responses to both the onset and offset of these tones.

Methodology

Juvenile and adult sea lamprey were captured from the Ocqueoc and Cheboygan Rivers, Michigan, in May and June of 2018 and 2019 and held at Hammond Bay Biological Station, MI, (USGS station) until transport to the University of Windsor Central Animal Care Facility in mid-June. The juvenile sea lamprey had completed metamorphosis but were still small in size and recently had become parasitic, while the adult sea lamprey were not yet spawning but of the right size and age to spawn. Sea lamprey were maintained prior to experimentation in 560 L covered tanks at University of Windsor Animal Quarters where the room was maintained at 4-6⁰ C with lights off. Experiments and animal care were conducted following Canadian Council for Animal Care (CCAC) protocols (University of Windsor AUPP 17-11).

Behavioural trials were conducted in a 1020 L fiberglass tank (243 x 91 x 91 cm) with a water depth of 35 cm at the University of Windsor with water temperatures around 5-10°C (Mickle et al. 2018). Transferring sea lamprey from the holding tank to the experimental tank took less than one minute and the sea lamprey were left in 50% holding tank water and 50% experiment tank water for 5 minutes to reduce shock. One third of the tank was sectioned off for experiments to aid camera visibility using a barrier made of Plexiglas with 1 cm holes to ensure water flow and reduce acoustic reflection (Mickle et al. 2018). To determine the behavioural response of sea lamprey to low frequency tones, an underwater speaker (Clark Synthesis Diluvio AQ339; Lubell Labs) connected to an amplifier (Scosche SA300), a 12 Volt PBS car battery for power and a Motorola phone were used to present acoustic stimuli (Mickle et al. 2018). Sea lamprey are nocturnal so trials were conducted with a limited light source used for camera visuals,

tested prior for effect on lamprey behaviour, for more natural responses (Wigley 1959). A demonstration of the setup is shown in Figure 3.1 A and B.

Sea lamprey were kept in the experiment tank for at least 1 hour to acclimate with no sounds and limited light source. Experimental tones, of 70 or 90 Hz, were played at varying amplitude levels (level 0-15, 129 – 169 dB re 1 μ Pa; Table 3.1) using an underwater speaker suspended off the bottom to reduce interference (Mickle et al. 2018). Sound levels of the experimental tones, and background noise were mapped within the study tank using a hydrophone (Inter Ocean system inc. – Acoustic 127 Calibration and System Model 902; Figure 3.2). Background noise levels were measured and averaged around 120 dB re 1 μ Pa. Lamprey were experimented individually, since lamprey do not school, to eliminate any group behaviour effects (Binder and McDonald, 2008). Frequencies of 70 Hz and 90 Hz were used for threshold testing due to previous experiments done by Mickle et al. (2018) that showed positive results to only low frequencies in this species. Sea lamprey were only used once then moved to a separate tank so that they were not used twice.

To determine behavioural reactions and thresholds, sea lamprey would have to stop moving for 1 minute uninterrupted, then sound would be played for 1 minute and a behavioural reaction would be recorded during the sound. The behavioural reaction of the sea lamprey directly after the sound played was also recorded and the process repeated with increased or decreased sound level. Trial duration times, depending on the reactivity of the sea lamprey, usually ranged from 30 min to 2 hours. A stepwise method was used for decibel changes, with sea lamprey responses leading to a higher decibel level for no responses and lower decibel levels or a repeated decibel level for behavioural responses

(movement) similar to Tavalga and Wodinsky (1965) and Lu et al. (1996). Behaviour of the sea lamprey were filmed with GoPro Hero 3+ and GoPro 5 (GoPro). Analysis of all trials examined the proportion of time spent swimming, resting or sucking (attached to tank) with and without sound.

Adult and juvenile sea lamprey behaviour was also compared by the immediate response to sound application. This “twitch” behaviour was quantified by tail or body movement that occurred in the first second of sound presented. Behaviour of the sea lamprey was compared during the minute right before sound was played and the response during the first second of sound to see if there was a change in behaviour, which could be described as a “twitch.”

Statistical analysis

There were 10 trials done on adult sea lamprey in 2018 with 5 trials each with 70 and 90 Hz tones. In 2019, 22 trials were done on juvenile sea lamprey with 11 trials each with 70 and 90 Hz tones. Finally, 22 more trials were done on different adult sea lamprey with 12 trials with 70 Hz and 10 trials with 90 Hz. In total, 54 sea lamprey were used with 22 juvenile sea lamprey and 32 adult sea lamprey used in this experiment with all experiments done separately and with different sea lamprey each time. All decibel (dB) references are re 1 μ Pa. Trial data for adult sea lamprey were analyzed together because there was no difference in the setup used for both years leading to no significant year effects on frequency for onset sound data ($F_{1,17}=0.151$, $p = 0.702$) and offset sound data ($F_{1,18}=1.050$, $p = 0.319$) and no year effect on level for twitch data ($F_{14,211} = 0.869$, $p = 0.593$).

Behavioural threshold levels were determined by quantifying the percent time swimming during or after sound compared to the sea lamprey not moving for a full 1 minute before playing sound. The behavioural threshold I look at here is the lowest decibel level of either frequency used (70 or 90 Hz) that elicits a “positive response” behaviour, which is described further, during or after sound. To determine the threshold of movement during sound playing, I examined the time spent swimming during the one minute of sound for a “positive response,” if a sea lamprey was moving more than 50% of the time (30 sec of 60 sec) then it was characterized as a “positive response” (example shown in Figure 3.3). To determine the threshold of movement directly after sound has stopped playing, I again examined the time spent swimming after the sound for a “positive response,” defined as a sea lamprey that moved within the first 10 seconds after the offset (example shown in Figure 3.3). The two separate thresholds (movement during sound and movement after sound) were then determined by looking at all positive responses and finding the first and, in all cases but three, the lowest sound level leading to, maximum, one threshold per sea lamprey for during sound and after sound. Statistically, I compared the different decibel levels of the determined threshold (determined for each responding sea lamprey) as the dependent variable between frequency (70 and 90 Hz) and life stage (adult and juvenile) as the independent variables, using 2-way ANOVA. Behaviour data were tested for normality using descriptive statistics in SPSS where it tested normally. Juvenile responses to sound offset varied wildly but only consisted of a total of 4 data points due to juvenile sea lamprey not responding to sound behaviorally after sound was played (one threshold recorded for 70 Hz, three thresholds recorded for 90 Hz).

Secondly, I tested for behavioural thresholds with twitch responses. While adult sea lamprey reacted positively by swimming when presented with sound, juveniles may have undeveloped systems or respond differently. To have a more in-depth study on behavioural thresholds, I included twitch responses, in case sea lamprey tended to avoid swimming in trials. Sound levels that were presented to sea lamprey less than three times were deleted within life stage and frequency tested (adult 70 or 90 Hz and juvenile 70 or 90 Hz) since all provided insufficient data for statistical analysis. Statistically, I compared the mean proportion of twitches as my dependent variable in adults and juveniles, separately, and frequency (70 and 90 Hz), also separately for each life stage, with differences between the different sound levels (0-15, 129 – 169 dB re 1 μ Pa; Table 3.1) as my independent variable which was evaluated using a Kruskal-Wallis one-way test. The data was not normal so a Kruskal-Wallis test was appropriate for statistically testing the overall difference between levels and for easier to interpret results, arbitrary levels were used in statistics which could then be translated to decibel level using Table 3.1. Further insight into the data was needed so a separate test specifically comparing each level with level 0 (no sound/129 dB re 1 μ Pa), was used to statistically determine the levels different from 0 or no movement. The biological significance of this test is evident by the benefit of knowing the levels applicable to field trials for a visible effect on sea lamprey. For juvenile sea lamprey, the comparison level 0 baseline (or no movement baseline) was added only for the comparison statistics while the adult sea lamprey already contained baseline comparison data (with the tests being done separate years, some changes were made for statistical advantage between the adult and juvenile trials). For adult sea lamprey at 90 Hz, since the KW statistical test data was not significant,

comparisons of levels to level 0 (no movement) was evaluated with simple contrasts relative to level 0 with a statistical difference showing a difference from zero for each level tested against.

