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Field assessments of anthropogenic noise effects on free-swimming fishes

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Field assessments of anthropogenic noise effects on free-swimming fishes

By

Rachel Pieniazek

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Field assessments of anthropogenic noise effects on free-swimming fishes

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

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

Chapters 2 and 3 of the thesis were co-authored with Megan Mickle and 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. Megan Mickle 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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This thesis includes 1 original paper that has been previously published/submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status*
Chapter 2	*Pieniazek, R. H., Mickle, M.F., Higgs, D. M. (2020). Comparative analysis of noise effects on wild and captive freshwater fish behaviour. Animal Behaviour*	*Accepted for Publication*

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ABSTRACT

Anthropogenic noise is globally increasing in aquatic ecosystems and causes adverse repercussions in many fish species, yet its effects in the field are not well understood. Here, I test the impact of boat noise on a number of wild freshwater and marine fish species as well as captive black bullhead (*Ameiurus melas*), a common species in the Laurentian Great Lakes with known hearing specializations. In a laboratory setting, black bullhead were exposed to boat noise, and a quiet control then monitored for changes in foraging behaviours and swimming patterns when presented with food. Black bullhead exposed to boat noise foraged less and startled more in comparison to trials without noise. Similarly, I exposed wild communities to boat noise and a quiet control in the field, then analysed videos for changes in both presence and foraging behaviours. Freshwater field experiments were consistent with results from the laboratory, showing fewer wild fish and foraging attempts during boat noise exposure. Marine experiments also yielded decreases in fish presence during boat noise, however there were no significant changes in foraging behaviours, and fish with previous boat noise exposure did not exhibit changes in behaviours. The effects of noise were highly variable by family though, with sensitive hearing freshwater fish in the Cyprinidae (or Leuciscidae) family predictably exhibiting significantly larger decreases in feeding events and presence compared to other families with more basic hearing abilities. While families in marine environments did exhibit varying degrees of responsiveness, they did not follow the predicted pattern based on hearing abilities. Therefore, while I did find evidence for anthropogenic effects on fish, the degree of impacts is dependent both on species differences as well as the degree of prior exposure, making clear the need for continued research to properly create sound exposure guidelines for conservation.

DEDICATION

I would like to dedicate my thesis to Megan Mickle, for I simply would not be here without you. We became friends the moment we met, and you quickly inspired me to completely change career paths, following my passion for marine research, and for this I am forever grateful. Thank you for sharing your love of stingrays and your passion for science and for being the best partner to have for fieldwork. Thank you for convincing me to rescue not one, but two dogs, because even though they can be menaces, they are often the best parts of my day. Thank you for helping me through some of the hardest times in my life and for contributing to some of the best times in my life. You are my sister, my mentor, and my role model. I cannot wait to see you continue to thrive as a scientist and a mother.

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CHAPTER I.

THE EFFECTS OF ANTHROPOGENIC NOISE ON FISH IN THE FIELD: A REVIEW

Introduction

Anthropogenic (man-made) noise has adverse impacts on fishes and has rightfully captured the attention of researchers and policy makers alike in recent years (Gedamke et al., 2016; Nolet et al., 2017; Popper et al., 2020; Hawkins et al., 2020). However, further investigation is necessary to better understand how such noise effects wild fishes for both parties to develop mitigation protocols and regulations. Thus far, laboratory experiments have substantially contributed to the current knowledge on the impacts of noise pollution on fish (e.g. Codarin et al., 2009; Simpson et al., 2015; Purser et al., 2016), however, there is growing concern that laboratory findings may not precisely capture the natural responses of wild fishes to underwater noise (Hawkins et al., 2020; Jones et al., 2020; Popper & Hawkins, 2020). Laboratory tanks are thought to distort the noise presented in them through the reverberations in the tank that unpredictably change the proportionality between sound pressure and particle motion, so the fish is not necessarily hearing ecologically-relevant sounds (Parvulescu, 1967; Akamatsu et al., 2002; Rogers et al., 2016; Jones et al., 2020). Captive fish are also confined within the tanks, so natural escape responses cannot be observed, and they may experience baseline stress levels which can conceal the magnitude of observed effects (Purser et al., 2016; Popper & Hawkins 2020). Additionally, captive fish can show genetic diversity to their wild counterparts through differing selective pressures (Jerem & Mathews, 2020), further altering their ability to represent wild fishes. Thus, there is a growing need for field-based experiments to meet the demand for real-world knowledge of the effects of underwater anthropogenic noise. The United Nations (UN) even acknowledges anthropogenic underwater noise as a cause for concern and is considering its addition in the Convention on the Law of the Sea, however these measures would mostly be precautionary as not enough information is available for countries to provide specific criteria to adhere to (Halvorsen et al., 2017; Nolet et al., 2017; Hawkins & Popper, 2016; Hawkins et al., 2020).

Under ideal circumstances, underwater sound is able to travel much farther and faster than in air (Rogers & Cox, 1988), making sound an incredibly important sensory modality and form of communication for fishes (Codarin et al., 2009). Noise, or unwanted sounds, created by human activity in aquatic ecosystems can be produced by recreational and commercial boats and vessels, resource-focused ocean exploration, such as pile driving and seismic testing, aquaculture, or construction (Slabbekoorn et al., 2010; Hawkins et al.,2020), and the addition of these sounds can interfere with ecologically important sounds to fish (Hawkins et al., 2015; Simpson et al., 2015). Noise exposure near the source can inflict hearing loss (McCauley et al., 2003; Codarin et al., 2009), while further away, the lower intensity sounds can mask acoustic cues and signals, alter fish physiology, inducing stress (Purser et al., 2016), or modify fish behaviour, such as driving them away from important resources, interrupting communication, disrupting migrations, affecting predator-prey interactions (Codarin et al., 2009; Slabbekoorn et al., 2010; Purser & Radford, 2011; Simpson et al., 2015) and altering their ability to communicate effectively in their environment (Popper & Hawkins, 2019; Putland et al., 2019). However, research gaps still remain, with little understanding of how anthropogenic noise affects fish communities, natural fish behaviour, how long term noise affects fish, and which sound levels (as pressure and particle motion) reveal such effects (Hawkins et al., 2015; Nedelec et al., 2016; Hawkins et al., 2020; Popper et al., 2020).

In light of recent reviews (Mickle & Higgs, 2019; Hawkins et al., 2020; Jerem & Mathews, 2020; Popper et al., 2020), it is becoming abundantly clear that more researchers need to conduct field-based studies in both marine and freshwater environments to better understand the impacts of noise on fish. Therefore, I aim to summarize the current literature and trends in studying the effects of anthropogenic noise on fish in field settings. My goal is to create a guide for researchers that are considering field-based methods and to provide a resource that unifies current efforts and highlights innovative techniques.

Methodologies

Bioacoustic studies investigating the impacts of anthropogenic noise in field settings have recently become more prevalent and have utilized a wide range of techniques that prove successful. Additionally, researchers solely focused on fish counts, community assemblages and fish populations have provided comparisons of popular methods to observe wild fish and have useful resources that are relevant to acoustic field studies (Bernard & Gotz, 2012; Lowry et al., 2012; Assis et al., 2013; Ghazilou et al., 2019; Schramm 2020; Wetz et al., 2020).

Cages, and similar enclosures typically equipped with cameras, appear to be the most-used option in field experiments (Picciulin et al., 2010; Fewtrell & McCauley, 2012; Holles et al., 2013; Johansson et al., 2016; Nedelec et al., 2016; Neo et al., 2016; Simpson et al., 2016; Holmes et al., 2017; Magnhagen et al., 2017; Neo et al., 2018), potentially for their elevated level of experimental control, as researchers can easily track the entire response of an individual fish (Neo et al., 2016), monitor schooling behaviours (Fewtrell & McCauley, 2012), collect fish for physiological samples (Johansson et al., 2016), as well as ask specific questions about avoidance using choice chambers (Holles et al., 2019). Conversely, enclosures are known to limit fish mobility which may affect their behaviours and increase baseline stress levels, and there are currently no set criteria for optimal cage sizes in field experiments so correlations between studies are difficult to establish (Fewtrell & McCauley, 2012).

Behavioural changes are effects that can be observed further from the source of anthropogenic noise than any other consequence of noise (Putland et al., 2019), so observing natural fish behaviour is vital for developing mitigation plans and regulations and thus cages may not be as efficient for behavioural studies as examining wild, free-swimming fishes (Hawkins et al., 2020). Traditionally, researchers have manually observed natural fish behaviours and assemblages through an underwater visual census (UVC). While there are more sophisticated methods, UVC is recognized in some studies to produce similar fish counts and species compositions to other techniques (Ghazilou et al., 2019; Wetz et al., 2020) and is beneficial for observing cryptic and rare fish species that are hidden within coral (Lowry et al., 2012), however, it does not yield similar results for diver-adverse species and may influence fish behaviour with the presence of a diver (Assis et al., 2013; Ghazilou et al., 2019).

Advancing technologies in recent times have allowed for the use of more accessible underwater camera systems (Ulrich & Bonar 2020), such as baited remote underwater video (BRUV; Lowry et al., 2012), as well as more sophisticated equipment such as sonar (Hawkins et al., 2014), animal-attached tags (Davidson et al., 2019), and underwater remote operated vehicles (ROV; Wetz et al., 2020). Often BRUVs are recognized as a more effective way of gathering data on natural fish behaviours and community compositions for they can last longer underwater than a diver (Ghazilou et al., 2019), use bait which increases the number of fish and species observed (Bernard & Götz, 2012; Schramm et al., 2020), are inexpensive, have accompanying video analysis programs (Ulrich & Bonar 2020), and, specifically for acoustic studies, do not produce sounds that could influence results. Alternatively, BRUVs are limited in fixed positions, over emphasize the carnivorous fish population, and require an extensive amount of time for video analyses. Mensinger et al. (2016) and Roberts (2018) further justified the use of BRUVs in studying the effects of boat noise for they both provided evidence that such techniques effectively capture changes in fish behaviours in response to noise.

Sonar systems offer a unique opportunity to study pelagic species and their schooling behaviours in response to anthropogenic noise for they produce ultrasonic sound pulses, which are outside of the range of fish hearing (200 kHz and 800 kHz), that reflect off fish and determine their approximate locations between the boat and the seafloor (Hawkins et al., 2014). Using sonar, Hawkins et al. (2014) successfully determined that schools of sprat (*Sprattus sprattus*) and mackerel (*Scomber scombrus*) respond to relatively low sound levels of impulsive sounds mimicking pile driving strikes, however, sonar alone is limited to detecting fish approximately larger than 100 mm and has difficulty distinguishing fish species (Egg et al., 2018). Aquatic telemetry can also benefit the field of underwater bioacoustics for this innovative technology can provide physiological information (e.g. heart rate, body temperature, etc.) through bio loggers, movement data (e.g. tailbeats, acceleration, migrations, etc.) through accelerometers, acoustic receivers, and GPS trackers, and environmental data (e.g. water temperature, depth, soundscapes, etc.) through sensors and hydrophones (Johnson & Tyack, 2003; Hussey et al., 2015). For example, Davidson et al. (2019) used bio loggers to show that fish may be capable of habituating to repeated exposures to high intensity sound pulses representing airguns for seismic exploration, and Neo et al. (2018) used acoustic tags to find potential diurnal effects at play by tracking individual swim patterns of fish in a net pen exposed to impulsive sounds. Similarly, researchers studying the effects of anthropogenic noise on marine mammals have taken to using novel archival DTAGs (Holt et al.,2017; Erbe et al., 2019; Parks et al., 2019; Christiansen et al., 2020) which have hydrophones within the tag, on top of accelerometers, magnetometers, and depth and temperature sensors (Johnson & Tyack, 2003), and would greatly benefit those studying how noise affects the movements of larger free-ranging fish species. Further use of hydrophones can be to passively monitor trends in fish communication and boat noise as demonstrated by Higgs & Humphrey (2020), and although they found no such correlations between calling behaviour of round gobies (*Neogobius melanostomus*) with boat traffic or ambient noise, this remains an enticing use of passive monitoring of free-swimming fishes. Drones and ROVs also present an appealing method for observing wild fishes from the perspective of the fish and from above which expand our realm of reasonable research questions, however, since these vehicles produce sound, they should be used with caution and appropriate measures should be taken to ensure they do not influence of alter fish responses (Wetz et al., 2020).

Sound Sources

The majority of field studies to date have assessed the effects of vessel noise on wild fish, which is appropriate considering boats are the most common form of anthropogenic noise in aquatic soundscapes (Simpson et al., 2016; Holmes et al., 2017; Nedelec et al., 2017; Slabbekoorn et al., 2010). Motorboat noise has led to reductions in parental care and larvae survival in wild spiny chromis (*Acanthochromis polyacanthus*; Nedelec et al., 2017) and largemouth bass (*Micropterus salmoides*; Maxwell et al., 2018), disruptions of orientation and settlement of coral reef fish (Holles et al., 2013; Simpson et al., 2016), and has altered short term behaviour causing local dispersion of juvenile common damselfish (*Pomacentrus amboinensis*; Holmes et al., 2017), Australian snapper (*Pagrus auratus*; Mensinger et al., 2018), and orange-fin anemonefish (*Amphiprion chrysopterus*; Milles et al., 2018), and increased hiding behaviour in threespot dascyllus (*Dascyllus trimaculatus*; Nedelec et al., 2016) as well as orange-fin anemonefish (Milles et al., 2018). Therefore, there seems to be a trend supporting previous laboratory findings with short-term boat noise exposure changing fish behaviour and physiology, however, recent papers also suggest wild marine fish may be more adept at enduring long term anthropogenic than initially thought (Nedelec et al., 2016; Holmes et al., 2017; Harding et al., 2018; Davidson et al., 2019). Wild fish have shown an increased tolerance to vessel noise after long term exposure, with both experimentally (Nedelec et al., 2016; Johanson et al., 2016; Holmes et al., 2017) as well as naturally occurring sounds in high boat traffic areas (Harding et al., 2018). Some studies have also explored the impacts of seismic airgun strikes, indicating that while fish initially exhibited significant changes in behaviour in response to such high intensity sound pulses, they also showed a diminished response after repeated exposure, again suggesting that fish may be able to habituate to anthropogenic noise (Fewtrell & McCauley, 2012; Davidson et al., 2019) or at least can no longer hear it due to hair cell damage. Moreover, fish regularly exposed to fishing pressures appeared to be more reactive to boat noise that those residing in protected areas; for example Mensinger et al. (2018) found that protected Australian snapper did not react behaviourally to boats passing whereas those in open fishing regions dispersed more and decreased foraging efforts.

