UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING AND PHYSICAL SCIENCES

International Centre for Ecohydraulics Research

Novel quantification of the reactions of a cypriniform fish to acoustic stimuli

By

Matthew Andrew Blake Short

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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NOVEL QUANTIFICATION OF THE REACTIONS OF A CYPRINIFORM FISH TO ACOUSTIC STIMULI

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Anthropogenic structures in the aquatic environment can cause mortality, damage and ecological exclusion of fish. These anthropogenic threats are traditionally mitigated for by physical means of excluding, diverting and deterring fish. These methods are not entirely effective for all species and all life stages of fish. Increasingly, fisheries managers are turning to behavioural deterrents to supplement and replace these traditional technologies; however, quite often these devices are deployed with little scientific basis.

Current understanding of fish behaviour in the presence of acoustic stimuli focuses on, often large-scale, experiments that aim to manipulate the placement of fish within a system. This management-led, deterrent-concentrated research misses many of the complex, and important, parts of the behaviour being displayed by these animals.

The fundamental-based approach of the research investigates the difference in reaction of individuals and groups of small cypriniform fish. Using finely measured acoustic treatments and high-definition video recording in an experimental flume, the European minnow (*Phoxinus phoxinus*) was exposed to differing acoustic treatments and the resultant footage was analysed. Results indicate that groups of fish are more uniformly impacted by sound than individuals and that they return to normal behaviour more slowly. The data also demonstrates that individuals within a group behave in a more coherent manner when initially exposed to sound. Where individuals did demonstrate changes in their behaviour, groups of fish did not demonstrate changes in the same metrics, namely swimming speed and persistence of swim paths.

The research presented within this thesis has contributed to scientific understanding of how the placement of fish can be influenced by sound and also the fine-scale behaviours that they present when exposed to an acoustic stimulus. Reactions of individual fish are compared with reactions of groups of fish and the significant differences between them are discussed. The information brought about by conducting this research will go towards furthering knowledge of the complex nature of animal behaviour and also the complexities of experimental acoustic work within water tanks.

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Declaration of Authorship

I, Matthew Andrew Blake Short declare that this thesis and the work presented in it is my own and has been generated by me as the result of my own original research.

NOVEL QUANTIFICATION OF THE REACTIONS OF A CYPRINIFORM FISH TO ACOUSTIC STIMULI

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission.

Signed:

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Date:

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Ethics

Ethical approval for this study was provided by the University of Southampton Animal Welfare and Ethical Review Board (ERGO ethics submission no. 19783). Permission to remove European minnow from the River Itchen was provided by the Environment Agency (permit reference EP/EW083-L-263/5065/02).

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Acronyms

ATF	Above tank floor, refers to the bottom of the flume tank floor as used as a reference when measuring the acoustic field.
BAFF	Bio-Acoustic Fish Fence, a behavioural device used to limit the movement of fish in water.
dB	Decibel, defined as the unit used to measure the intensity of a sound or the power level of an electrical signal by comparing it with a given level on a logarithmic scale.
FPS	Frames per second, refers to the rate at which still images are captured on a camera and presented as film footage for analysis.
ICER	The International Centre for Ecohydraulics Research. A research unit based at the University of Southampton where this thesis was conducted.
SPL	Sound pressure level which uses a logarithmic scale to represent the sound pressure of a sound relative to a reference pressure, and it is measured in units of decibels (dB). In this work the reference pressure is relative to 1 micropascal.
UV	Ultra-violet light, part of the electromagnetic spectrum with a wavelength from 10 nm to 400 nm.
WRST	Wilcoxon rank-sum test, refers to a nonparametric test of the null hypothesis that it is equally likely that a randomly selected value from one sample will be less than or greater than a randomly selected value from a second sample. It is a major feature of the analysis conducted within this thesis. Also referred to as the Mann–Whitney *U* test.

Glossary

Acclimation: To accustom or become accustomed to a new environment.

Anthropogenic: Of, relating to, or resulting from the influence of human beings.

Bar rack/ trash rack: Type of physical screen used to block fish entry to water intakes and divert them to a safe bypass. Usually consists of vertical oriented bars slotted into a frame.

Behavioural screen: Facility which exploits the fish's sensory system to deter them from a certain location (usually an intake).
Bypass: A safe route for downstream moving fish past riverine barriers.

Conservation: The principles and practice of the science of preventing species extinctions.

Conspecific: Referring to an organism belonging to the same species.

Effectiveness (in relation to fish passage): Effectiveness is a qualitative concept and concerns whether a structure is capable of passing its target species within the range of environmental conditions observed during the migration period (Larinier and Marmulla, 2004).

Efficiency (in relation to fish passage): The efficiency of a fish pass is a quantitative description of its performance. It may be defined as the proportion of stock present at an obstruction which then enters and successfully moves through the fish pass in an acceptable period of time (Larinier and Marmulla, 2004).

Elver: The juvenile life-stage of an eel between glass and yellow eel. Individuals are larger than glass eel and pigmented.

Entrain: To pull or draw along after itself.

Fish pass: A water passage around or through an obstruction, designed to dissipate the energy in the water in such a manner as to enable fish to ascend or descend without undue stress (Clay, 1995). Fish pass is synonymous with ‘fishway’, which is the more commonly used term in North America.

Fish passage facility: A fish pass plus any additional device/structure that aids in fish passage.

Freshwater fish: Fish that live all or a critical part of their life history in fresh, inland or brackish waters, including estuaries and mangrove swamps.

Habitat: The natural environment of an organism.

Habituation: See acclimation.

Hydraulic: Referring to liquids in motion, synonymous with ‘hydrodynamic ‘.

Hydrodynamic: Pertaining to forces in or motions of liquids.

Impingement: Prolonged physical contact of a fish with a structure, usually as a consequence of non-volitional entrapment.

Interspecific: In reference to something between species.