Results

1. Overall response frequency

A higher percentage of adult sea lamprey responded to sound in general while juvenile sea lamprey showed a higher percentage of no behavioural responses to sound (Figure 3.4 A,B,CandD). While adult sea lamprey showed an inclination for responding to sound both during and after sound presentations, for both frequency levels, with over 50% of reactions for 70 Hz and 40% for 90 Hz (Figure 3.4 AandB), juvenile sea lamprey had a higher percentage of unresponsive behaviour with both 70 Hz and 90 Hz causing no reactions over 40% of the time (Figure 3.4 CandD). Furthermore, adult sea lamprey displayed an equal percentage to only responding during sound or only responding after sound for 70 Hz (17%, 12%) and 90 Hz (20%, 27%; Figure 3.4 AandB). Juvenile sea lamprey responded 50% of the time for 90 Hz during sound and 27% of the time for 70 Hz, which for 90 Hz was the most likely response for juvenile sea lamprey (Figure 3.4 CandD).

2.1. Behavioural threshold of adult and juvenile sea lamprey to sound onset

There was no significant difference for adult and juvenile movement at sound onset between frequency ($F_{1,28}=0.007$, $p = 0.936$) and no significant interaction between frequency and life stage ($F_{1,28}=1.428$, $p = 0.242$). The statistical difference between life stages was on the boundary of significance ($F_{1,28}=3.655$, $p = 0.066$; Figure 3.5A), with adults tending to respond with a lower threshold. The frequencies (70 or 90 Hz) were

similar for both adult or juvenile threshold level with adult sea lamprey having an average threshold of 155 dB (+/- 3.48 SE) for 90 Hz and 151 dB (+/- 2.89 SE) for 70 Hz while juvenile showed a threshold decibel level of 158 dB (+/- 5.20 SE) for 90 Hz and 162 dB (+/- 2.84 SE) for 70 Hz. (Adults: 70 Hz n = 12, 90 Hz n= 9; Juvenile: 70 Hz n= 7, 90 Hz n= 4)

2.2. Behavioural threshold of adult and juvenile sea lamprey to sound offset

There was a significant effect of frequency for adult movement at sound offset with 70 Hz showing a lower threshold decibel level ($F_{1,20}=5.046$, $p = 0.036$; Figure 3.5B). The threshold level of adult sea lamprey was 155 dB (+/- 1.28 SE) for 90 Hz and 148 dB (+/- 2.79 SE) for 70 Hz. The results only include results of positively identified as behavioural responses. Juvenile sea lamprey only consisted of a total of 4 data points due to juvenile sea lamprey not responding to sound behaviorally after sound was played which was an insufficient amount of data for a statistical test to be valid and were therefore, left out. (Adults: 70 Hz n = 12, 90 Hz n= 10)

3.1. "Twitch" threshold of juvenile sea lamprey to 70 Hz

There was a significant difference between levels with higher sound levels (higher sound pressure) having higher proportion of the startle behaviour ($KW_{10}=21.292$, $p = 0.019$; Figure 3.6A). Levels 1, 2 and 6 were deleted since all provided only one response which is insufficient for statistical analysis, giving a total n of 83 cases. Follow-up testing (pairwise multiple comparison) showed the levels that were significantly different from level 0 or no sound were: 11, 12, 13, 14, 15 (shown in table 3.2; $p<0.05$) with none of the levels that were different from level 0 being statistically different from one another. The lowest decibel level sea lamprey significantly responded to being 160 dB (level 11).

3.2. “Twitch” threshold of juvenile sea lamprey to 90 Hz

There was no significant difference between sound levels ($KW_{10}=17.636$, $p = 0.061$; Figure 3.6B). Levels 1, 4 and 5 were deleted since all provided less than 3 responses which is insufficient for statistical analysis and level 2 had no responses, for a total n of 68 cases. Because the main effect was close to statistical significance follow up tests were conducted (pairwise multiple comparison) to examine if any levels did differ from level 0, those levels that were are: 14 and 15 (shown in table 3.3; $p<0.05$) with neither of the levels different from level 0 statistically different from one another. The lowest decibel level sea lamprey significantly responded to being 164 dB (level 14).

3.3. “Twitch” threshold of adult sea lamprey to 70 Hz

There was a significant main effect of level on twitch responses of adults at 70 Hz ($KW_{14}=34.298$, $p = 0.002$; Figure 3.7A). Level 1 was deleted since it provided less than 3 responses which is insufficient for statistical analysis, for a total n of 142 cases. Subsequent follow-up tests (pairwise multiple comparison) showed that levels: 10, 12, 14, and 15 were significantly different from level 0 or no sound (shown in table 3.4; $p<0.05$) with none of the levels different from level 0 statistically different from one another. The lowest decibel level sea lamprey significantly responded to was 157 dB (level 10).

3.4. “Twitch” threshold of adult sea lamprey to 90 Hz

There was no significant main effect of sound level ($KW_{12}=18.194$, $p = 0.110$; Figure 3.7B), although there was an apparent spike in proportion responding beginning at 154 dB re 1 uPa (level 10). Levels 1 and 4 were deleted since all provided less than three

responses which is insufficient for statistical analysis and level 2 had no response, for a total n of 120.

Discussion

In the current study, I found a behavioural threshold to sound by movement at onset in adult and juvenile sea lamprey of 150-162 dB re 1 μ Pa for both 70 and 90 Hz and similar behavioural threshold range for adults to sound by movement at offset. Adult sea lamprey showed a behavioural response (either during sound, after sound or both) while comparably, juvenile showed more responses that had an absence of behavioural changes to sound. In further experiments involving a “twitch” or startle-like behaviour, juvenile and adults both showed responses to 70 Hz with juveniles needing a higher decibel level (160 dB re 1 μ Pa) for a response while adult sea lamprey responded to decibel levels as low as 157 dB re 1 μ Pa.

Adult sea lamprey responded to sound behaviourally in the majority of the trials with either increased movement at the onset of sound or directly after sound compared to control. The response of adult sea lamprey to sound include the use of different increased movement reactions (i.e. swimming at the onset and offset of sound), this could be showing the development of diverse behavioural reactions to an aversive or new stimuli. Adult sea lamprey are not only responding to sound by increased movement during sound but also by movement after the sound is over (no movement during sound) which could be a version of the flight or freeze response. Sound can lead to different behavioural responses by individuals of the same species with some showing increased anti-predator behaviour while others are not affected (Voellmy et al. 2014; Maziarz et al. 2018). Some organisms react to novel sounds similar to other danger-related sounds, like predators,

showing an experience-independent ability for sounds (Blumstein et al. 2008). One possible explanation for the diversity of responses relates the response to the proximity of the sound or potential predator with research showing that distance from a predator leads to different behaviours from the potential prey (Hendrie et al. 1996). Eilam et al. (1999), suggests that the behaviour to freeze or flee could be a result of differences in individual normal behaviour and/or in stimulus interpretation. Sea lamprey's response to sound could be similar to responses to anthropogenic sound or, since the tones played were novel and organized by humans (the experimenters), the tones could be classified as anthropogenic sound. Sound or anthropogenic noise, has been shown to be a potential stressor in many fish with increases to glucocorticoids (stress related), changes to metabolic rate, and changes in behaviour (foraging, sheltering, avoidance behaviour; Mickle and Higgs, 2018). Moreover, auditory cues may not allow the prey to localize the predator accurately, and therefore the same cue could result in two different responses (Eilam et al. 1999). Another possible explanation is different reactions to stimuli can be a more advantageous decision for the individuals for risk of predators or risky conditions, this then leads to variability within the population leading to more stable population numbers overall (McIntosh and Peckarsky, 1996; Dall et al. 2004; Wilson and Stevens, 2005). Similar behavioural thresholds were seen when comparing adult sea lamprey thresholds for the movement after sound (no movement during sound) with threshold levels to adults' movement during sound (increased movement to sound). This shows that while movement after focused on a continued cessation of movement during the sound, leading to movement right after the sound stopped, this different behaviour to sound still had a similar threshold. Either behaviour (onset or offset movement) could be elicited by

the same lower decibel sound of either frequency, even though I do not know what triggers them to perform which behaviour. A previous study that used a similar setup, Mickle et al. 2019, found that sea lamprey adults respond to sound by increasing swimming activity during some frequencies and decreased swimming activity during other frequencies, which was similar to the results found in this study. The difference of responses to sound shown by adults shows the diversity of behaviours that sea lamprey have. There was no exclusive behavioural response to lower or higher frequency, which shows that frequency is not a factor controlling the type of behavioural response. The current study's adult sea lamprey showed both behaviours (onset and offset movement) more than showing individual increase or decrease to sound, this could be a survival technique to increase the chances of the individual to survive novel dangerous situations with multiple tactics, leading to better survival of the species (McIntosh and Peckarsky, 1996; Voellmy et al. 2014; Maziarz et al. 2018).