Additionally, Hawkins et al. (2014) used simulated pile-driving strikes in an attempt to establish a threshold of responsiveness from resource-based noise pollution which could assist in creating guidelines that policy makers can implement. They found that 50% of fish schools responded to single strikes at exposure levels of 135.0 and 142.0 dB re 1 lPa^2, with sprat (*Sprattus sprattus*) schools tending to disperse more and mackerel (*Scomber scombrus*) schools changing depths in response to noise (Hawkins et al., 2014). Simulated anthropogenic noise effects have also been studied in several species of sharks by Chapuis et al. (2019) which found that sharks display an aversion to the bait with fewer spotted during sound exposure and fewer interacting with the bait. Still, a broader spectrum of sound source should be investigated in future studies to assist in hastening mitigation processes. Recently, interest in sustainable energy options has led to the development converters that harness energy from waves, and although they produce sound within the hearing range of fish (< 1 kHz), little is known about how the noise from turbines may affect fish and surrounding ecosystems and therefore, supply an interesting new avenue of focus for future bioacoustic studies (Haikonen et al., 2013).

Discussion

Trends

Aquatic acoustic researchers seem to collectively agree that more field testing is necessary to realistically assess the effects of anthropogenic noise on fish as the number of papers published has considerably increased within the last decade (Neo et al., 2018; Mensinger et al., 2018; Davidsen et al., 2019; Mills et al., 2020; Staaterman et al., 2020). Many studies have taken to using enclosures for more experimental control or BRUV-like systems to study free-ranging fish. Boat noise was the most common sound source studied and there is now field evidence that such noise can negatively impact wild fish that encounter it. However, the results of field studies indicate there are more complex responses to noise than the overwhelmingly negative consequences found in laboratory studies (Table 1.1). Its impacts may depend on the location or habitat, behaviour, sex, life stage, fishing pressures, noise tolerance, and hearing abilities of fish, for some studies are finding varying levels of effects under variable conditions (Fewtrell & McCauley, 2012; Mensinger et al., 2018; Davidson et al., 2019; Mickle et al., 2020). As observed with laboratory studies (Mickle et al., 2018 & 2020), many more field studies have assessed the effects of anthropogenic noise on marine coral reef fishes than elasmobranchs which were represented by two studies (Chapuis et al., 2019; Mickle et al., 2020) and freshwater species, represented by four studies (Voellmy et al., 2014; Magnhagen et al., 2017; Maxwell et al., 2018; Higgs & Humphrey, 2020).

Novel studies

While there have been trends within the current collection of noise pollution studies focusing on fish, there are some notably innovative ones that are worth highlighting. Simpson et al. (2016), for instance, interestingly studied how boat noise impacted the settlement of coral reef fish larvae by establishing two reef patch clusters with one broadcasting typical sounds from coral reefs while the other one playing reef sounds and boat noise. Instead of using cameras or UVC, researchers used clove oil to physically collect and count all larval fish that had settled on each reef the night before, determining that fewer fish settled on reefs with boat noise playing and demonstrating a deterrence effect of boat noise on free-swimming fish (Simpson et al., 2016). Clove oil therefore offers a unique way to accurately collect fish assemblage data and have the potential to allow for physiological and morphological assessments of fish after noise exposure. Mickle et al. (2020) gauged response thresholds in southern stingrays (*Hypanus americanus*) using a net pen to observe behavioural changes in response to varying levels and frequencies of pure tones and yielded sex differences with females appearing more sensitive than males. Not only is this a unique experimental design that addresses the need for response thresholds, but it targeted an elasmobranch species which is a group of fish largely understudied in terms of anthropogenic noise (Chapuis et al., 2019), and it suggests that there may be discrepancies between sexes leading one to question if other species of fish have sex differences noise responses (Mickle et al., 2020). Additionally, Neo et al. (2018) outlined the possibility that fish may have stronger responses to noise at night when comparing swim patterns of fish over two days. To my knowledge, diurnal effects have not been studied in the response of fish to noise in the laboratory, with only one other field study finding no diurnal effects in response to noise (Ivanova et al., 2018) which thus suggests further investigation, for this could be useful information when developing protections plans if fish are more affected at night. Neo et al. (2018) also showed how to avoid the constraints that cameras put on field enclosure sizes by using acoustic tags and receivers to track fish movements in 3D. Thus, the enclosure can be much larger and avoid constraining the fish too much while still supplying an element of experimental control.

Future directions

Existing underwater acoustic field studies have provided valuable evidence for methodologies that function well and have furthered our understanding of how noise can affect fishes, however, much more investigation is required to appropriately use such findings for real world applications. Reviews such as Hawkins et al. (2015 & 2020) and Popper et al. (2020) already describe in detail the areas in which research is lacking in the present field, but here I will highlight a few main points to help focus future field studies. Firstly, the propagation, levels, characteristics, and types of sound that impact fish the most need to be investigated in order for quantifiable sound criteria to be incorporated in mitigation efforts and monitoring (Hawkins et al., 2020). Particle motion therefore needs to be reported in all studies going forward, for it is how most fish detect sound but is not proportional to sound pressure, so it is imperative information when establishing sound criteria (Hawkins et al., 2014; Popper & Hawkins, 2018; Hawkins et al., 2020). That being said, there are currently no easily accessible sensors to measure particle motion in the field, so advancement in such hydrophone technology would be beneficial, however particle motion can also be calculated from sound pressure gradients measured at known distances (Popper & Hawkins, 2018). Response thresholds are often much higher than hearing thresholds because while an animal may hear a stimulus it may not react until it is at a higher threshold, (Hawkins et al., 2020), thus field behavioural audiograms like those demonstrated by Mickle et al. (2020) and Hawkins et al. (2014) would benefit our understanding of noise pollution impacts. Furthermore, there appears to be a great deal of variation in the responses of fish in distinct species and habitats (Fewtrell & McCauley, 2012; Mensinger et al., 2018; Davidson et al., 2019) and understanding where such differences exist is imperative for future mitigation.

Conclusions and Thesis Objectives:

Anthropogenic noise has a range of effects on fishes, but our limited understanding of their natural responses prevents us from establishing appropriate guidelines and criteria. A variety of options exist when studying wild fish in terms of data collection and experimental design with all of them presenting their own advantages and drawbacks. Some techniques even provide the ability to expand our understanding and observe rare, cryptic, schooling, pelagic, and/or even deep-water species in the wild. While each method alone can assist in answering specific research questions, a combination of techniques would provide a more comprehensive and accurate representation of natural fish responses to noise and community compositions and is recommended by many studies (Lowry et al., 2012; Schramm et al., 2020; Wetz et al., 2020). Therefore, there is still a substantial amount of research that needs still needs to take place, so hopefully this review can help focus future efforts and inform researchers of past successes and downfalls, and hopefully this thesis can provide insights into how field studies can be carried out and what results can be discovered through their use.

Furthermore, I contribute to the growing body of knowledge surrounding the natural responses of fish to noise through the use of laboratory and field experiments as well as through the investigation of both freshwater and marine species. The diversity of environments helps expand the reach of my collected data while exposing commonalities between marine and freshwater habitats. My objective was to address a variety of literature gaps, including the effects of habituation, prior noise exposure, and community compositions on the responses of free-ranging fishes to boat noise, in hopes of furthering our understanding of how noise affects wild fishes.

In my first data chapter, I compared the foraging behaviours and escape responses of captive and wild fishes to noise to better understand the relationship of their results, which could translate to better interpretations of laboratory findings in the literature to natural settings. This chapter also focused on freshwater species in recognition of the deficit of information encompassing the effects of anthropogenic noise on freshwater habitats. My second data chapter aimed to understand how wild marine fish respond to boat noise and how their responses might be mediated from previous boat noise exposure by conducting experiments in a high boat disturbance area and a low disturbance area.

FIGURES:

Topic	Technique	No. of Studies	Effects Seen*	Sound Source**	Reference
Caged	Schooling	4	1,4	1,2,3	Neo et al., 2018; Neo et al., 2016; Johansson et al., 2016; Fewtrell & McCauley, 2012
	Individual fish	5	1,2,3,4	1	Harding et al., 2018; Holmes et al., 2017; Picciulin et al., 2010; Nedelec et al., 2016; Staaterman et al., 2020
	Tags	1	1,2,4	2	Davidsen et al., 2019
	Choice Chamber	2	1	1	Holles et al., 2013; Mickle et al., 2020
Free-Swimming	Diver Census	3	1,5	1	Nedelec et al., 2017; Simpson et al., 2016; Mills et al., 2020
	Underwater Video	4	1,4	1	Manna et al., 2016; Mensinger et al., 2016 & 2016; Roberts et al., 2018
	Sonar	1	1,4	3	Hawkins et al., 2014

*Effects seen:
1= Short term behavioural effects
2= Short term physiological effects
3= No short-term effects
4= Signs of Habituation
5= Long term physiological effects

**Sound sources:
1= Boat
2= Seismic air guns
3= Artificial sound impulses

Table 1.1: A summary of methodologies and results from saltwater studies investigating the effects of noise on wild fishes.

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CHAPTER II.

COMPARATIVE ANALYSIS OF NOISE EFFECTS ON WILD AND CAPTIVE FRESHWATER FISH BEHAVIOUR

Introduction

Auditory stimuli play vital roles in aquatic environments as sound has high propagation rates and low attenuation over large distances in open-water habitats (Bass & Clark, 2003; Popper & Hastings, 2009; Tyack, 1998). Sound therefore can supply a reliable, fast and long-range mechanism for transmitting and receiving information and has prompted the evolution of diverse hearing sensitivities amongst fish species (Bass & Clark, 2003; Ladich, 2013; Putland et al., 2018; Tyack, 1998). Accessory hearing structures, such as Weberian ossicles and swim bladder extensions, evolved in some fish to allow for more sensitive and broader frequency range sound detection (Fritzsch, 1999; Ladich, 2013; Popper & Fay, 2011; Putland et al., 2018). The ability to detect acoustic stimuli drives important interactions and behaviours (Popper, 2003) and allows fish to analyse the entire ‘auditory scene’ (Popper & Fay, 2011) to obtain information about predators, acquire mates and locate resources (Popper, 2003; Popper & Hawkins, 2019; Slabbekoorn et al., 2010).

Anthropogenic noise created by human activities threatens the usefulness of acoustic cues to fish, as noise has significantly increased ambient sound levels around the world (Popper & Hastings, 2009; Slabbekoorn et al., 2010; Whitfield & Becker, 2014). Animals must isolate biologically important signals from the ambient, or background, noise in their environment to detect relevant cues, and high anthropogenic noise outputs are disrupting these sensory processes (Rogers & Cox, 1988). Anthropogenic noise is masking animal communication (Codarin et al., 2009), increasing stress levels (Smith et al, 2004), causing hearing loss (McCauley et al., 2003) and diverting animals' attention, which can disrupt important signals such as predator–prey interactions (Chan et al., 2010; Frisk, 2012; Slabbekoorn et al., 2010; Whitfield & Becker, 2014). For example, zebrafish, *Danio rerio*, show slower attack times and more handling errors when foraging near noisy areas (Shafiei Sabet et al., 2015), and ambon damselfish, *Pomacentrus amboinensis*, exposed to noise are predated twice as often as control fish due to high stress levels and distraction (Simpson et al., 2016). With noise levels predicted to continue to increase due to human activity (Popper & Hawkins, 2019), it is imperative to fully understand the potential effects of noise pollution throughout global waterways.

One challenge to assessing impacts of anthropogenic noise on fish is the ongoing debate over the validity of using laboratory tanks for experiments, as tanks obstruct natural sound propagation patterns (Parvulescu, 1967; Rogers et al., 2016), but it is still unclear whether the distortion in tanks differs enough from natural settings to discredit such studies (Cox et al., 2018). Tank experiments also receive criticism for limiting a fish's ability to move and escape unwanted sounds (Popper & Hastings, 2009), so while a number of recent studies have performed in situ experiments, few have used wild, free-swimming fish to do so (Bracciali et al., 2012; Jacobsen et al., 2014), potentially skewing perceived natural behaviours. Therefore, there is currently a call for bioacoustic researchers to conduct experiments in natural environments for more ecologically relevant results (Gray et al., 2016; Rogers et al., 2016), yet the limitations in fully controlling these environments suggest that laboratory-based experiments may still be necessary to answer research questions and provide controlled settings to conduct experiments. Baited underwater video (BUV) represents a compelling technique to assess community responses to noise of a range of species while maintaining their free-swimming status (Follana-Berná et al., 2019) and has been used to show that passing boats can impact the behavioural response of marine species to bait (Mensinger et al., 2016). Freshwater environments offer easily accessible and quiet locations to experimentally explore the effects of noise on fish species, particularly with the use of BUV (Slabbekoorn et al., 2010), yet few studies have focused on the effects of noise on wild freshwater species (Bolgan et al., 2016; Mickle & Higgs, 2017).

Here, we observed changes in foraging and swimming behaviours of freshwater fish species found in the Laurentian Great Lakes in response to boat noise. We first conducted a series of captive experiments using black bullhead, *Ameiurus melas*, which possess accessory hearing structures (Weberian ossicles; Weiss et al., 1969) to assess the effects of boat noise on their behaviours, then we conducted field experiments on fish with a range of hearing abilities to test the relevance of our findings from the laboratory and also examined how fish might respond differently in a free-swimming community setting. We hypothesized that noise would affect the frequency of foraging behaviour and alter swimming patterns in fish dependent upon an individual species' hearing ability, and thus predicted that black bullhead would forage less often when exposed to noise. Our free-swimming design allowed us to analyse the natural behaviours of fish as they were not enclosed or trapped. Our analysis of foraging behaviours allowed us to gain insights into the effects of boat noise across multiple fish species attracted to the food sources, and the varying field conditions used offer real-world estimations of the possible extent of noise effects.