Lateral line: Mechanosensory organ, unique to fish and amphibians, that serves to detect movement and pressure changes in surrounding water.

Migration: The round-trip, seasonal movement of organisms among two (or more) locations (Shaw, 2016).

Mitigation: The action intended to reduce the adverse impact of a specific project, development, or activity.

Rheotaxis: Movement of an organism in relation to a current of water. Positive rheotaxis describes fish that are oriented facing into the current. Negative rheotaxis describes fish that are oriented facing with the current.

Rheotactic: Of or relating to Rheotaxis.

School: A set of individuals adopting shoaling behaviours, living in a group and adopting a significant degree of synchronisation of displacements (in speed and polarity terms) resulting from social interaction between these individuals.

Self-organisation: A process in which a pattern at the global level of a system emerges solely from numerous interactions among the lower-level components of the system (Camazine *et al.*, 2003).

Shoal: A set of individuals presenting a significant degree of cohesion, limited in a relatively small portion of space, a consequence of a social interaction between these individuals.

Swim bladder: internal gas-filled organ that allows fish to control their buoyancy. The swim bladder can also function as a resonating chamber to produce or receive sound.

Utricle: otolith organ located in the inner ear.

Weberian apparatus: anatomical structure connecting the swim bladder to the auditory system of fish. It is only present in species belonging to the superorder *Ostariophysi*.

1 Introduction

In a world of finite resources, ecosystem services and their value are of increasing importance, and concern, to conservationists (Costanza & Kubiszewski, 2012; Costanza *et al*., 2014). Freshwater ecosystems cover 1% of the surface of the earth and make up 0.01% of all water worldwide (Costanza *et al*., 1997; Dudegon *et al*., 2006; Costanza *et al*., 2014) and yet contribute £1.3 trillion to the world economy every year in ecosystem services (Costanza *et al*., 2014). In many communities around the world, the freshwater environment can be a reliable source of income and sustenance (Easterling, 2007; Rice & Garcia, 2011; Merino *et al*., 2012). The human population and many, more intricate ecosystems, rely on the freshwater habitat for nutrient import and export (Dudgeon *et al*., 2006; Abell *et al*., 2015). Fresh water is also a valuable commodity; therefore, humans have modified the freshwater environment for their own gain for millennia (Beveridge & Little, 2002) creating stock ponds for aquaculture (Beveridge & Little, 2002), weirs for irrigation and nourishment of farm land and flood control (Michael, 2009), pumps for abstraction of water for human consumption (Petts, 1996) and, increasingly, generation of power through hydropower installations (Postel *et al*., 1996; Sternberg, 2008; Nelson *et al*., 2013).

Anthropogenic structures in rivers, streams and lakes provide potential hazards to fish (Schilt, 2007; Kemp & O'Hanley, 2010; Goudie, 2013) and change the nature of watercourses through reservoirs and flood-plain modification (Goudie, 2013). Mitigating for these anthropogenic threats, using current information, is a key part of supporting fish conservation worldwide through research and management. To understand how to enhance and support fish populations, these animals and the threats they face must be first understood.

Threats to fish populations can come in the form of physical barriers, such as dams and weirs, or more immediate dangers such as hydropower turbines and water abstraction pumps (Figure 1.1), (Richter *et al*., 1997; Schilt, 2007). Fish can be impinged against a trash rack or bar screen (Figure 1.2) that was designed to protect them from the turbine housing or entrained in a turbine or pump. Smaller fish and those that are migrating down-stream are at particular risk of entrainment where traditional mitigation technologies are deployed

(Godinho & Kynard, 2009). These dangers pose immediate risk of injury or mortality, but barriers are also a serious threat as they can prevent migration of fish within a river system which can affect abundance due to reduced spawning activity or impeded access to nursery and feeding areas (Caudill *et al.*, 2007; Matzinger *et al.*, 2007; Agostinho *et al.*, 2008). An unintended consequence of blocking fish movement within a river system is increased predation, reduced access to good habitat, and reduced individual fitness where fish aggregate in large numbers at a barrier. Poor implementation of mitigation technology can also decrease individual fitness and recruitment; this is known as an ecological trap (Pelicice & Agostinho, 2008).

Figure 1.1 Low-head irrigation dam in the Pacific North-west of the USA. This dam provides a barrier to fish passage if no passage structures are implemented.

In the United Kingdom (UK) there are high and low head anthropogenic barriers that have the potential to halt migration pathways or delay groups of fish in a single location (Kemp & O'Hanley, 2010). There are also hydropower turbines and water abstraction pumps that pose a risk of mortality or injury to aquatic vertebrates. It is therefore important that these anthropogenic threats are mitigated, and that research is conducted to reduce the risk of the threats listed above.

Fisheries scientists have traditionally attempted to mitigate for the threats outlined above by providing bypass channels and alternative routes around these threats and introducing physical barriers, such as screens (Michaud & Taft, 2000; Lemasson *et al*., 2008; Noonan *et al*., 2011). These techniques often involve further modifying the aquatic environment to provide fish with an alternative means of navigating a hazard, but such methods are not always successful (Noonan *et al*., 2011; Kerr *et al*., 2015;). Fish may miss the bypass channel or display rejection behaviour at the mitigating structure. Hydraulic engineering is also used to improve the efficiency of these bypass facilities, but it is not always entirely effective (Piper *et al*., 2012).

Modifying the aquatic environment from a hydraulic perspective also has negative consequences for the power and water companies that operate the dams, weirs and turbines. Finely spaced barriers prevent fish from seeking entrance to a turbine, but they can become clogged with debris from the water column quickly and can reduce water flow rates, even when not clogged, depending on the size of the gaps between bars. Changing the orientation or position of an intake can limit the chances of fish becoming impinged by it but that also comes at the cost of a reduced water intake rate. The cost of large-scale structural changes to dams and abstraction points also reduces the likelihood of these measures being put into practice by power and water companies. To address this issue fisheries scientists are increasingly turning to other mitigation technologies; the most popular of these is the behavioural barrier (Zielinski *et al*., 2014).