Adult sea lamprey respond to sound with an increase of movement at the onset or offset of sound, while juveniles mostly respond with increased movement at the onset of sound, indicating that in earlier life stages, sea lamprey have a less diverse range of behavioural responses. Juvenile sea lamprey may be focusing on one behavioural response or strategy, indicated here by swimming when a novel sound appears. Sabet et al. (2016) induced a significant reduction in swimming speed when exposing cichlids, *Haplochromis piceatus*, to experimental sound while zebrafish, *Danio rerio*, showed an increase in swimming speed. Atlantic salmon, *Salmo salar*, showed increased swimming activity when exposed to infrasound where speeds could triple but returned to normal after sound had stopped (Bui et al. 2013). The choice of swimming response between

juvenile and adult sea lamprey evidently vary with juvenile triggered to increase their swim movement when exposed to noise while adults are triggered to either increase swimming movement during or after sound. In the largemouth bass (*Micropterus salmoides*), juveniles survived better when using the opposite strategy of reproductive adults (Ballew et al. 2017). A large portion of the juvenile sea lamprey showed no swimming behavioural response to sound as well. Juvenile sea lamprey have been shown to hear low frequency sounds in past experiments by Mickle et al. 2019, so they are likely hearing the sound but not responding with movement. The juvenile sea lamprey may not have had time to develop a behavioural swimming response to sound since lamprey larvae are poor swimmers (Sutphin and Hueth 2010). The selective behavioural responses may vary for different life stages due to neural mechanisms, brain or muscle development. Another possible reason is that selective pressure on the sea lamprey at different stages of life could lead to increased swimming response to predators or higher sensitivity to discern sounds as danger (Bell et al. 2010; Toscano 2017). Regardless, fewer juvenile sea lamprey responding by swimming meant a lower sample size for threshold testing, meaning that the results are hard to verify for the population as a whole. Also, although there was no significant difference between life stages (adult and juvenile) for behavioural threshold to onset of sound by swimming, it was close to significance ($p < 0.1$) indicating that further studies may show a difference in threshold between juveniles and adults that was not seen in the current study. Comparing the average juvenile and adults threshold levels shows that juvenile sea lamprey have a non-significant higher (less sensitive) average threshold level for behavioural responses.

Similar experiments have also looked at the behavioural response thresholds of other underwater organisms by exposing them to differing intensity levels, showing the lowest threshold with the highest sensitivity (Stanley et al. 2011). Present data showed that only 70 Hz lead to a difference in sound level for startle or twitch behaviour in adult and juvenile sea lamprey but both frequencies showed levels that were significantly different from zero with higher proportion of twitch at louder sounds. This is similar to the overall behavioural threshold to sound by swimming for adults where 70 Hz had a lower average threshold than 90 Hz for both behaviours (onset and offset movement), showing that 70 Hz is the more effective frequency to use to elicit any behavioural reaction with sound (twitch or swimming). Juvenile sea lamprey, in comparison to adults, had a lower threshold for 90 Hz for swimming behaviour but had a larger standard error and lower total number of trials to compare leading to some validity questions. The threshold for swimming and startle response for adults and juveniles was around 150 to 162 dB re 1 μ Pa for both frequencies compared to the behavioural threshold for anguilliform fish for rejecting sound equipped passage ways which was 100 to 150 dB re 1 μ Pa (Deleau et al. 2020). Three marine species were exposed to playback noise, with behavioural responses to impulsive sound at 163–171 dB re 1 μ Pa and continuous sound of 142.7 dB re 1 μ Pa showing directional changes and movement (Roberts et al. 2016). The behavioural threshold range found within the current study falls within the range of behavioural responses by other aquatic organisms. The behavioural thresholds for twitch and swimming movements shown in this study for adult and juvenile sea lamprey can be used in future studies with using a low frequency range with higher decibel sounds to

efficiently elicit the needed behaviour. The additional use of continuous sound and/or shorter bursts of sound, with a similar range, may lead to more pronounced effects.

Sea lamprey seem to have a similar hearing ability as adults and juveniles shown by a similar behavioural response for both younger and older life stages. Similar results were found in Mickle et al. 2019, who found that the hearing threshold for juvenile and adult sea lamprey with auditory evoked potentials differed by around 5 dB. Both results with swimming behavioural threshold and twitch behavioural threshold showed similar thresholds to sound for both age groups. A study by Higgs et al. (2002) also showed that the difference between life stages does not always result in a difference in sensory abilities. Although Zeddies and Fay, 2005, predicted that the acoustically evoked behavioural responses (AEBR) thresholds in adult zebra fish would be lower than the thresholds in larvae, Zeddies and Fay instead found a similar threshold for adult and larval fish. Juvenile sea lamprey's behavioural threshold for twitch behaviour was higher at both frequencies when compared to adults' by 10 dB from the lowest threshold for adults (90 Hz) compared to the highest for juvenile (90 Hz) which does show a decibel significance. Although there may be no difference behaviourally between the two life stages, indicating that juveniles are equipped to functionally respond to sound with a startle response, there is a large (10 dB) difference in decibel level needed for a similar reaction. Larval zebrafish experiments have shown that free swimming larval zebrafish can perform a startle response in reaction to sudden acoustic stimuli and a similar response by touch can be induced, meaning that there is no limit from motor development (Kimmel et al., 1974). Sea lamprey are also able to perform the same behavioural startle response to sound as juvenile and adult showing no restriction by movement but, like the

zebrafish, may be dependent on the development of morphological specializations formed around the same time as the development of the startle response to sound (Eaton and DiDomenico, 1986). Another possibility is that the age gap between the study groups (juvenile and adult) were very close in age so while there was a louder sound needed for juvenile to respond, both age groups were able to behaviourally respond by twitching. Future studies building off this research could use more trials or a wider age range, such as comparing sea lamprey before metamorphosis and reproductive adults, this may show a larger difference in behavioural threshold or compare on a scale that incorporates the log ratio of decibel levels into the statistical comparison.

One possible limitation of the study is that the responses from juvenile sea lamprey for twitch responses could be due to the vibrations from the sound stimuli. Larval lamprey has shown to respond to low frequency vibrations, through lateral line detection, with responses to as low as 20 Hz vibrations (Popper and Carlson 1998). Many of the sea lamprey in the present experiment reacted to sound after a few seconds of no reaction, which could indicate that instead of immediately being affected by the vibration, a continued presence of sound set off a behavioural reaction. The current experiment did not have the equipment to compare behaviours to sound with and without vibrations or test just vibrations, future experiments should consider this. Lateral line sensitivity can lead to bursts of movement, which could also be due to sea lamprey being sensitive to weak low frequency electric fields, (Dubuc et al. 2008) but sea lamprey exposed to electrical fields swam constantly until it was turned off which is not the type of behaviour I saw in my experiment (Chung-Davidson et al. 2004).

In this experiment, I waited for sea lamprey to stop moving before playing sound, which means this study has a possible bias. The methodology used allows for different acclimation times meaning that there are different times in between the sound exposure which could affect the behaviour of sea lamprey. In addition, the practical aspects of comparing sea lamprey not moving as a self-control to movement or continued non-movement during sound could bias the type of results I can see. If the sea lamprey started as swimming, I might see more examples of sea lamprey stopping movement due to sound. For the present experiment, I needed a control for the sea lamprey behaviour and assuming that sound would lead to swimming behaviour, as this would be the most practical use of a behavioural reaction to sound. Future studies could look at different starting or comparison behaviours of sea lamprey.