Methods

Laboratory Experiment Design

Laboratory experiments were first conducted to validate claims of foraging behaviours being affected by noise exposure and establish hypotheses for future experiments to be conducted in the field. Black bullhead were used to estimate the maximum effects of anthropogenic noise on swimming behaviour and foraging efficiency in a controlled setting, for they have specialized hearing structures called Weberian ossicles (Weiss et al., 1969) and were readily available and adjusted to captive conditions. All procedures were conducted in accordance with Canadian Council for Animal Care (CCAC) protocols (University of Windsor AUPP 17-11). Black bullhead were supplied by a local fish farm (Leadley Environmental Corp., Essex County, ON, Canada; 42°6′11′′N, 82°55′45′′W) and measured 13–17 cm in total length. All fish (N = 81) were housed at the University of Windsor in two 90-litre tanks maintained on a reverse 12:12 h dark:light cycle at ~17 °C and a pH level of ~7. We covered the sides of each tank with black garbage bags to reduce disturbances and stressors. All experiments were performed from January to early March 2018 between 1100 and 1600 hours to reduce diurnal variation. Experiments were conducted in a separate experimental room, to avoid excess noise produced from housing conditions, equipped with a 100-litre opaque plastic tank that was held off the ground by a metal frame to avoid vibrations from the floor. The experimental tank contained a barrier to separate the staging area from the underwater speaker (Electro-Voice UW-30) (Fig. 2.1a). Sound files were played from a Nexus 5X android smartphone (LG Electronics), which was connected to an amplifier (Schosche SA300), and, subsequently, the underwater speaker, which output the sounds into the tank. Ambient sound levels were measured in the middle of the experimental tank before each trial using a hydrophone (Inter Ocean System Inc., Acoustic Calibration and System Model 902), with a range of 128–132 dB re 1 µPa. All sound files were played, so the average sound level in the tank was 160 dB re 1 µPa, with little variation across different tank locations, and all sound measurements are given in pressure, as opposed to particle motion, to correspond to levels presented in the field trials. All trials were recorded using a GoPro HERO+ camera (GoPro), and the fish were exposed to a randomly assigned sequence of the sound treatments, which were determined using a random number sequence generator (random.org).

Four treatments were used throughout the experiments: boat noise (80–10 000 Hz), band-pass filtered white noise to include the hearing range of fish (10–5000 Hz; Nichols et al., 2015; Fig. 2.2), and two treatments of no sound files playing, with the speakers either connected or disconnected. Adding a treatment of no sound with the speaker connected (NSC) allowed us to control for electrical interference, while the treatment with the speaker disconnected (NSD) acted as a baseline control. A single boat noise file was played on a continuous loop to provide a constant sound level. The boat noise was collected from a 20 HP Honda boat engine (model no. 20DK0SHC), at mid-throttle, but held in neutral, using a hydrophone (High Tech, Inc., model no. HTI-96-Min, sensitivity -180.6 dB re 1 µPa, frequency range 2 Hz – 30 kHz) anchored to a concrete block approximately 2 m deep and 1 m from the boat in the Detroit River (Windsor, ON), thus ensuring its ecological relevance.

We used an array of earthworms (*Lumbricina terrestris*) as prey species, allowing us to quantify responses of predatory fish to noise. The array was suspended above the experimental tank and was constructed from a perforated Plexiglas board (60 cm long × 30 cm wide), with nine monofilament fishing lines (8 pound test; 0.02 mm; 80 cm long pieces) tied to it, and small fishing weights holding the fishing lines straight in the water. Earthworms were cut just before each trial into nine, approximately 1 cm long pieces for each of the fishing lines. Each fishing line was then threaded through a piece of worm and tied off in a loop to prevent the fish from easily removing and ingesting the worms, thus avoiding consumption effects and acting as a substitute for hooks to avoid harming the fish. The array was added to the tank prior to the study; however, worms were pulled out of the water to rest outside of the tank before commencing each trial. The worms were then introduced after acclimation by letting them swing back into place in the tank, so the fish could adjust to their new environment before being presented with food.

Pilot trials revealed that black bullhead did not adjust well to individual experimentation, and since they lived in large groups in their housing tanks, they were kept in groups of three throughout each experiment to reduce additional stress and were only experimented on once. Three black bullhead were therefore removed from their housing tanks and transferred to the experimental tank at the start of each of the 27 trials. After assessing acclimation periods during pilot trials, we determined that 30 min was a sufficient adjustment period for black bullhead as they assumed normal swimming and explorative behaviours well before these times expired. Once acclimation time had elapsed, the worms were returned to the tank and the fish were given an additional 10 min to notice the food and recover from any startle response that the process may have caused. After 10 min, we played the first treatment, predetermined by the random number sequence generator, for 2 min, followed by a 5 min rest period. This pattern of 2 min of treatment and a 5 min rest period was repeated until all four treatments were complete.

Laboratory analysis

The videos from each experiment were analysed with the sound muted and by an individual blind to the study to avoid potential bias. Foraging behaviours were recorded if one of two behaviours occurred: a foraging attempt or a foraging attack. Foraging attempts were recorded when a fish approached the worm with an open gape, yet failed to procure the worm, while foraging attacks were recorded when the fish successfully latched onto the worm. The amount of time, in seconds, each fish spent erratic was also recorded, to allow for examination of potential stress responses during noise exposure. Erratic swimming was characterized by bursts of high-speed swimming interlaced with startle responses and swimming into tank walls. The four treatments, 2 min each, were compared to the 2 min period that occurred directly before each treatment (pretreatment control) to detect changes in foraging and swimming behaviours and therefore these data were analysed as differences. Once both foraging and erratic swimming difference metrics of the three fish in a single trial were averaged and found to be normally distributed (Shapiro–Wilk test: $P > 0.05$), we performed a within-subjects repeated measures ANOVA using SPSS Statistics (v.24, IBM, Armonk, NY, U.S.A.). Significant treatment effects were further analysed using a simple contrast to the treatment of no sound with the speaker disconnected (NSD).

Field Experimental Design

Field experiments were conducted in the Detroit River at Riverdance Park in LaSalle, Ontario (42°14′13.9′′N, 83°6′21.1′′W) during July–August 2018 between 1000 hours and 1600 hours. Two sites within the park were chosen to conduct experiments simultaneously, with one located next to a fishing dock and the other located against a break wall, however, no significant difference was found in fish behaviour between sites (Kruskal–Wallis independent samples test: $P > 0.05$). Both sites consisted of rocky bottoms with some submerged aquatic vegetation, were measured to have a depth of 1–1.2 m and contained an abundance of minnows (Cyprinidae; also denoted as Leuciscidae; Schönhuth et al., 2018), round gobies, *Neogobius melanostomus* (Gobiidae), sunfish and bass (Centrarchidae), as well as yellow perch, *Perca flavescens* (Percidae). Water temperatures fluctuated between 23.4 °C and 26.3 °C. We also determined water clarity visually and conducted experiments only when the rocky bottom was visible to allow video analysis to be conducted.

We used the same worm array from the laboratory experiment to quantify foraging attempts in the field, except that we secured two Styrofoam 'pool noodles' with zip-ties to the edges of the array to keep it afloat (Fig. 2.1b). We constructed a second worm array so that trials could simultaneously be conducted at both study sites within Riverdance Park. Ambient sound levels ranged from 116 to 121 dB re 1 µPa, and the same boat noise file from the laboratory experiments was used and played in the field at a sound level of 160 dB re 1 µPa. Laboratory experiments for boat noise and white noise revealed only minor differences in behaviours measurable in the field (see below), so only boat noises were used in the field experiments because of their higher ecological relevance. The underwater speaker wires were threaded through the arrays, so the arrays were anchored in place with the worms hanging 30 cm above the speakers (Electro-Voice UW-30). Again, the speakers were connected to an amplifier (Schosche SA300) and an MP3 player (Apple iPod Nano). All trials were recorded using a GoPro HERO+ and the sound file used was boat noise (80–10 000 Hz). We conducted 16 experiments; each experiment consisted of a 30 min boat noise treatment and a 30 min no-noise treatment with the speaker electrically connected throughout, with the presentation sequence randomized for every experiment and with 30 min of rest separating the two treatments. Only one set of trials was conducted per day at each site, making it less likely the same fish was tested over multiple experiments.

New worms were cut and attached to the worm array before each experiment using the same methods from the laboratory experiments. The speaker was placed in the water for 10 min before the worm array, to allow the fish to acclimate to the speaker's presence before being presented with food and sound. Either boat noise or no noise was then played, and the array was placed in the water for 30 min, at which point the sound was shut off and the array and speaker were retrieved. A 30 min rest period was given for the fish to resume normal swimming behaviour, during which the worms were removed from the array and fresh worms were attached. Once 30 min expired, the speaker was placed in the water 10 min before and then the opposite sound from the first treatment was played for 30 min once the array was in the water. The conclusion of both 30 min sound treatments was the end of an experiment. All fish observed in these field experiments were not handled to minimize stress and experimental impact.

Field analysis

The videos from both sites were analysed twice by two different individuals blind to the study to avoid bias, and the analysis focused on fish presence and foraging behaviours. Fish presence was monitored by counting the number of fishes in the camera frame of a screenshot every 30 s throughout the trial to check for potential habituation effects. Foraging events were quantified both when the fish physically touched or bit a worm and when a fish approached and inspected a worm but did not physically touch the worm. The species or family of the fish exhibiting each of the behaviours was also recorded so further analyses could take hearing abilities into account. Four families of fish were ascertained during analyses based on the fish observed: (1) Percidae, comprised of yellow perch; (2) Centrarchidae, composed of *Lepomis* species, such as bluegill sunfish, *Lepomis macrochirus*, green sunfish, *Lepomis cyanellus*, and pumpkinseed sunfish, *Lepomis gibbosus*, and of *Micropterus* species, such as largemouth bass, *Micropterus salmoides*, and smallmouth bass, *Micropterus dolomieu*; (3) Cyprinidae, composed mostly of species from the Leuciscinae subfamily, including spotfin shiners, *Cyprinella spiloptera*, spottail shiners, *Notropis hudsonius*, and bluntnose minnows, *Pimephales notatus*; and (4) Gobiidae, which was represented only by the invasive round goby. First, we performed a Kruskal–Wallis test using SPSS to ensure there was no significant variation in the behaviours and fish observed between the two different sites ($P > 0.05$). Next, we used a Mann–Whitney *U* test to assess whether the treatment type influenced the number of fish present per 30 s, and we used a linear regression to determine the effects of habituation throughout each trial. Finally, we tested the effects of boat noise on foraging behaviour using a Mann–Whitney *U* test in SPSS.

Results

Laboratory Analysis

There was a significant decrease in black bullhead foraging behaviour in the boat noise ($F_{1,26} = 6.91$, $P = 0.01$; Fig. 2.3) treatments compared to the NSD treatment, however no such difference was found when comparing NSD to white noise ($F_{1,26} =$

1.958, $P > 0.05$; Fig. 2.3). Black bullhead foraged an average of 0.48 times more in the 2 min NSD treatment than in the pretreatment control as opposed to 0.45 times less during boat noise. There was a significant increase in erratic swimming behaviour during boat noise ($F_{1,41} = 60.58$, $P < 0.001$) and white noise ($F_{1,41} = 8.11$, $P = 0.008$) compared to the NSD treatment (Fig. 2.4). Fish exhibited erratic swimming for an average of 23.33 s more during 2 min boat noise exposure than during pretreatment controls and 4.59 s more during white noise compared to 0.49 s more and 0.03 s less for NSC and NSD, respectively. The assumption of sphericity was violated when conducting a repeated measures analysis for erratic behaviour, so we used the Greenhouse–Geisser correction for this analysis. No statistical differences were detected between the NSD and the NSC for foraging behaviours ($F_{1,26} = 0.14$, $P = 0.71$) or erratic swimming ($F_{1,26} = 1.75$, $P = 0.19$), indicating no effect of electrical interference.

Field Analysis

Fish presence

Discerning the number of fish seen within the camera's field of view at each 30 s mark yielded a significant overall effect of treatment on fish presence, with an average of 0.58 fish spotted each 0.5 min during boat noise treatments, compared to an average of 1.2 fishes seen during the control ($U = 369891.5$, $N_1 = 915$, $N_2 = 915$, $P < 0.001$; Fig. 2.5). Further contrasts revealed significant declines in the number of Cyprinidae ($U = 420428.0$, $N_1 = 915$, $N_2 = 915$, $P < 0.001$) and Gobiidae ($U = 421948.5$, $N_1 = 915$, $N_2 = 915$, $P < 0.001$) present during boat noise treatments compared to control treatments and no significant changes were found for Centrarchidae ($U = 466614.0$, $N_1 = 915$, $N_2 = 915$, $P = 0.20$) and Percidae ($F_{1,1707} = 1.22$, $P = 0.27$; Fig. 2.5). There was no relationship between the fish present in each time interval of a trial and the number of fish present for control trials ($r^2 < 0.001$, $P = 0.54$) and a weak downward trend for fish presence during the boat noise trials ($r^2 = 0.012$, $P = 0.001$; Fig. 2.6).

Foraging

Wild fish significantly decreased foraging behaviours from an average of 31.45 foraging events during the 30 min control treatments compared to an average of 16.92 events during boat noise treatments ($U = 75.5$, $N_1 = 16$, $N_2 = 16$, $P = 0.047$; Fig. 2.7). Cyprinidae displayed the most foraging events, with an overall average of 60.57 behaviours observed during control treatments compared to averages of 7.73 for Gobiidae, 24.00 for Centrarchidae and 4.43 for Percidae. There were only statistically significant differences in the number of foraging events between boat noise and control treatments for Cyprinids ($U = 71.0$, $N_1 = 16$, $N_2 = 16$, $P < 0.031$; Fig. 2.7).

Discussion

In the current study, we assessed the effects of boat noise on foraging behaviours, first, in captive black bullhead to ensure a highly controlled environment, and then in wild freshwater fish to validate behaviours observed in the laboratory and to further assess differential responses across species with varying hearing sensitivities. Our results demonstrated significant changes in behaviour between control and boat noise treatments in both the laboratory and the field. The decrease in black bullhead foraging and the increase in erratic swimming during boat noise treatments are consistent with previous research showing increased startle and avoidance behaviours and reductions in foraging throughout exposures to boat noise (Mickle & Higgs, 2017; Slabbekoorn et al., 2010).

