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Behavioural, Morphological and Physiological Impacts of Anthropogenic Noise on Freshwater Fish

by

Megan Mickle

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Behavioural, Morphological and Physiological Impacts of Anthropogenic Noise on Freshwater Fish

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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:

Chapter 1 of the thesis was co-authored with Dennis Higgs. The key ideas, primary contributions, interpretation, and writing were performed by the author, and the co-author included contribution of scientific thought, suggestion of papers to read/include and overall editing of the manuscript. Chapter 2 of this thesis was a result of joint collaboration between Oliver Love, Dennis Higgs and Christopher Harris, collaborators provided feedback on experimental design and editing experimental protocol (ELISA protocol viewed in the appendix).

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Thesis Chapter	Publication title/full citation	Publication status*
Chapter [1]	*Integrating Techniques: A Review of the Affects of Anthropogenic Noise on Freshwater Fish*	*Submitted: Canadian Journal of Fisheries and Aquatic Sciences*

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ABSTRACT

There is increasing concern about the effect of underwater noise on fish behavior due to the increasing elevations of anthropogenic noise levels — primarily caused by the expansion of urban developments, transportation networks and freshwater resource extraction methods. Despite this increasing study, there is conflicting evidence on the impact of underwater noise to fish behaviour, morphology and physiology and its possible role as an environmental stressor. I performed experiments on two local Great Lakes fish, the black bullhead *(Ameiurus melas)*; a species with known hearing specializations, and the yellow perch (*Perca flavescens*); a species with basic sound detection. Choosing these species allowed us to test and compare the morphological, physiological and behavioural response of these fish to local noise sources. Even at relatively low sound levels we saw clear behavioural effects of enhanced noise levels despite no obvious morphological or physiological stress. At higher noise levels we observed apparent indications of both behavioural and morphological impacts of noise on bullhead. Here we also determined that after both acute and chronic exposure of noise, bullhead were less active and sheltered more, and also exhibited hair cell damage in the saccule region of the auditory organ. These results suggest that there are sublethal effects of anthropogenic noise on fish behaviour and physiology which may have direct implications on species fitness. Our current understanding of noise effects on freshwater fish is lacking and it is imperative to study a diversity of species to fully understand the impacts of noise on fish both for environmental and aquaculture concerns.

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INTEGRATING TECHNIQUES: A REVIEW OF THE AFFECTS OF ANTHROPOGENIC NOISE ON FRESHWATER FISH

Introduction:

Sound is an important sensory modality for fish as it can be used actively for reproduction, prey/predator detection and territory defense as well as for identification of important habitat parameters (Fay & Popper, 2000; Slabbekoorn & Bouton, 2008; Sluijs et al., 2010). Sound propagates very efficiently in deep water but is difficult to model in shallow environments due to interaction with surfaces and sediments (Kuperman & Ingenito, 1998; Akyildiz et al., 2005) but it is a critical sensory modality in most environments (Popper & Fay, 1973). Many fish species are particularly reliant on sound as a form of communication (Sluijs et al., 2010), especially as visual cues can be obstructed in dark or turbid environments (Heuschele et al., 2012; Fisher & Frommen, 2013). Some sounds in underwater environments are more harmful than they are helpful, particularly anthropogenic noise, which is a common underwater abiotic disturbance for aquatic species (Popper & Hastings, 2009; Radford et al., 2014; Solan et al., 2016) primarily caused by urban developments, the expansion of shipping transportation networks, underwater resource extraction and seismic exploration devices (Hildebrand, 2009; Frisk, 2012). There has been a notable increase in anthropogenic noise, specifically ambient sound budgets – patterns of biological activity, shipping and recreational boating - which have doubled every decade for the last six decades (Frisk, 2012; Solan et al., 2016). Most aquatic studies have focused on high-power, acute noise sources such as sonar, airguns and pile driving due to the direct damage they can cause on animals (Popper & Hastings, 2009); however, shipping is the most dominant source of anthropogenic noise which propagates at low underwater frequencies and overlaps with the hearing range/vocal outputs of many aquatic species (Ross, 1976; Dyndo et al., 2015; Solan et al., 2016). The majority of documented impacts of such noise pollution on aquatic species have focused on detecting perceptible behavioural changes in an animal, including changes to their foraging efficiency (Purser & Radford, 2011; Sabet et al., 2015; McLaughlin & Kunc, 2015) or resulting in physiological changes, such as increasing stress levels or causing a hearing impairment (Smith et al., 2004; Wysocki et al., 2006; Nichols et al., 2015). While individual effects can be important, most aquatic noise research lacks integration of multiple techniques within each study when determining the impacts of anthropogenic noise on animals.

While effects of anthropogenic noise are well studied in marine species, particularly focusing on marine mammals (Weilgart, 2007; Heide et al., 2013; Dyndo et al., 2015); there are far fewer studies that examine the effects of noise pollution on freshwater species (Popper, 2003; Slabbekoorn et al., 2010). The acoustic landscape of marine vs. freshwater environments differs quite markedly; oceanic environments are in general much deeper, which allows sound to travel further than it can in freshwater environments, where sound scatters more readily due to shallow depths (Kupperman, 1977; Rogers & Cox, 1988). Freshwater systems may be less efficient at sound transmission than marine environments and only comprise 1% of the water on the globe, however they harbour a disproportionately high proportion of earth’s biodiversity (Combes, 2003). Biodiversity in freshwater habitats is especially vulnerable to human-induced environmental change due to the high human populations around freshwater ecosystems along with their high species richness (Abell, 2002; Dudgeon et al., 2005).

Freshwater ecosystems are experiencing a decline in biodiversity greater than those in terrestrial environments and with a global demand for freshwater; this is arguably one of the most important ecosystems to study (Dudgeon et al., 2005). In particular, fish are an important occupant of freshwater ecosystems and represent over half of all of the vertebrate species on the planet (Thomson & Shaffer, 2010) and dominate global aquaculture production (Radford et al., 2014), highlighting their importance to humans and the need for further research. Noise pollution research in marine ecosystems is studied quite extensively, generally indicating that the impacts of noise can range from a behavioural change in an animal to death (Weilgart, 2007). We can use these studies as a marker and guideline for future freshwater noise pollution research. Due to the outsize importance of freshwater habitats for fish diversity and the dearth of studies on noise effects in these habitats this review will focus on what is known about anthropogenic noise and freshwater fish and suggest ways forward on these sets of research questions. The observed impacts of noise levels on freshwater fish can be broadly categorized into behavioural changes and physiological changes, and listed below are common techniques used to determine the impacts noise has on aquatic animals and a summary of overall findings and results. This is not intended to be an exhausted review as they can be found elsewhere (Popper & Hastings, 2009; Kight & Swaddle, 2011) but instead to be used as a resource when determining which scientific technique best fits a given study species or research question.

Physiological studies:

Glucocorticoids

Glucocorticoids (GC) are used as an indicator of stress in a wide array of animals and chronic increases in GC levels can have detrimental effects on survival and reproduction (Sheriff et al., 2011; Narayan, 2016; Dantzer et al., 2014). The mechanisms behind GC response include dysregulation of the hypothalamic pituitary axis or the hypothalamic pituitary interrenal axis in the brain brought on by environmental challenges (Bronson, 2008; Dantzer et al., 2014). Often chronically-stressed individuals exhibit higher baseline plasma GC levels and an increased amount of time taken to return back to baseline levels (Sapolsky et al., 2000; Dantzer et al., 2014). Anthropogenic disturbances, such as noise, are consistently associated with increased GC regardless of the type of human disturbance, ranging from habitat fragmentation to climate change (Dantzer et al., 2014). Glucocorticoid measurements can be collected from blood, saliva, faeces/urine, hair, feathers (for birds) and water (fish) (Sheriff et al., 2011; Dantzer et al., 2014). Cortisol, a glucocorticoid that is indicative of a stress response, has been shown to increase in three European freshwater fishes when exposed to noise (Wysocki et al., 2006). Two fish species capable of hearing a wide range of frequencies — the common carp (*Cyprinus carpio)* and the gudgeon (*Gobio gobio*) — and one species that hears primarily lower frequencies of sound — the European perch (*Perca fluviatilis*) — exhibit an increase in cortisol when exposed to ship noise but no increase in cortisol when exposed to Gaussian noise, indicating all three species are stressed when exposed to anthropogenic noise (Wysocki et al., 2006). Blacktail shiner (*Cyprinella venusta*) exhibit both an increase in cortisol and a shift in hearing threshold when exposed to acute levels of road traffic noise which can ultimately have negative consequences on the fishes' fitness (Crovo et al., 2015). Research should include both acute and chronic measures when studying physiological stressors to determine if habituation comes into play, as this could be important when determining if fitness will be impacted or if animals can habituate to the stressor. Johansson et al. (2016), exposed motorboat noise to Eurasian perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) in their natural environment and determined after short-term noise exposure both species exhibited an increase in cortisol, whereas during the long-term exposure (11 days) fish no longer had elevated cortisol levels, suggesting the role of habituation. As outlined in a review by Madliger and Love (2014) there are two main advantages to GC measurements; first, baseline levels can be obtained in one sample, therefore it is not always essential for the animals to be sacrificed. Secondly, GC exhibit an essential role in energy regulation, as anthropogenic disturbances may influence general energy expenditures they can provide a good insight on the organisms overall state (Madliger & Love, 2014). However, there are considerations associated with this method, notably, individual differences in physiological stress responses, seasonal and diurnal variations in GC production and the time sensitivity related to collection of GC (Madliger & Love, 2014). However, under natural circumstances animals may modify their lifestyle characteristics without an alteration in GC levels, for example, while nesting during Antarctic winter, king penguins (*Aptenodytes patagonicus*) fast for weeks without experiencing a rise in their GC levels (Sapolsky et al., 2000). This may be considered a stressful situation for humans, however it is perfectly natural for these animals. Glucocorticoid measurements are a common technique used to detect a physiological stress response in fish but it is important to take careful baseline measurements and show clear links to other integrative measures before assuming that elevations of GC in response to noise demonstrates an actual stressor. These considerations have not always been taken into account in previous research on noise as a physiological stressors but are critical to truly understand chronic and acute responses to noise in fish.

Body and Tissue Samples

A second physiological measure to indicate impacts of noise on freshwater fish involves examination of body and tissue samples. Loud intensities of noise can significantly alter the auditory system or physiology of animals (Welch & Welch, 1970; Smith et al., 2004*b*; Popper et al., 2014). Noise exposure can result in a temporary hearing loss, termed “temporary threshold shift”, which affects the audibility of signals and can prevent normal behavioural responses to signals, or permanent threshold shift which can lead to injury (Popper & Hawkins, 2012). Previous work has determined that intense sounds can cause temporary changes to the hearing thresholds of fish, or cause damage to sensory hair cells in the ear (Smith et al., 2003; Smith et al., 2004*a*). Goldfish (*Carassius auratus*) exposed to white noise (160-170 dB re 1 µPa) for a long period of time exhibit a decrease in hearing threshold and an increase in cortisol and glucose levels compared to control fish (Smith et al., 2003). When exposed to three increments of decibel levels (115, 130 and 150 dB re 1 µPa) cultured juvenile rainbow trout (*Oncorhynchus mykiss*) exhibit a significant difference in hearing threshold when compared to fish exposed to ambient noise (Wysocki et al., 2007). Rainbow trout are a member of the salmonid family and have no known hearing specializations, unlike goldfish, so it was somewhat surprising that even trout can exhibit a shift in hearing threshold when exposed to noise (Wysocki et al., 2007). Oscars (*Astronotus ocellatus*) exposed to differing frequencies and intensities of sound show clear evidence of auditory hair cell damage when exposed to sound at 400 Hz and 180 dB re 1 µPa and allowed to survive for four days after treatment (Hastings et al., 1996). Hybrid striped bass (Cross between *Morone chrysops* and *Morone saxatilis*) and Mozambique tilapia (*Oreochromis mossambicus*) exhibit swim bladder ruptures, herniations and some instances of hair cell damage when exposed to loud playbacks (210-216 dB re 1 µPa) of pile driving noise (Casper et al., 2013). Hair cell density following loud noise exposure has been shown to have regenerative characteristics in some regions of the auditory system but not others (Smith et al., 2006). When goldfish were exposed to 170 dB re 1 µPa for two days, hair cells regenerated in the central saccule region after 8 days, however hair cells in the caudal saccule did not return to pre-exposure hair cell counts in this time frame, suggesting evidence for tonotopic organization (Smith et al., 2006). Following noise exposure, goldfish exhibit a significant shift in hearing threshold, however, 7 days post-exposure, their hearing recovered significantly, indicating that only a subset of hair cells are required for auditory response (Smith et al., 2006).

With relatively few studies examining anthropogenic influences on auditory damage in fish (but see Casper et al., 2013), more research is needed to determine the extent of hair cell damage when fish are exposed to differing levels of noise frequency and intensity found in their natural environment. Measuring physiological damage or a shift in hearing threshold is a powerful method when determining the extent to which noise impacts animals. For example, if a researcher uncovers that a fish species has damage or a threshold shift after exposure to 180 dB re 1 μPa, this could provide pertinent information for conservation methods to protect the species by limiting human activities in at-risk areas. The limited data on actual damage in freshwater fish with anthropogenic noise makes regulatory and mitigation techniques limited in their effectiveness; therefore in order to properly set up for conservation methods the first step is to collect evidence regarding noise impacts on freshwater species (Popper et al., 2014).

Metabolic/Ventilation Rate

The final physiological measure that is studied in aquatic ecosystems, although not as commonly, is the impact of noise on metabolic rate. An example of increased metabolic rate was observed when European eels (*Anguilla anguilla*) were exposed to motorboat noise as they displayed a significant increase in oxygen usage compared to those in the control experiment, leading to a physiological impairment of the eels in the treatment group (Simpson et al., 2014). This method is non-invasive, as determining oxygen content in water can be done through a dissolved oxygen (DO) reader. Measuring ventilation rate of fish species is another method used to indicate stress levels, usually measured by counting opercular beat rate (OBR). Nedelec et al. (2016) discovered that short-term boat noise exposure resulted in an increase in OBR in the coral reef fish (*Dascyllus trimuculatus*), however the effect decreased over long-term exposure, indicating possible habituation to the noise. While measuring ventilation rate is a robust and easy method to carry out, it can also be subjective based on the audience analyzing the response and has some logistical issues. Ventilation frequency (VF) was used as an indicator of stress in Nile tilapia, *Oreochromis niloticus* and based on inconsistency of results researchers concluded that VF is not a good indicator of stress and caution should be used when using this measure alone (Barreto & Valpato 2004). Using metabolic rate and ventilation frequency to determine a stress response in fish can be considered powerful as it is non-invasive and relatively easy to carry out, however, few studies use these methods as indicators of stress in freshwater fish, therefore more research is needed to determine the validity of his method.