Behavioural barriers in aquatic environments are a currently employed technology (Scruton *et al*., 2003; Taylor *et al*., 2005; Zielinski *et al*., 2014). This is partly due to high maintenance and installation costs of conventional barriers around water abstraction points and hydropower facilities (Schilt, 2007); these barriers can also cause high mortality rates in fish. For the above reasons, there is increasing interest in alternative methodologies that can be used to mitigate for negative anthropogenic effects on fish. The main fields that are being investigated rely on the sensory capabilities of the fish themselves; these are electric, magnetic, acoustic, hydraulic and light stimuli. These different approaches can be deployed independently or combined with other techniques to reduce mortality at barriers, intakes and turbines (Figure 1.2), (Popper & Carlson, 1998; Zielinski *et al*., 2014). However, because these forms of mitigation measures rely on eliciting a behavioural response, it is important that the mechanism by which the behaviour of fish changes is understood.

Reception of stimuli by anatomical features of a fish is largely understood (electroreception, sight, hearing etc.). However, the sensitivity of different fish species, and their reactions to these stimuli, is not fully understood, other than that it varies greatly between fish species (Zielinski *et al*., 2014). The response of fish to the varying levels of stimuli that they can receive also requires more research. To ensure the effectiveness of behavioural barriers, the response of fish to given stimuli needs to be quantified.

Figure 1.2 Impingement of fish at a screen. Notice the fish pressed against the screen in the upper section of the photograph and the fish swarming in the lower section. (Credit FAO)

Behavioural deflection methods rely on fish trajectories of movement being changed by the stimulus. Hydraulic mitigation can manipulate these trajectories in a waterway either by changing the flow to force a fish in a certain direction, or to elicit a behavioural response from the fish such as upstream migration or rejection of accelerating flows (Russon & Kemp, 2011; Piper *et al*., 2012). Electroreception is typically associated with sharks and the marine environment in terms of behavioural barriers (Hart & Collin, 2015). However, electric signals in water also form the foundation of electrofishing, a recognised and widely used fisheries tool (Roset *et al*., 2007).

Much of the work carried out so far in fisheries science concerning mitigation methods has focused on the hydraulic element of a waterway. It is thought that the other stimuli discussed previously can cause similar changes to fish placement, but the mechanism by which they effect change will be mainly behavioural and not a physical obstruction; an obvious exception to this would be the Bio-Acoustic Fish Fence (BAFF), (Figure 1.3) sometimes referred to as an acoustic bubble curtain (Terry *et al*., 2005; Perry *et al*., 2014). The BAFF is thought to work by not only providing an acoustic stimulus, but also by manipulating the hydraulic flow of the system in the upwelling of the bubbles and the restriction of the fish's vision through the line of bubbles (Patrick *et al*., 1985; Zielinski & Sorensen, 2015). In this way the BAFF can be considered, from a behavioural point of view, a semi-physical barrier. The acoustic element of BAFFs and other behavioural devices is still yet to be fully quantified (Terry *et al*., 2005; Perry *et al*., 2014; Bowen, 2015).

Figure 1.3 Operating bubble curtain at a diversion channel. The path of the bubbles is visible spreading from the lower right-hand corner of the photograph and spreads upstream. The purpose of the barrier is to divert fish into the bypass channel at the bottom left of the photograph.

Using acoustics as a tool to manipulate the behaviour of fish is a relatively new field (Popper & Carlson, 1998) and relies heavily on published audiograms (Kenyon *et al*., 1998) that quantify the extent of hearing in certain fish species. These audiograms do not, however, demonstrate the level to which a fish will react when in the presence of a given acoustic signal. Because of the nature of the acoustic wave and its ability to travel through water quickly, and largely without interference from external factors such as chemical cues, temperature and turbidity (Kinsler & Frey, 1962), the auditory system can provide the clearest information among the senses used by fish (Popper & Coombes, 1980; Popper *et al*., 2003a; Rogers & Cox, 1988). It should be noted that due to these properties the sound field in an aquatic environment can be complex and has the potential to be confusing.

Therefore, the design of the sound field should be considered and mapped in any potential research, as there is a lack of finely measured acoustic fields in current literature (Popper, 1972; Bui *et al*., 2013; Perry *et al*., 2014; Bowen, 2015).

A fish's ability to sense and communicate using sound has been the topic of many papers (Popper & Coombs, 1980; Jerkø *et al*., 1989; Kenyon *et al*., 1998; Scholz & Ladich, 2006; Popper & Fay, 2011) and the precise level of hearing in some fish has been firmly established (Jerkø *et al*., 1989; Scholik & Yan, 2001). Existing knowledge of fish hearing and its potential for behavioural manipulation in aquatic environments has made acoustics a key area in fisheries management and guidance methods (Popper & Carlson, 1998; Sand *et al*., 2000; Simmonds & MacLennan, 2008). However, there is a gap in the number and variety of fish species for which there is a published audiogram (Kenyon *et al*., 1998; Ladich, 2014; Bhandiwad & Sisneros, 2016).

To date, research has primarily focused on the impacts of acoustic stimuli on an individual fish, and not on multiple fish (Slabekoorn *et al*., 2010; Popper & Fay, 2011). This is important to note as many species of fish are most frequently encountered in schools and aggregations. These aggregations may occur both naturally and as a result of bunching at anthropogenic barriers (Keenleyside, 1955; Pitcher, 1986; Fukushima *et al*., 2007). Knowledge of how fish react to acoustic fields is vitally important in developing applied management tools and also in understanding how fish react to current passage structures due to the noise they can generate through vibration and turbulence (Hawkins & Popper, 2016). The introduction of groups as a variable may affect the measured reaction of fish under acoustic treatment; this has not been tested in previous research despite having the potential for important management implications as many fish exist in groups at sites where mitigation technologies are used.