Our results suggest a general trend of fish with more sensitive hearing exhibiting a greater decrease in foraging during boat noise exposure. Magnhagen et al. (2017) found similar results with Eurasian perch, *Perca fluviatilis*, and roach, *Rutilus rutilus*, both decreasing foraging efforts, but perch, a species that possesses lower hearing sensitivities, displayed a less dramatic decline in foraging that was not significant. Cyprinidae have Weberian ossicles and therefore represent a family with more sensitive hearing in our study than the other three families observed (Chardon & Vandewalle, 1991; Holt & Johnston, 2011a), which do not possess known specialized hearing structures other than their auditory epithelia and otoliths (Holt & Johnston, 2011b; Magnhagen et al., 2017; Scholik & Yan, 2002; Wysocki et al., 2007). Cyprinids followed our proposed trend for they were the only family whose foraging and presence significantly decreased during boat noise in the field. Additionally, we observed only minute decreases in foraging in taxonomic families not possessing accessory hearing structures, which suggests an increase of concern for species with specialized hearing structures in terms of boat noise pollution. Although a decrease in foraging could simply result from a decrease in presence (e.g. cyprinids), there were opposing trends seen in Gobiidae, which showed decreases in presence but not in foraging, suggesting they were present but not foraging during boat noise exposure. Trends observed in Gobiidae may also suggest that responses to boat noise are highly species dependent and potentially community dependent, for there may be resource competition motives that outweigh the avoidance of a stressor (Hubert et al., 2018). Hubert et al. (2018), found a negative correlation between crab and shrimp located near a food source during noise exposure, indicating that community effects may be moderating a species' response to noise in such a way that single-species experiments would not detect (Hubert et al., 2018).

Although we did not directly quantify bullhead behaviour in the field, because we observed only a few (3 in total) during field experiments, all evidence indicates that minnows and bullheads should have similar hearing abilities due to their shared possession of Weberian ossicles and, therefore, should experience comparable effects from noise exposure (Holt & Johnston, 2011a; Weiss et al., 1969), as also shown by the similarity between our laboratory and field trials. The agreement in responses between laboratory and field trials for fish with specialized hearing illustrates the validity of laboratory trials and suggests applicability of laboratory findings to broader community effects. Black bullhead, for example, spent more time erratically swimming into tank walls during noise exposure, which is not part of their natural behavioural repertoire, but this behaviour could potentially be perceived as an escape response, for the wild fishes with similar hearing simply leave the area. Therefore, even though there is a debate between tank acoustics and wild experiments (Parvulescu, 1967; Rogers et al., 2016), laboratory experiments may be more relatable to natural settings than sometimes assumed. It is understood that laboratory experiments do not reflect the natural behaviour of fish in the wild, however, laboratory conditions are still worth performing for more controlled experiments and for gaining insight into approximate responses. Wild fish did not display signs of habituation in this study, as observations of fish were consistent throughout each experiment, indicating that (1) fish did not reduce interest in the provided food source during the control and (2) fish did not acclimate to boat noise, suggesting temporary distributional shifts (Neo et al., 2018). However, ours was a fairly short-term study, and longer exposure to noise may reveal some effects of habituation; thus, further investigation into this consequence of noise is necessary.

Our finding that boat noise negatively affects fish behaviour is not unique (Cox et al., 2018; Kunc & Schmidt, 2019), as many previous studies have reported declines in foraging due to boat noise exposure (Bracciali et al., 2012; Magnhagen et al., 2017; Mensinger et al., 2016; Purser & Radford, 2011; Shafiei Sabet et al., 2015; Voellmy et al., 2014). However, our study helps to fill an exceedingly large deficit of knowledge surrounding the impacts of boat noise on freshwater species (Mickle & Higgs, 2017) and falls within a small group of studies that have successfully conducted such acoustic experiments on wild free-swimming fish (Jacobsen et al., 2014; Mensinger et al., 2016). Our approach offered us a unique opportunity to investigate natural fish behaviour in response to noise and to observe community interactions, allowing us to make hypotheses on how competition may moderate responses to such stimuli (Hubert et al., 2018). Although we present our findings from the present study as a consequence of hearing ability, differences in feeding and antipredator strategies between species may also have contributed to the varying responses we observed between species. It would be interesting to assess differential noise responses in fish with similar hearing abilities but different behavioural strategies, although this would have to be investigated in more artificial experimental set-ups. Because playback experiments have been critiqued for not having completely ecologically relevant acoustics (Magnhagen et al., 2017), we aimed to ensure that our experiment had the utmost control and that the fish were responding to the playback sound and not to the presence of a motor or to water disturbance. Future experiments would however benefit from comparing speaker playbacks and live motorboat engines to assess possible differential effects on fish behaviour. Lastly, we recognize the limitations of using only one boat noise file (Slabbekoorn & Bouton, 2008).

Nevertheless, we chose this method to increase internal validity between trials, and we think that it was appropriate since the file was recorded within 200 m of the field testing sites and produced clear effects; however, we will pursue the effects of subsequent sound files in future studies.

Conclusion

Boat noise influenced fish behaviour in both laboratory and field settings, and we believe these consistencies give merit to combining both approaches when testing effects of anthropogenic noise on fish behaviour. Fish with a more sensitive hearing range exhibited a greater response to noise than other species, suggesting that species-specific responses are at play. Future studies are advised to consider sampling fish species with a range of hearing abilities to ensure results are not over- or underestimating the potential effects of boat noise. Overall, the observed decline in foraging behaviour and the increase in escape responses in some species further suggests that boat noise negatively affects fish, but these effects must be carefully interpreted relative to the species of interest.

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FIGURES

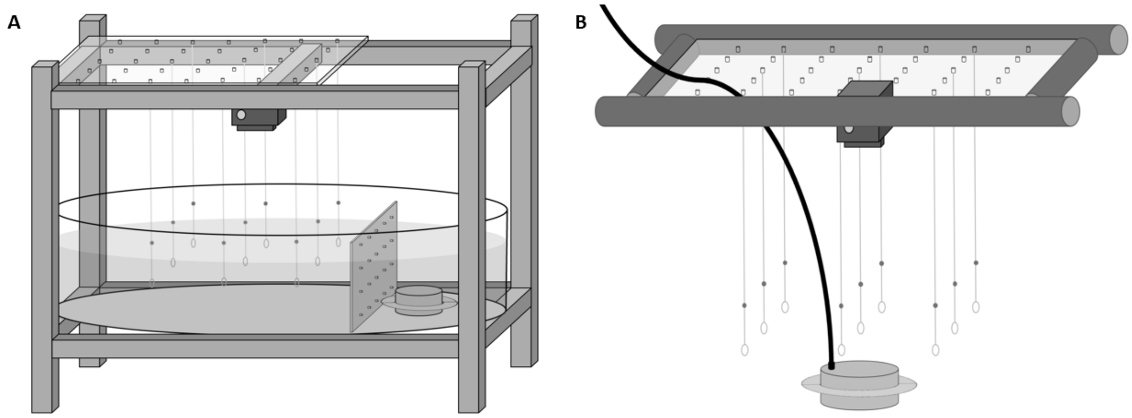

Figure 2.1: A) The experimental tank set-up for laboratory experiments equipped with a metal frame, worm array, underwater speaker, a GoPro HERO+, and perforated barrier. B) The experimental set up for the field experiment. Equipped with a worm array, underwater speaker, pool noodles and a GoPro HERO+.

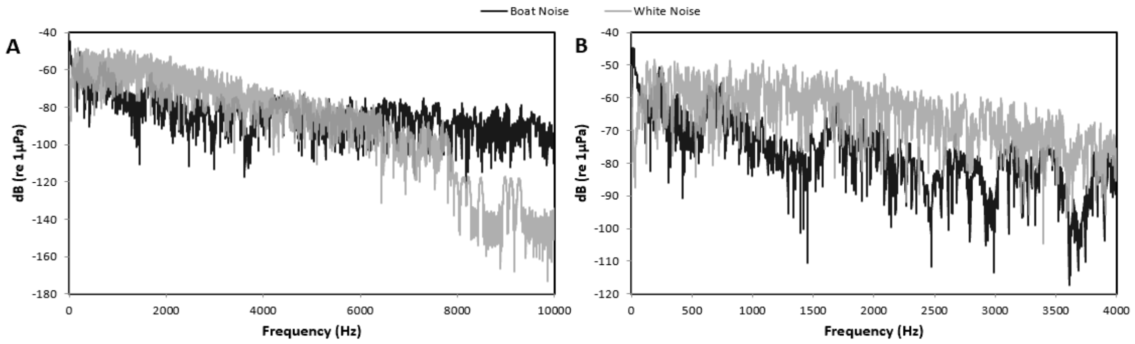

Figure 2.2: Sound spectra of experimental boat noise (black line) recorded in the Detroit River, and white noise (grey line) with A) depicting a frequency range of 0-10kHz and B) zooming in on the frequency bandwidth of 0-4kHz. Spectrograms created in Excel from Adobe Audition (version 12.1) with a Hanning window type, fast Fourier transforms (FFTs) of 32768.

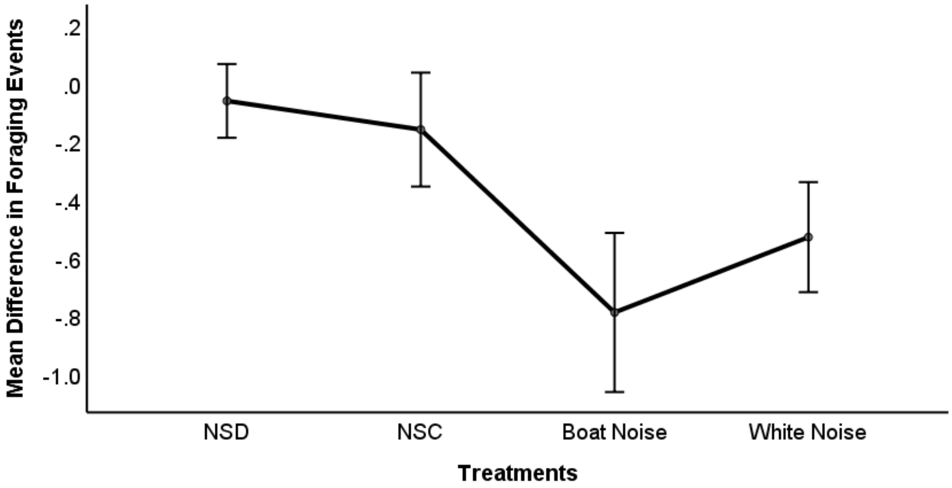

Figure 2.3: Foraging behaviours presented as the mean difference of events for each sound treatment relative to the two-minute controls directly before treatment for 81 captive black bullhead, tested in groups of 3 (N = 27), with +/- 1 standard error. NSD denoting the baseline control treatment with no sound playing and the speaker disconnected and NSC denoting no sound playing with the speaker connected.

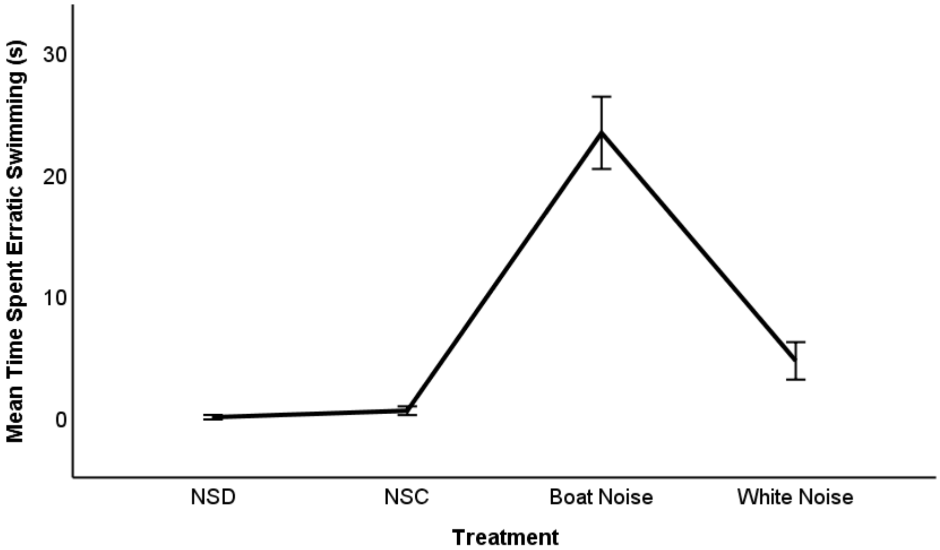

Figure 2.4: The mean time spent erratically swimming for each sound treatment relative to the two-minute controls directly before them for 81 captive black bullhead, tested in groups of 3 (N = 27), with +/- 1 standard error. NSD denoting the baseline control treatment with no sound playing and the speaker disconnected and NSC denoting no sound playing with the speaker connected.

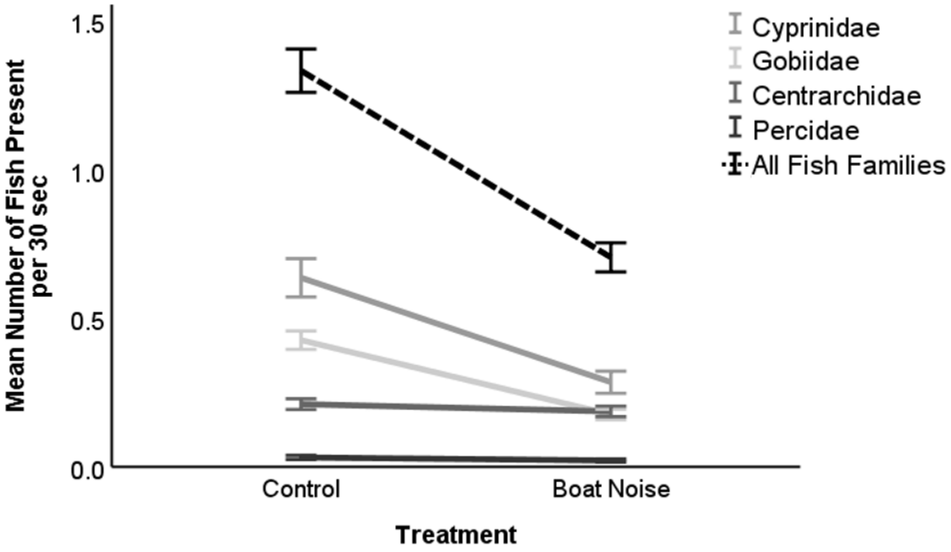

Figure 2.5: Mean number of fish present in each 30 second screenshot during 16 boat noise treatments and the 16 no-noise treatments for each family and for all fish overall with +/- 1 standard error.