The purpose of the current study was ultimately to answer the question: what is the lowest sound that sea lamprey can detect and react to behaviourally. Specifically, if there is a difference for hearing in juveniles and adults and what this has done to their behavioural responses. Using two different strategies: change in swimming behaviour and twitch or startle behaviour due to sound, I can more accurately measure the possible behavioural thresholds for adult sea lamprey and for juvenile sea lamprey, who prefer to respond with startle behaviour. Juvenile sea lamprey compared to adults did not respond as much with movement to novel sounds indicating a different strategy is used at different life stages for sea lamprey, this may be to combat the different environments they would find themselves in. Juvenile sea lamprey are moving downstream after metamorphosing and adults are moving upstream to begin spawning leading which happen at different times of the year and different environmental factors (Manion and

Piavis 1977). The life cycle of sea lamprey may lead to different hearing ability at different points in development. Sea lampreys go through a phase known as metamorphosing, which dramatically change the structure, physiology, chemistry, and behaviour of the larvae to adult (Manion and Stauffer, 1970). On the other hand, sea lamprey behaviour has not been well studied, and can be very subjective which could lead to a variety of responses available (Miehls et al. 2017b). Behavioural responses to sound can take on a variety of types: startle responses, increased swimming speed, increased group cohesion and bottom diving (Neo et al. 2014) or by participants responding differently from one another to the same sound (stopping or remaining unaffected; Moorhouse et al. 1987). Responses to sound can lead to habituation if no immediate danger is detected or can depend on the properties of the sound stimuli (Talling et al. 1996). For example, Chapuis et al. (2019) found that white sharks (*Carcharodon carcharias*) were affected by artificial sound but not by natural orca call sounds. Further studies on the type of sound that elicits a specific response from sea lamprey are needed with an emphasis on the repertoire of sea lamprey at different life stages and the possibility of behavioural flexibility in sea lamprey.

Invasive species are considered to be the leading cause of animal extinctions worldwide (Clavero and Garcia-Berthou, 2005). Sound is applicable in many settings including fish exclusion or protection; this can be used with attraction or repelling fish, which can be important for invasive species (Popper and Schilt, 2008). While water turbidity can provide a host of problems for visual communication, it leaves an opportunity to use sound as an alternative option (Popper and Schilt, 2008). Startle responses, which were examined in this study, have been observed in larval sea lamprey (Currie and Carlsen,

1985) and frequently among fish, when an impulsive acoustic stimulus is introduced even in low visibility water (Richard 1968). The current study is important since the applicability of sound or noise on different life stages of animals are rarely studied (Popper and Schilt 2008). This current study is also applicable in the use of sound as a behavioural control in conjunction with trapping. Adult sea lamprey are vulnerable and easy to capture during spawning season (as they were for this experiment) while juvenile sea lamprey are vulnerable during their migration downstream after metamorphosis (Katopodis et al. 1994; Miehl et al. 2017a). Detailed knowledge of the target species' behaviour can affect trapping efficiency and is required for effective implementation of trapping programs (Bravener and McLaughlin 2013). The use of sound, knowing the threshold levels for lamprey, can be used to guide sea lamprey into traps and increase the efficiency of traps leading to a new future of integrative approaches for invasive sea lamprey control. Heath et al. (In press) used acoustic barriers in an applied setting with sea lamprey and found sound to be an adequate deterrent for adult migrating sea lamprey. Using sound as a deterrent can be used to guide sea lamprey into traps, leading to a new age of more efficient trap capture rate. Future studies could include multiple sensory modalities for sea lamprey control, using multiple senses together with their environmental cues and the behaviours associated to elicit stronger responses (Ferrari et al. 2008; Johnson et al. 2015). For example, light as an attractant with more traps becoming more effective with lighted entrances or pheromone signals in combination with acoustic or light attractants (Purvis et al. 1985, Sorensen and Stacey 2004, Stamplecoskie et al. 2012). Future scientific experiments should look into the extension

of sound deterrence into new areas of lamprey control with juvenile or adult sea lamprey in conjunction with other control methods or tools.

Figures

A)



B)



Figure 3.1. Side view and top-down view of experimental setup. A) The red oval indicates the camera attachment where the camera would have a birds-eye view of the tank. The red circle indicates the speaker set up in the middle of the tank. The red square shows the battery and amp that are attached to the speaker to provide the power and the arrow points to the lamp that provides the light so that the camera can pick up movement. B) An example frame of the view from the experiment camera.

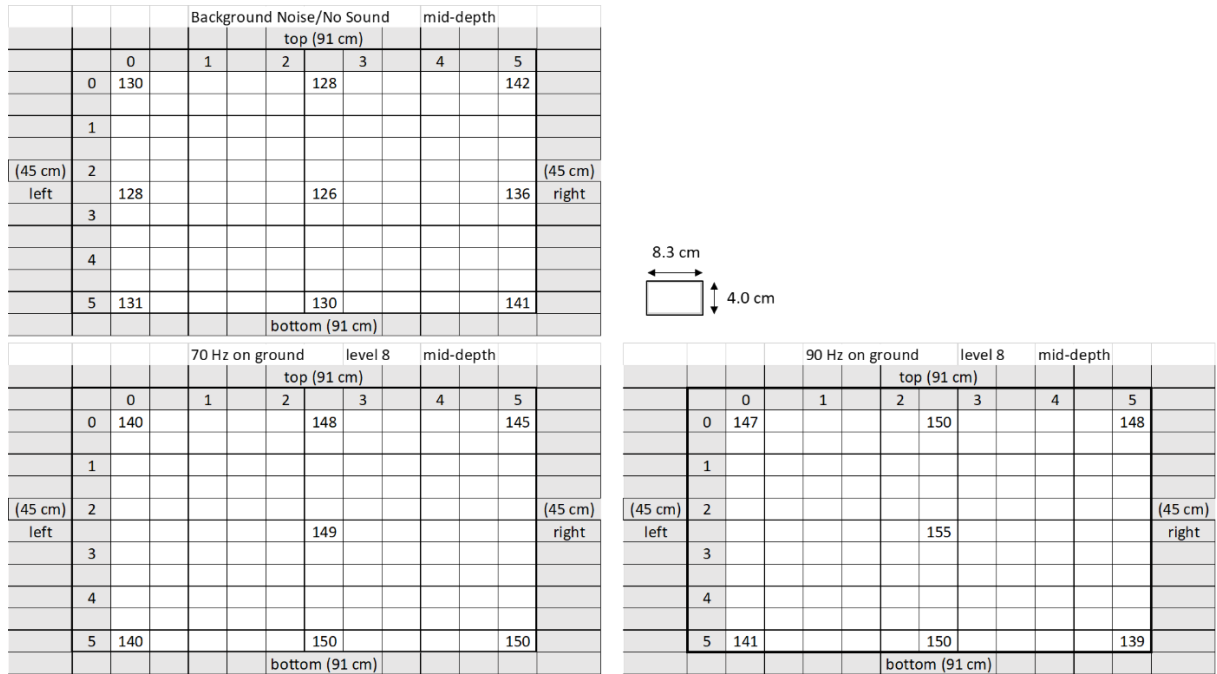


Figure 3.2. Sound map of experiment tank with distance covered by each box shown in the small box diagram. Sound pressure level was measured in all corners, middle and top (away from experiment equipment table, Figure 3.1. A) and bottom for 70 Hz and 90 Hz (shown at the bottom of figure) for “level 8” which was used as mid-range loudness. Background noise map used same setup as 70 and 90 Hz with decibel readings from the left and right of tank for more precise reading. Levels were within 16 dB range for background noise and within 11 dB for 70 and 90 Hz. Map sound levels were taken at mid-depth in the tank with speaker placed on the tank bottom (center) where it remained for all trials (Figure 3.1. B).