This research will focus on decreasing the impact of the anthropogenic threats faced by fish in aquatic environments by increasing the knowledge of fish behaviour when they encounter mitigation technology. This research seeks to enhance and support fish populations by contributing to knowledge so that more effective management strategies and devices may be deployed in the future. This PhD will therefore focus on acoustic stimuli and how these affect the behaviour of fish in groups.

1.1 Aims and objectives

Many of the studies that have been conducted so far have focused on the reaction of one fish to an acoustic stimulus (Jerkø *et al.*, 1989; Sand *et al.*, 2000; Zielinski & Sorensen, 2015). The aquatic environment is a complex one but introducing more elements to an artificial environment will help develop knowledge and hence conclusions that are more appropriate to field situations as they will be closer to those experienced in-situ. The interactions between fish in a school may affect their response to stimuli; it is for this reason that the affect acoustic stimuli have on a school will be tested. Knowledge of individual fish response to an acoustic stimulus cannot be applied to an in-situ situation if that fish would naturally school or be encountered in groups. It is for this reason that the research will be conducted.

The knowledge gained could eventually be used to develop an acoustic deterrent that may be deployed at anthropogenic structures in aquatic environments that pose a threat to fish. The ultimate aim of this research is to quantify the difference in response of individuals and groups of fish to acoustic cues. There are important fisheries management implications that will come from this research, as many acoustic deterrent devices currently on the market for hydropower companies have little available quantified data behind them (Michaud & Taft, 2000; Taylor *et al.*, 2005; Schakner & Blumstein, 2013).

There are a number of problems caused by anthropogenic structures in aquatic ecosystems that are faced by current fish populations. Fish mortality due to impingement and entrainment at water abstraction points and hydropower installations is a problem for many fish species (Cada *et al.*, 1997), but this is not the only danger these facilities pose. Blocking of migratory pathways is another problem for fish (Dudgeon *et al.*, 2006), some of which only require a limited migratory range within a river system. Secondary mortality at migratory barriers and intake stations is another problem that occurs when large numbers of fish congregate in the same area due to a barrier. Predators and disease can quickly reduce the numbers of fish present in the entire reach of a river if all the fish congregate in a single area (Dudgeon *et al.*, 2006).

The blocking of migratory pathways, and impingement and entrainment, are such a threat for some species, such as the European eel (*Anguilla anguilla)*, that their population numbers are suffering as a result (Calles *et al*., 2010). Barriers to migration, impingement and entrainment are also a threat to fish that display schooling behaviour (Enders *et al*., 2009). Schooling interactions between fish can affect age class sizes due to breeding and spawning success, chance of predation and foraging success (Paull *et al*., 2010; Katz *et al*., 2011, Vasconcelos *et al*., 2011). If a migratory pathway is blocked but can be navigated by other means, such as a bypass facility, then it is possible that the chances of successful identification and navigation of a bypass will be different for schools of fish compared to individual fish, perhaps due to differences in exploration. In the wider scientific literature, there is also a lack of information regarding the difference in animal, specifically fish, behaviour in group settings. The reaction of animals to acoustic treatment when in aggregations has the potential to be different to that of solitary animals and so this behaviour needs to be understood. The aim of this thesis is therefore to enhance scientific understanding of how fish react to acoustic fields by answering the question highlighted below in section 1.1.1.

1.1.1 Research question

The main research question addressed by this PhD is ""To what extent is the behaviour of individual and shoals of fish different in response to acoustic stimuli?". This question will drive the topic of research towards understanding the effect sound has on the behavioural reactions of fish. In future work this knowledge could inform better experimental design for research into fish behaviour in response to acoustic stimuli. This research could also be used to develop acoustic deterrents that can be strategically deployed at anthropogenic structures e.g. water abstraction points and hydropower inlets. Finally, the findings presented in this thesis enables a better understanding of how anthropogenic noise pollution affects behavioural change in fish. The research question will be answered by following the objectives outlined next, in section 1.1.2.

1.1.2 Objectives

The objectives of this PhD research are as follows:

1. To quantify an acoustic field within a flume tank.
2. To quantify reaction to the acoustic field of a shoaling fish, through video tracking within a flume tank, individually and as a group.
3. To compare the fine-scale behaviours of individual and groups of fish in response to acoustic treatment.
4. To determine the effect the acoustic field has on the behaviour of fish.

1.2 Thesis overview

The introduction to this thesis (chapter 1) has briefly highlighted the value of freshwater ecosystems worldwide and summarised some of the anthropogenic threats that fish within these systems face. Traditional screening techniques have also briefly been discussed in relation to the literature and a conclusion, with the aim and objectives of this study reached. This thesis will now review the literature (chapter 2), covering collective behaviour and fish group behaviour. Physical acoustics will also be discussed alongside hearing in fish. The materials and methods required to answer the research question (1.1.1) will be explained in chapter 3. Work completed in pursuing these objectives is then documented in chapters 4, 5 & 6. The findings of these pieces of research are discussed individually at the end of these chapters (4, 5 & 6). Chapter 7 discusses the relationship between the findings in chapters 5 & 6. The thesis concludes, at the end of chapter 7, with a discussion of future research options, and the potential impact this research may have for the wider scientific community.

2 Literature review

Knowledge of fish physiology, ecology and general behaviour is essential in conducting the research for this PhD. However, anthropogenic structures in the aquatic environment can reduce individual fitness and recruitment and force fish into groups and aggregations. The ability to manipulate the behaviour of these fish could result in reduced mortality at these high-threat sites through increased efficiency of behavioural deterrents (Noatch & Suski, 2012). If fish react differently to these behavioural deterrents when in groups, as opposed to individually, then this needs to be accounted for in their design and implementation. Therefore, understanding the links between anthropogenic activity, fish physiology and collective behaviour will ultimately lead to the creation of tools that can be used to mitigate for anthropogenic disturbances in aquatic environments.