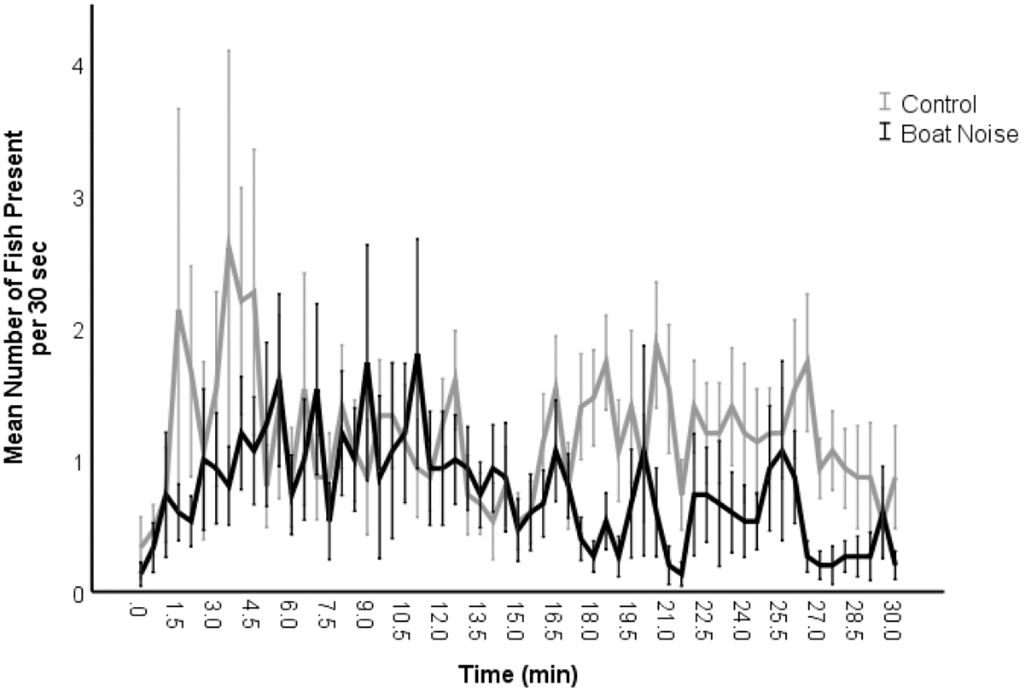

Figure 2.6: Mean number of fish present in each 30 second screenshot throughout the 16 30-minute boat noise treatments and the 16 30-minute no-noise treatments.

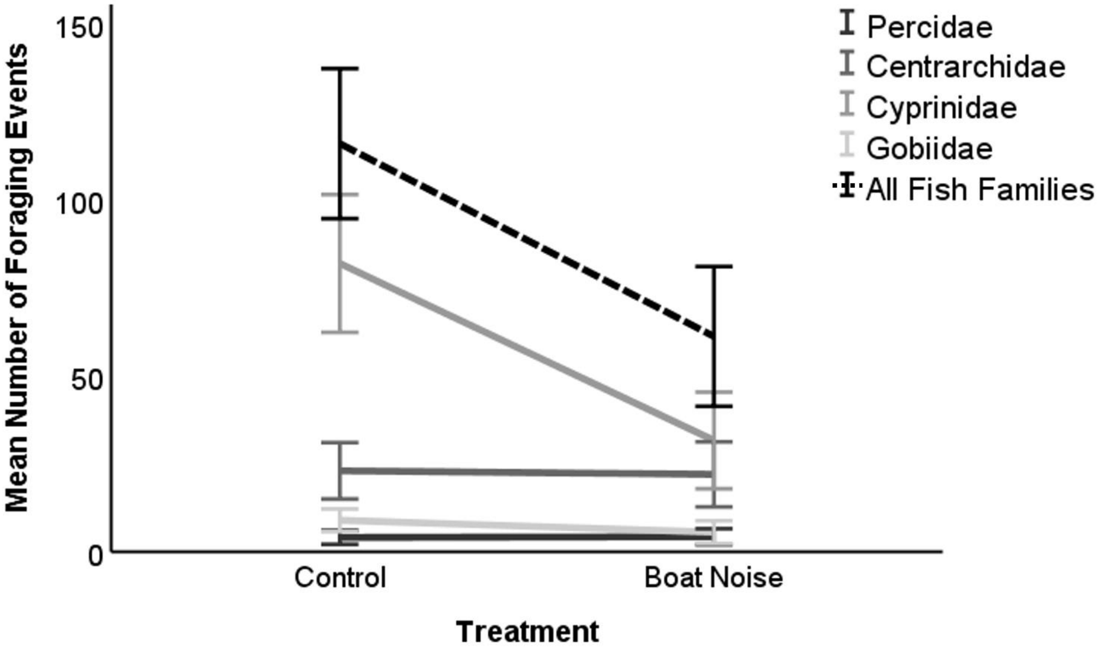

Figure 2.7: Mean number of foraging events that occurred during 16 boat noise treatments and the 16 no-noise treatments for each family and for all fish overall with +/- 1 standard error.

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CHAPTER III

FIELD ASSESSMENT OF THE EFFECTS OF NOISE ON FREE-SWIMMING FISH COMMUNITIES

Introduction

All fish species studied thus far can detect sound through either or both sound pressure and particle motion by using a wide variety of hearing structures (Popper & Hawkins, 2018; Putland et al., 2019). Fishes can hear some sounds with the basic inner ear structure, while others also possess highly specialized hearing structures that connect the swim bladder to the inner ear; allowing for the detection of sound pressure and a much broader range of hearing (Hawkins, 1981; Popper & Hawkins, 2019; Putland et al., 2019). Sound propagates faster, and typically farther, in water than in air (Hawkins, 1986) and is thus an important mechanism of communication, navigation, and predator-prey detection for fish (Higgs, 2005; Putland et al., 2019). The addition of sound in aquatic environments may then hinder the ability to detect such acoustic cues and can cause behavioural and physiological consequences (Putland et al., 2019), making anthropogenic noise a recognized global problem (Slabbekoorn et al., 2010; Hawkins et al., 2020). Activities such as seismic surveying, commercial shipping, offshore drilling, and construction heavily contribute to rapidly rising ambient sound levels (Slabbekoorn et al., 2010; Putland et al., 2019). Coastal habitats are particularly affected by noise as these regions accommodate high densities of sea life and boat traffic which results in an increased number of interactions and elevated noise levels (Hildebrand, 2009). Recreational and commercial boats produce low frequency noise (20-1000 Hz) that overlaps with the hearing ability and vocal range of most fish species (50-1000 Hz) and warrants increasing concern for how it might be affecting fish that dwell in coastal regions (Hildebrand, 2009; Putland et al., 2019). At lower intensities, boat noise can increase stress levels and startle responses in fish (Purser et al., 2016), cause deviations from their normal behaviour (Purser & Radford, 2011), and mask communication and signals from other fish or their environment (Naguib, 2013) while higher intensities of vessel noise can lead to temporary or permanent threshold shifts (Codarin et al., 2009) and injury (Govoni et al., 2003; McCauley et al., 2003; Putland et al., 2019). Noise can affect large areas of aquatic environments and behavioural changes can be observed throughout such an area, hindering predator-prey interactions, rerouting migrations, and deterring fish from important resources, making behavioural consequences particularly concerning compared to physical impacts from noise, which only burden fish immediately surrounding the noise source (Popper et al., 2020, Slabbekoorn et al., 2010).

Studies hitherto have often conducted acoustic experiments of fish responses to noise in laboratory tanks, and while laboratory studies are excellent for answering specific questions, observing cryptic fish species, and providing controlled environments, their results are not necessarily predictive of how wild fish will behave and respond (Hawkins et al., 2020). Captive conditions pose problems when trying to emulate natural settings for the tank reverberations skew the propagation of sound (Parvulescu, 1967; Rogers et al., 2016), the baseline stress levels may vary between captive and wild fish (Wright et al., 2007), and confining animals within enclosures has been shown to effect their natural behaviours (Popper & Hawkins, 2019; Hawkins et al., 2020). Technological advances in underwater monitoring have greatly improved the ability to study wild fish and allow for high-definition behavioural analyses (Ulrich & Bonar, 2020; Mallet &

Pelletier, 2014; Mensinger et al., 2016; Roberts et al., 2016). Sonar, for example, has previously allowed for the successful tracking of free-swimming fish movement in response to sound stimuli (Hawkins et al., 2014), but improvements to underwater cameras have since proven to be a much less expensive and easy-to-use alternative that offers similar results (Ulrich & Bonar, 2020). Consequently, baited remote underwater video (BRUV) systems have recently gained recognition among acoustic researchers with Mensinger et al. (2016) and Roberts et al. (2016) both demonstrating their effectiveness in collecting behavioural responses to noise from wild fish and Mensinger et al. (2018) showing that fish decrease their feeding behaviours and scatter more in response to noise but only with those located outside of the Cape Rodney–Okakari Point Marine Reserve, not the fish located within it. Therefore, anthropogenic noise likely affects many fish species; however, the degree of its impact in natural settings is still debated and field studies offer a way to resolve such uncertainties (Hawkins et al., 2015; Hawkins et al., 2020).

The current study aims to address gaps in the literature by trying to 1) understand how free-swimming fish respond to boat noise exposure, 2) investigate how habituation might affect a fish's long-term behaviour with noise present, and 3) examine how community effects influence fish behaviour, as these have been frequently outlined in the literature (Hawkins et al., 2015; Mickle & Higgs, 2018; Popper & Hawkins, 2019; Putland et al., 2019; Hawkins et al., 2020). Therefore, BRUV systems were deployed off both well-trafficked and abandoned boat docks to assess the effects of boat noise playbacks on wild, free-ranging fish communities that differ in their degree of exposure to boat traffic. Overall, fish were hypothesized to avoid the BRUV during boat noise trials and forage more often during control treatments, but since the fishes at the active boat docks are regularly exposed to noise, we hypothesized to see a lesser degree of behavioural changes during noise treatments compared to fishes at abandoned docks. There were fewer free-swimming fish present at the abandoned docks during boat noise exposure than during the controls with different families of fish showing varying degrees of responses, however, the fish at the active docks did not change their behaviours due to noise. Therefore, I showed how the impacts of boat noise are dependent on the location of the fish communities and species and my design allowed for the analysis of natural fish responses to noise and for insights into how boat noise may affect community compositions. Within the field of aquatic bioacoustics, there are still many information gaps that require attention, but here we hoped to address the effects of habituation, free-ranging fish behaviour, and communities on mediating responses to anthropogenic noise to help make clearer inferences that can hopefully contribute to regulation development and thus actionable change.

Methods

To explore the impacts of boat noise on the foraging efficiency and behavioural response of wild marine fishes, a field study was conducted at two differing sites near the Bimini Biological Field Station (BBFS, hereforth referred to as Shark Lab) on South Bimini Island, The Bahamas in February 2020. The first site was located at the Shark Lab's boat dock (25.6998°N, 79.2968°W) which is frequented by boats and bait castoff resulting in a community of fish that are quite conditioned to humans. The second site was located off docks at the former Beach Club Resort (25.6930°N, 79.3040°W) that was severely damaged and ultimately closed by Hurricane Mathew in 2016, leaving it abandoned and rarely used by humans since. More specifically, Shark Lab receives regular boat traffic with a minimum of two boats leaving and returning to the docks each day, whereas the Beach Club site is unlikely to have a single boat in the marina per week and none coming within 15 m of the particular dock where experiments were conducted. A metal, baited remote underwater video (BRUV) structure (95l x 83w x 98h cm; Fig. 3.1) equipped with a GoPro HERO+ camera (GoPro) above the bait for recording behavioural responses, another GoPro Hero+ on the side of the BRUV for identification purposes, and an underwater speaker (Electro-Voice UW-30) which was used to capture the responses of wild, foraging marine fish to sound stimuli. A mesh bag with 5 mm holes was filled with about 1.75lbs of Atlantic Menhaden (*Brevoortia tyrannus*) chum (Bionic Bait) equaling approximately a 7.5 x 19 x 4 cm bait block and secured with plastic ties to a wooden platform at the base of the BRUV. The platform ensured that all foraging attempts were within view of the GoPro, which had a bird's eye view in the BRUV, by blocking any foraging behaviours that might have occurred underneath the bait bag. A collection of three 2-stroke outboard engine boat noise files were randomly assigned to each trial and presented to the fish at a pressure level of 160 dB re 1µPa and a particle motion metric of -0.835 dB re 1m/s^2 which was well above the range of ambient sound levels of 111 – 116 dB re 1µPa at the sites. Sound pressure was measured with a hydrophone (Inter Ocean System Inc.- Acoustic Calibration and System Model 902) and particle motion was calculated with the Euler equation by taking two measurements of sound pressure 1 m apart and estimating the pressure gradient (Mann, 2006). One of the presented sound files was recorded with a hydrophone, in Bimini to ensure ecological relevance, 2 m from a boat that was approaching and then idling, and two of the files were purchased from soundsnap.com (Fig. 3.2).

After the BRUV was lowered into the water and secured approximately 30 cm off the sand substrate, to avoid interference from blue crabs (*Callinectes sapidus*), the wild fish were given an acclimation period of 1 hour, providing more than enough time for fish to resume normal behaviours based on a previous BRUV study conducted in Bimini which found that 10 minutes was a sufficient enough time for fish numbers to plateau (Grimmel et al., 2020). After acclimatization, a pattern of 10 minutes of no sound followed by a 10-minute period of boat noise was repeated for 1 hour per day. The 10-minute no-sound period served as the pre-treatment control and was determined to be a sufficient amount of time between noise treatments for fish to resume normal behaviours during pilot trials. In total, 38 10-minute video clips were recorded with 19 treatments of boat noise, and 19 pre-treatment controls (recorded directly before treatment trials) collected between the 2 sites. All experiments were conducted according to Canadian Council for Animal Care (CCAC) protocols (University of Windsor; AUPP 17-11).