Noise as a Physiological Stressor

Stress data collected from aquatic species can have a direct relation with conservation efforts and determining the appropriate habitat for aquaculture production (Pickering 1992; Smith et al., 2003). Research regarding suitable acoustic environments needed for a fish's optimal growth or survivorship in an aquaculture setting may also have direct implications on human demand for fish (Smith et al., 2004). For example, goldfish (*Carassius auratus*) exhibit a shift in hearing threshold and masking of sounds when exposed to four different types of filters in aquaria, however, there was no shift in threshold when goldfish were housed in ponds (Gutscher et al., 2011). Detection of stress response is not always cut and dry as it is important to determine the "context, severity and duration" of the challenge presented (in this case noise), when indicating if the animal is indeed impacted (Bronson, 2008). For example, if the stress response of the animal lasts for only one hour, is growth rate or fitness actually impacted? Future research should include the collection of glucocorticoid levels at different time intervals to determine a stress vs. time gradient which would also indicate if habituation has occurred. Future research may also benefit from integrating physiological techniques to determine if the animal is indeed stressed and if so, to what extent. For example, Flodmark et al. (2002) collected cortisol and glucose levels of brown trout (*Salmo trutta*) exposed to fluctuating water levels and flow to indicate a stress response. Furthermore, it is important to determine if the stress response is a result of natural diurnal or seasonal changes in gluccocorticoid levels, as opposed to the stressor. To determine noise impacts on fish, it is also possible to measure cardiac output as a measure of stress, as it has similar mechanisms to humans (Graham & Cooke, 2008). Graham and Cooke (2008) subjected Largemouth bass (*Micropterus salmoides*) to three different boat noise disturbances and discovered that fish exposed to canoe paddle noise increased their heart rate 29%, 44% when exposed to trolling motor and 67% when exposed to a combustion motor. The increase in cardiac output bass experience is consistent with an increasing magnitude of noise (combustion engine being the loudest). Measuring cardiac output is seldom performed to determine stress response of fish to noise, therefore more research should be done on this topic to increase validity. However, when determining if anthropogenic stressors cause damage to an animal it is often invasive, so it is also advantageous to develop less invasive physiological measures or to use behavioural mechanisms first.

Behavioural studies:

Examining a change in behaviour to indicate the state of an animal's well-being is readily accessible, but can be easily misinterpreted without special knowledge of the species of interests' "normal" behaviour. Behavioural responses to sound are influenced by cognitive processes such as detecting, classifying and decision making; therefore any form of disturbance in the environment can compromise this process and cause a decrease in fitness of the animal (Slabbekoorn et al., 2010). For example, if acoustic information is masked by noise pollution, important communication methods can be negatively impacted (Amoser et al., 2004; Slabbekoorn et al., 2010). To fully comprehend the extent of noise influence on behavioural characteristics of an animal, consideration of the species' full behavioural repertoire is needed as the response of the animal is dependent on the current state they are in (Bruintjes & Radford 2013). To determine boat noise impacts on cichlids (*Neolamprologus pulcher*), Bruintjes and Radford (2013) studied nest-digging behaviours, anti-predator defense, and social interactions in cichlids by taking into account breeding context, sex and dominance hierarchy. The results of this study indicate that the behavioural repertoire of the animal did impact their reaction to noise. The following are different behavioural changes observed in freshwater fish species when exposed to noise.

Foraging Efficiency

Fish can be impacted by noise through masking important acoustic signals (Codarin et al., 2009; Slabbekoorn et al., 2010), causing a change in normal movement or activity which can ultimately decrease the time spent foraging. Noise may also impact foraging efficiency as it is a stressor which can alter behaviour of animals and cause a narrowing in attention (where animals focus on a smaller area) or focusing their attention on the noise itself (Slabbekoorn et al., 2010; Purser & Radford, 2011). Currently there is a poor understanding of how noise pollution affects wild populations of fish as it is easier to track and quantify their behaviour in a manipulated experimental setting. However, Payne et al. (2015) examined the impact of anthropogenic noise on wild mulloway (*Argyrosomus japonicus)* populations using two experimental factors. In the first experiment researchers captured and tagged 10 mulloway and placed noise receivers at multiple positions along their aquatic habitat. The researchers also caught and dissected 278 mulloway on weekdays and 83 on the weekends over a three year period to compare gut content. Mulloway were less active and inhabited greater depths on the weekend compared to the week which is consistent with boat activity records. Stomach fullness was also significantly lower on weekends compared to weekdays, displaying an impact of boat noise on foraging efficiency. Studying animals in their natural environment is beneficial as it decreases the need to control for multiple variables that experimental manipulations can include, however finding and tracking the animals can be difficult and quite expensive.

The addition of brief white noise (10sec) to an acoustic habitat has been shown to increase performance errors and ultimately decrease foraging efficiency in three-spined sticklebacks (*Gasterosteus aculeatus*) (Purser & Radford, 2011), demonstrating the large range of detriments noise can have on aquatic species. Predator-prey interactions in zebrafish (*Danio rerio*) are impacted when exposed to differing levels of noise; zebrafish display an increase in handling error and a delayed response to food as noise increases (Sabet et al., 2015). Besides the obvious consequences exhibited by a decrease in foraging efficiency, if animals were to consistently increase effort needed to forage, their “net energetic gains” may decrease, impacting reproductive success or survival (Purser & Radford, 2011). Determining a change in foraging status or efficiency is a good indicator of health status for an animal as it is an essential component of survival for all animal species. However, often during experimental manipulations other confounding factors can cause stress for the animal and affect their foraging abilities; it is therefore essential to form a strong control.

Startle and Sheltering Response

An increase in startle response when anthropogenic noise is present has been shown to negatively impact the escape response of some marine organisms (McLaughlin & Kunc, 2015; Nedelec et al., 2016; Sabet et al., 2016) and the same effects would be expected for freshwater fish. Increases in noise caused a reduced startle response in juvenile eels, resulting in an increased predator vulnerability (Simpson et al., 2014). As previously mentioned in this review, eels also displayed a significant increase in oxygen usage compared to fish in the control experiment. Coral reef fish (*Dascyllus trimaculatus)* exhibited an increase in sheltering when exposed to two days of motorboat noise, but stopped responding after one week, uncovering evidence for behavioural and physiological attenuation (Nedelec et al., 2016). Sheltering behaviour and a significant increase in OBR were no longer observed in the fish after chronic exposure (1 week), indicating animals that continually respond to anthropogenic stressors may be negatively impacted in terms of growth, reproduction and survival, whereas those that habituate may have a decreased impact of noise and a better chance of survival (Nedelec et al., 2016). Zebrafish exhibit a startle response and a brief increase in swimming speed when exposed to anthropogenic noise (Sabet et al., 2016). Behavioural responses, such as an increase in startle events, sheltering and a change in swim speed can impact predation risks (Sabet et al., 2016). Measuring sheltering and startle response as an indicator of stress is easy to recognize, non-invasive (particularly of benefit to endangered or at risk species) and can be necessary when physiological measures are not always feasible.

Change in Activity Levels/Avoidance Behaviour

A change in activity level in response to noise may have repercussions on lifestyle characteristic in animals, such as increasing predation levels (Simpson et al 2016). Using activity levels as an indicator of stress or impact created by anthropogenic disturbances can be useful as it is easy to record/and interpret. However, it is necessary to have a strong background knowledge on the normal behaviour exhibited by an animal, which requires observation and analyses of multiple controls to ensure a change in behaviour is present due to the stressor and not the experimental set up or design. When presented with noise, fish may simply respond through evasion techniques. Cod (*Gadus marhua*) hear low-frequency sounds and can discriminate engine/propeller noise at distances up to 2.0km away (Ona & Godø, 1990). Cod exhibit avoidance behaviours (vertical or horizontal movements away from noise source) during trawling events and even demonstrate pre-vessel avoidance at depths less than 200m (Ona & Godø, 1990). A review by De Robertis and Handegrad (2012) shows fish often avoid approaching boats/vessels which can lead to a potential bias in fishery surveys. To contest the issue of boat noise impacting fishery surveys, noise-reduced research vessels have been constructed and implemented in some areas (DeRobertis & Handegrad 2012). Noise-reduced vessels have been shown to represent a more accurate measure of walleye pollock (*Gadus chalcogrammus*) detection (DeRobertis & Wilson 2011); however more research is needed to determine the impact on other fish species, especially freshwater species. Using activity levels as an indicator of stress in freshwater fish is not commonly performed but it is a powerful method to ascertain natural responses of fish and will allow a better understanding of true anthropogenic impacts.

Behavioural techniques provide a good measure of anthropogenic influences on animals, however, as with all methods, there are caveats with using this technique. For example, when using fish as a model species it is common to perform these studies in an artificial setting. The housing condition itself may be stressful to the animal and can potentially confound the results of physiological or behavioural measures of stress. Therefore, variables that may impact the results, such as pH levels, background noise, and lighting conditions, must all be accounted for. The acoustics of experimental tanks can also be problematic (e.g. Akamatsu et al., 2002; Rogers et al., 2016). Having said that, experimental manipulation is important as it is a powerful tool to pinpoint the exact cause of stress, where some of the field studies, such as those performed by Heide and colleagues (2013), can be considered correlation as some confounding variables cannot be controlled for. As long as important caveats are kept in mind, both laboratory and field experiments can provide useful insight into noise as a possible behavioural stressor in freshwater fish.

What can we gain from integrating?:

While using individual behavioural or physiological techniques as a measure of stress is often used as a proxy for impacts on growth and survival (Pickering, 1992; Ellis et al., 2004; Huntingford et al., 2006), a more integrative approach would better assess the true impacts of noise as a potential stressor. Most documented impacts of noise pollution exhibited in studies look at specific behavioural or physiological characteristics of a species, for example determining the impacts of noise on hair cell damage in goldfish. This is important as it increases our knowledge base on the topic of noise pollution; however the majority of studies lack integration within their design. Future studies should incorporate integrative examinations of noise on freshwater fish species, for example, using behavioural characteristics such as foraging efficiency and physiological measures such as glucocorticoid levels. Data collected from integrative studies can provide critical information on the extent of noise impacts, if cortisol data was collected and no significant differences were found, this can be attributed to a lack of sensory hair cells due to noise exposure. However, this finding would not occur without the presence of an integrative study. Understandably, such integrative studies require more work and knowledge on the topic, however the results attained will be stronger and more comprehensive.

More research is also needed to determine the hearing threshold of freshwater fish species, and background noise levels in the freshwater environments in which they reside to better understand possible anthropogenic influences. Amoser et al. (2004) were one of the first researchers to estimate hearing thresholds of species both with and without known hearing specializations in a freshwater lake (Lake Traunsee) and determine noise levels during boating activities to predict impacts this noise may have on these species. Boat noise overlaps within the most sensitive hearing range of cyprinids in Lake Traunsee, thus possibly masking sounds present in their natural habitat and impairing signal detection (Amoser et al., 2004). Braun (2005) argues that although there is increasing concern and documentation of noise pollution on fish, research should include data on how measures of stress affects the sensory system function, again furthering the need for integration. When determining the impacts of anthropogenic influences, it is important to describe the background noise level first (Codarin et al., 2009). As described by Mann and colleagues (2009), to create regulations of anthropogenic noise the following information is needed: the amount of noise created, the audiograms of fish in the surrounding area, data on sound propagation of particular source and finally an assessment of the impact noise may have on surrounding species. Before regulations are implemented, further research needs to be conducted to determine the hearing range/vocal output of a number of fish species and finally, what sort of impact does noise have on their lifestyle characteristics. Improvements to the field should also include: a deeper focus into low frequency chronic stressors commonly found underwater, more research on freshwater ecosystems, further research examining habituation (as exhibited by Nedelec & Radford 2016) and to conduct studies based in the field rather than exclusively in a lab setting.

To summarize the results from this perspective, a stress response can be visualized through behavioural characteristics such as a change in: foraging efficiency, avoidance response, startle/shelter response or activity levels and physiological changes in: glucocorticoid levels, body/tissue samples and metabolic rate. Some techniques contain more drawbacks than others and have not been researched as extensively, however, the type of technique used is ultimately dependent on the study species, resources available and experimental setup. Here we suggest using at least one behavioural and physiological measure when studying noise impacts on freshwater fish to determine the full extent of the impact, which can further lead to predictions on animal welfare. As mentioned in this review all of the techniques used to determine anthropogenic influences on aquatic species include strengths and weaknesses, therefore to create a more powerful study and avoid confounding variables, it should be common protocol to include integration of multiple techniques within each study.

Table 1: A comparison of the effects and techniques used in noise pollution research in freshwater and marine ecosystems.

	Species	**Techniques Used**	**Integration within study**	**References**	**Title**
Marine Environments	Coral reef fish (*Dascyllus trimaculatus*)	Physiological: ventilation rate Behavioural: Sheltering behaviour	**Yes:** incorporating both behavioural and physiological measures	Nedelec et al., 2016	Repeated exposure to noise increases tolerance in a coral reef fish
	Mulloway (*Argyrosomus japonicus)*	Behavioural: decrease in activity, inhabited greater depths (avoid sound) Physiological: decrease in stomach content	**Yes:** looking at two behavioural response and one physiological to noise	Payne et al., 2015	Foraging intensity of wild mulloway *Agryrsomus Japonicus* decreases with increasing anthropogenic disturbance
Freshwater Environments	Rainbow trout (*Oncorhynchus mykiss*)	Physiological: hearing threshold shift	**Partial:** using three physiological markers to determine noise impact	Wysocki et al., 2007	Effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout *Oncorhynchus mykiss*
	Common carp (*Cyprinus carpio*), gudgeon (*Gobio gobio*), European perch (*Perca fluviatilis*)	Physiological: increase in cortisol	**No:** using one physiological marker	Wysocki et al., 2006	Ship Noise and Cortisol Secretion in European Freshwater Fishes

	Blacktail shiner (*Cyprinella venusta*)	Physiological: increase in cortisol, shift in hearing threshold	**Partial:** Using two physiological measures	Crovo et al., 2015	Stress and Auditory Responses of the Otophysan Fish, *Cyprinella venusta*, to Road Traffic Noise
	Eurasian perch (*Perca fluviatilis*), Roach (*Rutilus rutilus*)	Physiological: increase in cortisol	**No:** only using one physiological measure to indicate stress	Johansson et al., 2016	Stress Response and Habituation to Motorboat Noise in Two Coastal Fish Species in the Bothnian Sea
	Goldfish (*Carassius auratus*)	Physiological: increase cortisol/glucose levels, shift in hearing threshold	**Partial:** Using two physiological measures when determining impact of noise	Smith et al., 2003	Noise induced stress response and hearing loss in goldfish (*Carassius auratus*)
	Hybrid striped bass, Mozambique tilapia (*Oreochromis mossambicus*)	Physiological: damage to hair cells, swim bladder ruptures, herniations	**Partial:** Looking at multiple tissues to determine damage from noise	Casper et al., 2013	Effects of exposure to pile driving sounds on fish inner ear tissues
	Zebrafish (*Danio rerio*), Lake Victoria cichlids (*Haplochromis piceatus*)	Behavioural: startle response, increase in swimming speed	**Partial**: using two behavioural responses	Sabet et al., 2016	Behavioural responses to sound exposure in captivity by two fish species with different hearing ability
	Three-Spined Stickelback (*Gasterosteus aculeatus*)	Behavioural: attention shift, decreasing foraging efficiency	**Partial**: using two behavioural responses	Purser & Radford 2011	Acoustic noise induces attention shifts and reduces foraging performance in three-spines sticklebacks (*Gasterosteus aculeatus*)
	Oscars (*Astronotus ocellatus*)	Physiological: hair cell damage.	**No:** using one physiological measure	Hastings et al., 1996	Effects of low-frequency underwater sound on hair cells of the inner ear and lateral line of the teleost fish *Astronotus ocellatus*
	Goldfish (*Carassius auratus*)	Physiological: damage to hair cells	**No:** using one physiological measure	Smith et al., 2006	Anatomical and functional recovery of the goldfish (*Carassius auratus*) ear following noise exposure

	Daffodil Cichlids (*Nedamprologus pulcher*)	Behavioural: anti-predator, social interactions	**Partial:** using two behavioural markers	Bruintjes & Radford 2013	Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish
	Largemouth bass (Micropterus salmoides)	Physiological: cardiac output	**No:** one physiological marker	Graham & Cooke 2008	The effects of noise disturbance from various recreational boating activities common to inland waters on the cardiac physiology of a freshwater fish, the largemouth bass (*Micropterus salmoides*)
	Zebrafish (*Danio rerio*)	Behavioural: predator prey interaction, foraging efficiency	**Partial:** using two behavioural markers	Sabet et al., 2015	The Effect of Temporal Variation in Sound Exposure on Swimming and Foraging Behaviour of Captive Zebrafish
	Cod (*Gadus marhua*)	Behavioural: avoidance behaviour	**No:** one physiological measure	Ona & Godø 1990	Fish reaction to trawling noise: the significance for trawl sampling

References:

Abell, R. (2002). “ Conservation Biology for the Biodiversity Crisis: A Freshwater Follow-up.” *Society for Conservation Biology*, **16**: 1435-1437.