In this literature review there will be a focus on collective behaviour. This will be discussed in a wider context alongside collective behaviour of fish. A short summary of the fish at the centre of this research will also be provided. Threats in the aquatic environment and how they are mitigated for are examined and physical and underwater acoustics will be briefly discussed in conjunction with hearing in fish and its application to mitigation technologies.

2.1 Collective behaviour

In nature many animals exist in groups or aggregate at certain points in their life cycle (Davies *et al.*, 2012). Understanding the interactions that occur between individuals in these groups and how their behaviour changes when in an aggregation is key to understanding how the group as a whole, functions and reacts to opportunities and threats.

Collective behaviour and the various advantages and disadvantages that grouping can provide an individual has been the subject of a large quantity of research (Couzin *et al.*, 2002; Krause & Ruxton, 2002; Robbins *et al.*, 2013). With human behaviour, literature traditionally focuses on psychological influences and impacts on a group; lesser importance is given to sensory factors (Wagner & Hollenback, 2014). In modern animal based behavioural studies there is generally a greater focus on sensory factors as a deeper psychological analysis is not always possible (Carter *et al.*, 2013; Buss, 2015). Animal group behaviour has been studied in numerous ways either in-situ or ex-situ. The driving factor behind this in many cases is the ease with which the data can be obtained without clouding or influencing any signal the animals give or receive and how important contributing factors, influences and behaviours are deemed to be (Nelson, 1998; Krebs & Davies, 2009). Many animals are frequently observing for potential threats and opportunities (feeding, mating etc.). In-situ studies can manipulate these threats and opportunities to focus on the reaction of an animal and to explore the behaviour of an individual or a group within its wider context (Bytheway *et al.*, 2013).

Certainly, there are behaviours that animals display that change when in a group (Davies *et al*. 2012). The range of behaviours and influences an animal can display in this context is wide: from anti-predator types, such as vigilance (Childress & Lung, 2003; Creel *et al.*, 2014), and dilution effects through group size (Dehn 1990), to foraging efficiency (Charnov, 1976; Day *et al.*, 2001; Creel *et al.*, 2014), information transfer (De Bie *et al.*, in press), hunting (Whiteside *et al.*, 2015) and mate selection (Thornhill & Alcock, 1983; Krebs & Davies, 2009). The behaviours animals choose to exhibit affect factors such as foraging efficiency and predation risk (Lima *et al.*, 1985). The environment that an animal is in can also affect the behaviours displayed. In high flow regimes some fish display increased dominance over their conspecifics; it is assumed that this is for a hydraulic benefit (reduced drag), although recent research argues that this may not be for hydraulic benefit, but to increase information transfer between individuals (De Bie *et al*., in press).

Avoidance of an unwelcome stimulus is a well-recognized group behaviour, but it is not unique to groups (Morse, 1977; Durant, 2000; Childress & Lung, 2003; Creel *et al*., 2014). Individuals display avoidance behaviour when confronted by an unwelcome stimulus (Durant, 2000), although, their reaction time and directional response frequently differs from that observed in group behaviour (Deneubourg & Goss, 1989; Gueron *et al*., 1996). Reaction time and directional response varies according to the level of individual vigilance (an animal's anti-predator precautionary behaviour) whether in solitary conditions or in a group. Some animals even involve groups containing different species in their vigilance routine (Pays *et al*., 2014). It is vigilance that many behavioural scientists exploit when attempting to modify an individuals' learned behaviour.

It is assumed that most individuals, whether in a group or not, display some level of vigilance (Beauchamp, 2001; Creel *et al*., 2014). This is especially true of lower-trophic animals such as elk (Childress & Lung, 2003) and prey fish (Pitcher, 1986). This vigilance level varies with group size (Roberts, 1996, Godin *et al*., 1988; Childress & Lung, 2003) as dilution effects change survival rate of an individual within a group in the event of a predator encounter (Morgan & Godin, 1985; Dehn, 1990; Roberts, 1996). It is then logical to assume that the instantaneous effect a stimulus has on an individual changes with the size of the group that the individual is in. These behaviours therefore affect how individuals behave and how the group responds to a stimulus.

Vigilance may assist an individual in avoiding a predator, but it is not the only important behaviour that individuals require to survive. Foraging and hunting are other group behaviours which are key to an individual's survival (Creel *et al*., 2014; Whiteside *et al*., 2015). Prey animals must be vigilant to avoid being hunted but they must still forage for food; finding the optimum trade-off between these two conditions is vital for survival (Thaler *et al*., 2012). For apex predators, vigilance is of a lower priority (Ordiz *et al*., 2013); however, other behaviours then become more important. Hunting, a type of foraging, is a behaviour that such predators exhibit by pursuing and ambushing prey (Scheel & Packer, 1991; Creel & Creel, 1995; Dickman & Newsome, 2015). Ambush predators frequently hunt alone due to the risks associated with prey detection and competition for food (Benhaiem *et al*., 2008); although research has highlighted that, in some species, there is information transfer occurring even though the individuals hunt alone (Clark, 2007). Predators that chase and trap their prey can work alone, such as the domestic house cat *Felius catus* (Dickman & Newsome, 2015), but they can also work in groups, for example the African Wild Dog *Lycaon pictus* (Creel & Creel, 1995). Hunting in groups is beneficial for these animals if they co-ordinate their actions and work together using their experience and cues from their conspecifics (Scheel & Packer, 1991). The threat of being hunted induces a reaction from the prey animal (Benhaiem *et al*., 2008; Schultz & Kruschel, 2010; Lone *et al*., 2015). This reaction is of particular interest to ecological managers as it might be possible to manipulate it to deter animals from certain spatial zones (Howery *et al*., 2013; Greggor *et al*. 2014; Polajnar *et al*., 2015). Behaviour of groups can also change when under threat of predation (Hager & Helfman, 1991).