To create a blind study and avoid bias, videos were assigned random numbers for analysis and examined by three individuals with the sound muted in the video files. The videos were analysed in video editing software that allowed for frame by frame analysis and for the event time to be recorded in centiseconds (QuickTime Player by Apple Inc. and Microsoft Video Editor by Microsoft Corporation). The first minute of each boat noise video and the last minute of each pre-treatment control video were analysed for five behaviours and metrics of interest: First, the number of fish and the species of each fish was identified, as specified by Humann & Deloach (2014), and crossed checked to those found in Grimmel et al. (2020), then the time each fish entered and exited the frame was recorded. Next, foraging behaviours were quantified when a fish physically touched the bait with their mouth and then startles were counted when a fish contracted its head and tail to the same side of its body and propelled itself quickly in a different direction, known as a C-start (Blaxter & Hoss, 1981; Eaton & Emberley, 1991). Lastly, each fish was assigned 1 of 3 overall behavioural statuses for their time in frame: 1) If a fish simply swam through the BRUV, they were given a "swimming" behavioural status; 2) if the fish swam in view, and then positioned their head and hovered at the bait bag, they were denoted as "investigating"; 3) and finally, if a fish swam up to the bait bag and foraged, they were assigned a "foraging" status. Behavioural statuses were used to gain a snapshot of the intention each fish had within the BRUV as well as to gauge the overall interest in foraging throughout each treatment. The entirety of each 10-minute video was more broadly analysed to gauge changes in community compositions, signs of habituation to noise, or potential changes in food interest throughout each treatment, by stopping the video every 30 seconds and counting the number of fish in the frame as well as logging their species.

Statistical Analyses

To ensure the results were minimally biased by daily environmental conditions, the number of fish recorded, the amount of time spent in view, the percentage of fish foraging, and the number of times fish startled during pre-treatment controls and boat noise treatments were analysed as difference metrics subtracting boat noise treatment data from the pre-treatment control that occurred directly before it, and then were compared to a dummy variable of 0 to represent the null hypothesis of no effect. Since these data were not normal a nonparametric Mann-Whitney U test was conducted during analysis to assess the main effects of site and treatment on the presence, foraging, startles, and time fish spent within view, which was then followed up with repeated measures pairwise comparisons between the families on SPSS (version 27, IBM SPSS Statistics, Chicago, IL). To assess the changes in individual family data, each one was compared to zero with a Mann-Whitney test. The data containing the number of fish recorded every 30 seconds were normal however, so these data were analysed with a one-way ANOVA to determine the main effects of site and treatment and was then followed by Tukey post hoc to uncover where differences occurred between the families.

Results

Frequency Table

There were a total of 21 species identified within the 1848 fish observed over 38 videos (Table 3.1), however only 11 species individually accounted for more than 1% of the total number of fish, so these 11 were subsequently grouped into 5 families and used in further analyses: wrasse (Labridae: slippery dick; *Halichoeres bivittatus),* snappers (Lutjanidae: schoolmaster snapper (*Lutjanus apodus*), gray snapper (*Lutjanus griseus*), lane snapper (*Lutjanus synagris*)), grunts (Haemulidae: French grunt (*Haemulon flavolineatum*), white grunt (*Haemulon plumierii*), blue striped grunt (*Haemulon sciurus*)), damselfish (Pomacentridae: cocoa damselfish (*Stegastes variabilis*), longfin damselfish (*Stegastes diencaeus*), threespot damselfish (*Stegastes planifrons*)), and squirrelfish (Holocentridae: *Holocentrus adscensionis*). There were 9 videos of each treatment that took place at Shark Lab and 10 videos of each treatment that took place at Beach Club. All behaviours and trials were also tested for differences between the days in which they occurred with no significant date effect for any of them.

Fish Presence

The difference in the total number of fish spotted between treatments had a main effect of site ($U = 495$; $p = 0.01$), but only Beach Club exhibited a treatment effect that was significantly different from zero ($U= 400$; $p < 0.001$; Fig. 3.3). Furthermore, at Beach Club all families except for squirrelfish had significantly fewer fish observed during boat noise ($U = 0.0 – 20.0$; $p \leq 0.005$) with grunts showing a significantly greater decrease than all other families ($p \leq 0.027$) and wrasse exhibiting significantly reduced responses from damselfish ($p = 0.037$). Fishes at Shark Lab exhibited no overall difference between treatments in the number of fish present per family even though there were significantly more grunts in the pre-treatment control than boat noise treatments ($U = 5$; $p = 0.001$; Fig. 3.4).

Time Spent in View

There was a main effect of site on the difference in time fish spent within the camera's view between treatments ($U = 336.5$; $p < 0.001$; Fig. 3.5), where the time fish spent in the frame at Beach Club was significantly decreased by the presentation of noise compared to Shark Lab ($p < 0.001$), and only Beach Club was significantly different from zero ($p < 0.001$; Fig. 3.5). All families except snappers at Beach Club spent significantly less time within view of the camera during boat noise exposure than during the pre-treatment control (U = 0.0 – 18.0; $p < 0.05$).

Foraging

The overall differences in the percentage of fish foraging between pre-treatment controls and noise treatments revealed a significant difference between the sites (U = 504.0; $p = 0.015$), but only Beach Club had the percentages of fish foraging significantly decrease during boat noise exposure (U = 799.0; $p = 0.004$). Beach Club separately had significant effects between families ($p < 0.001$), however, further analyses revealed that only grunts had significantly higher foraging percentage differences than all other families (U = 0.0; $p < 0.001$: Fig. 3.6).

Startles

The difference measures in number of times fish startled between treatments showed no significant effect between sites (U= 531.5; $p = 0.226$) nor families ($\chi^2_{(4)} = 8.897$; $p = 0.064$), and no significant differences when compared to zero at Shark Lab (U = 658.0; $p = 0.508$) with minor differences from zero detected at Beach Club (U = 822.5; $p = 0.08$; Fig. 3.7). While snappers appeared to startle more in response to noise at Beach Club, the high degree of variance exhibited (from -0.07 to -0.25) resulted in no significant differences.

Habituation

Overall, the number of fish recorded every 30 seconds throughout each 10-minute trial revealed main effects from the sites ($F_{(5,680)} = 113.05$; $p < 0.001$) and the treatments

($F_{(5,680)} = 20.273$; $p < 0.001$; Fig. 3.8), as well as an interaction between sites and treatments ($F_{(5,680)} = 6.138$; $p < 0.001$), but no significant effect of time at either site. Comparisons further revealed significantly more fish at Shark Lab than Beach Club overall ($F_{(1,684)} = 284.638$; $p < 0.001$) as well as separately for wrasse ($F_{(1,684)} = 19.954$; $p < 0.001$), damselfish ($F_{(1,684)} = 28.179$; $p < 0.001$), and snappers ($F_{(1,684)} = 447.691$; $p < 0.001$) with only grunts exhibiting similar numbers of fish between the sites over time ($F_{(1,684)} = 3.85$; $p = 0.050$).

There was no significant difference in the number of fish present at 30-second intervals between treatments when including all fish observed at Shark Lab, however, wrasse ($F_{(1,306)} = 10.711$; $p = 0.001$), damselfish ($F_{(1,306)} = 8.478$; $p = 0.004$), and grunts ($F_{(1,306)} = 9.307$; $p = 0.003$) individually appeared significantly more often during pre-boat controls then noise treatments, whereas snappers did not ($F_{(1,306)} = 0.123$; $p = 0.726$; Fig. 3.9). Collectively, there were more fish at Beach Club during pre-boat controls over time than boat noise treatments ($F_{(1,378)} = 81.664$; $p < 0.001$) with damselfish ($F_{(1,378)} = 30.717$; $p < 0.001$), grunts ($F_{(1,378)} = 71.635$; $p < 0.001$), squirrelfish ($F_{(1,378)} = 12.517$; $p < 0.001$) and snappers ($F_{(1,378)} = 15.618$; $p < 0.001$) also separately showing similar trends, whereas wrasse did not ($F_{(1,378)} = 1.154$; $p = 0.253$; Figure 3.10).

Discussion

In the current study, I investigated the effects of boat noise on wild, foraging Caribbean reef fish. My results give evidence that suggests boat noise can have negative effects on wild fish populations, but the magnitude of its effects may depend on the amount of previous boat noise exposure. The mean differences in both the total number of fish and the number of fish per family counted between the boat noise and the control at Beach Club were significantly greater than those at Shark Lab suggesting that fish at Beach Club were more affected by boat noise.

Beach Club represented a population of fish that had little previous boat noise exposure, and here, I found a general trend of fish changing their behaviour during short-term noise treatments. Not only were fish entering the BRUV structure less during boat noise playbacks, but the fish that did appear were spending significantly less time there yet foraging at similar rates to the controls. Several papers have also described behavioural changes after short term boat noise exposure (Popper & Hawkins, 2018; Putland et al., 2018), however, interestingly, there was no relative interference with fishes' motivations to forage in the current study. We were only able to track the foraging attempts that were made though, as it was not possible to tell whether a fish was successful in procuring the bait, so there may have been effects on the success rate of foraging that went undetected. Purser & Radford (2011), for example, found that three-spine sticklebacks (*Gasterosteus aculeatus*) displayed more handling errors with their food and attention shifts when exposed to noise. Moreover, the lack of foraging differences, yet changes in the amount of time spent within the BRUV may suggest shifts in foraging strategies; as fish made quick and concise trips into the BRUV to feed during boat noise treatments whereas they seemed to linger around the bait longer during controls. Furthermore, there was no variation in the number of times fish startled in boat noise treatments compared to control nor between sites, which was unexpected, however, this could potentially be because we quantified the startle behaviours over the full 1-

minute analyses when the differences are likely only observed within the first 10 seconds at the onset of noise (Kastelein et al., 2008).

Shark Lab had significantly higher populations of fishes observed during trials, likely attracted by the consistent supply of bait castoff, however, they showed contradicting results to Beach Club with fish presenting no overall changes to any of their behaviours between the sound treatments. Therefore, fishes at Shark Lab seem to have become desensitized to the presence of boat noise or have experienced hearing threshold shifts leading to desensitized responses. Such results are validated through previous work by Harding et al. (2018) which also demonstrated that fishes in areas of high boat disturbance were less affected by boat noise playbacks than fishes in areas of low disturbance. Although, Mensinger et al. (2018) appear to have found opposing results in that fish in protected areas responded more to boat noise than those in fished areas, which suggests that the stresses of fishing pressures may play a greater factor in moderating fish behaviour than the boat noise itself. Furthermore, studies such as Nedelec et al. (2016), found that threespot dascyllus (*Dascyllus trimaculatus*) no longer hid during boat noise playbacks after 1 to 2 weeks of exposure, and Holmes et al. (2017) found that Ambon damsels (*Pomacentrus amboinensis*) reverted back to pre-noise behaviours after just 20 minutes of boat noise exposure. Therefore, my work provides additional evidence that fish can haituate to noise despite previous belief that they could not (Wright et al., 2007), and suggests that noise effects are exposure dependent and thus can greatly affect guidelines with respect to noise damage.

Damselfish and snappers both displayed avoidance responses to noise at Beach Club, however, damselfish presented as more sensitive than snappers for they also spent less time within frame during noise exposure, which was unexpected for snappers have slightly poorer hearing abilities (100 - 1000 Hz) than damselfish (100 – 1200 Hz; Tavolga & Wodinsky, 1963; Wright et al., 2011). Slippery dicks were the only species observed from the wrasse family which have similar hearing abilities to damselfish and grunts (100 – 1000 Hz; Tavolga & Wodinsky, 1963), but did not appear to be as responsive to boat noise playbacks as the other families when looking at presence data at Beach Club. However, slippery dicks significantly decreased the amount of time they spent within the BRUV, suggesting they did not leave the area as other fish did, but they made quicker trips within the BRUV when boat noise was playing, again suggesting a potential shift in foraging strategies. Furthermore, grunts, a family that consisted of white grunts, bluestriped grunts and French grunts, were disproportionately more affected by boat noise at both sites suggesting they may be more sensitive to noise than the other fish. Therefore, we found no evidence of hearing thresholds moderating the responses of fish to noise for squirrelfish possess the hearing abilities to detect the largest bandwidth of sound frequencies compared to all the families observed (100 – 2800 Hz; Tavolga & Wodinsky, 1963), for they have extensions that connect their inner ear to their swim bladder which allows for the detection of sound pressure as well as a broader range of sounds (Popper, 1977; Coombs & Popper, 1979), yet they did not display the most sensitive responses. Squirrelfish were the only family of fish to not display an avoidance-like behaviour as their numbers did not change between treatments, suggesting there may be factors other than hearing ability involved in arbitrating fish responses to noise.

While I understand the limitations of my field experiments, as we were not able to control weather conditions or if the fish populations were consistent or different every trial, our data was analysed as difference metrics between treatments that happened consecutively and there was no effect of day on the treatment pairings, thus allowing for more persuasive conclusions to be made from the collected data. I successfully addressed literature gaps concerning habituation and community effects of free-ranging fish responses to noise, which are currently important for better understanding the natural response of fish to noise, and for improving the development of future guidelines and regulations. I experimentally tested changes in behavioural responses of wild unrestrained fishes to noise which allowed us to gain an ecologically relevant view of the effects of boat noise and allowed us to challenge the current perception that all fish are negatively affected by boat noise. Additionally, I was able to study the community of fish present and their collective behaviours which allowed us to focus on multiple species of fish instead of just a single species as most current studies do. Finally, I further established that wild fish may in fact be able to habituate to boat noise after long term exposure for the fish exhibited differential responses depending on their previous exposure. While the present study is certainly not the only one to have looked into these questions, hopefully it can be an example of how to conduct integrative studies that address multiple research questions in one.