Akamatsu, T., Okumura, T., Novarini, N., and Yan H.Y.(2002). “Empirical refinements applicable to the recording of fish sounds in small tanks.” *J Acoust Soc Am*. **112**(6): 3073-3082.

Akyildiz, I., Pompili, D., Melodia, T. (2005). “Underwater acoustic sensor networks: research challenges.” *Elsevier*, **3**(3): 257-279.

Amoser, S., Wysocki, L., and Ladich, F. (2004). "Noise emission during the first powerboat race in an Alpine lake and potential impact on fish communities." *The Journal of the Acoustical Society of America*, **116**(6): 3789-3797.

Barreto, R & Valpato, G. (2004). “Caution for using ventilatory frequency as an indicator of stress in fish.” *Behavioural Processes*, **66**: 43-51.

Braun, C. (2015). "Signals and noise in the octavolateralis systems: What is the impact of human activities on fish sensory function?" *Integrative zoology*, **10**(1): 4-14.

Bruintjes, R., & Radford, A.N. (2013). "Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish." *Animal Behaviour*, **85**(6): 1343-1349.

Casper , B., Smith, M., Halvorsen, M., Sun, H., Carlson, T., & Popper, A. (2013). “Effects of exposure to pile driving sounds on fish inner ear tissues.” *Comparative Biochemistry and Physiology*, **166**: 352-360.

Codarin, A., Wysocki, L., Ladich, F., Picciulin, M. (2009). "Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy)." *Marine pollution bulletin*, **58**(12): 1880-1887.

Combes, S. (2003). “Buying Time: A Users Manual.” *Protecting Freshwater Ecosystems in the Face of Global Climate Change*. 203-242.

Crovo, J., Mendonça, M., Holt, D., Johnston, C. (2015). "Stress and Auditory Responses of the Otophysan Fish, *Cyprinella venusta*, to Road Traffic Noise." *PloS one*, **10**(9): 1-9.

Dantzer, B., Fletcher, R & Sheriff, M. (2014). “Measures of Physiological Stress: A Transparent or Opaque Window into the Status, Management and Conservation of Species.” *Conservation Physiology*, **2**(1): 1-18.

De Robertis, A., & Handegard, N. (2013). “Fish avoidance of research vessels and the efficacy of noise-reduced vessels: a review.” *ICES Journal of Marine Science*, **70**: 34-45.

De Robertis, A., & Wilson C. (2011). “Silent ships do not always encounter more fish (revisited): comparison of acoustic backscatter from walleye pollock recorded by a noise-reduced and a conventional research vessel in the eastern Bering Sea.” *ICES Journal of Marine Science*, **68**: 2229-2239.

Dudgeon, D., Arthington, A., Gessner, M., Kawabata, Z., Knowler, D., Leveque, C., Naiman, R., Prieur-Richard, A., Soto, D., Stiassny, M., & Sullivan C. (2005). “Freshwater Biodiversity: Importance, Threats, Status and Conservation Challenges.” *Biological Reviews*, **81**(2): 163-182.

Dyndo, M., Danuta, M., Rojano-Donate, L. & Madsen, P. (2015). “Harbour Porpoises React to Low Levels of High Frequency Vessel Noise.” *Scientific Reports*, **5:** 1-9.

Ellis, T., James, D., Stewart, C & Scott, P. (2004). “A Non-invasive Stress Assay Based Upon Measurement of Free Cortisol Released into the Water by Rainbow Trout.” *Journal of Fish Biology*, **65**: 1233-1252.

Erulkar, S.D. (1972). “Comparative Aspects of Spatial Localization of Sound.” *Physiological Reviews,* **52**: 237-254.

Fay, R.R.,& Popper, A.N. (2000). “Evolution and hearing in vertebrates: The inner ears and processing.” *Hearing Research*, **149**: 1-10.

Fischer, S., & Frommen, F.J. (2013). "Eutrophication alters social preferences in three-spined sticklebacks (*Gasterosteus aculeatus*)." *Behavioral ecology and sociobiology,* **67**(2): 293-299.

Flodmark, L., Urke, H., Halleraker, J., Arnekleiv, J., Vøllestad, L., & Poléo, A. (2002). “Cortisol and glucose responses in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream.” *Journal of fish biology*, **60**: 238-248.

Frisk, G.V. (2012). “Noiseonomics: The relationship between ambient noise levels in the sea and global economic trends.” *Scientific Reports*, **2**: 1-14

Graham, A.L., & Cooke, S.J. (2008). "The effects of noise disturbance from various recreational boating activities common to inland waters on the cardiac physiology of a freshwater fish, the largemouth bass (*Micropterus salmoides*)." *Aquatic Conservation: Marine and Freshwater Ecosystems,* **18**(7): 1315-1324.

Gutscher, M., Wysocki, L.E., & Ladich, F.(2011). "Effects of aquarium and pond noise on hearing sensitivity in an otophysine fish." *Bioacoustics,* **20**(2): 117-136.

Hastings, M.C., Popper, A.N, Finneran, J.J., & Lanford, P.J. (1996). “Effects of low-frequency underwater sound on hair cells of the inner ear and lateral line of the teleost fish *Astronotus ocellatus.*” *Journal of the Acoustical Society of America,* **99**: 1759-1766.

Heide, M.P., Hansen, R.G., Westdal, K., Reeves, R.R., & Mosbech, A. (2013). "Narwhals Seismic Exploration: Is Seismic Noise Increasing the Risk of Ice Entrapments?" *Biological Conservation*, **158**: 50-54.

Hildebrand, J.A (2009). "Anthropogenic and natural sources of ambient noise in the ocean." *Marine Ecology*, **395**: 5-20.

Heuschele, J., Salminen, T., & Candolin, U. (2012). "Habitat change influences mate search behaviour in three-spined sticklebacks." *Animal Behaviour,* **83**(6): 1505-1510.

Huntingford, F.A., Adams, C., Braithwaite, V., Kadri, S., Pottinger, G., Sandøe., Turnbull, J. (2006). "Current Issues in Fish Welfare." *Journal of Fish Biology*, **68**(2): 332-372.

Johansson, K., Sigray, P., Backström, T., Magnhagen, C. (2016). "Stress Response and Habituation to Motorboat Noise in Two Coastal Fish Species in the Bothnian Sea." *The Effects of Noise on Aquatic Life II*. Springer New York: 513-521.

Kight, C. R., & Swaddle, J. P. (2011). "How and why environmental noise impacts animals: an integrative, mechanistic review." *Ecology letters*, **14**(10), 1052-1061.

Kiley-Worthington, M. (1989). "Ecological, ethological, and ethically sound environments for animals: Toward symbiosis." *Journal of Agricultural Ethics,* **2**(4):323-347

Kuperman, W.A. & Ingenito .(1998). "Attenuation of the Coherent Component of Sound Propagating in Shallow Water with Rough Boundaries." *The Journal of the Acoustical Society of America*, **61**(5): 177.

Madliger, C.L & Love, O.P. (2014). "The Need for a Predictive, Context-Dependent Approach to the Application of Stress Hormones in Conservation." *Conservation Biology*, **28**: 283-287.

Mann, D., Cott, P., & Horne, B. (2009). "Under-ice noise generated from diamond exploration in a Canadian sub-arctic lake and potential impacts on fishes." *The Journal of the Acoustical Society of America*, **126**(5): 2215-2222.

Mclaughlin, K.E, & Kunc, H.P. (2015). "Changes in the Acoustic Environment Alter the Foraging and Sheltering Behaviour of the Cichlid *Amititlania nigrofasciata.*" *Behavioural Processes*, **116**: 75-79.

Narayan, E.J. (2013). "Non-invasive reproductive and stress endocrinology in amphibian conservation physiology." *Conservation Physiology,* **1**: 1-16.

Nedelec, S., Mills, S., Lecchini, D., Nedelec, B., Simpson, S & Radford, A. (2016). "Repeated Exposure to noise increases tolerance in a coral reef fish." *Environmental Pollution*, **216**: 428-436.

Nichols, T.A., Anderson, T.W. & Sirovic, A. (2015). "Intermittent Noise Induces Physiological Stress in a Coastal Marine Fish." *Plos One*, **10**(9): 1-10.

Ona, E., & Godø, O. (1990). "Fish reaction to trawling noise: the significance for trawl sampling." *Rapports et Procès-Verbaux des Réunions*, **189**: 159-166.

Payne, N., Van DerMeulen, D., Suthers, I., Gray., C., & Taylor, M. (2015). "Foraging Intensity of Wild Mulloway *Argyrsomus Japonicus* Decreases with Increasing Anthropogenic Disturbance." *Marine Biology*, **162**(3): 539-546.

Pickering, A.D. (1992). "Rainbow trout husbandry – management of the stress response." *Aquaculture*, **100**: 125-139.

Popper, A.N. (2003). "Effects of Anthropogenic Sounds on Fish." *Fisheries*, **28**(10): 24-31.

Popper, A.N., & Fay, R.R. (1973). "Sound Detection and Processing by Teleost Fish: A Critical Review." *The Journal of Acoutsical Society of America*, **53**:1515-1529.

Popper, A.N., Hawkins, A., Fay, R., Mann, D., Bartol, S., Carlson, T., Coombs, S., Ellison, W.T., Gentry, R., Halvorsen, M., & Lokkeborg, S. (2014). "ASA S3/SC1. 4 TR-2014 Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report Prepared by ANSI-Accredited Standards Committee S3/SC1 and Registered with ANSI." Springer

Popper, A.N., & Hastings, M.C. (2009). "The Effects of Anthropogenic Sources of Sound on Fishes." *Journal of Fish Biology*, **75**(3): 455-489.

Popper, A.N., & Hawkins, A. (2012). *The Effects of Noise on Aquatic Life*. New York: Springer.

Popper, A.N. (2011). "Effects of Anthropogenic Sounds on Fish." *Fisheries*, **28**: 24-31.

Purser, J., & Radford, A. (2011). "Acoustic Noise Induces Attention Shifts and Reduces Foraging Performance in Three-Spined Sticklebacks (*Gasterosteus aculeatus*)." *PLoS ONE*, **6**(2): 1-8.

Radford, A., Kerridge, E., & Simpson, S. (2014). "Acoustic Communication in a Noisy World: Can Fish Compete with Anthropogenic Noise?" *Behavioural Ecology*, **25**(5): 1022-1030.

Rogers, P., Cox, M. (1988). Underwater Sound as a Biological Stimulus. In: Atema J., Fay R.R., Popper A.N., Tavolga W.N. (eds) *Sensory Biology of Aquatic Animals*. New York: Springer.

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

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

Sabet, S.S., Wesdorp, K., Campbell, J., Snelderwaard, P., Slabbekoorn, H. (2016). "Behavioural responses to sound exposure in captivity by two fish species with different hearing ability." *Animal Behaviour*, **116**: 1-11

Sabet, S.S., Neo Y.Y., Slabbekoorn, H. (2015). "The Effect of Temporal Variation in Sound Exposure on Swimming and Foraging Behaviour of Captive Zebrafish." *Animal Behaviour*,**107**:49-60.

Sapolsky, R.M., Romero, L.M., & Munck, A.U. (2000). "How do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions." *Endocrine Reviews*, **21**(1): 55-89

Simpson, S.D., Purser, J., & Radford, A.N. (2014). "Anthropogenic noise compromises antipredator behaviour in European eels." *Global Change Biology*, **21**(2): 586-593.

Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, MCO., Chivers, D.P., McCormick, M., & Meekan, M.G. (2016). " Anthropogenic noise increases fish mortality by predation." *Nature Communications,* **7**: 1-6.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). "Measuring stress in wildlife: techniques for quantifying glucocorticoids." *Physiological Ecology,* **166**: 869-887.

Slabbekoorn, H., & Bouton, N. (2008). Soundscape orientation: a new field in need of sound investigation."*Animal Behaviour,* **76**:5-8.

Slabbekoorn, H., Bouton, N., Opzeeland, I., Coers, A., Cate, C., & Popper, A. (2010). "A Noisy Spring: The Impact of Globally Rising Underwater Sound Levels on Fish." *Trends in Ecology & Evolution*, **25**(7): 419-427.

Smith, M.E, Kane, A.S., & Popper, A.N. (2004). "Noise-induced Stress Response and Hearing Loss in Goldfish (*Carassius auratus*)." *Journal of Experimental Biology*, **207**(3): 427-435.

Smith, M.E., Kane, A.S., and Popper, A.N. (2004). "Acoustical stress and hearing sensitivity in fishes: does the linear threshold shift hypothesis hold water?" *Journal of Experimental Biology,* **207**: 3591-3602.

Smith, M.E., Coffin, A.B., Miller, D.L., & Popper, A.N. (2006). “Anatomical and functional recovery of the goldfish (*Carrasius auratus*) ear following noise exposure.” *Journal of Experimental Biology,* **209**: 4193-4202.

Solan, M., Hauton, C., Godbold, J., Wood, C., Leighton, T., & White, P. (2016). “Anthropogenic Sources of Underwater Sound Can Modify How Sediment-dwelling Invertebrates Mediate Ecosystem Properties.” *Scientific Reports*, **6:** 20540

Thomson, R.C., & Shaffer, H.B. (2010). “Rapid progress on the vertebrate tree of life.” *BMC Biology,* **8**:1-9.

van der Sluijs, I., Gray, S.M., Amorim, MCP., Barber, I., Candolin, U., Hendry, A.P., Krahe, R., Maan, M.E., Utne-Palm, A.C., Wagner, H.J., and Wong, BBM. 2010. Communication in troubled waters: responses of fish communication systems to changing environments. Evol. Ecol. **25**(3): 623-640.

Weilgart, L. (2007). “A Brief Review of Known Effects of Noise on Marine Mammals.” *International Journal of Comparative Physiology,* **20**: 159-168.

Welch, B. L. and Welch, A. S. (ed.) (1970). Physiological Effects of Noise. New York: Plenum Press

Wysocki, L.E., Dittami, J.P., and Ladich, F. (2006). "Ship Noise and Cortisol Secretion in European Freshwater Fishes." *Biological Conservation,* **128**(4): 501-508.

Wysocki, L.E., Davidon, J.W., Smith, M.E., Frankel, A.S., Ellison, W.T., Mazik, P., Popper, A.N., Bebak, J. (2007). “Effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout *Oncorhynchus mykiss.” Aquaculture,* **272**: 687-697.

CHAPTER II.