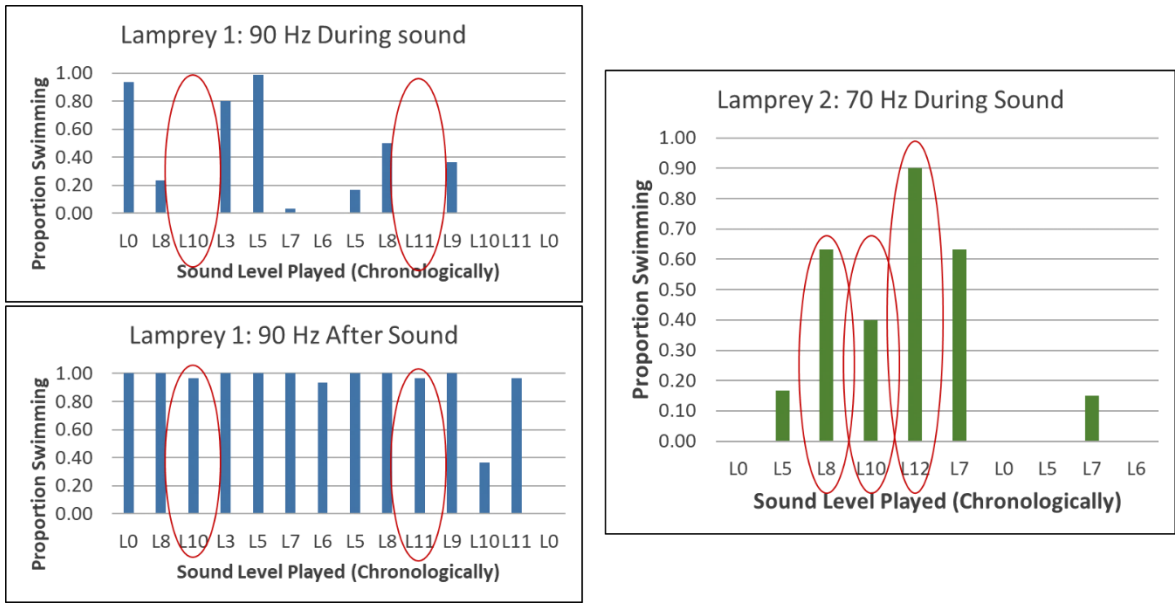


Figure 3.3. Example diagram of sea lamprey 1 and 2 showing the determination of movement at offset of sound (sea lamprey 1) and movement at onset of sound (sea lamprey 2). X-axis shows the sound level played during the trial for the individual sea lamprey (in chronological order) and the Y-axis shows the proportion of time spent swimming during the 1 minute during sound or after sound. Red circles indicate the area of interest that indicate “positive results” or are used for comparison for movement after sound.

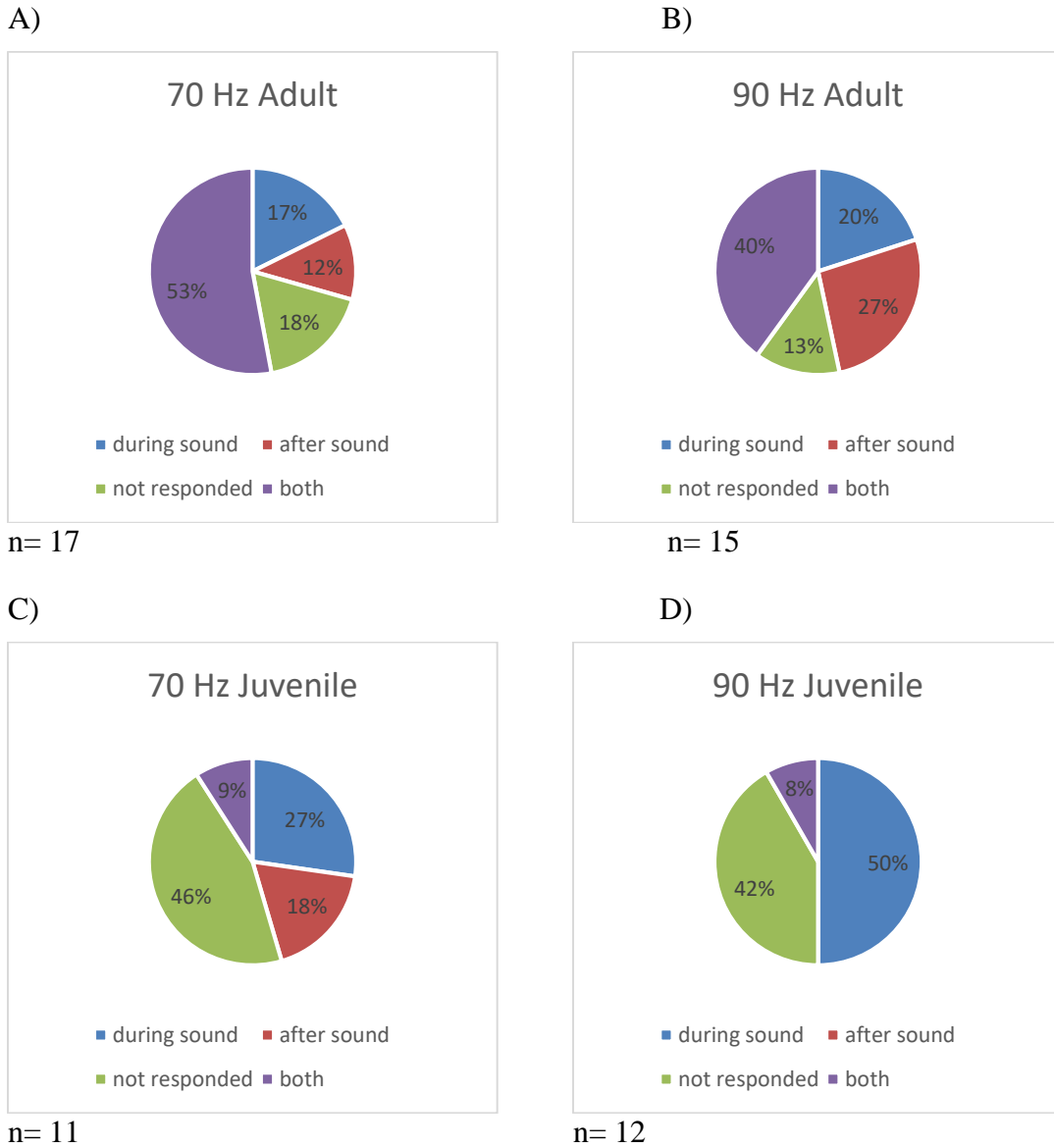
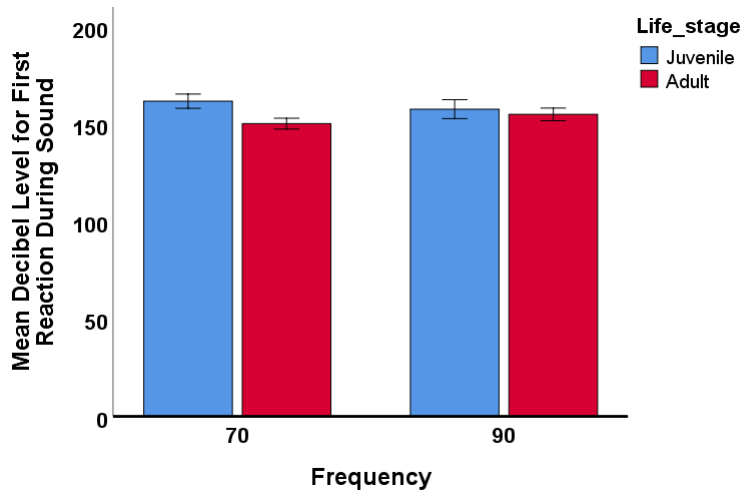


Figure 3.4. The percentage of adult (A, B) and juvenile (C,D) sea lamprey reacting to 70 Hz (A,C) or 90 Hz (B,D) tones by either: moving during the sound played (during sound), moving only after the sound has stopped (after sound), both, or not showing any movement (not responded). The symbol n represents the total number of trials represented by each diagram.

A) Mean decibel level for first reaction during sound



B) Mean decibel level for first reaction after sound (just adults)

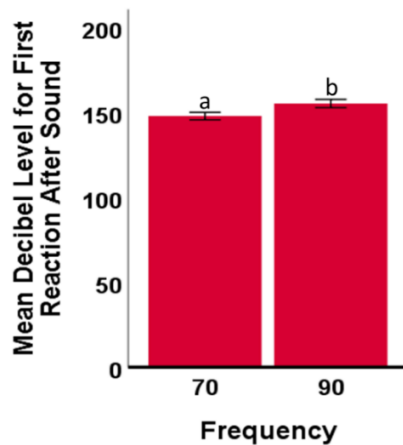


Figure 3.5. Threshold bar graphs comparing both juvenile to adult sea lamprey and 70 Hz to 90 Hz frequency level for the sound played by looking at the first sound level that sea lamprey responded to. A) Threshold to move shows the average sound level found for juveniles or adults to respond to while sound was playing (Adults: 70 Hz n = 12, 90 Hz n = 9; Juvenile: 70 Hz n = 7, 90 Hz n = 4). B) Threshold to move after shows the average sound level found for adults to respond by moving directly after sound has ceased (Adults: 70 Hz n = 12, 90 Hz n = 10). The results only include results of positively identified as behavioural responses. Lower case letter represent significant differences of $p < 0.05$. Error bars are representative of the mean (± 1 SE).