Exploration is another key behaviour to consider when examining the ecology and management of an animal or ecosystem. Exploration is defined as the area that an animal occupies in a specific period of time. In experimental work where an animal is exposed to a treatment it is important to consider the exploration of an animal through the experimental area; this is discussed in more detail later in this thesis (chapter 4).

Among the animals commonly found in groups, or more organized collectives, birds and fish are commonly the subject of research. For this particular research there will be a focus on fish and their collective behaviour. This is because of the value of fish populations worldwide (Costanza *et al*., 1997) and the potential implications of this work on management strategies that can assist in mitigating for anthropogenic threats. For this research to take place it is important first to define what a school of fish is, and then its place within fisheries ecology.

2.2 Schooling behaviour

The terms school, shoal, aggregation and group are used to describe a collection of fish. To continue the discussion of the collective behaviour of fish these terms must first be defined. A shoal is defined as a group that remain together for social reasons (Pitcher, 1983; Delcourt & Poncin, 2012). This differs from a school which is defined as a group swimming in a synchronised and polarised manner (Pitcher, 1983; Delcourt & Poncin, 2012). Groups and aggregations are considered to be less organised collections of animals with aggregations retaining some form of interaction between conspecifics and a group consisting of more than one individual of a given species that are spatially and temporally assembled (Krause & Ruxton, 2002). For the purposes of this research a school of fish is representative of what would occur at a barrier such as a hydropower installation or a waterfall. The difference in behaviours between an individual and a shoal will be the foundation of this research.

A group of fish is often considered as a school but there are differences in behaviour between the two (Pitcher, 1986). A school of fish may react to an unwelcome stimulus by avoiding it; this will usually involve a locomotive effort and then a propensity to avoid the area where the unwelcome stimulus was first experienced (Verheijen, 1956). A group of fish, however, may not share this avoidance as a collective; they may avoid the area, but their behaviour may not otherwise change (Pitcher, 1986; Viscido *et al.*, 2004). It has also been shown that groups of fish demonstrate inspection behaviour when in the presence of a predator or other danger (Magurran, 1986). A school will change its dynamics in the presence of an unwelcome stimulus; this may involve changing orientation or proximity relative to each other (Krause, 1993; Godin, 1997). These are metrics that will be used during the analysis of the experiments as described later in this thesis (section 4.5).

Many fish species exhibit social interactions and exist in large congregations and schools, even if only at certain points in their life cycle (Pitcher, 1986). These group interactions have the potential to influence how fish identify, approach and react to bypass facilities and other physical and behavioural barriers. A large percentage of the information that currently exists regarding the behavioural response of fish to sound focuses on individual fish (Jerkø *et al.*, 1989; Sand *et al.*, 2000). Combining a knowledge of fish reaction to sound and group behaviour is therefore key to developing an understanding of behavioural mitigation tools in the aquatic environment. Consequently, it is important to understand what effect acoustic stimuli have on fish that are acting in schools. This will prove to what extent schooling interactions change a fish’s reaction to an acoustic stimulus. Fundamentals-based research will provide the fine scale information required to enhance general understanding of collective behaviour in the aquatic environment.

2.3 Underwater acoustics

An understanding of physical and underwater acoustics will help to complete the research in two ways. Firstly, it provides an understanding of what is happening in aquatic acoustic environments in relation to the physical movement of sound waves and their effects. Second, this knowledge helps the researcher to consider how the physical and acoustic environment will influence the behaviour of any fish involved in the experiments.

2.3.1 Physical properties of the acoustic wave

An acoustic wave is a form of energy that is transmitted mechanically by compressing and expanding the medium it is travelling through (Kinsler & Frey, 1962). The number of compression/rarefaction cycles measured at a spatially fixed point, per second, represents the frequency (f) of the wave and is expressed in Hertz (Hz). The length of this cycle in the spatial domain (λ) is referred to as the wavelength and is related to f by $\lambda = c/f$ where c is the speed of sound in the medium in which the wave is propagating.

There are two properties of this wave that are interesting in fisheries acoustics. Firstly, the wave consists of fluctuations of acoustic pressure that propagate from the point of creation (Kinsler & Frey, 1962). Second there is particle motion; this is the movement of particles during the compression and rarefaction stages of the acoustic pressure component of the wave (Figure 2.1), (Kinsler & Frey, 1962). It is important to differentiate these two parts of an acoustic wave as fish perceive them in different ways, or not at all (depending on the hearing capabilities of the particular fish). It is also important to note that in underwater acoustics the wave propagates at approximately 1500 m/s which is roughly 4.3 times faster than in air (Kinsler & Frey, 1962).

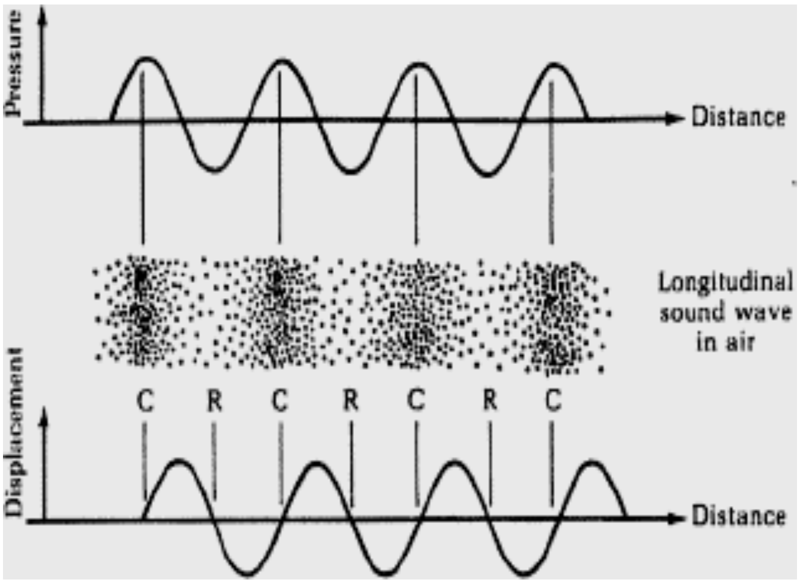


Figure 2.1 The pressure and particle motion components of an acoustic wave. Notice the periods of compression and rarefaction as indicated by the C and R. (Credit – John Askill)