AN INTEGRATIVE STUDY: THE EFFECTS OF DIFFERING NOISE LEVELS ON FRESHWATER FISH

Introduction:

Anthropogenic noise is a common occurrence in aquatic ecosystems but the effects this has on aquatic animals, particularly freshwater fish, remains unclear (Slabbekoorn *et al.,* 2010; Popper & Hawkins, 2012). Sound travels very efficiently underwater (Rogers & Cox, 1988) and common sources of natural background noise are weather, wave action, and animal communication (Popper & Hastings, 2009; Radford et al., 2014; Solan et al., 2016). There has been a notable increase in anthropogenic noise due to industrialization primarily caused by the expansion of transport networks, various resource extraction methods and seismic exploration devices (Wale et al, 2013; Solan et al., 2016); all of which have been hypothesized to disrupt acoustic communications and have far-reaching effects on aquatic species (Wysocki et al., 2006; Wale et al, 2013; Popper & Hastings, 2009). There is a greater focus on high-power noise sources such as sonar, airguns and pile driving; however, shipping is the predominant source of anthropogenic noise at low frequencies underwater (20-1,500 Hz) (Ross, 1976; Dyndo et al., 2015; Nichols & Širović, 2015; Solan et al., 2016) and considerably overlaps with the output and hearing range of most tested fish species (20-1000 Hz) (Kasumyan, 2005; Ladich & Fay, 2013; Nichols & Širović, 2015). Aquatic animals produce and hear different frequencies of sound (Atema, 1988); therefore the effects of noise pollution will depend on the noise source as well as species-specific communication abilities (Peng et al., 2015). For example, an aquatic animal that hears lower frequencies of sound may be impacted by ship noise, while an animal that hears higher frequencies of sound may be more likely impacted by military SONAR.

Hearing is an important adaptation in fish for purposes of communication and orientation (Aalbers & Drawbridge, 2008; Popper & Hawkins, 2012; Nichols & Širović, 2015). Fish are impacted by noise either behaviourally (e.g. impacting foraging, prey detection) (Popper & Hawkins, 2012) physiologically (e.g. increase in stress levels) (Barcellos et al., 2007) and through physical damage (e.g. hair cells, swim bladder and other tissues) (Popper & Hastings, 2009). The majority of research regarding noise pollution incorporates either behavioural, morphological or physiological measures, and seldom integrate techniques. Here, I argue that to fully understand the extent that fish are influenced by noise, research should focus on more integrative methods. One widespread and commonly used method to determine a physiological stress response in fish in response to anthropogenic stressors is cortisol detection (Mommsen et al., 1999; Barton, 2002) as it is an important corticosteroid produced in animals and it is released in response to stress (Mommsen et al., 1999). High cortisol levels can cause a number of physiological detriments to an animal such as: an increased chance of mortality, suppressed immune function, increased susceptibility to disease/infection, and decrease in growth rates, reproduction and sexual maturation (Pickering & Pattinger, 1989; Pickering, 1992; Ellis et al., 2002; Anderson et al., 2010). Fish exposed to loud noise may suffer from morphological impairments such as hair cell damage or a shift in their hearing threshold (Enger et al.,1981; Smith et al., 2006). Hair cells are a critical component of fish hearing as they bend in response to sound and send signals to the brain, alerting the fish of a change in their environment (Lu & Popper, 2001), therefore damage to these hair cells will negatively impact the fish's abilitiy to detect sound. A temporary threshold shift generally affects the audibility of signals by causing a decrease in the hearing range of the fish, which can have negative implications on predator detection or audibility of important communication or soundscape signals (Enger et al., 1981; Smith et al., 2006). A permanent threshold shift can cause injury in the fish and may lead to permanent deafness (Popper & Hawkins 2012). The second method commonly used to determine the impacts of noise on fish is behavioural analysis. As behavioural responses to sound are influenced by cognitive practices, a disturbance in the environment may potentially compromise this process and lead to a decrease in fitness of the animal (Slabbekoorn et al., 2010). Behavioural analyses of fishes are often an effective measurement of welfare (Martins et al., 2012), however a careful examination of a fish's routine behaviour is necessary to determine the extent of the stressor's impact. Common behavioural practices quantified to determine a stress response include: foraging efficiency, activity levels (time spent moving), avoidance behaviours, sheltering and startle response (Popper & Hawkins, 2012; Payne et al., 2014; McLaughlin & Kunc, 2015).

A noise response is also influenced by the duration of exposure, commonly characterized as an acute or chronic stressor. Acute stressors such as noise can negatively impact a fish's ability to react to such stressors (Wingfield et al., 1998), while chronic increases in glucocorticoid levels are thought to inhibit reproduction and may also reduce survival (Sapolsky et al., 2000; Dantzer et al., 2014). To determine the length and full impact of the stressor it is important to consider both acute and chronic exposure. For example, when brown trout (*Salmo trutta)* and rainbow trout (*Oncorhynchus mykiss*) are exposed to one hour of handling and confinement (acute stressor) cortisol levels rise but return to basal levels within 48 hours while chronic stressors can cause cortisol elevations for 4 weeks in these species (Pickering & Pattinger, 1989). Cortisol responses have been measured for a number of fish species after exposure to handling stress, pollutants, temperature changes and predator simulations (Barton & Iwama, 1991; Brown, 1993; Donaldson, 1981; Bonga, 1997) but little is known about the long term stress effects of noise on fish species (Popper & Hastings, 2009).

In general, research regarding noise impacts on freshwater fish species is lacking as compared to marine species and the majority of documented effects in freshwater fish focus on either a behavioural change, such as detecting changes in foraging efficiency (Voellmy et al., 2014; McLaughlin & Kunc, 2015; Sabet et al., 2015) or a physiological change such as increasing stress levels (Smith et al., 2003; Wysocki et al., 2006; Nichols & Širović, 2015). However, few studies integrate both behavioural and physiological mechanisms within their design. Here, we conducted an integrative study to determine the behavioural, morphological and physiological impacts of noise on two fish species, the black bullhead (*Ameiurus melas*) and yellow perch (*Perca flavescens*). We chose bullhead as a study species as they are a common Laurentian Great Lakes fish with specialized hearing capabilities, and we used perch as a comparison species as they have no known hearing specializations (Amoser & Ladich, 2005; Ladich & Fay, 2013). There are two goals in the current study; the first is to determine behavioural and physiological impacts of low noise levels (140 dB re 1µPa) on two Laurentian Great Lakes species with different hearing capabilities. The second is to determine behavioural and physiological impacts of noise on black bullhead when exposed to noise at differing intensities (160 and 170 dB re 1µPa) for both acute (1 hour) and chronic (24 hour) exposure. We hypothesize that bullhead exposed to low levels of noise (140 dB re 1µPa) will exhibit behavioural changes, while bullhead exposed to high levels of noise (160 and 170 dB re 1µPa) will exhibit behavioural, physiological and morphological changes in response to noise. More specifically, we hypothesize that bullhead exposed to acute noise stressors will exhibit behavioural and physiological responses to noise, while those exposed to chronic exposure will exhibit morphological changes (physical tissue damage). Finally, we hypothesize that yellow perch will not be impacted by low levels of noise. Using an integrative approach we determine that freshwater fish are impacted by anthropogenic noise.

Methods:

Experiment 1: Low Intensity Noise Exposure

Black bullhead and yellow perch were used to evaluate behavioural and physiological responses to anthropogenic noise. These two species were chosen as they exhibit different hearing capabilities: black bullhead are a member of the Ostariophysi, all of whom have specialized abilities to detect sound (Hoar & Randall, 1971) whereas yellow perch lack specialized hearing structures and exhibit basic sound detection (Amoser & Ladich, 2005; Ladich & Fay, 2013). Black bullhead (n= 100), ranging from 22.02-117.60g were obtained from a fish farm in Harrow, Essex County, Ontario (42°01'14.5"N 83°00'04.1"W) and yellow perch (n = 60), ranging from 14.68-132.0g were caught locally at Chewitt Bay, Windsor ON (42°18'23.7"N 83°04'30.2"W). These fish were housed at a temperature of 22.2°C and a pH of 6.5-7 in animal quarters at the

University of Windsor. Due to the constraints of catching wild/farmed fish these species exhibited a range in size. The fish were fed daily and kept at a 12:12 light-dark cycle to mimic natural conditions, however as both species prefer dark environments all housing tanks were covered with black garbage bags and opaque tank covers. Background noise levels were collected from housing tanks and consistently measured from 110-122 dB re 1 µPa. To start a trial, six individual bullhead or perch, similar in size within a species, were randomly collected from housing tanks and added into separate plastic experimental tanks (55 liters) in a dark room equipped with red light, an underwater speaker and single air stone set up in each tank. The test tanks were fitted into a Styrofoam holding structure and placed on top of two acetal plastic sheets, 2 cm thick, to minimize acoustic disturbance and vibrations from the floor. The fish were given an acclimation time of one hour after which a control trial took place for four hours and sheltering and general swimming behaviours were quantified during a one hour video during this time. To quantify a change in behaviour, trials were recorded and analyzed using a GoPro Hero3+ (Go Pro). We quantified a sheltering response when the fish were residing in Polyvinyl chloride (PVC) tubing (1 tube in each tank) and activity levels were quantified as a measure of time spent moving throughout the videos. After the control trial, boat noises recorded from the perch's native habitat (Chewitt bay, ON) were played through the underwater speaker at approximately 140 dB re 1µ Pa; a common noise level recorded for local waters (unpub data). The experimental setup included an underwater speaker (Electro-Voice UW-30), connected to an amplifier (Scosche SA300), a 12 Volt PBS car battery for power and an mp3 player to play the noise (Sony Walkman NWZ-E464). The speaker was placed in the middle of the tank and background noise was measured at 3 different locations along the tank using a hydrophone (Inter Ocean system inc. – Acoustic Calibration and System Model 902) and averaged around 120 dB re 1µPa. The control video trial started approximately 1.5 h after fish entered the tank, and the second behavioural video occurred approximately 5 h into the experiment. At the end of each trial, bullhead and perch were anaesthetized using 2-phenoxy ethanol (SIGMA-ALDRICH: 1 mL of 2-phenoxy ethanol per 2 L of water) and tails were removed so that blood from the caudal artery could be collected using a heparanized capillary vial. Once blood was collected, the fishes' heads were decapitated and preserved in paraformaldehyde (4%) for further dissection of ears. Plasma was isolated via centrifugation and cortisol was subsequently extracted from the plasma using a standard ELISA protocol (see appendix for specific protocol). Cortisol levels in both black bullhead and yellow perch were determined using a commercial cortisol EIA kit (Cayman Chemical #500360, Ann Arbor, MI, USA). Assay plate wells have been previously coated with goat polyclonal anti-mouse IgG. The cortisol in the sample and a kit-provided cortisol tracer, itself composed of cortisol linked to acetylcholinesterase, compete to be bound by an antibody to the IgG on the wells. After binding, plates are washed to remove unbound agents and Ellman's reagent is added. The Ellman's reagent enzymatically reacts with the acetylcholinesterase of the cortisol tracer to produce a yellow colour. The intensity of this yellow colour is proportional to the amount of tracer bound to the well and is therefore inversely proportional to the concentration of cortisol in the sample.

Saccules were further dissected (using a Leica L2 10445930 dissecting scope) from fish ears from 1 of every 6 bullhead or perch randomly selected from each trial (giving us a total of 10 saccules for each species). After saccules were collected, they were preserved in paraformaldehyde (4%) until stained with 12.5 µl of fluorescent green phalloidin in 200 µl of phosphate buffer (Higgs et al., 2001). Once saccules were properly stained, hair cells were visualized through images collected from a Leica microscope, (Leica DM IRB inverted fluorescence microscope, Las A.F. 4.5). Images were then imported into Adobe Photoshop (V3.0; Adobe Systems) to create 3 identical boxes of 15 μm^2 in size, which were placed along the top, middle and bottom section of the saccule image. Hair cells within each box were then counted using Image J software (NIH).

Experiment 2:High Intensity Noise Exposure

In the second experiment we further analyzed behavioural and physiological responses of black bullhead when exposed to 160 dB re 1µ Pa and 170 dB re 1µ Pa of boat noise. Three bullhead were added to a 55 litre plastic tank at a time (to avoid sound interference) equipped with a bubbler and an underwater speaker. Fish were allowed to acclimate for one hour before sound trials (at either 160 or 170 dB re 1µ Pa) began, fish were either exposed an acute (1 hour) or chronic (24 hour) stressor. Two control trials took place for both the acute and chronic experiments where fish were exposed to the same experimental conditions, however no sound was played from the speakers. There were two experimental replicates (n=6) for both the short and long sound exposure experiments, at 160 and 170 dB re 1µPa totalling 35 fish (including the controls). Control and sound experiments started at approximately the same time each day to avoid diurnal differences in behaviour. To keep consistency in behavioural videos, we recorded the fishes' behaviour at the 23rd hour for the long duration trial and after the one hour acclimation period for the short duration trial. We quantified sheltering response and activity levels using the same methods presented in experiment 1, however there were 3 PVC tubes in each tank, one for each fish. Cortisol collection and analysis protocol was also consistent with experiment 1. Saccules were collected and analyzed from each fish during this experiment.

Statistical Analyses:

Experiment 1: Low Intensity Noise Exposure

To allow each fish to act as its own behavioural control, comparisons were made before and after the sound was played in each experimental trial. Comparisons were also made between the pure control trials and experimental noise trials. Both comparisons yielded the same results, therefore for the purposes of this study, only statistics are reported for the fish acting as their own control. Once data was collected, a one-way analysis of variance (ANOVA), designating fish ID as a random factor, was performed using SPSS (IBM, 2014) to analyze behavioural differences of black bullhead and yellow perch when exposed to noise.

Hair cell data for both the yellow perch and bullhead were compared using an independent sample t-test, setting hair cell position as a random factor. To determine differences in cortisol levels, data was log-transformed (as cortisol data was not normally distributed) and compared using a one-way ANOVA test through SPSS. The dependent variables in this experiment were: behavioural markers (activity/sheltering response), cortisol levels and hair cell count. The fixed factor in experiment 1 consisted of sound exposure (control or 140 dB re 1µPa),

Experiment 2: High Intensity Noise Exposure

Multivariate tests were used to examine behavioural differences of black bullhead in the control and noise trials for activity and sheltering behaviours. We used a Tukey-post hoc test to further investigate where the differences were present. Again, to determine differences in cortisol levels, data was log-transformed and compared using a one-way ANOVA through SPSS. Hair cell data was analyzed using a one-way ANOVA for both acute and chronic stressors, again designating hair cell position and fish ID as a random factor. The dependent variables in experiment 2 were consistent with those described in experiment 1. The fixed factor in experiment 2 consisted of sound exposure (control or 160 or 170 dB re 1µPa) and time of exposure (acute or chronic).

Results:

Experiment 1: Low Intensity Noise Exposure

When exposed to boat noise black bullhead exhibited changes in behavioural characteristics. Foraging efficiency was initially expected to be quantified but no foraging attempts were made by either fish species throughout the entire experiment. Activity levels decreased from approximately 2.5 to 1 minute per hour when bullhead were exposed to boat noise played at 140 dB re 1µPa noise $F_{(1,5)=}8.401$, p=0.034 (Fig 1). Sheltering behaviour increased from approximately 21 to 25 minutes per hour when fish were exposed to noise $F_{(1,5)}$=8.588, p=0.033 (Fig 2). Yellow perch did not exhibit a change in activity levels when exposed to noise $F_{(1,5.020)}$ =2.830, p=0.153 (Fig 1), however, they did demonstrate a significant difference in sheltering behaviour $F_{(1,5.048)}$ =

15.961, $p=0.010$ (Fig 2) demonstrating higher levels of sheltering in the control than the noise trial.

There was no significant difference in cortisol levels for either the black bullhead $F_{(8,50)}=4.192$, $p=0.184$ or yellow perch $F_{(8,49)=}0.422$, $p=0.902$ (Fig 3) exposed to 140 dB re 1μPa for four hours, indicating these fish are not physiologically stressed by this noise level. Cortisol levels collected from yellow perch were much higher on average than bullhead, 162.27 ng/ml in perch and 7.30 ng/ml in bullhead.

When comparing hair cell data in the control and experimental trial there was no significant difference in hair cell counts for either bullhead t(6)= 0.784, $p=0.902$ or perch t(4)= -1.380, $p=0.182$ (Fig 4). While hair cells in bullhead appeared lower after noise exposure, ranging from about 700 hair cell per 200μm box to 500 hair cells per 200 μm box, the high variability in these counts resulted in no significant differences.