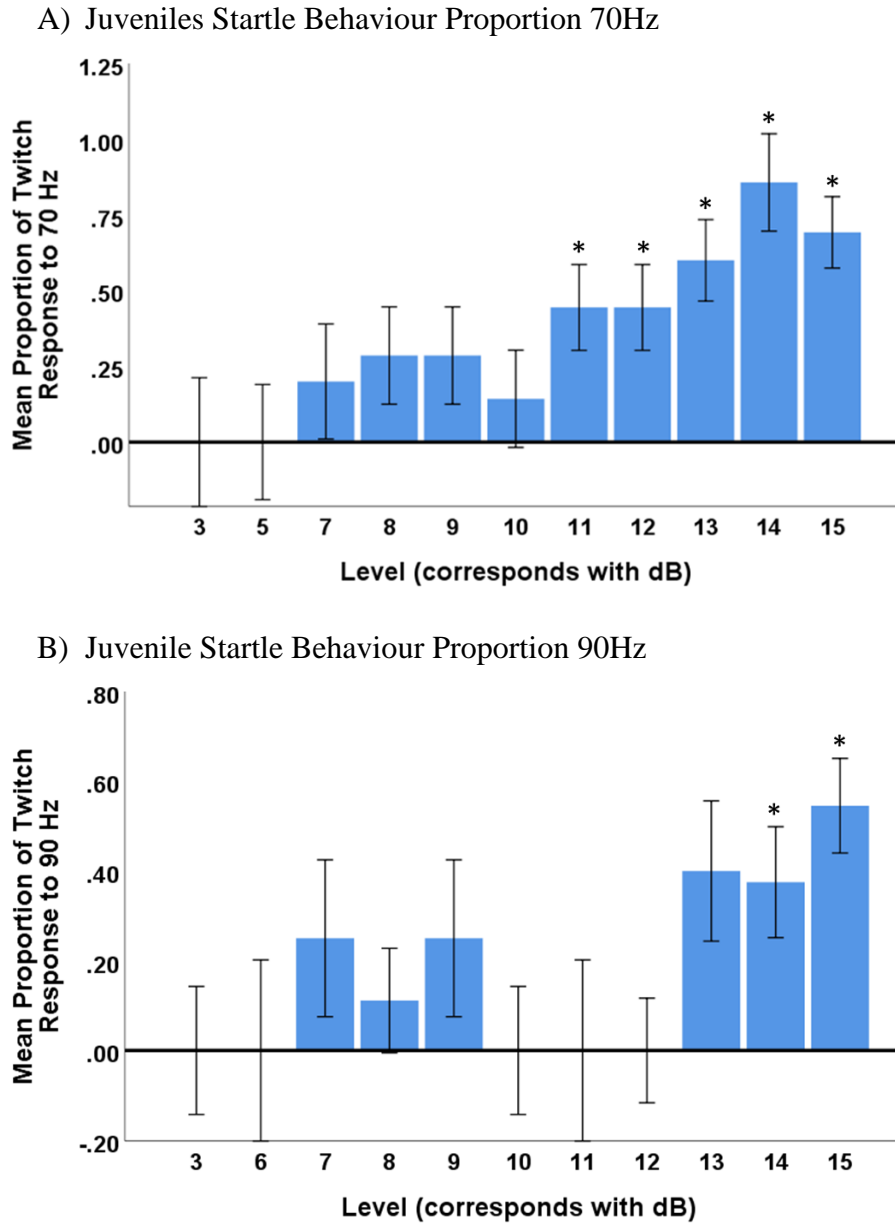
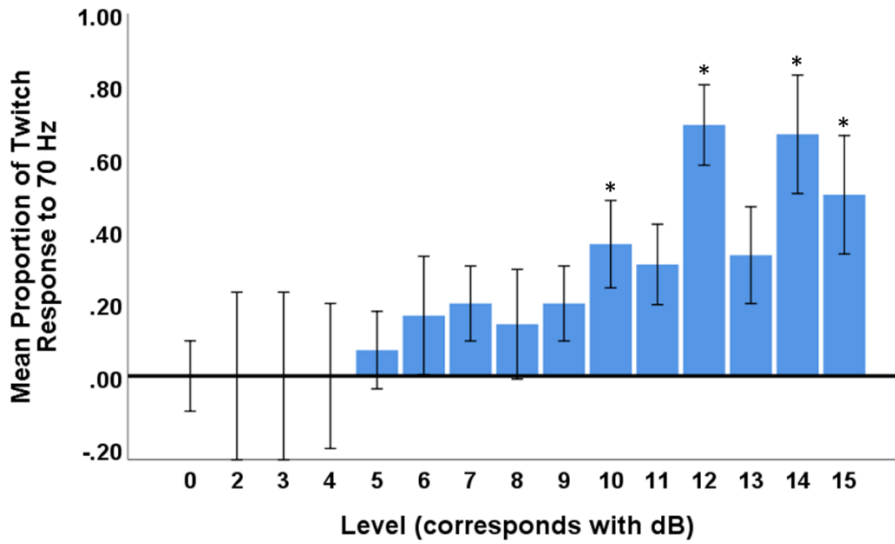


Figure 3.6. Proportion of juvenile sea lamprey twitching (startle behaviour) in response to sound (70 Hz or 90 Hz) at different sound levels (0-15, 129 dB-169 dB). Results show higher sound levels (higher sound pressure) leading to higher percentage of transformer performing a startle behaviour. A and B. (70 Hz n=83, 90 Hz n=68). Asterisks represent significant differences from level 0 or no movement of $p < 0.05$. Error bars are representative of the mean (± 1 SE).

A) Adult Startle Behaviour Proportion 70Hz



B) Adult Startle Behaviour Proportion 90Hz

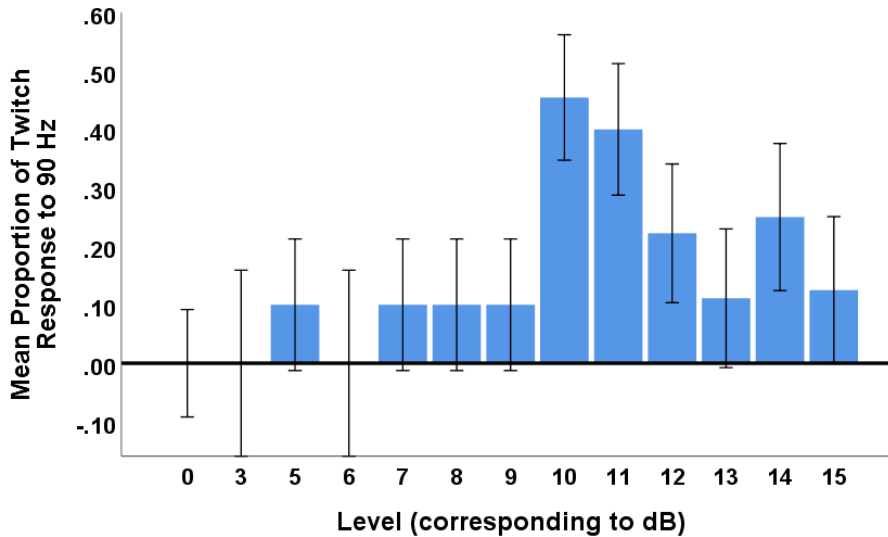


Figure 3.7. Proportion of adult sea lamprey twitching (startle behaviour) in response to sound (70 Hz or 90 Hz) at different sound levels (0-15). Results show higher sound levels (higher sound pressure) leading to higher percentage of adults performing a startle behaviour. A and B. (70 Hz n=142, 90 Hz n=120). Asterisks represent significant differences from level 0 or no movement of $p < 0.05$. Error bars are representative of the mean (± 1 SE).

Tables

Table 3.1. Sound levels and their corresponding decibel levels, specifically dB re 1 μ Pa, for both 70 and 90 Hz.

	L0	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15
90 Hz (dB)	129	129	130	131	134	138	140	144	149	151	154	157	160	161	164	167
70 Hz (dB)	129	129	130	133	137	140	143	147	150	154	157	160	161	164	166	169

Table 3.2. Juvenile 70 Hz twitch data. This table represents a summary of statistical data for juvenile 70 Hz proportional twitch data comparing all sufficient levels to level 0 baseline (no movement) with levels 11, 12, 13, 14, and 15 showing a significant difference from level 0 ($p < 0.05$).

Level	Std. Error	Difference Estimate	Sig
3	13.299	<0.001	1.000
5	12.261	<0.001	1.000
7	12.261	-9.500	.438
8	10.955	-13.571	.215
9	10.955	-13.571	.215
10	10.955	-6.786	.536
11	10.157	-21.111	.038
12	10.157	-21.111	.038
13	9.863	-28.500	.004
14	10.955	-40.714	<0.001
15	9.221	-32.885	<0.001

Table 3.3. Juvenile 90 Hz twitch data. This table represents a summary of statistical data for juvenile 90 Hz proportional twitch data comparing all sufficient levels to level 0 baseline (no movement) with levels 14 and 15 showing a significant difference from level 0 ($p < 0.05$).