Conclusion

Wild free-ranging Caribbean reef fishes at abandoned docks either avoided the BRUV structure or spent less time swimming within it during boat noise than the controls, however, fishes at regularly trafficked docks did not exhibit such differences in behaviour, suggesting they have habituated to the boat noise. Variations in the responses of each family supports the idea that the effects of boat noise are specie-dependent, so the composition of fish communities should be taken into consideration in future studies and policymaking. In summary, boat noise demonstrated the ability to significantly affect short-term fish behaviour, but fishes having undergone long-term noise exposure presented the ability to habituate. While habituation is encouraging for the well-being of current ecosystems exposed to regular boat traffic, further investigation is necessary to establish whether that is sustainable in a long-term capacity, and if this is true for all taxa within an ecosystem.

FIGURES

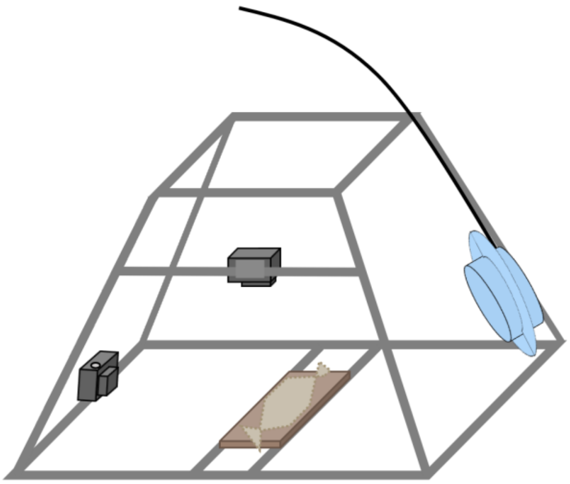

Figure 3.1: The experimental set-up with two GoPros (Hero+), positioned on the side and above the bait, a bait bag positioned on a wooden platform, and an underwater speaker attached to the baited underwater video (BRUV) structure (95l x 83w x 98h cm).

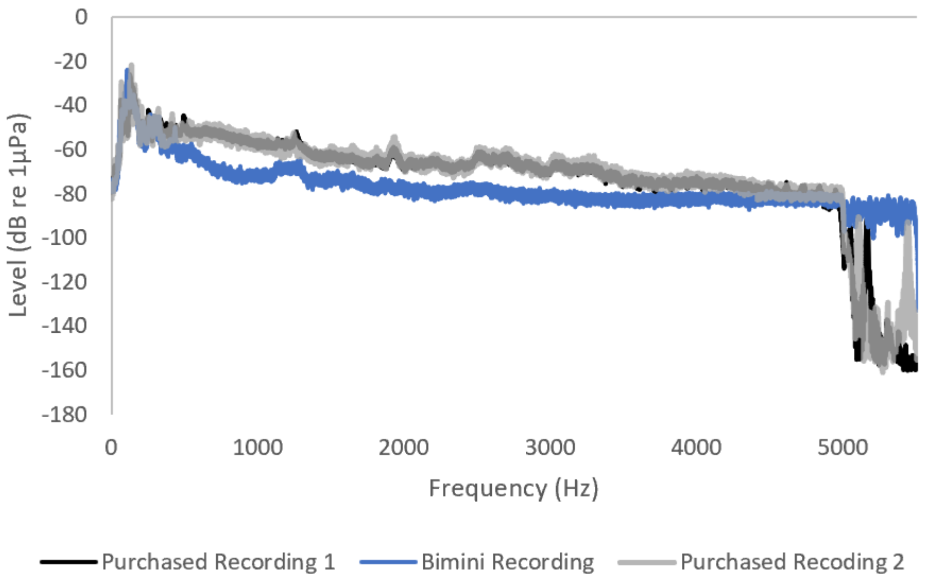

Figure 3.2: The spectral outputs of the 3 boat noise files used during the experiments up to 5000 Hz to encompass the hearing range of fishes. Spectrograms were created in Excel from Audacity (version 2.3.3) with a Hanning window type, and fast Fourier transforms (FFTs) of 32768.

Family	Species	N	%
	Slippery Dick	87	2.8
Wrasse		**87**	**2.8**
	Longfin Damselfish	259	8.4
	Cocoa Damselfish	118	3.8
	Threespot Damselfish	46	1.5
Damselfish		**423**	**13.7**
	Gray Snapper	632	20.6
	Schoolmaster	437	14.2
	Lane Snapper	142	4.6
Snapper		**1211**	**39.4**
	Squirrelfish	86	2.8
Squirrelfish		**86**	**2.8**
	French Grunt	764	24.9
	Bluestriped Grunt	145	4.7
	White Grunt	268	8.7
Grunt		**1177**	**38.3**
	Parrotfish spp.	24	0.8
	Foureye Butterflyfish	5	0.2
	Bermuda/Gray Chub	15	0.5
	Doctorfish	3	0.1
	Brown Chromis	2	0.1
	Bigeye Mojarra	3	0.1
	Unidentified spp. 1	20	0.7
	Unidentified spp. 2	6	0.2
	Unidentified spp. 3	6	0.2
	Unidentified spp. 4	1	0
Not Analysed		85	2.9

Table 3.1: A frequency table depicting the number of fish per species and per family and the percentage each species and family contributed to the total number of fish.

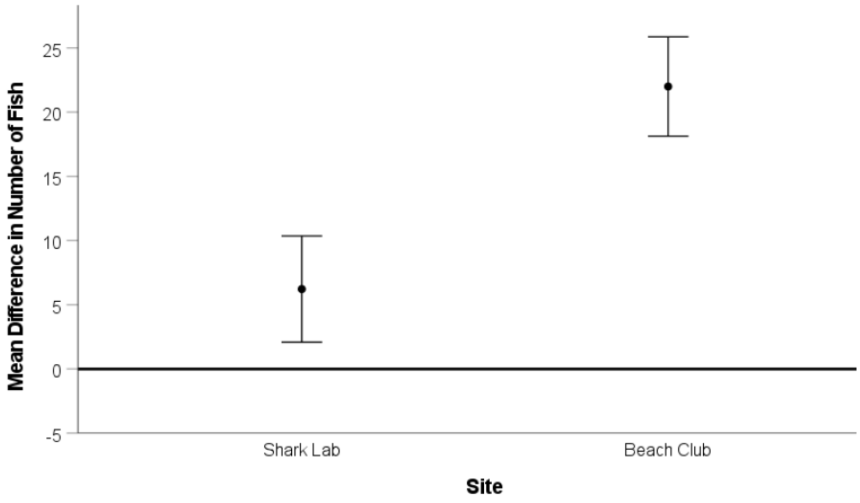

Figure 3.3: The mean differences between pre-boat and boat noise treatments in the total number of fish observed at Shark Lab and Beach Club compared to a dummy variable of 0 with the error bars depicting ±1 standard error.

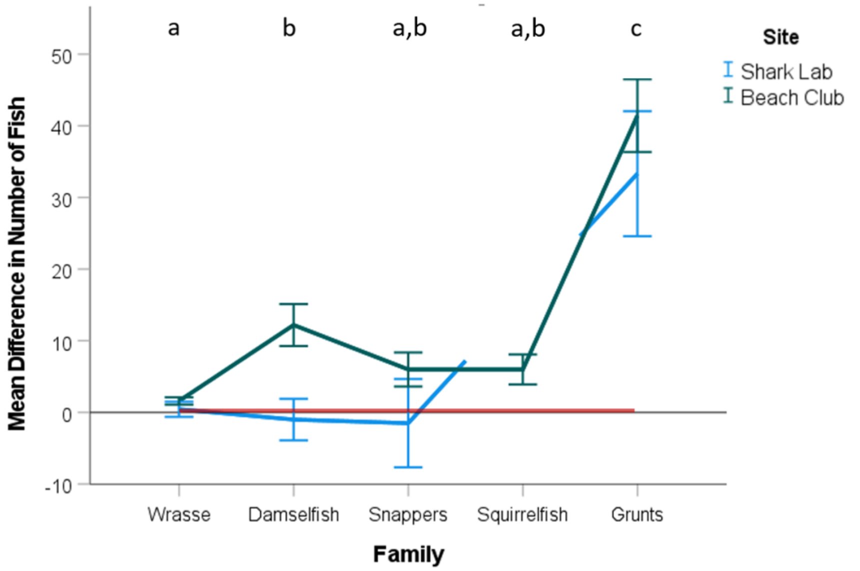

Figure 3.4: The mean differences between pre-boat and boat noise treatments in the number of fish observed per family at Shark Lab and Beach Club compared to a dummy variable of 0 with the error bars depicting ±1 standard error and letters showing significant differences between the sites. No squirrelfish were observed at Shark Lab, hence the missing value.

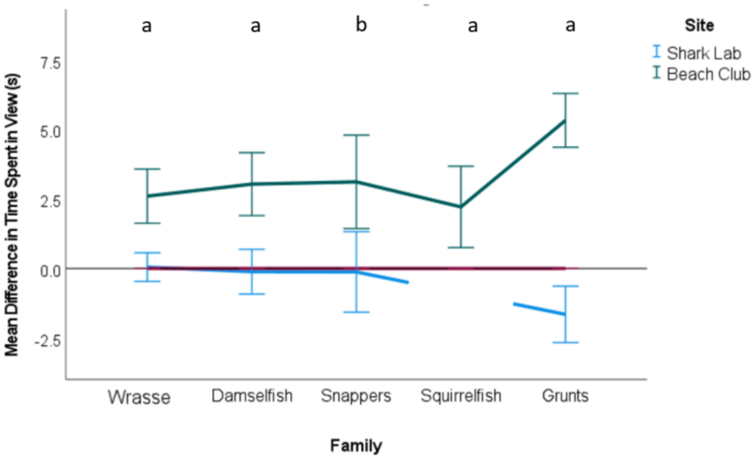

Figure 3.5: The mean difference between pre-boat and boat noise treatments in the amount time of each fish per family spent within the camera's view at Shark Lab and Beach Club compared to a dummy variable of 0 with the error bars depicting ±1 standard error and letters showing significant differences within Beach Club. No squirrelfish were observed at Shark Lab, hence the missing value.

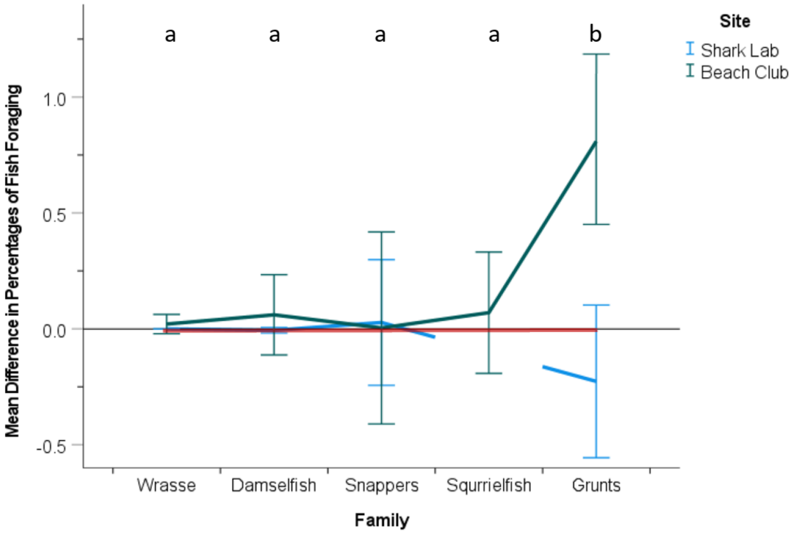

Figure 3.6: The mean difference between pre-boat and boat noise treatments in the percentage of fish foraging per family at Shark Lab and Beach Club compared to 0 with the error bars depicting ±1 standard error and letters showing significant differences within Beach Club. No squirrelfish were observed at Shark Lab, hence the missing value.

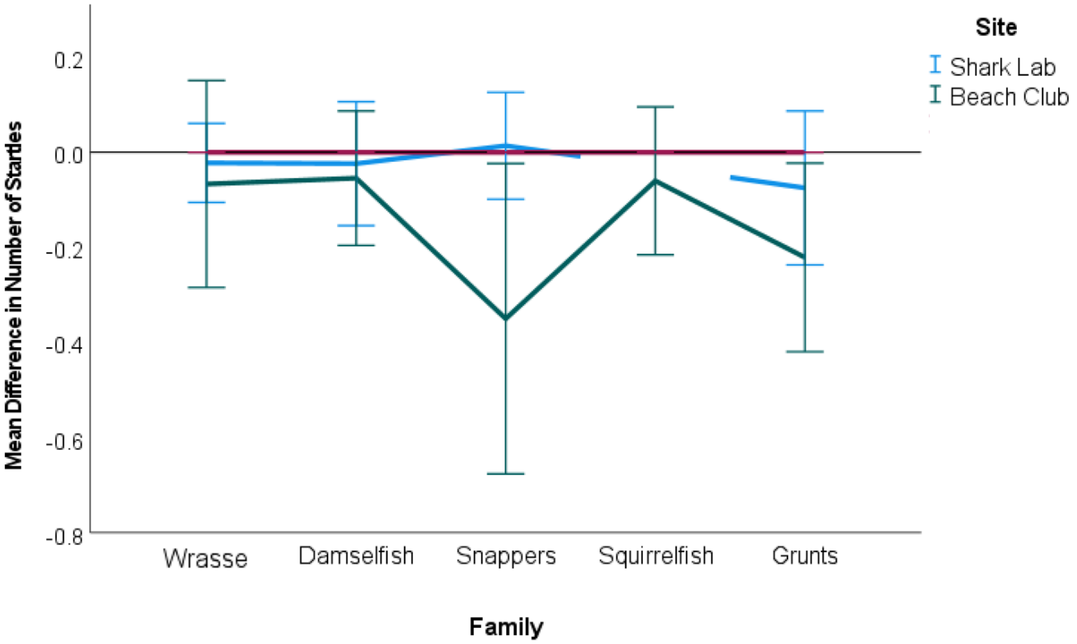

Figure 3.7: The mean difference between pre-boat and boat noise treatments in the number of c-start startles performed by each fish per family at Shark Lab and Beach Club compared to a dummy variable of 0 with the error bars depicting ±1 standard error. No squirrelfish were observed at Shark Lab, hence the missing value.