Experiment 2: High Intensity Noise Exposure

Multivariate tests indicated significant differences within the acute trial (1 hour) for both activity and sheltering (Wilks lambda $\Lambda = 11.427$, $p = 0.001$). Activity levels significantly decreased from approximately 22 to 2 minutes per hour during 160 dB re 1μ Pa exposure and from 22 to 8 minutes at 170 dB re 1μPa, with an overall significance of $F_{(2,12)} = 32.987$, $p<0.001$ (Fig 5). Sheltering behaviour increased from approximately 28 to 58 minutes during 160 dB re 1μ Pa and from 28 to 50 minutes per hour during 170 dB re 1μ Pa when bullhead were exposed to noise, $F_{(2,12)} = 11.236$, $p<0.001$ (Fig 6). Post-hoc tests indicated that the control trial was significantly different from both 160 and 170 dB re 1µPa (all $p<0.005$) and 160 was significant different than 170 dB re 1µPa (all $p<0.007$).

During chronic (24 hour) noise exposure multivariate tests also indicated some significant differences of activity and sheltering behaviours (Wilks lambda $\Lambda = 0.338$, $p = 0.003$). Overall, activity levels significantly decreased from approximately 2 to 0 minutes per hour at 160 dB re 1µ Pa trial and increased from 2 minutes to 8 minutes at 170 dB re 1µ Pa, $F_{(2,12)} = 9.989$, $p=0.002$ (Fig 5). Post-hoc tests demonstrated significant differences in activity levels between the control trial and 170 dB re 1µPa of noise ($p=0.020$) and between 160 and 170 dB re 1µPa ($p=0.002$), indicating that fish were more active during chronic exposure of 170 dB re 1µPa compared to the control trial. However, there was no significant difference in activity levels when comparing the control trial and 160 dB re 1µPa trial ($p=0.442$). Sheltering behaviour differed overall when bullhead were exposed to noise, $F_{(2,12)} = 10.799$, $p= 0.001$ (Fig 6). Post-hoc tests indicated a non-significant difference in sheltering between the control trial and 170 dB re 1µPa ($p=0.072$) of noise and also between control trial and 160 dB re 1µPa ($p=0.095$). However, the bullhead sheltered more at 160 dB re 1µPa (60 minutes per hour) compared to 170 dB re 1µPa (50 minutes per hour) ($p=0.001$).

During acute exposure of both noise levels of 160 and 170 dB re 1µPa bullhead did not exhibit a change in cortisol levels compared to the control, $F_{(2,14)} = 1.305$, $p=0.302$ (Fig 7). Cortisol data collected during the chronic noise trial also uncovered marginal significant results $F_{(2,15)} = 3.268$, $p=0.066$ (Fig 7). Post-hoc analyses revealed no significant difference between cortisol levels in the control trial compared to 160 dB re

1µPa (*p*=0.147) and 170 dB re1µPa (*p*=0.992) and between the control and 170 dB re 1µPa (*p*=0.075), however cortisol results seemed to be lower at 160 dB re 1µPa.

There was a significant effect of noise exposure on hair cell number $F_{(2,30)}$ =18.458, *p* <0.001, resulting in fewer hair cells present in noise treatments than controls (Fig 8). Post-hoc tests further uncovered an insignificant difference between the control trial and 160 dB re 1µPa (*p*=0.062), however there was a significant difference between the control and 170 dB re 1µPa trials (*p*<0.001) during acute exposure. During chronic exposure of noise post-hoc analyses determine a significant difference between the control and 170 dB re1µPa (*p*<0.001),160 vs 170 dB re 1µPa (*p*=0.001), however there was not a significant difference between the control and 160 dB re 1µPa *(p*=0.062) (Fig 8). Noise had no significant effect on ear region (*p*=0.0727).

Discussion:

Anthropogenic noise negatively impacted both behavioural and morphological characteristics in black bullhead. Bullhead exhibit changes in behavioural characteristics such as an increase in sheltering and a decrease in activity levels even when exposed to low noise levels. Bullhead exposed to high noise levels at both acute and chronic time points showed evidence of hair cell damage. Using an integrative approach we demonstrate the wide array of impacts noise pollution has on bullhead, and we can use this study as a model for other Great Lakes fish with similar hearing capabilities. Noise pollution research is not commonly studied in freshwater environments, even though these environments are species rich and important for human survival as we rely on fish as a major source protein for the world’s population (16%) (Tidwell & Allan, 2001).

More resources should be attributed to trying to preserve these species, which starts by determining the impact anthropogenic stressors have on their fitness.

The decreased activity patterns demonstrated by bullhead in the first experiment when exposed to 140 dB re 1µPa suggest even this lower level of anthropogenic noise drove behavioural responses often associated with stressors (Eriksson, 1979; Valdimarrson & Metcalfe,1998). Contrary to some literature (Smyly 1957; Lelek 1987), bullhead are not normally sedentary in nature but instead mobilize (usually under dark conditions) to detect prey species and find suitable spawning habitats (Eriksson, 1979; Knaepkens, 2004). Research has indicated that fish can often exhibit avoidance behaviours (Ona & Godø, 1990; Fewtrell & McCauley, 2012) in response to noise, however, due to constraints of tank size, we used activity levels to indicate a change in behavioural characteristics. McLaughlin and Kunc (2015) examined the behavioural impacts of boat noise on the convict cichlid (*Amatitlania nigrofsciata*) and found the presence of a boat noise did indeed increase the fish's time spent sheltering, and decreased their time foraging, but did not alter their activity level. Activity effects in bullhead at even low noise levels may be due to the enhanced hearing capability of bullhead and suggests caution in extrapolating effects between species with different hearing abilities.

Cortisol levels were used as a measure of physiological stress as it is a widely accepted method within the field of biology (Mommsen et al., 1999; Barton, 2002) and has been used to detect a stress response when fish are exposed to noise (Wysocki et al., 2006; Smith et al., Barcellos et al., 2007). While our behavioural results suggested that fish were showing a stress response, this was not indicated by the physiological marker of cortisol level. This apparent discrepancy can be explained by the principle that animals first respond to stress through a behavioural mechanism (Dawkins, 2003; Moberg, 2005). For example, if an animal is exposed to a stressor in nature, it can simply evade the area in attempts to avoid further physiological impacts (Moberg, 2005). It is also possible that there was no clear pattern associated with cortisol data (and why cortisol was lower at 160 dB re 1µPa) due to sampling cortisol at the end of the trial. If bullhead exhibited a spike in cortisol at the beginning of the trial when fish were first introduced to the noise, it is possible cortisol levels returned back to baseline levels after a certain amount of time had passed. Baseline cortisol levels in siluriformes have be consistently reported under 10 ng/mL (Davis & Small, 2006; Manuel et al., 2013), which is comparable to our cortisol data. However, baseline cortisol levels in yellow perch generally range between 3.4-8.3 ng/mL (Barton, 2002; Haukenes & Barton, 2004) which is higher than cortisol levels detected in our experiment (average of 167.27). Here we show that bullhead exhibit behavioural signs of stress indicating boat noise is indeed a stressor, however, noise at this level does not act impact cortisol levels indicative of a physiological stressor. Yellow perch did not show the same responses as bullhead during the first experiment and this may have been due to their reduced hearing ability relative to bullhead (Amoser & Ladich, 2005; Ladich & Fay, 2013) or due to difficulties adjusting to life in captivity. As perch exhibit basic hearing capabilities we did not have strong predictions for behavioural response to noise in this species but it was key to test them as they are an important species in our freshwater ecosystem. European perch *(Perca fluviatilis)* are also not sensitive to noise and researchers suggest that they are only able to detect the first harmonic of boat noise (Amoser et al., 2004). Alternatively our lack of response from yellow perch may have been due to their difficulty to adapt to captivity, which is also indicated by their high baseline levels of cortisol (Brown et al., 1996). When exposed to 140 dB re 1µPa yellow perch did not exhibit a change in activity levels, but did exhibit higher sheltering behaviours in the control compared to the experimental trial. Higher sheltering levels in the control trial could be explained by timing of the experiment, the control took place an hour after acclimation which may not be a sufficient amount of acclimation time for this species. Cortisol levels were extremely high in both the control and manipulation experiments making it difficult to detect a physiological stress response. While it is possible the lack of response was simply due to the inability of perch to detect our boat noise 'stressor' it also seems likely that handling stress outweighed these considerations.

During the second experiment bullhead were exposed to acute and chronic noise at levels of 160 and 170 dB re 1µPa to determine if these fish were impacted behaviourally and physiologically by boat noise. The boat noise was played to the bullhead at 160 and170 dB re 1µPa as this noise level is ecologically relevant, small ships generally produce noise ranging from 140-167 dB re 1µPa and merchant ships produce noise, ranging from 178-192 dB re 1µPa up to an approximate distance of 2 m away (Amoser et al., 2004). The behavioural effects may be attributed to the hair cell data; if hair cell damage occurs after both acute and chronic stressors of noise played at 170 dB re 1µPa, bullhead hearing sensitivity will likely be affected (Smith et al., 2003). If fish are no longer sensitive to the noise it is likely they will no longer exhibit signs of stress, explaining why bullhead exposed to 170 dB re 1µPa for 24 hours did not exhibit a change in cortisol levels and were more active during this trial. As 160 dB re 1µPa is still an intense noise level, fish showed signs of behavioural stress, but this intensity did not cause hair cell damage. A change in 10 dB re 1µPa (from 160 -170 dB re 1µPa) caused morphological tissue damage in bullhead, this can be explained as an increase in signal amplitude by 6 dB re 1µPa which doubles the distance of sound propagation (Forrest, 1994). The behavioural data for chronic noise exposure at 160 dB re 1µPa showed a pattern of difference, however this data was not statistically significant, suggesting the future need for a larger sample size.

There are a few considerations to take into account when analyzing these data. First, some fish have higher baseline cortisol levels than others. Second, cortisol levels fluctuate seasonally and diurnally (Laidley & Leatherland, 1988); to avoid this confounding variable, all experiments were started at approximately the same time each day, over the period of three months. Bullhead were collected from a pond in Harrow, ON, using seining techniques, whereas perch were collected at Chewitt bay in Windsor, ON, through both seining and angling techniques. These capture techniques can influence short-term fluctuation in cortisol levels, however fish were allowed to acclimate to their new environment for approximately three weeks before experimentation commenced. Due to the capture of live fish, we had a large size range in both species, however the fish were non reproductive (fish were dissected to visualize presence of gonads) to avoid the impacts reproduction could have on behaviour. As we did not test other sources of noise, we cannot definitively say fish are responding to the boat noise specifically, however, we can conclude that bullhead display behavioural and morphological changes when exposed to noise.

As our research includes loud intensities of noise (at 160 and 170 dB re 1µPa), we need to determine the relevance of these noise levels by determining if these data are ecologically relevant. Are their local aquatic environments this loud and, if so, do they habituate or does this ultimately impact their fitness and life expectancy? Most research involving soundscaping data is carried out in marine environments (McWilliam & Hawkins, 2013; Staaterman et al., 2014; Erbe et al., 2015). Martin and Cott (2016) performed one of the first long-term soundscaping freshwater studies by measuring acoustic data under ice in the Northwest territories, located close to a road and an airport. Researchers determined a mean noise level ranging from 88-96 dB re 1µPa (dependent on time of day) with the loudest noise level recorded at 161 dB re 1µPa (Martin & Cott, 2016). As 160 dB re 1µPa of noise was detected in freshwater habitats, we can hypothesize that this noise level may not be as uncommon as we think and further use the data collected in our study to predict behavioural and physiological impacts of this noise level on fish. Amoser and Ladich (2010) collected year round data on ambient noise levels in 7 European freshwater habitats including lakes, rivers and streams and discovered noise levels were lower on average in lakes (91.6-111.7 dB re 1µPa) compared to rivers/streams (111.2–133.4 dB re 1µPa), and significantly varied throughout the year (Amoser & Ladich, 2010). Lakes and streams had higher variation in noise throughout different time periods (mean: 9.9-14.9 dB re 1µPa) compared to rivers (4.2-4.4 dB re 1µPa). As sound levels significantly vary throughout the year, some periods of time being noisier than others, we can use this data to make predictions on the time of year fish may be most impacted by noise.

Here we determine that bullhead exhibit behavioural changes when exposed to low noise levels and both behavioural and morphological changes when exposed to high levels of noise. Bullhead exhibit a decrease in activity levels and an increase in sheltering levels when exposed to both low and high noise levels for a short duration of time. Bullhead also exhibit hair cell damage at high intensity of noise (170 dB re 1µPa) during both acute and chronic exposure. As bullhead have specialized hearing structures, we can apply this research to other Great Lakes fish species with similar hearing capabilities and possibly implement noise pollution restrictions at or above 170 dB re 1µPa of noise in areas of concern. The next steps for this study should be to measure sound levels in local areas across the Great Lakes and determine what the noise levels are and how long it lasts. Another component to consider is that the fish were kept in captivity and could not escape, therefore in the wild fish may simply leave the area to avoid the noise. However, depending on the noise source, health status of the animal and how loud it is, this may not always be possible. Noise pollution research is not commonly studied in freshwater environments, even though these environments are species rich and important for human survival (Tidwell & Allan, 2001). More resources should be attributed to trying to preserve these species, which starts by determining the impact of anthropogenic stressors on their fitness.

Figures for Experiment 1:

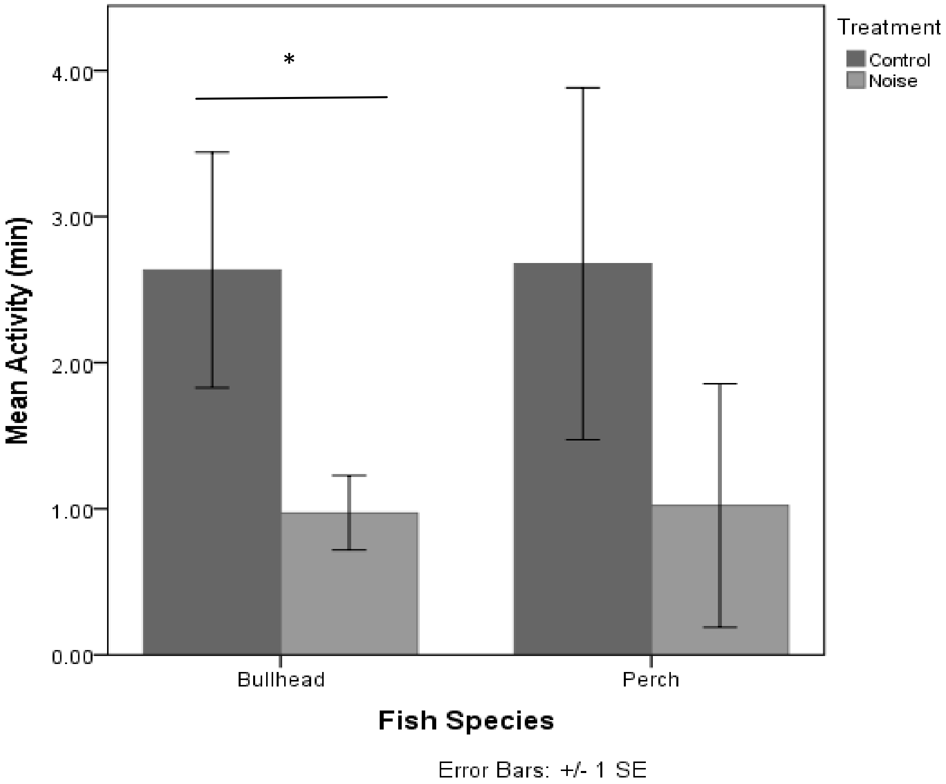

Figure 1: Mean activity levels of black bullhead ($F_{(1,5)=}$8.401, *p*=0.034) and yellow perch ($F_{(1,5.020)}$ =2.830, *p*=0.153) when exposed to 4 hours of 140 dB re 1µPa of boat noise. Significant differences are indicated with an *.