Level	Std. Error	Difference Estimate	Sig
3	7.704	<0.001	1.000
6	9.887	<0.001	1.000
7	8.863	-9.875	.265
8	6.823	-4.389	.520
9	8.863	-9.875	.265
10	7.704	<0.001	1.000
11	9.887	<0.001	1.000
12	6.823	<0.001	1.000
13	8.187	-15.800	0.054
14	7.053	-14.812	0.036
15	6.473	-21.545	0.001

Table 3.4. Adult 70 Hz twitch data. This table represents a summary of statistical data for adult 70 Hz proportional twitch data comparing all sufficient levels to level 0 baseline (no movement) with levels 10, 12, 14, and 15 showing a significant difference from level 0 ($p < 0.05$).

Level	Std. Error	Difference Estimate	Sig
2	19.411	0.0	1.0
3	19.411	0.0	1.0
4	17.225	0.0	1.0
5	11.187	-5.071	.650
6	14.719	-11.833	.421
7	10.980	-14.200	.196
8	13.920	-10.143	.466
9	10.980	-14.200	.196
10	11.944	-25.818	.031
11	11.420	-21.846	.056
12	11.420	-49.154	<0.001
13	12.778	-23.667	0.064
14	14.719	-47.333	0.001
15	14.719	-35.500	0.016

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CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

Summary

Sea lamprey (*Petromyzon marinus*) are invasive in the Laurentian Great Lakes and parasitically feed on valued/important fishes for fisheries and recreational fishing. Sea lamprey lower the abundance of native fish and change the structure of fish populations (Farmer and Beamish 1973; Smith, and Tibbles 1980). Sea lamprey populations are present in all the Laurentian Great Lakes and maintaining populations below management targets is a significant challenge. For over 60 years, the sea lamprey control program has been entirely dependent on chemical pesticides and migration barriers to suppress sea lamprey populations to target levels (Miehls et al. 2020). Managers are increasingly calling for additional control measures in an integrated approach (McLaughlin et al. 2007). Sea lamprey require a multinational control effort to suppress their population and allow native species recovery (Christie and Goddard 2003). Capture and removal of migratory adults may hold promise as a control measure (Velez-Espino et al. 2008; Miehls et al. 2020), however capture rate has been low at many trap locations, perhaps due to low encounter rates (Bravener and McLaughlin 2013). Other non-physical cues used with traps have been used to increase trap efficacy but each possess drawbacks (Johnson et al. 2013; Hume et al. 2015; Johnson et al. 2016). Sound has been used to control behaviour in other fishes (Ross et al. 1993; Knudsen et al. 1994, Sand et al. 2000, Maes et al. 2004; Pegg and Chick 2004; Sonny et al. 2006; Vetter et al. 2015). Sea lamprey can hear at low frequencies of sound and respond to acoustic stimuli by showing increased activity levels with exposure to 70 Hz and 90 Hz specifically (Mickle et al. 2019). The use of sound to guide sea lamprey into traps, block sea lamprey from

vulnerable streams, or deter sea lamprey entrance in fish passage scenarios has great potential for sea lamprey control in the Great Lakes. However, how sea lamprey behaviorally respond to low frequency sounds in natural streams and their behavioural thresholds to sound has yet to be studied.

Here I first monitored the distribution of upstream migrating sea lamprey to understand if low frequency sounds would produce a response in a natural stream setting and if that response could be used to manipulate sea lamprey movement. Behavioral responses of upstream migrating adult sea lamprey in response to low frequency sounds was tracked in a small stream (8 m wide) using Passive Integrated Transponder (PIT) telemetry for 33 nights with either no sound (control), 70 Hz or 90 Hz tones playing. Two underwater speakers were placed on cement plates in front of the one of the four antenna in the stream. To determine if sound influenced the distribution of sea lamprey in the stream, two behavioral metrics were investigated: (1) What antenna an individual sea lamprey was first detected on (First Detection) (2) which antennas an individual sea lamprey was detected on throughout the night (Proportion Overall Detections). We compared the differential proportions of detections per night among antennas as a function of treatment (70 Hz, 90 Hz) using Generalized Linear Model with a Tukey HSD post hoc analysis. Sea lamprey moved away from low frequency sound sources in both years when first moving upstream with sea lamprey first detected more at the adjacent antenna compared to control trials. A similar pattern emerged with mean proportion of detections during both years, especially for 70 Hz, with sea lamprey having lower detection near a sound source and higher detection at the adjacent antenna even though it was not significant (compared to control trials). My results indicate that sound may affect sea lamprey

behaviour even with multiple interactions from sea lamprey returning to the same spot (similar results for first detection and overall detection). Taken together, my results do show that sound deters sea lamprey the first time they swim past speakers and has potential as a stimulus to direct sea lamprey towards existing traps. Sound as a barrier can be used to guide sea lamprey behaviour while not impacting the environment like many physical deterrents.

My second set of experiments examined the acoustic behavioural threshold for sea lamprey with a behavioural comparison of life stages. This was to contribute to the growing knowledge and use of sound as a deterrent for invasive species and use for conservation biology as a sensory-based method for invasive species control. Behavioural trials were conducted with adult and juvenile sea lamprey's behavioral responses to low frequency sounds of, again, 70 or 90 Hz recorded. Experimental tones were played at varying amplitude levels (level 0-15, 129 – 169 dB re 1 μ Pa). To determine behavioural reactions and thresholds, sea lamprey have a control period of no movement followed by sound and the behavioural reactions would be recorded during and after sound. A stepwise method was used for decibel changes, with the process repeated with increased or decreased sound level determined by sea lamprey responses (Tavolga and Wodinsky 1965; Lu et al. 1996). The behavioural threshold I look at here is the lowest decibel level of either frequency used (70 or 90 Hz) that elicits a “positive response” behaviour during or after sound. The two separate thresholds (movement at onset and offset of sound) were then determined by looking at all positive responses and finding the first threshold per sea lamprey. Statistically, I compared the different decibel levels of the determined threshold (determined for each responding sea lamprey) as the dependent variable between

frequency (70 and 90 Hz) and life stage (adult and juvenile) as the independent variables, using 2-way ANOVA. I included twitch responses, in case sea lamprey tended to avoid swimming in trials. Statistically, I compared the mean proportion of twitches as my dependent variable in adults and juveniles, separately, and frequency (70 and 90 Hz), also separately for each life stage, with differences between the different sound levels (0-15, 129 – 169 dB re 1 μ Pa) as my independent variable which was evaluated using a Kruskal-Wallis one-way test. Adult sea lamprey respond to sound by different increased movement reactions (i.e. swimming at the onset and offset of sound) but both juvenile and adult sea lamprey had similar behavioural thresholds, this could be showing the development of diverse behavioural reactions to an aversive or new stimulus. The varied responses by adult sea lamprey could be a version of the flight or freeze response. Either behaviour (onset or offset movement) could be elicited by the same lower decibel sound of either frequency, even though I do not know what triggers them to perform which behaviour. Juveniles, in comparison, mostly respond with increased movement at the onset of sound, indicating that in earlier life stages, sea lamprey have a less diverse range of behavioural responses. Juvenile sea lamprey may be focusing on one behavioural response or strategy, indicated here by swimming when a novel sound appears. A large portion of the juvenile sea lamprey showed no swimming behavioural response to sound and fewer juvenile sea lamprey responding by swimming meant a lower sample size for threshold testing, meaning that the results are hard to verify for application. Sea lamprey seem to have a similar hearing ability as adults and juveniles with similar thresholds for swimming and startle response at both frequencies. Although the startle-like behaviour, juveniles needed a higher decibel level while adult sea lamprey responded to lower

decibel levels. There is no difference behaviourally between the two life stages indicating that juveniles are equipped to functionally respond with a startle response but there is a significant difference in decibel level needed for a similar reaction. By using two different strategies for threshold testing, I can more accurately measure the possible behavioural thresholds for adult sea lamprey and for juvenile sea lamprey, who prefer to respond with startle behaviour. Juvenile sea lamprey compared to adults did not respond as much with movement to novel sounds indicating a different strategy is used at different life stages for sea lamprey. Juvenile sea lamprey are moving downstream after metamorphosing and adults are moving upstream to begin spawning leading which happen at different times of the year and different environmental factors (Manion and Piavis 1977). The life cycle of sea lamprey may lead to different hearing ability or different reactions to sound at different points in development.