Throughout this research an experimental area will be mapped under treatment to build up a spatial understanding of the acoustic environment. This will be completed by using a hydrophone and plotting sound pressure levels (SPL) in decibels (dB) against a location (Figure 2.2). This map will only display the acoustic pressure component of the acoustic wave, due to the relationship between acoustic pressure and particle motion (Kinsler & Frey, 1962). It is a reasonable assumption that a map of particle motion of the same field would look similar, but with opposite values.

2.3.2 Room acoustics and its role in fish/turbine interactions

The way acoustic waves respond to physical barriers, such as dams and bypass channels, is important for understanding and manipulating fish behaviour in the aquatic environment. At a boundary the energy from the acoustic wave is both absorbed and reflected; the extent to which it is absorbed depends on the material the boundary is constructed from and the incident angle of the wave front. The reflected waves can create a highly complex acoustic field in the vicinity of an obstacle, depending on its shape, structure and position (Leighton, 2012). In the case of pure tones, sound cancellation may occur leading to quieter zones of low acoustic pressure; however, reflected waves may also lead to areas of increased intensity (Leighton, 2012).

This difference in reflected noise between a general acoustic wave and that of a pure tone of a single frequency is important to note as it may allow fisheries scientists to create deterrent and guidance devices that are customised to the hearing of the target species. For example, pure tone noise cancellation can be used to create areas of low acoustic pressure relative to the surrounding acoustic field but with high particle motion. This may allow the diversion of target fish species away from turbines and other types of intakes.

The impacts of room acoustics and the difficulties of recording and playing sound to conduct experiments in small water tanks is well documented and discussed (Rogers *et al.*, 2016). Within this thesis, each piece of experimental work quantifies the sound field within the tank before conducting any trials. The methods for this are discussed in more detail in chapter 3. Having a better understanding of the sound fields enables informed discussion as to what the fish within the experimental structures have experienced. This is discussed in more detail in relation to the findings within each of the results chapters (4, 5 & 6). This has also lead to the fine-scale mapping of Sound Pressure Levels (SPL) and Particle Acceleration (PA) at a range of frequencies and depths to ensure any anomalous features are identified and their role in invoking a behavioural response is considered.

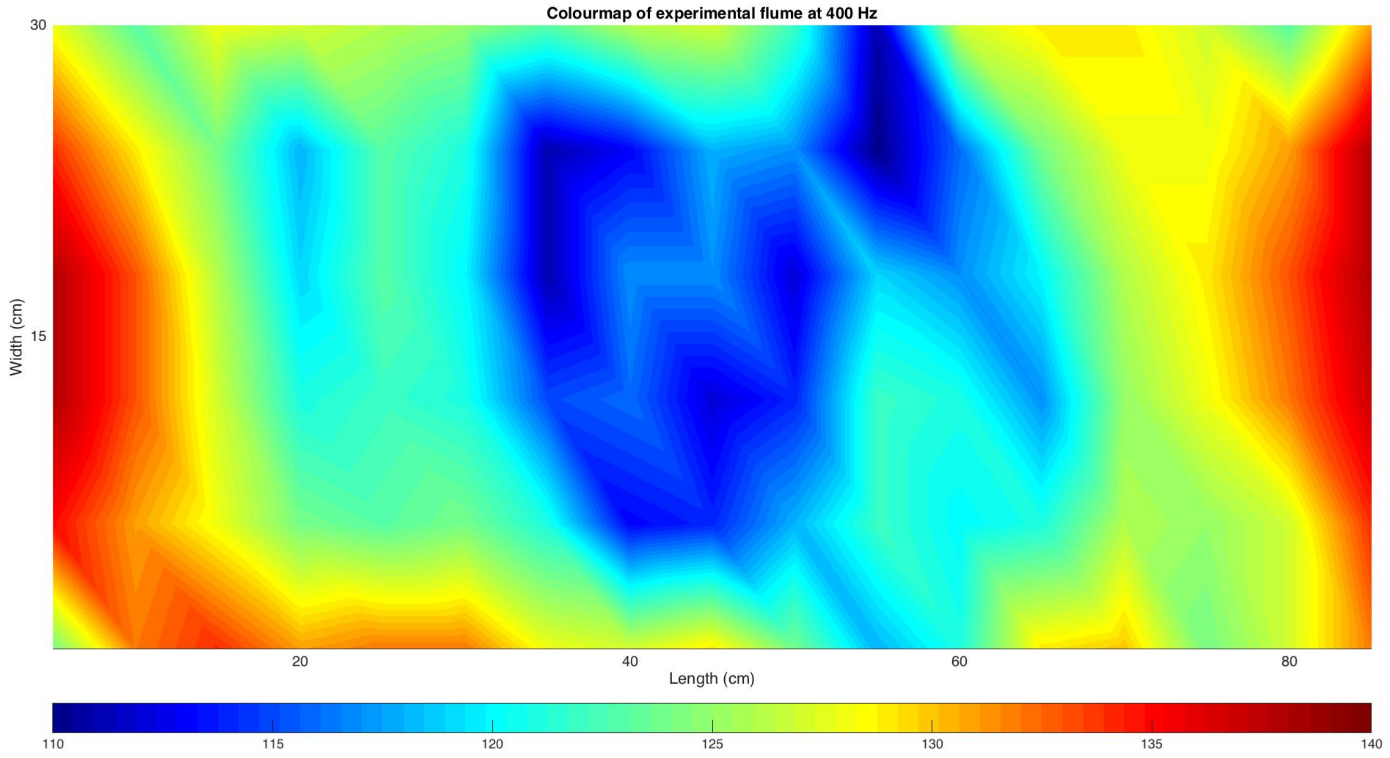


Figure 2.2 SPL Colour Map showing sound pressure level in dB re 1 μPa against location plotted. The map was taken from an acrylic tank with a water depth of 26cm. The map was measured at 8cm above the bottom of the tank and the scale on the X and Y axis is in cm. The colour map is plotted in dB as calculated by the code in MATLAB as discussed in chapter 3.