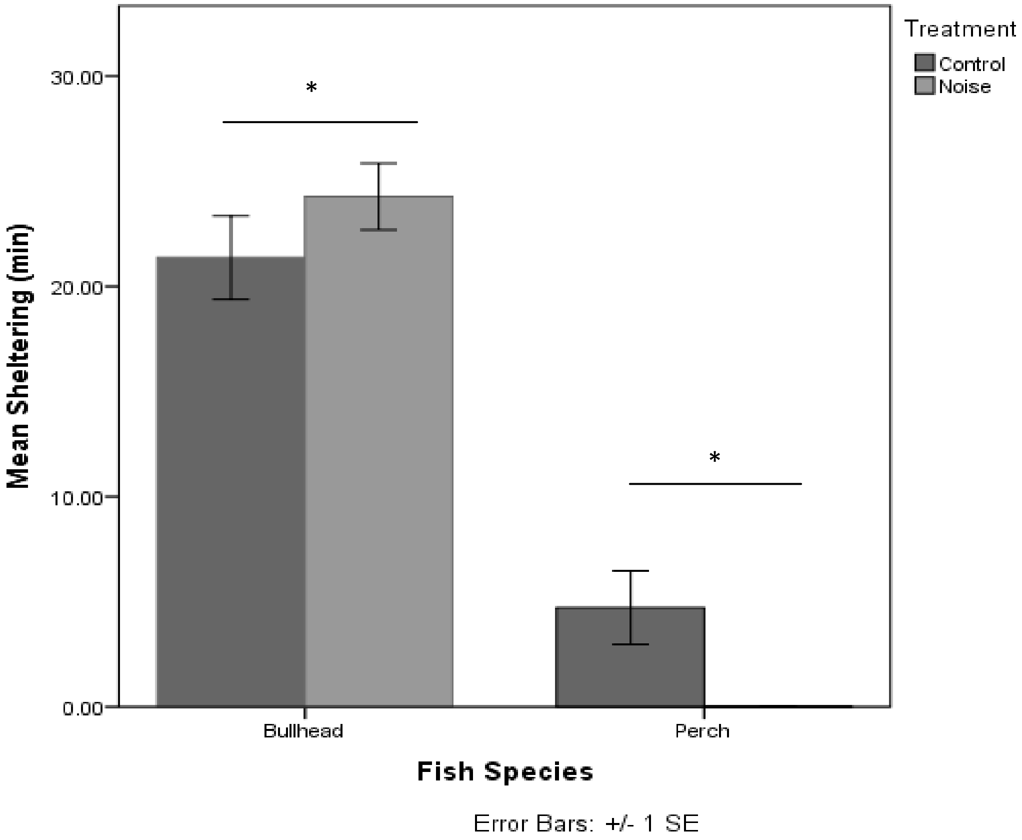

Figure 2: Mean sheltering levels of black bullhead ($F_{(1,5)}$=8.588, *p*=0.033) and yellow perch $F_{(1,5.048)}$ = 15.961, *p*=0.010 when exposed to 4 hours of 140 dB re 1µPa of boat noise. Significant differences are indicated with an *.

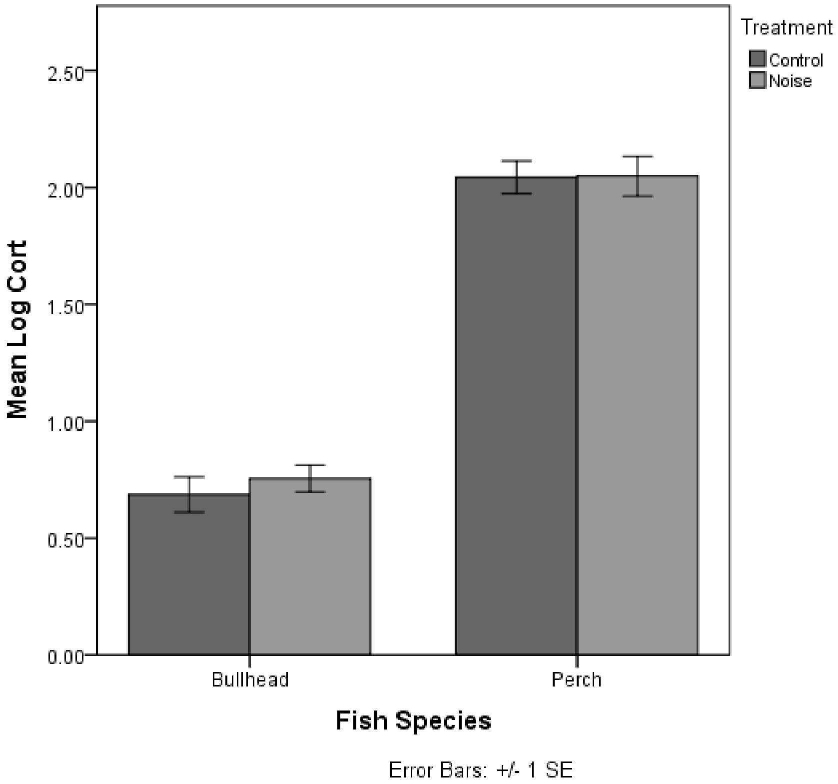

Fig 3: Log cortisol levels of black bullhead ($F_{(8,50)}$=4.192, *p*=0.184) and yellow perch ($F_{(8,49)=}$0.422, *p*=0.902) after 4 hours of 140 dB re 1 µ Pa of boat noise exposure.

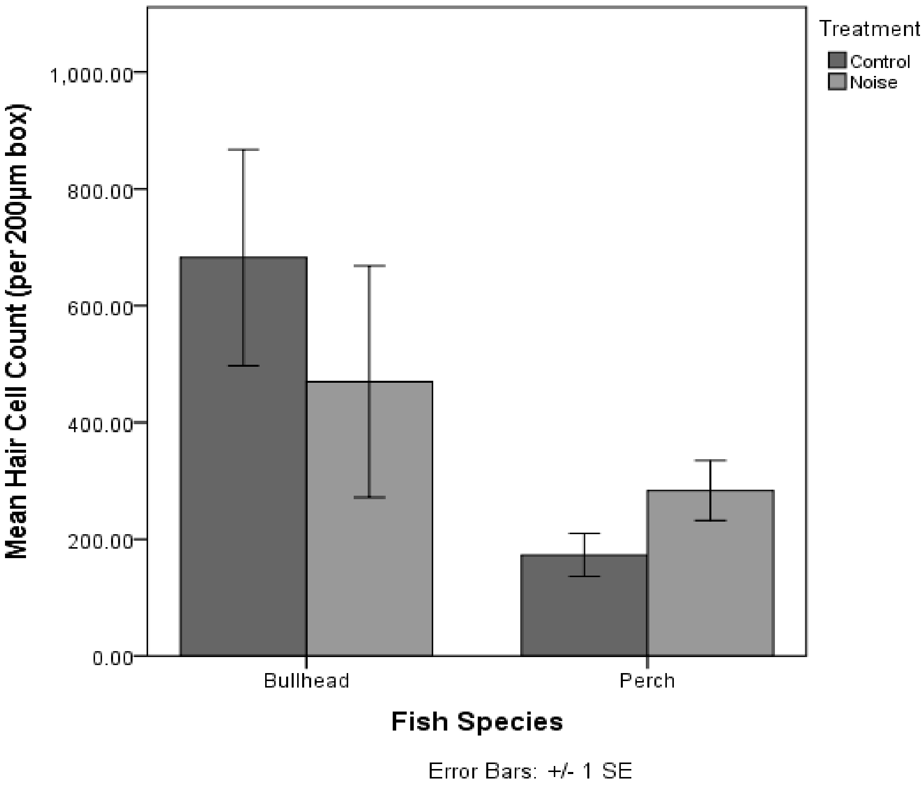

Fig 4: Hair cell data, characterized by hair cell counts in 3 (200µm) boxes of the saccule region of black bullhead (t(6)= 0.784, *p*=0.902) and yellow perch (t(4)= -1.380, *p*=0.182) after 4 hours of 140 dB re 1µPa of boat noise exposure.

Figures for Experiment 2:

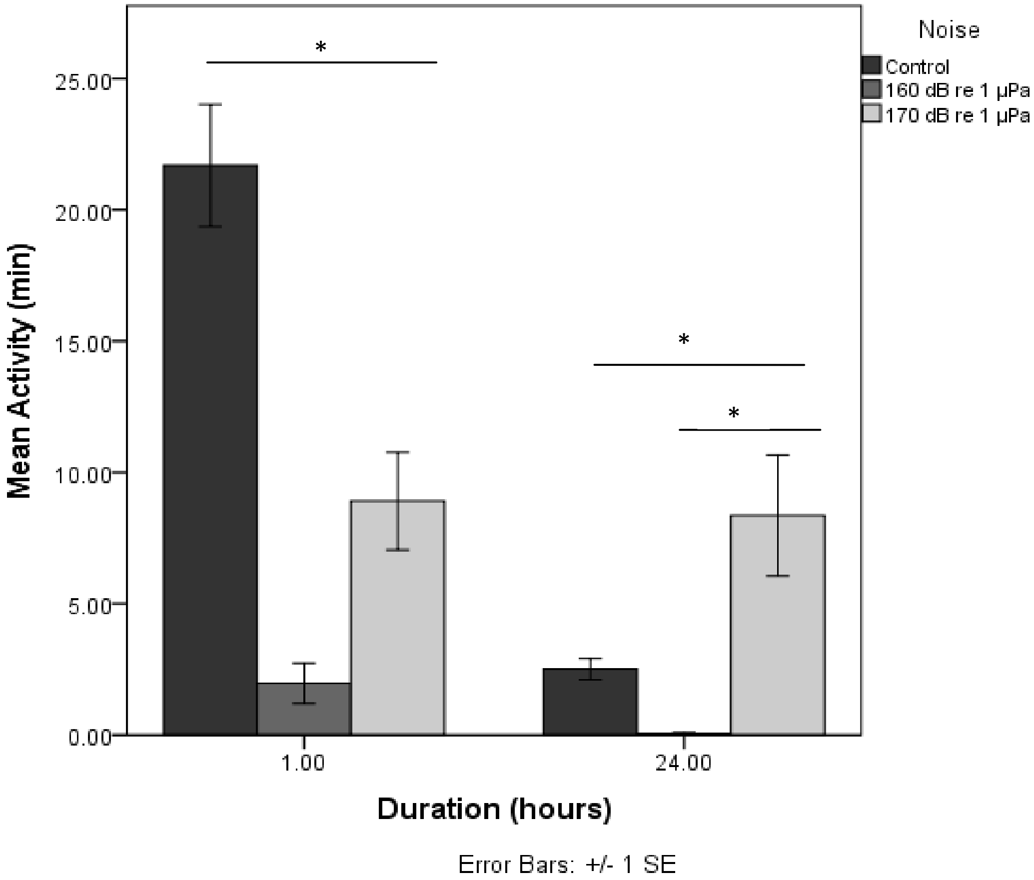

Fig 5: Activity levels of bullhead exposed to boat noise for both acute (1 hour) and chronic (24 hours) exposure. After acute noise exposure bullhead are impacted at both noise levels, Wilks lambda Λ = 11.427, *p* = 0.001. Post-hoc analyses indicate: control vs 160 dB re 1µPa, *p*<0.001; control vs. 170 dB re 1µPa, *p*=0.004;160 vs. 170 dB re 1µPa , *p*=0.007. Bullhead exposed to a chronic stressor exhibit some significant differences in activity levels, Wilks lambda Λ = 0.338, *p* = 0.003. Post-hoc tests indicate: control vs 160 dB re 1µPa, *p*= 0.422; control vs. 170 dB re1µPa, *p*=0.020; 160 vs. 170 dB re 1µPa, *p*=0.002. Significant differences are indicated with an *.

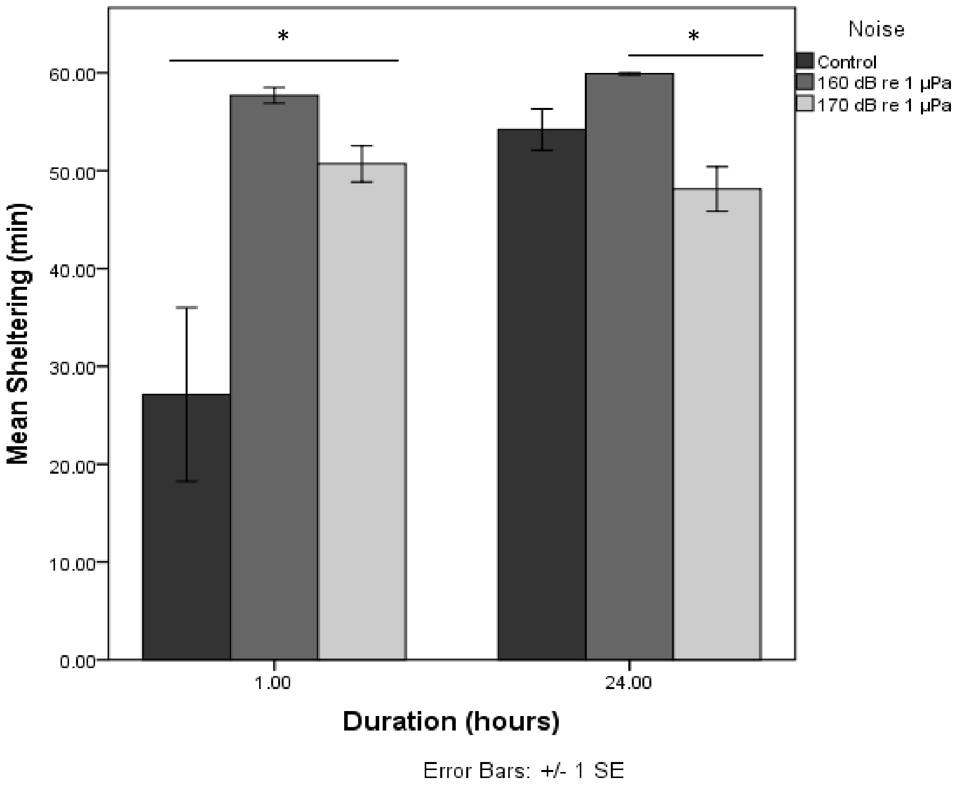

Fig 6: Sheltering behaviour when bullhead are exposed to an acute stressor of boat noise (1 hour) and a chronic stressor (24 hours). During acute noise exposure sheltering levels increase when fish are exposed to noise Wilks lambda Λ = 11.427, *p* = 0.001. During chronic exposure we see some differences in sheltering levels, Wilks lambda Λ = 0.338, *p* = 0.003. Post-hoc analyses indicate: Control vs 160 dB re 1µPa, *p*=0.095; control vs. 170 dB re 1µPa; *p*=0.072, 160 vs. 170 dB re 1µPa, *p*=0.001. Significant differences are indicated with an *.

