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Integrating Techniques: A Review of the Effects of Anthropogenic Noise on Freshwater Fish

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Abstract:

In recent years, the effects of anthropogenic noise on freshwater fish has been of increasing interest for fishery managers due to rising levels of this background noise. While it is clear that anthropogenic noise can have important impacts on mammals and marine fish, much less is known about these effects in fresh water. The influence of anthropogenic noise on freshwater fish can be quantified using the same methods as with marine species — through measuring changes in behavioural and physiological outputs. Here, we briefly review the literature regarding behavioural and physiological impacts of noise pollution on freshwater fish and further note the lack of incorporation of both behavioural and physiological measures within current studies. We call for an increased research emphasis on possible effects of anthropogenic noise on freshwater fish and further suggest that the integration of behavioural and physiological techniques is critical for a full understanding of these effects. While freshwater fish face many stressors, it is unclear how important anthropogenic noise really is and this issue can only be properly resolved through careful study.

Introduction:

Sound is an important sensory stimulus 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; van der 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 1977; Akyildiz et al. 2005) yet it is a critical sensory stimulus in most environments (Popper & Fay 1973). Many fish species are particularly reliant on sound as a form of communication (van der 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 manmade disturbance for aquatic species (Popper & Hastings 2009; Radford et al. 2014; Solan et al. 2016). Anthropogenic noise is primarily caused by urban developments, the expansion of shipping transportation networks, underwater resource extraction and seismic exploration devices and has been increasing in the past six decades (Hildebrand 2009; Frisk 2012; Solan et al. 2016; Vazzana et al. 2017). These sources of anthropogenic noise are hypothesized to disrupt acoustic communications and have far-reaching effects on aquatic species (Wysocki et al. 2006; Popper & Hastings 2009). 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). Soundscape data collected from a marine protected area for one year in the Mediterranean Sea indicates that vessel traffic masks fish choruses

46% of the time during peak vocalization hours (7:30-11:30 pm) therefore fish may be protected from some human impacts like overfishing in these refuge areas but can still be negatively impacted by anthropogenic noise (Buscaino et al. 2016). With some exceptions (Buscaino et al. 2010; Celi et al. 2013), 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 generally fewer studies that examine the effects of noise pollution on freshwater species (Popper 2003; Slabbekoorn et al. 2010; see Table 1). The acoustic landscape of marine vs. freshwater environments differs quite markedly. Sound transmission in the open ocean can be effectively modeled as an unbounded medium but, especially for shallow freshwater environments, acoustic modelling is much more difficult when depth is often very shallow and substrates poorly defined (Kupperman 1977; Rogers & Cox 1988), although coastal marine environments can also be difficult to properly model. 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; Popper & Hawkins 2012). 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 (Table 1) 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 and as an attempt to stimulate more research and possibly guidelines on acceptable levels of anthropogenic noise in freshwater environments (Popper et al. 2014).

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; Dantzer et al. 2014; Narayan 2016). The mechanisms behind GC response are now well understood (e.g. Vazzana et al. 2010 and references therein) and include dysregulation of the hypothalamic pituitary axis or the hypothalamic pituitary interrenal axis in the brain brought on by environmental challenges (Bronson 1995; 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 and colleagues, presented 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 GC 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 just 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 stressor 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 controls (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). Halvorsen et al. (2012) discovered that lake sturgeon (*Acipenser fulvescens)* and Nile tilapia (*Oreochromis niloticus*), species with two different types of swim bladders, both exhibited damage to their swim bladder after exposure to pile driving. 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 freshwater 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 regulate noise levels 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 a 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, and based on inconsistency of results it was 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 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.

Is Noise 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). 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 an electric trolling motor and 67% when exposed to a combustion motor. 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 1995). 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 and colleagues (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). The increase in cardiac output that the bass experienced 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. In some studies, researchers use biomarkers such as glucose, lactate and heat shock protein to determine a stress response (Celi 2016; Vazzana et al. 2017). For example, Vazzana and colleagues (2017) discovered that damselfish (*Chromis chromis*) experienced an increase in levels of glucose, lactate, proteins present in plasma and heat shock protein (HSP70) when exposed to low frequencies of noise. 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 their current state (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, showing that the full 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 and colleagues (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 showing higher activity on the weekend. 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 (Purser & Radford 2011), demonstrating the large range of detriments noise can have on aquatic species. Predator-prey interactions in zebrafish (*Danio rerio*) are also 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 (Purse & 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 an appropriate control and maintain consistencies in all environmental conditions.

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 cause 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 display a significant increase in oxygen usage in noise conditions compared to fish in control environments (Simpson et al. 2014). Coral reef fish (*Dascyllus trimaculatus)* exhibit an increase in sheltering when exposed to two days of motorboat noise, but stop responding after one week, showing 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 and is often the first signs of stress an animal exhibits. 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 are also problematic (e.g. Parvulescu, 1967; 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 correlational 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 effects of noise on Mauthner-mediated startle responses (Zottoli et al. 1977) or the impacts of noise on hair cell damage in goldfish (Smith et al. 2006). This is important as it increases our knowledge base on the topic of noise pollution; however the majority of these studies lack integration within their design. Future studies should incorporate integrative examinations of noise on freshwater fish species to determine the extent to which noise affects them. For example, when studying the impacts of stress on a local freshwater species, it may be beneficial to measure behavioural characteristics such as foraging efficiency and avoidance response but also look at physiological responses such as glucocorticoid levels. Data collected from integrative studies can provide critical information on the extent of noise impacts; for example if cortisol data was collected and no significant differences were found after noise exposure it could be that hair cell damage occurred rendering fish deaf to the noise and therefore no longer physiologically stressed by a noise they can no longer hear. However, this finding would not occur without the presence of an integrative study that examined noise impacts at multiple levels. Understandably, such integrative studies require more work and knowledge on the topic, however the results attained will be stronger and more comprehensive. When interpreting findings from each technique it is important to form a strong control to have a good comparison of "normal" behaviours to determine what constitutes a stress response.

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 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 affect 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 well argued by Mann et al. (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 noise has 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 such as 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 one 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 perspective 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.

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Table 1: A partial review of effects and techniques used in noise pollution research in freshwater and ecosystems, outlining the need for more integration across studies.

Species	Techniques Used	Integration within study	References	Title
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	Stress Response and Habituation to Motorboat Noise in Two Coastal Fish Species in the Bothnian Sea
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

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