Conclusion and Future Directions

Therefore, sound does affect sea lamprey. The use of sound as a behavioural control is a viable option with lower frequencies leading to a change in behaviour from sea lamprey. The behavioural threshold of sea lamprey to low frequency sounds is similar in both juvenile and adult sea lamprey meaning that downstream and upstream migrating sea lamprey can be targeted with sound. There will be different behavioural responses from the different age groups with adults tending towards a swimming-based approach and juvenile sea lamprey responding with a startle response. Adults are more likely to either move at the onset or offset of the sound, which may be shown as fleeing or freezing to sound, while juveniles move at the onset or show no movement in response. In the context of traps, adults may be more likely to flee away from sound and into traps, while

juveniles may need a different conservation strategy. Another application of sound is to use it to deter sea lamprey away from vulnerable areas, which could work for juveniles as they have shown a behavioural response to sound. In an applied scenario, sound was shown to be applicable in a stream environment with the same low frequencies deterring adult migrating sea lamprey away from a sound source. Although the sound did not push the sea lamprey very far, with sea lamprey being detected a short distance away from the sound source, the application for more precise control on the animal's movements are available. With multiple years and different placements of the sound source, a similar pattern of sea lamprey detection decreasing around low frequencies sound output can be used for control and management. Vulnerable streams and lakes can be guarded with speakers or speaker's setup as a directional push to congregate many invasive sea lamprey into one area for a better chance of capture. Sound has been shown as an important link between sea lamprey and their interaction with their environment that we can use to our advantage for controlling or guiding their behaviour.

Using sound as a management strategy is cost effective, easy to use, and simple to set up and move if needed. The speakers that one can use are able to change places with seasons and times and adding or removing speakers is a viable option depending on the soundscape of the area. Although sound will not completely block sea lamprey from coming upstream, by adding more speakers, one is able to easily increase the impacted area and influence more sea lamprey (or other possible target species), which is flexible to use with other methods (traps and barriers). A possible set up of multiple speakers could line an entire stream with sound playing as a deterrent in smaller streams or

specifically direct sea lamprey into particular areas. By using it in specific locations, small speakers would not get in the way of the environment, making it very practical.

To use sound effectively, a sound level of around 150-162 dB re 1 μ Pa with either 70 Hz or 90 Hz would be effective in a similar small river or stream known to have adult sea lamprey spawning with 70 Hz being more effective at lower decibel levels but most speakers are able to play 90 Hz louder. Smaller streams and rivers with soft-bottomed areas are best for the sound to propagate (Rogers and Cox 1988). Sound should be used as a multimodal method, such as using with traps or barriers or with other sensory methods such as olfactory options that can work as a push and pull method (aversive stimuli and attractive stimuli). As another optional use for sound, speakers can be set up downstream from juvenile sea lamprey migration patterns to direct juveniles into traps or impede transition into open water. Sound has many different uses as a control, making it a very versatile option for researchers.

The use of sound as a deterrent may have an effect on native fish. More studies are needed, specifically with low frequency noise, on fish present at night, since sea lamprey are nocturnal, which will be most affected. Also, a focus on species present in similar streams and rivers during the spring to migrate upstream will be important as this is the same technique sea lamprey use and when most control techniques are used. In France, native sea lamprey are threatened by dams, increased temperature, pollution, habitat degradation and by predation by invasive species (Bouletreau et al. 2020). Since landlocked sea lamprey in the Great Lakes do not have the same pressures, it is imperative that we continue to control their population since they could negatively impact the biodiversity and environment present in the Great Lakes.

Taken together, sound has potential as a stimulus to direct sea lamprey towards existing traps. Sound as a barrier can be used to guide sea lamprey behaviour while not impacting the environment like many physical deterrents. Further studies should continue to look at possibilities of sound use as a control for invasive species. Specifically for sea lamprey, the applied use of sound with traps seems the next obvious step to see if the number of captured sea lamprey can be increased. In an applied setting, sound systems can be used by being set up at the sides of traps, where sea lamprey would be guided to the middle of two speakers where the trap is, leading to a higher encounter rate and a higher catch rate (Bravener and McLaughlin 2013). One potential problem of the use of sound is that it may impact other organisms that are present in rivers or streams during the sea lamprey spawning season. To avoid issues, sound could be only used during peak sea lamprey spawning times then the impacts on native species could be lessened as the sound will have no long-lasting effects and will not propagate farther than the speaker array.

Acoustic-based conservation has already been attempted in a range of species including marine animals (Friesen et al. 2017). Sound is applicable in many settings including fish exclusion by attracting or repelling fish, which can be important for invasive species (Popper and Schilt, 2008). The use of sound, knowing the threshold levels for lamprey and the application of sound, can be used to guide sea lamprey into traps and increase the efficiency of traps leading to a new future of integrative approaches for invasive sea lamprey control. Future scientific experiments should look into the extension of sound deterrence into new areas of lamprey control in conjunction with other control methods or tools.

Other potential future studies building off this research could use more trials or a wider age range, such as comparing sea lamprey before metamorphosis and reproductive adults, this may show a larger difference in behavioural threshold. An interesting comparison can also be used for upstream and downstream sea lamprey capture rate with the addition of sound to see how or if sound would make a larger difference for capture for younger or older sea lamprey. Adult sea lamprey are vulnerable and easy to capture during spawning season (as they were for this experiment) while juvenile sea lamprey are vulnerable during their migration downstream after metamorphosis (Katopodis et al. 1994; Miehl et al. 2017a). The current study is important since the applicability of sound or noise on different life stages of animals are rarely studied (Popper and Schilt 2008).

The use of sound in a number of different underwater landscapes should be investigated. Future studies could also continue testing low frequency sounds in other larger rivers with larger, more advanced speakers for use as a natural deterrent at sea lamprey barriers to push sea lamprey toward traps with louder tones played. Sound can also be used as an inexpensive diversion in rivers and streams where they have a smaller radius of effect, which can be an advantage when shaping an effective range, or be used at the mouth of important spawning rivers and streams where water is deeper and sound would attenuate less (Sand et al. 2001). Barriers, like sound, do not affect water flow and may be the only practical option in some cases (Miehl et al. 2017b). Sound also provides a more flexible system that can be adjusted seasonally or daily to help protect non-target species (Johnson et al. 2016; Miehl et al. 2017b). Future directions can look at the extension of sound deterrence into new areas of sea lamprey control either in conjunction with other tools or alone.

Finally, understanding the behaviour of any species can be extremely important. The majority of research conducted on lamprey is focused on relatively few species with 60% of all records through Web of Science for 1864-2013 involve directly or indirectly sea lamprey (Docker et al. 2015). That said only 4.5-6.7% of lamprey studies in 2004-2013 were related to “Ecology/environmental science,” “Marine/freshwater biology,” and “Behavioral science” with even lower percentage in years prior to 1943 most of which was conservation or management related. In comparison “Zoology,” “Developmental biology,” and “Anatomy/morphology” represented as respectively, of all lamprey records they made up 13.6, 3.8, and 2.5 % of retrieved papers in 2004–2013. While “neuroscience/neurology,” “Biochemistry/molecular biology” and “genetics/heredity” made up 6.6-9.2% in 2004-2013 (Docker et al. 2015). For an invasive species such as the sea lamprey, understanding behaviour can lead to crucial increases in success of control methods, where information about how close and when sea lamprey enter could lead to better results (Bravener and McLaughlin 2013). The current experiment did not have the equipment to compare behaviours to sound with and without vibrations or test just vibrations, future experiments should consider this. The lateral line can lead to bursts of movement, by sea lamprey detecting vibrations in their surroundings or could be due to sea lamprey being sensitive to weak low frequency electric fields, (Dubuc et al. 2008). Further studies on the type of sound that elicits a specific response from sea lamprey are needed with an emphasis on the repertoire of sea lamprey at different life stages and the possibility of behavioural flexibility in sea lamprey. Sea lamprey movements around traps varied with a number of different factors with other unknowns still existing (Johnson et al. 2020). Much is still unknown about the behaviour of sea lamprey, and the

data provided by the research here, emphasizes the need for a deeper look into the drivers and circumstances leading to the differing behaviour of the invasive sea lamprey.

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