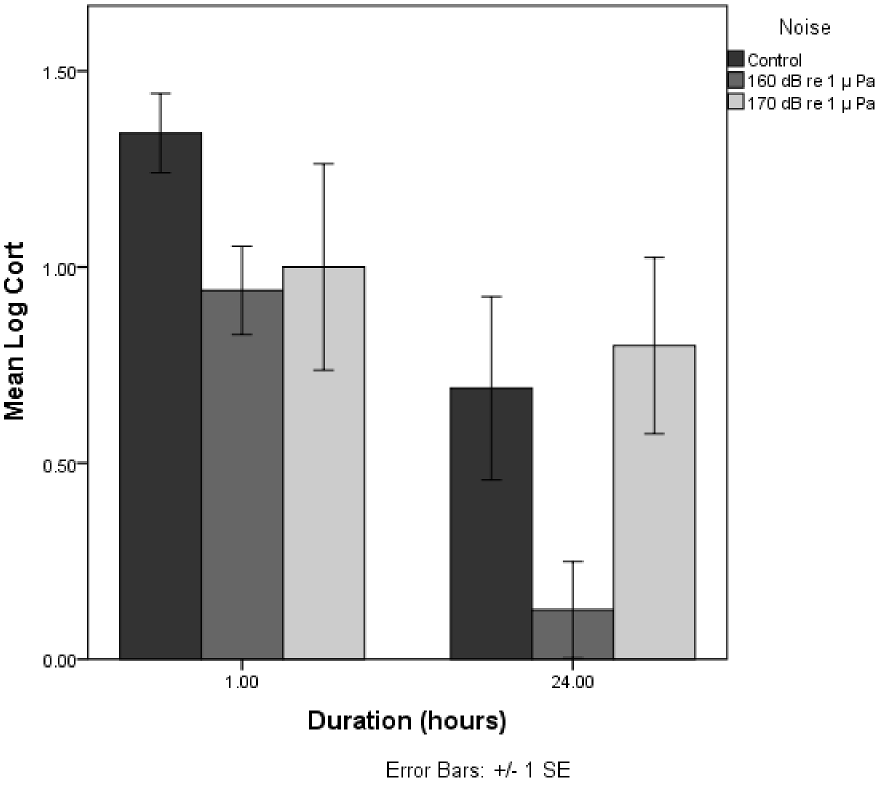

Fig 7: Log-cortisol levels of bullhead after acute and chronic noise exposure. There was no significant differences during acute exposure $F_{(2,14)} = 1.305$, $p=0.302$. There was also no significant difference (possibly marginal) during chronic exposure $F_{(2,15)} = 3.268$, $p=0.066$, and between the 160 and170 dB re 1µPa ($p=0.075$).

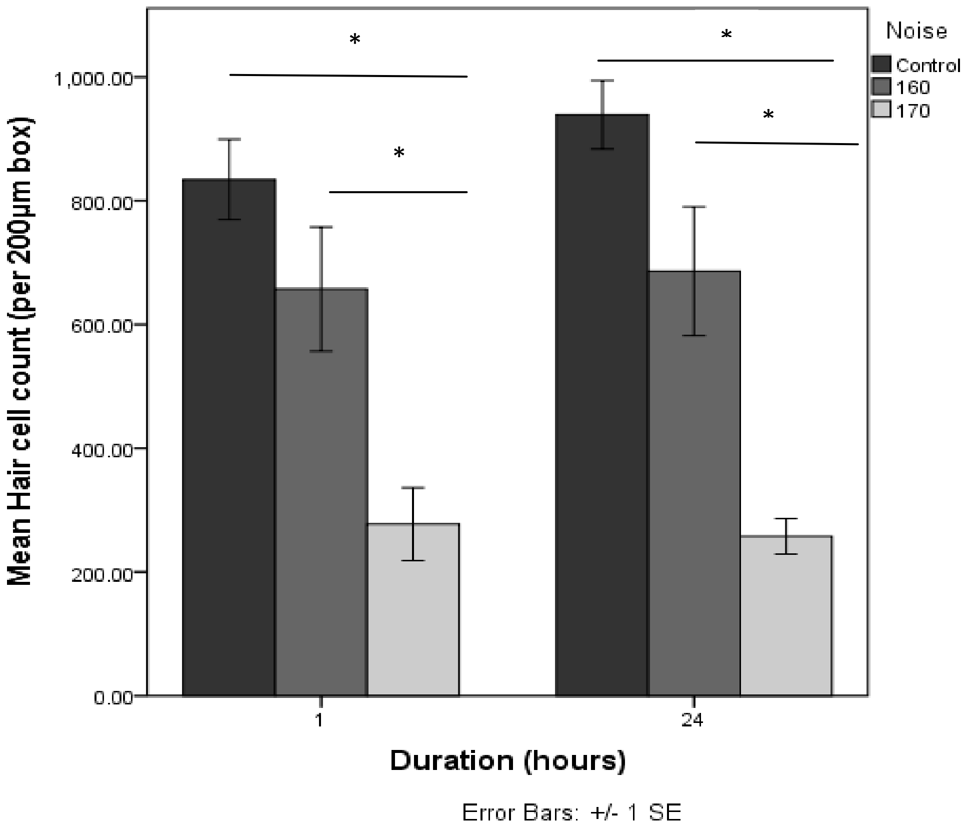

Fig 8: Hair cell counts in 3 (200µm) boxes across the saccule in bullhead, after 1 hour noise and 24 hour exposure. After acute noise exposure we see evidence of hair cell damage, $F_{(2,30)}$ =18.458, *p* <0.001. Post-hoc analyses indicate significant differences in hair cell counts between the control and 170 dB re1µPa, *p*=0.002;160 and 170 dB re 1 µPa, *p*=0.007. However there were no significant differences between the control and 160 dB re 1 µPa. After chronic noise exposure we also see evidence of hair cell damage, $F_{(2,30)}$ =18.458, *p* <0.001. Post-hoc tests indicate: control vs 160 dB re 1µPa, *p*=0.062; control vs. 170 dB re1µPa; *p*<0.001;160 vs 170 dB re 1µPa, *p*=0.001. Significant differences are indicated with an *.

References:

Aalbers, S., & Drawbridge, M. (2008). “White Seabass Spawning Behavior and Sound Production.” *Transactions of the American Fisheries,* **137**: 542–550.

Amoser, S., & Ladich, F. (2005). Are hearing sensitivities of freshwater fish adapted to the ambient noise in their habitats? *Journal of Experimental Biology,* **208**(18), 3533-3542.

Amoser, S., Wysocki, L., & Ladich. F. (2004) "Noise emission during the first powerboat race in an Alpine lake and potential impact on fish communities." *The Journal of the Acoustical Society of America***, 116**(6): 3789-3797.

Amoser, S., & Ladich, F. (2010). "Year-round variability of ambient noise in temperate freshwater habitats and its implications for fishes." *Aquatic sciences,* **72**(3): 371-378.

Amoser, S., Wysocki, L., & Ladich, F. (2004). "Noise emission during the first powerboat race in an Alpine lake and potential impact on fish communities." *The Journal of the Acoustical Society of America,* **116**(6): 3789-3797.

Anderson, P., Berzins, I., Fogarty F, Hamlin, H., Guillette, L. (2011). “Sound, stress, and seahorses: The consequences of a noisy environment to animal health.” *Aquaculture,* **311**: 129–138.

Atema, J. *Sensory Biology of Aquatic Animals*. New York: Springer-Verlag. 1988

Barcellos, L., Kreutz. L., Quevedo, R.,. Bolognesi, da Silva, L., and Bedin, A. (2007). "Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*." *Aquaculture,* **272**(1): 774-778.

Barton, B., & Iwama, G. (1991). “Physiological changes in fish from stress in aquaculture with emphasis on the responses and effects of corticosteroids.” *Annual Review of Fish Diseases,* 1: 3-26.

Barton, B. (2002). “Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids.” *Integrative and Comparative Biology,* **42**: 517–525.

Bonga, S. (1997). “The stress response in fish”. *Physiological Reviews,***77**:591-625.

Brown, J. Endocrine responses to environmental pollutants. In: Fish Ecophysiology, edited by J. C. Rankin and F. B. Jensen. London: Chapman and Hall, 1993, p. 276-296.

Brown, P., Dabrowski, K., & Garling, D. (1996). “Nutrition and Feeding of Yellow Perch”. *Journal of Applied Ichthyology,* **12**: 171-174.

Danzter, B., Fletcher, Q., Boonstra, R., & Sheriff, M. (2014). “Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species?” *Conservation physiology,* **2**(1): 1-18.

Dawkins, M. (2003). “Behaviour as a tool in the assessment of animal welfare.” *Zoology,* **106**: 383–387.

Donaldson, E. The pituitary-interrenal axis as an indicator of stress in fish. In: Stress in Fish, edited by A. D. Pickering. London: Academic, 1981, p. 11-47.

Enger, P. (1981). In: Hearing and Sound Communication in Fishes. Tavolga WN, Popper AN, Fay RR, editor. New York: Springer Verlag. Frequency discrimination in teleosts-central or peripheral? pp. 243–255.

Eriksson, L., & Van Veen, T. (1980). “Circadian rhythms in the brown bullhead, *Ictalurus nebulosus* (Teleostei). Evidence for an endogenous rhythm in feeding, locomotor, and reaction time behaviour.” *Canadian Journal of Zoology*, **58**: 1899-1907.

Erbe, C., Verma, A., McCauley, R., Gavrilov, A., & Parnum, I. (2015). “The marine soundscape of the Perth Canyon.” *Progress in Oceanography,* **137**: 38-51.

Fewtrell, J., & McCauley, R. (2012). “Impact of air gun noise on the behaviour of marine fish and squid.” *Marine Pollution Bulletin,* **64**(5): 984-993.

Forrest, T. G. (1994). “From sender to receiver: propagation and environmental effects on acoustic signals”. *American zoologist*, **34**(6), 644-654.

Graham, A., & Cooke, S. (2008). “The effects of noise disturbance from various recreational boating activities common to inland waters on the cardiac physiology of a freshwater fish, the largemouth bass (*Micropterus salmoides*).” *Aquatic conservation,* **18**(7): 1315-1324.

Haukenes, A.H., & Barton, B. (2004). “Characterization of the cortisol response following an acute challenge with lipopolysaccharide in yellow perch and the influence of rearing density.” *Journal of Fish Biology*, **64**:851-862.

Higgs, D.M., Souza, M., Wilkins, H.R., Presson, J.C., Popper, A.N. (2002). "Age-and size-related changes in the inner ear and hearing ability of the adult zebrafish (*Danio rerio*)." *JARO-Journal of the Association for Research in Otolaryngology,* **3**(2) : 174-184.

Hoar, W., & Randall, D. (1971). “Sound Production and Detection.” *Fish Physiology* , **5**: 135-192.

Iwama, G., Pickering, A., Sumpter, J., Schreck, C. (1997). Fish stress and health in aquaculture. Society for Experimental Biology Seminar Series 62. Cambridge University Press, Cambridge. 278pp.

Kasumyan, A. (2005). “ Structure and Function of the Auditory System in Fishes.” *Journal of Ichthyology,* **45**: 223–270.

Knaepkens, G., Bruyndoncx, L., Eens, M. (2004). “Assessment of residency and movement of the endangered bullhead (*Cottus gobio*) in two Flemish rivers.” *Ecology of Freshwater Fish,* **13**: 317–322.

Ladich, F., & Fay, R. (2013). “Auditory evoked potential audiometry in fish.” *Reviews in Fish Biology and Fishieries,* **23**: 317–364.

Laidley, C., & Leatherland, J. (1988). “Circadian studies of plasma cortisol, protein, glucose and ion concentration, liver glycogen concentration and liver and spleen weight in rainbow trout, *Salmo gairdneri* Richardson.” *Comparative Biochemistry and Physiology*, 8**9**: 495-502.

Lanford, P., Presson, J., & Popper, A. (1996). “Cell proliferation and hair cell addition in the ear of the goldfish, *Carassius auratus*.” *Hearing Research,* **100**: 1-9.

Lelek, A. 1987. The freshwater fishes of Europe. Threatened fishes of Europe. Wiesbaden: Aula-Verlag

Lu, Z., & Popper, A. N. (2001). “Neural response directionality correlates of hair cell orientation in a teleost fish.” *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology,* **187**(6), 453-465.

Martins, C., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J., Carter, C., Planellas, S., & Kristiansen, T. (2011). “Behavioural indicators of welfare in farmed fish.” *Fish Physiology and Biochemistry*, **38**(1), 17-41.

Martin, B., &. Cott, P. (2016). "The under-ice soundscape in Great Slave Lake near the city of Yellowknife, Northwest Territories, Canada." *Journal of Great Lakes Research*, **42**(2): 248-255.

Mclaughlin, K., & Kunc, H. (2015). “Changes in the Acoustic Environment Alter the Foraging and Sheltering Behaviour of the Cichlid *Amititlania nigrofasciata.*” *Behavioural Processes*, **116**: 75-79.

McCormick, S., Shrimpton, J., Carey, J., O’Dea, M., Sloan, K., Moriyama, S. (1998). “Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol.” *Aquaculture,* **168**: 221–235.

McWilliam, J.N., & Hawkins, A.D. (2013). “A comparison of inshore marine soundscapes.” *Journal of experimental marine biology and ecology,* **446**:166-176.

Mommsen, T., Vijayan, M., & Moon, T. (1999). “Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation.” *Reviews in Fish Biology and Fisheries*, **9**: 211–268.

Moberg, G. (2005). The biology of animal stress: basic principles and implications for animal welfare. Wallingford: CABI publication.

Nichols, T., & Širović, A. (2015). “Intermittent noise induces physiological stress in a coastal marine fish.” *PLOS ONE*, **10**(9): 1-10.

Payne, N., Van Der Meulen, D., Suthers, I., Gray., C., & Taylor, M. (2014). “Foraging Intensity of Wild Mulloway *Argyrsomus Japonicus* Decreases with Increasing Anthropogenic Disturbance.” *Marine Biology*, **162**(3): 539-546.

Peng, C., Zhao, X., & Liu, G. (2015). “Noise in the Sea and Its Impacts on Marine Organisms.” *International Journal of Environmental Research and Public Health*, **12**(10): 12304-12323.

Pickering, A., & Pattinger, T. (1989). “Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol.” *Fish Physiology and Biochemistry*, **7**: 253-258.

Popper, A., Fay, R., Platt, C., & Sand, O. (2003). “Sound detection mechanisms and capabilities of teleost fishes. In Sensory Processing in Aquatic Environments.” Edited by: Collin SP, Marshall NJ. New York: Springer-Verlag: 3-38.

Popper, A & Fay, R. Fish Hearing and bioacoustics: An Anthology in Honour of Arthur Popper and Richard Fay: Volume 87 of advances in experimental medicine and biology. Editor Joseph Sisneros, Springer 2015.

Popper, P. & Hastings, M. (2009). “The effects of anthropogenic sources of sound on fishes.” *Journal of Fish Biology*, **75**: 455–489.

Popper, A., & Hawkins, A. (2012). *The Effects of Noise on Aquatic Life*. New York: Springer.

Popper, A. and Schilt, C. (2008). Hearing and acoustic behavior (basic and applied). In: Webb, J. F., Fay, R. R., and Popper, A. N. (eds). *Fish Bioacoustics*. Springer Science+Business Media, LLC, New York, pp. 17-48

Radford, A., Kerridge, E., & Simpson, S. (2014). “Acoustic Communication in a Noisy World: Can Fish Compete with Anthropogenic Noise?” *Behavioural Ecology*, **25**(5): 1022-1030.

Ramcharitar, J., Gannon, D., & Popper, A. (2006). "Bioacoustics of fishes of the family Sciaenidae (croakers and drums)." *Transactions of the American Fisheries Society,* **135**(5): 1409-1431.

Ramsay, J., Feist, G., Varga, Z., Westerfield, M., Kent, M., & Schreck, C. (2009). “Whole-body cortisol response of zebrafish to acute net handling stress.” *Aquaculture,* **297**(1-4): 157–162.