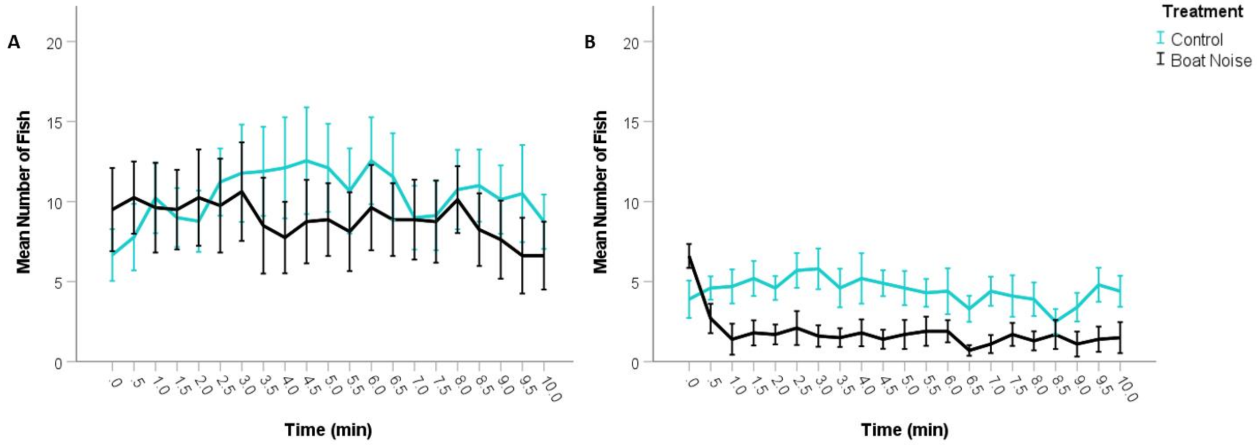

Figure 3.8: The overall mean number of fish observed over the course of each 10-minute treatment, with error bars depicting ±1 standard error, where A) illustrates counts at Shark Lab and B) illustrates the counts at Beach Club.

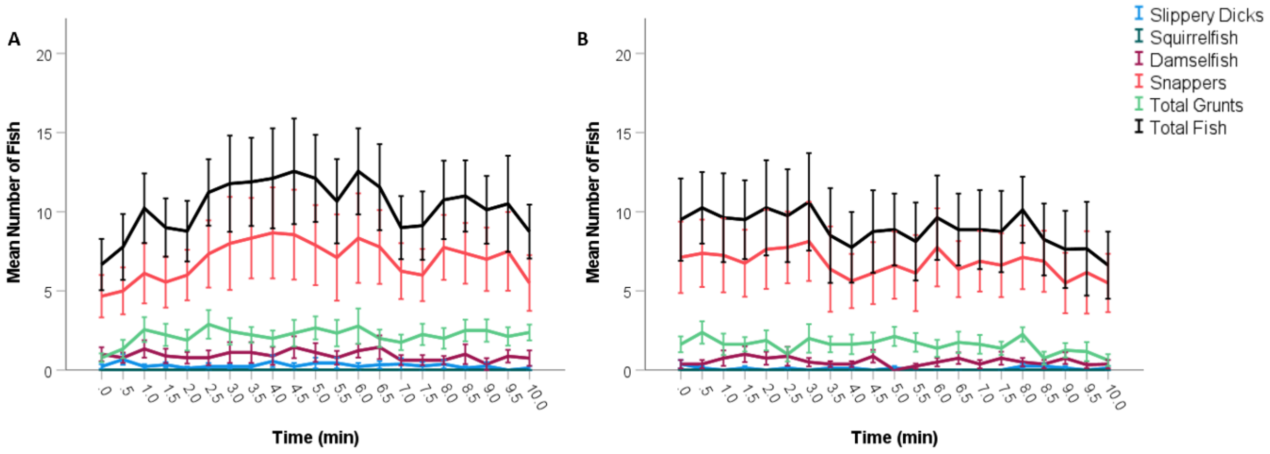

Figure 3.9: The mean number of fish per family observed at the Shark Lab site per 30 seconds over the course of each 10-minute treatment, with error bars depicting ±1 standard error and letters showing significant differences between the sites. Where A) illustrates the pre-treatment control and B) illustrates the boat noise treatment.

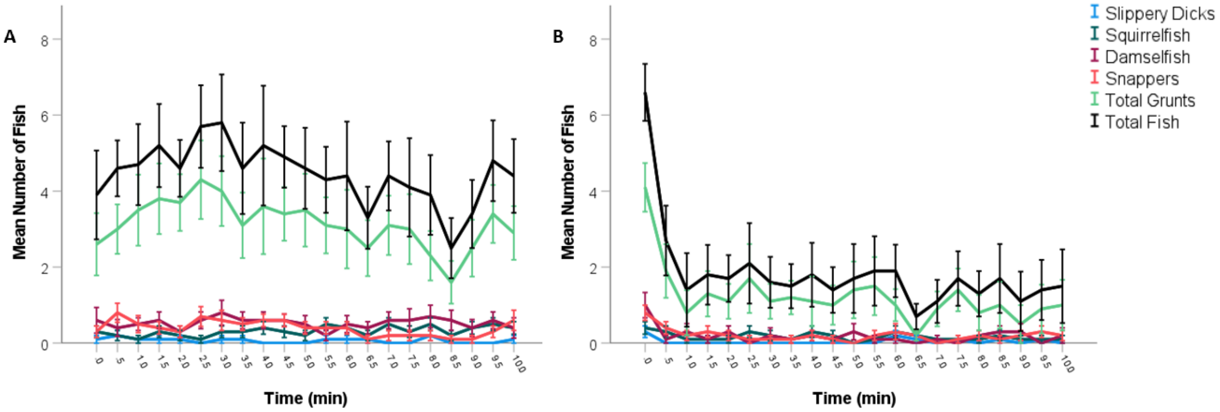

Figure 3.10: The mean number of fish per family observed at the Beach Club site per 30 seconds over the course of each 10-minute treatment, with error bars depicting ±1 standard error. Where A) illustrates the pre-treatment control and B) illustrates the boat noise treatment.

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CHAPTER IV

CONCLUSION AND RECCOMENDATIONS

Summary

Anthropogenic noise can have adverse effects on fishes, but its impacts in natural settings are not well understood (Slabbekoorn et al., 2010; Hawkins et al., 2020). The majority of current studies have analysed the effects of noise on captive fish, but experimental tanks have been continuously shown to skew sound propagation and inhibit natural fish responses (Parvulescu, 1967; Rogers et al., 2016), and thus may not accurately represent the effects of noise. Laboratory experiments have demonstrated overwhelmingly negative effects on fish due to noise (Slabbekoorn et al., 2010; Popper & Hawkins, 2019; Putland et al., 2019), however, recent field experiments are revealing more diverse findings with fish showing variable noise tolerances depending on their previous noise exposure, local fishing pressures, species, sex, and hearing sensitivities (Nedelec et al., 2016; Holmes et al., 2017; Magnhagen et al., 2017; Harding et al., 2018; Davidsen et al., 2019; Mickle et al., 2020). Thus, further investigation into the responses of fish to anthropogenic noise is necessary to accurately describe the consequences of such pollution and develop appropriate mitigation plans.

Here, I investigated the effects of boat noise in the field on communities of freshwater and marine fishes. First, I was interested in comparing field experiments to those conducted in the lab to determine the level of similarity between the responses of captive and wild fish in hopes of shedding light on how previous laboratory studies may be able to predict natural responses. I used a similar experimental design in both settings, with black bullhead (*Amerius melas*) as my captive species to compare to all species spotted in the wild. Black bullhead have specialized hearing abilities and therefore were expected to be more sensitive to noise and ultimately form an overestimation how free-swimming fish might response. They were exposed to four randomly sequenced sound treatments (no sound with speaker connected, no sound with speaker disconnected, boat noise, and white noise), where noise was played at 160 dB re 1 µPa, and monitored for foraging behaviours and changes in swimming activity. Black bullhead exhibited significant deceases in their foraging behaviours and increases in time spent erratically swimming during boat noise exposure compared to the controls. In the field, fish communities were exposed to boat noise and control treatments consecutively with foraging behaviours, quantified by physical touches of the worms, the presence of fish, and the species of each fish. Similar to laboratory findings, I found a decrease in overall foraging behaviours, but instead of erratic swimming there was an overall decrease in the number of fish present, suggesting the usual swimming observed in the lab may have been indicative of an escape response. The overall results, however, were heavily skewed by the responses of minnows, a fish family with specialized hearing structures, for they were significantly more sensitive to the noise playbacks than all other fish families, which had poorer hearing in comparison, suggesting that fishes with broad ranges of hearing should be studied to avoid over- or underestimation of the effects of noise.

The second set of experiments I conducted were in Bimini, The Bahamas, and were intended to investigate the responses of wild marine fishes to boat noise with contrasting amounts of previous noise exposure. One site, at the Bimini Biological Field Station (Shark Lab), was located at docks that were regularly trafficked by boats and therefore previously exposed to boat noise, the second site, called Beach Club, was situated at docks within an abandoned marina that received minimal, if any, boat traffic. Using a BRUV system, I exposed fishes to 10 minutes of boat noise at a time, played at 160 dB re 1 µPa, and compared their behaviours during treatments to their behaviours in the 10 minutes directly before the onset of sound, as a pre-treatment control. I compared fish behaviours in the first minute of each trial to the minute of no sound just before noise was played, and quantified foraging behaviours, when fishes physically touched the bait bag with their mouths, and startle behaviours, when fishes performed a C-start escape response, and tracked the amount of time each fish spent within the BRUV while also documenting the species of fish that performed each behaviour. Fish assemblages were also documented throughout the 10-minute exposure to detect any signs of habituation to the noise; however, no indication of such short-term desensitization was found. While there were significantly more fish dwelling at Shark Lab, they were significantly less affected by boat noise than those at Beach Club, with no overall changes uncovered in any of the observed behaviours. At Beach Club however, there were significantly less fish present during boat noise exposure, with minimal fish that were present, spending significantly less time within the BRUV. Fishes within the grunt family were more affected by boat noise at both sites then all other families with similar numbers of grunts detected between the two sites as well. The results suggest that while some fish responded negatively to boat noise, fish may habituate to long-term noise exposure and the responses of wild fish to noise are thus highly dependant on where the fish is located. Harding et al. (2018) found similar results where fish from high boat traffic areas were less sensitive to the noise experimentally presented compared to fish from quieter habitats.

Conclusions and Future Directions

Therefore, I found that laboratory experiments may be able to help anticipate the initial reaction of fish to noise for my freshwater results were consistent between the lab and field with both captive black bullhead and wild cyprinids decreasing the number of times they foraged during boat noise treatments compared to the controls. While the black bullhead were not able to escape and disperse like fish in the wild, they still displayed escape-like behaviours through the increase of erratic swimming during boat noise. Additionally, freshwater and marine fishes showed no desensitization throughout the duration of the trials suggesting the fish did not experience any short-term habituation. Similar short-term responses were observed in marine environments with fewer fish seen during boat noise treatments and the remaining fish spending less time within the camera’s view. However, marine fish that resided in high boat traffic areas were not as responsive to boat noise playbacks as the fishes living in relatively low boat traffic areas, which suggests that the fishes may be able to habituate to boat noise after extended exposure.

Based on my work, there are a number of suggestions that I would make and questions that should be asked to further our understanding of the impacts of noise on fishes. Future studies should continue to shift experiments into the field for the natural responses of fish will be valuable when developing mitigation plans. However, the previous wealth of knowledge from laboratory studies is not to be disregarded as they provide some reference to how wild fish may respond and can cautiously be used to make real-world predictions. In developing practical guidelines though, there needs to a clear understanding of the thresholds in which fish are negatively impacted by noise, so studies such as Hawkins et al. (2014) and Mickle et al. (2020), where they systematically recorded the responses of fish to varying levels and frequencies of sound, with the addition of testing the distances from the sound sources, need to be more commonplace within the field to establish thresholds in which fishes are affected.

Conducting more multi-species analyses would maximize the amount of information researchers can gain from natural habitats for the responses of fish with diverse hearing abilities can be observed and community effects will already be accounted for, negating the need to guess how they might affect fish behaviours. Increasing the diversity of fish species used, with a special emphasis on using fish of varying hearing abilities allows for better evaluations of the range of response thresholds within a community and avoids misleading results that could under- or overestimate the affects of noise. Future studies would also benefit from considering the habitat and prior noise exposure the fishes may have endured before making conclusions on the effects of noise as responses to noise appear to be highly dependent on many factors including species, sex, location, noise tolerance, hearing ability, and behaviour (Fewtrell & McCauley, 2012; Mensinger et al., 2018; Davidson et al., 2019; Mickle et al., 2020). Such variability also brings into question the importance of noise as a stressor in general, compared to other anthropogenic impacts. For example, Mensinger et al. (2018) found that fish within a protected area were less impacted by boat noise than fish in fished waters. The question here is, why? It may be that fish have higher baseline cortisol levels with more fishing pressures imposed, thus making them scare easier, or potentially the increased fishing pressures naturally selected for more shy fish while protected areas selected for bold personalities (Mensinger et al., 2018). While it is hard to make any conclusions about why such things occurred, studies like Mensinger et al. (2018), could provide insight of the significance of anthropogenic noise compared to other stressors. Moreover, while climate change is usually the biggest concern in most environments, studying and minimizing other forms in their environment is also necessary to ensure the welfare of wildlife.

While some studies have investigated the effects of noise on freshwater fish in the laboratory they tend to focus on a select few species (Mickle & Higgs, 2018), so the minimal data we have on freshwater species is unlikely to represent the extent to which noise can impact these fish and thus multi-species field investigations could greatly improve our understanding of such ecosystems. Freshwater environments are dynamically complex, from waterfalls to quiet lakes, and so understanding anthropogenic noise effects in all such habitats is vital to developing recommendations on sound exposure guidelines. Therefore, the increasing emergence of field studies is encouraging for this field of bioacoustics, but there are still so many unknowns. How does anthropogenic noise affect the fitness of fishes? Studies have shown decreased parental care and larval survival (Nedelec et al., 2017; Maxwell et al., 2018), but does noise select for and thus alter certain characteristics within exposed communities? In order to regulate aquatic noise pollution and propose mitigation strategies there needs to be a clear understanding of how it impacts fish in the wild and such data is simply not yet available.

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