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

Rogers, P., & Cox M. (1988) Underwater Sound as a Biological Stimulus. In: Atema J., Fay R.R., Popper A.N., Tavolga W.N. (eds) Sensory Biology of Aquatic Animals. Springer, New York.

Sabet, S., Neo Y., Slabbekoorn, H. (2015). “The Effect of Temporal Variation in Sound Exposure on Swimming and Foraging Behaviour of Captive Zebrafish.” *Animal Behaviour,***107**: 49-60.

Sapolsky, R., Romero, L., & Munck, A. (2000). “How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions.” *Endocrinology Reviews,* **21**:5–89.

Scnnrcr, C., & Lonz,W. (1978). “Stress response of coho salmon (*Oncorlrynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress.” *Journal of the Fisheries Research Board of Canada,* **35**: 7124-1129.

Schwarz, A., and Greer, G. (1984). “Responses of Pacific herring, *Clupea harengus pallasi,* to some underwater sounds.” *Fish Aquatic Science,* **41**: 1183-1192.

Slabbekoorn, H., Bouton, N., Opzeeland, I., Coers, A., Cate, C., & Popper, A. (2010). “A Noisy Spring: The Impact of Globally Rising Underwater Sound Levels on Fish.” *Trends in Ecology & Evolution,* **25**(7): 419-427.

Smith, M., Coffin, A., Miller, D., & Popper, A. (2006). “Anatomical and functional recovery of the goldfish (*Carassius auratus*) ear following noise exposure.” *Journal of Experimental Biology,* **209**:4193–4202.

Smith, M., Kane., A., & Popper., A. (2003). “Noise-induced Stress Response and Hearing Loss in Goldfish (*Carassius Auratus*).” *Journal of Experimental Biology,* **207**(3): 427-435.

Smith, M., Schuck, J., Gilley, R., & Rigers, B. (2011). “Structural and functional effects of acoustic exposure in goldfish: evidence for tonotopy in the teleost saccule.” *BMC Neuroscience,* **12**: 1-17.

Smyly, W. (1957). “The life-history of the bullhead or Miller’s thumb (*Cottus gobio*).” *Proceedings of the Zoological Society London,* **128**: 431–453.

Solan, M., Hauton, C., Godbold, J., Wood, C., Leighton, T., & White, P. (2016). “Anthropogenic Sources of Underwater Sound Can Modify How Sediment-dwelling Invertebrates Mediate Ecosystem Properties.” *Scientific Reports,* **6**: 20540.

Staaterman, E., Paris, C. B., DeFerrari, H. A., Mann, D. A., Rice, A. N., & D’Alessandro, E. K. (2014). “Celestial patterns in marine soundscapes.” *Marine Ecology Progress Series,* **508**: 17-32.

Tidwell, J., & Allan, G. (2001). “Fish as food: aquacultures contribution.” *EMBO reports,* **2**(11): 958-963

Voellmy , I., Purser, J., Flynn, D., Kennedy, P., Simpson, S., & Radford, A. (2014). “Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms.” *Animal Behaviour,* **89**: 191-198.

Valdimarrson, S., & Metcalfe, N. (1998). “Shelter selection in juvenile Atlantic salmon, or why do salmon seek shelter in winter?” *Journal of fish biology,* **52**(1):42-49.

Vasconcelos, R., Amorim, M., & Ladich, F. (2007). “Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish.” *Journal of Experimental Biology*, **210**(12): 2104-2112.

Wale, M., Simpson, S., & Radford, A. (2013). “Noise Negatively Affects Foraging and Antipredator Behaviour in Shore Crabs.” *Animal Behaviour*, **86**(1): 111-118.

Wingfield J., Maney., D, Breuner., C, Jacobs, J., Lynn, S., Ramenofsky, M., Richardson, R. (1998). “Ecological bases of hormone behavior interactions: the emergency life-history stage.” *American Zoologist,* **3**:191–206

Wysocki, L., Dittami,J., and Ladich, F. (2006). "Ship Noise and Cortisol Secretion in European Freshwater Fishes." *Biological Conservation,* **128**(4): 501-508.

CHAPTER III

CONCLUSION AND RECCOMENDATIONS

Summary:

Anthropogenic noise impacts behavioural and physiological characteristics of marine animals (Popper & Hastings, 2009), which can ultimately impact their fitness. However, there is little research regarding the impacts of anthropogenic noise on freshwater animals, particularly fish (Slabbekoorn et al., 2010). Freshwater fish are an important component of our ecosystem and are considered a major source of protein for humans (Tidwell & Allan, 2001), yet little work has been performed regarding their appropriate acoustic habitat (Smith et al., 2003). The majority of research regarding the impacts of noise on freshwater fish often focus on either a behavioural change, such as detecting changes in foraging efficiency (Voellmy et al., 2014; McLaughlin & Kunc, 2015) or a physiological change such as increasing stress levels (Smith et al., 2003; Nichols & Širović, 2015). Research regarding noise pollution in freshwater ecosystems commonly lacks integration within the design as researchers often use one measure to indicate stress impacts on fish (Barcellos et al., 2007; Purser & Radford, 2011). The use of integrative studies in the field would allow us to make better predictions on welfare and fitness characteristics of fish in response to acoustic stressors.

Here, I use an integrative approach to determine the impacts of anthropogenic influences on local freshwater fish found in the Laurentian Great Lakes. I performed two studies to illustrate the effects of differing noise levels on behavioural and physiological characteristics of the fish. When determining a behavioural response to noise I used activity and sheltering levels as indicators of stress, and cortisol and hair cell damage when determining a physiological stress response. In the first experiment I exposed both black bullhead (*Amerius melas*) and yellow perch (*Perca fluvescens*) to boat noise played at an ecologically relevant noise level based on soundscape data (unpub) collected the previous year. After four hours of noise exposure at 140 dB re 1 µPa bullhead exhibited a decrease in activity levels and an increase in sheltering levels, however they did not exhibit a change in physiological characteristics. Here I indicate that bullhead are behaviourally stressed by relatively low noise levels, but not impacted physiologically. However, yellow perch did not exhibit the same behavioural patterns as bullhead and it was also difficult to detect a physiological stress response in perch as cortisol levels were high in both the control and manipulation experiments. The results from our perch data may be explained by their lack of hearing specialization (Amoser & Ladich, 2005; Ladich & Fay, 2013) or to difficulty housing in captivity. During the second experiment I exposed bullhead to higher levels of boat noise (160/170 dB re 1µPa) for both an acute (1 hr) and chronic (24 hr) time point. Bullhead exhibited an increase in sheltering and a decrease in activity levels when exposed to an acute stressor, however they displayed a higher behavioural response to noise at 160 dB re 1µPa compared to 170 dB re 1µPa. Bullhead did not exhibit an increase in cortisol at either noise level during chronic exposure, however hair cell damage occurred at 170 dB re 1µPa. During chronic exposure of noise bullhead exhibited some behavioural changes in activity and sheltering levels, but most notably a significant difference between 160 dB re 1µPa and 170 dB re 1µPa where fish were most stressed at 160 dB re 1µPa yet again. Hair cell damage occurred at both acute and chronic time points when fish were exposed to 170 dB re 1 µPa of noise, therefore it is predicted that fish exhibited a decrease in hearing sensitivity at this level due to damage (Smith et al., 2003). As fish are predicted to be less sensitive to noise at 170 dB re 1 µPa this explains the behavioural pattern observed at this noise level, which further explains the behavioural differences between 160 and 170 dB re 1 µPa.

If only behavioural characteristics of bullhead were studied, it would be difficult to understand/explain why fish are more stressed at a lower noise level (160 dB re 1 µPa) compared to the higher noise level (170 dB re 1 µPa). This coincides with my argument on the importance of integration within noise pollution research. Here I use an integrative approach to further the field of noise impacts on freshwater fish. I discover that a local species native to the Laurentian Great Lakes is impacted behaviourally at relatively low noise levels and physiologically after short exposure of high noise levels. To my knowledge this is one of the first studies to discover hair call damage in a local freshwater fish species, as most research pertaining to hair cell damage focuses on goldfish (*Carassius auratus*) (Smith et al., 2003; Smith et al., 2006) or zebrafish (*Danio rerio*) (Harris et al., 2003; Ou et al., 2007). As bullhead exhibit specialized hearing capabilities we can use this study to model potential impacts of noise on other fish local to the area with similar hearing capabilities. This research can be used for conservation purposes and to further the need for noise restrictions in certain breeding areas of keystone or endangered species with similar hearing capabilities.

Future Directions:

There are a number of future questions we can ask regarding noise pollution impacts on freshwater fish species as little is known about this topic. Below, I have listed a few suggestions on future research projects with an aim to move the field forward. Future studies should conduct a similar integrative study on another model fish species with general hearing capabilities, this was my aim at the start of the project but perch did not do well in a captive setting. It is also important to determine if the noise levels played to the fish are ecologically relevant, are fish exposed to this noise in nature? If so for how long and how do they react to this stressor? Future research should include collecting soundscaping data in local areas of boat traffic to determine the validity of this noise level in nature. Analysis of unpublished data collected the previous year provides us with an approximate range of background noise from 110-137dB re 1 μPa across eight locations along the Great Lakes. Long-term acoustic data should be collected in both low and heavy boat traffic areas around the Great Lakes to determine noise levels fish are exposed to in their natural environment. Future studies should focus on determining the hearing range and vocal output of a number of local Great Lakes fish so we are able to further predict potential behavioural and physiological impacts of noise on these species. As we have determined a range of behavioural and physiological impacts of both low and high levels of noise on black bullhead we can use this data to model potential impacts on other local species with specialized hearing capabilities. It would be beneficial to conduct a field study in both noisy and quiet locations of the Laurentian Great Lakes to determine the presence of fish in different acoustic environments. This would allow researchers to determine if certain fish species avoid noisy areas, what fish actually reside in these areas? Are they fish with general hearing capabilities? and finally determine if adaptation has occurred.

Few studies have examined the presence of hair cell damage in freshwater fish when exposed to noise, especially fish residing in local areas along the Great Lakes. Future research should examine if hair cell damage occurs in both the lateral line and inner ear when fish are exposed to differing levels of noise. Future research should also determine if/when hair cells regenerate and if certain noise levels can cause permanent damage to fish hearing. If fish exhibit permanent hearing damage, does this impact courtship, predator/prey detection and overall survivorship? There are many questions we can ask in the field of freshwater noise pollution as research regarding this topic is severely lacking. We know very little about noise influences on fish and need to continue to study these impacts to prevent future population crashes and preserve our freshwater ecosystems.

References:

Amoser, S., & Ladich, F. (2005). Are hearing sensitivities of freshwater fish adapted to the ambient noise in their habitats? *Journal of Experimental Biology,* **208**(18): 3533-3542.

Barcellos, L., Kreutz. L., Quevedo, R.,. Bolognesi, da Silva, L., and Bedin, A. (2007). "Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*." *Aquaculture,* **272**(1): 774-778.

Harris, J.A., Cheng, A. G., Cunningham, L. L., MacDonald, G., Raible, D. W., & Rubel, E. W. (2003). "Neomycin-induced hair cell death and rapid regeneration in the lateral line of zebrafish (*Danio rerio*)." *Journal of the Association for Research in Otolaryngology,* **4**(2): 219-234.

Ladich, F., & Fay, R. (2013). "Auditory evoked potential audiometry in fish." *Reviews in Fish Biology and Fishieries,* **23:** 317–364.

Mclaughlin, K., & Kunc, H. (2015). "Changes in the Acoustic Environment Alter the Foraging and Sheltering Behaviour of the Cichlid *Amititlania nigrofasciata.*" *Behavioural Processes,* **116**: 75-79.

Nichols, T., & Širović, A. (2015). "Intermittent noise induces physiological stress in a coastal marine fish." *PLOS ONE,* **10**(9): 1-10.

Ou, H., Raible, D.W., Rubel, E.W. (2007). "Cisplatin-induced hair cell loss in zebrafish (*Danio rerio*) lateral line." *Hearing research,* **233**(1): 46-53.

Popper, P. & Hastings, M. (2009). "The effects of anthropogenic sources of sound on fishes." *Journal of Fish Biology,* **75**: 455–489.

Purser, J., and Radford, A.N. 2011. Acoustic Noise Induces Attention Shifts and Reduces Foraging Performance in Three-Spined Sticklebacks (*Gasterosteus aculeatus*). PLoS ONE, **6**(2): 1-8.

Ramcharitar, J., Gannon, D., & Popper, A. (2006). "Bioacoustics of fishes of the family Sciaenidae (croakers and drums)." *Transactions of the American Fisheries Society,* **135**(5):1409-1431.

Slabbekoorn, H., Bouton, N., Opzeeland, I., Coers, A., Cate, C., & Popper, A. (2010). "A Noisy Spring: The Impact of Globally Rising Underwater Sound Levels on Fish." *Trends in Ecology & Evolution,* **25**(7): 419-427.

Smith, M., Kane., A., & Popper., A. (2003). Noise-induced Stress Response and Hearing Loss in Goldfish (*Carassius Auratus*). *Journal of Experimental Biology,* **207**(3): 427-435.

Smith, M., Coffin, A., Miller, D., & Popper, A. (2006). “Anatomical and functional recovery of the goldfish (*Carassius auratus*) ear following noise exposure.” *Journal of Experimental Biology*, **209**:4193–4202.

Tidwell, J., & Allan, G. (2001). “Fish as food: aquacultures contribution.” *EMBO reports*, **2**(11):958-963.

Voellmy , I., Purser, J., Flynn, D., Kennedy, P., Simpson, S., & Radford, A. (2014).

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APPENDIX

Elisa Protocol:

The assay was performed according to manufacturer instructions with minor modifications to optimize for the study species as summarized below. Before beginning assays, a pool of black bullhead plasma was assayed both raw and after dichloromethane extraction. Serial dilutions of both were found to be parallel to the standard curve. As extracted samples showed reduced values due to recovery losses and raw plasma showed no indication of interference, samples were run on raw plasma without extraction. To ensure sample cortisol values fell within the kit detection range, black bullhead samples were assayed at 1:20 dilution (10uL of plasma and 190uL of assay buffer) while yellow perch plasma samples were assayed at a 1:120 dilution (5uL of plasma and 595uL of assay buffer).

Sample concentrations were determined using an 8 point standard curve run in duplicate on each assay plate. Standards ranged in concentration from 4000pg/mL to 6.6pg/mL while the minimum detection limit of the assay is 35pg/mL. The curve was created individually for each plate by serial dilution from a kit-provided concentrated cortisol EIA standard:

In addition to a standard curve, each plate contained non-specific binding wells (assay blank), maximum binding wells (maximum colour), and a control sample (pooled bullhead plasma). The remaining plate wells were loaded with 50uL of diluted sample in triplicate. Cortisol tracer was then added to all wells except the non-specific binding blank wells using a repeater pipette. Monoclonal antibody was added to all wells in the same way. Plates were covered with an adhesive plate cover and incubated overnight in the refrigerator to allow hormone to bind to the wells. The next morning, plate contents were dumped, wells washed 5 times with wash buffer, and then plates were tapped sharply on paper towel to remove all liquid. Ellman's reagent was added using the repeater pipette. Plates were again covered with a plate cover, placed in a covered plate shaker, and incubated to develop colour for 1 hour at 25°C and 450RPM. At the end of incubation, the absorbance values for each well were measured at 412nm using a BioTek Synergy H1 plate reader. The absorbance value of the maximum binding wells was checked to ensure sufficient plate development, the mean value of the assay blank wells was subtracted from the values of all wells, and sample concentrations were determined by fitting a 4-parameter logistic regression through the blanked absorbance values of the standard curve.

All samples were assayed across 7 plates yielding an inter-assay variation of 19.96% and intra-assay variation of 5.3% for black bullhead and 3.0% for yellow perch.

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