2.3.3 Characteristics of anthropogenic noises in freshwater

Although this review has so far concentrated on group behaviour and underwater sound as a controlled phenomenon, it is important to consider the nature of existing underwater sound, as driven by humans. Throughout the remainder of this thesis, this type of sound will be referred to as anthropogenic sound or anthropogenic noise. This is also a good point in the thesis to define the differences between noise and signal. Although both may be considered sound, noise is defined as any undesired sound that is either unwanted or interferes with the desired acoustic environment (Carlson, 1968). Whilst a signal is defined as constituting a desired part or parts of a soundscape that may have the potential to contain information or to aid in communication (Carlson, 1968).

The structure of the aquatic systems that we are interested in also plays an important part of this picture. The anthropogenically modified and engineered nature of most river systems in the UK and wider (Kemp and O'Hanley, 2010), (Figure 2.3) means that increasing consideration should be given to how these modifications may impact the acoustic environment of (typically shallow) freshwater habitats (Leighton *et al.* 2019). In addition to this, the modifications and anthropogenic structures around these environments mean that anthropogenic noise is far more likely to be generated (Slabbekoorn *et al.* 2010). Finally, compounding this issue is that these engineered environments couple the aquatic and terrestrial environment in a much more efficient manner than in a natural river system (Leighton and Evans, 2008). Whilst the increase in attention to this area of shallow, freshwater acoustics and the impact of anthropogenic sound is welcome (Vračar and Mijić, 2011). There is still a long way to go in understanding the impacts of anthropogenic driven noise pollution on fish, their behaviour and subsequent consequences.

Another consideration for the freshwater environment is that the shallow (less than 20 metres) and typically narrow (less than 50 metres) nature of rivers and streams, combined with the prevalent engineered banks and other structures (Figure 2.3) mean that fish that are exposed to noise disturbance are in close proximity to the source of the radiating feature (Leighton *et al.* 2019). This may be particularly problematic for various fish species depending on their specific hearing adaptations (section 2.4) and whether or not they are able to migrate away from the disturbance.

Figure 2.3 A photograph displaying the engineered nature of the Itchen Navigation, the site of collection for the subject fish species in this thesis.

2.4 Hearing in fish

Interpreting their surroundings and detecting threats and opportunities is key for all animals (Godin, 1997; Durant, 2000; Sand *et al*., 2000; Childress & Lung, 2003). Fish use sound in a number of different ways, such as communication and to detect predators and prey. Fish can perceive their environment using different components of the acoustic wave and they communicate across large distances using signals of different wavelengths (Popper *et al*., 2003a). There are two main parts to an acoustic wave (see section 2.3.1) and fish are capable of detecting these two components using two different physiological features (Fay & Popper, 2000). The first of these features is common to all fish (Fay & Popper, 2000) and is called the lateral line system. This system is used to detect particle motion and consists of an internal canal system and a series of sensory hair cells called kinocilia inside a dome-like structure called the cupula (Figure 2.4), (Platt & Popper, 1981). This system detects changes in the movement of the water surrounding the fish. Some fish have systems which are more complicated than others and can detect movement from multiple directions and dimensions (Platt & Popper, 1981; Fay & Popper, 2000).

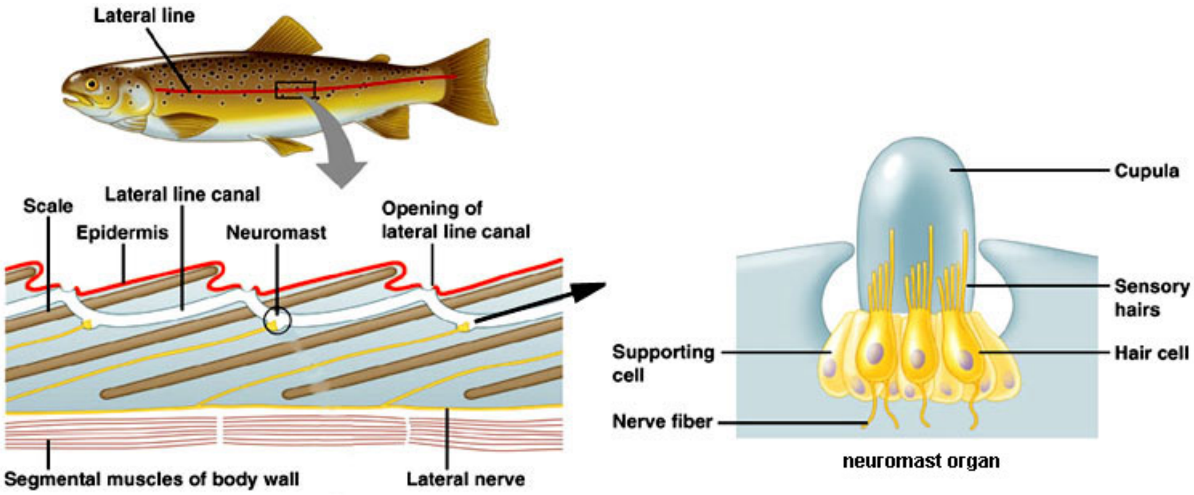


Figure 2.4 Structure and placement of the lateral line system of a salmonid. (Copyright – University of Miami, Department of Biology)

The second of these features is a system that is not dissimilar to the hearing structures in other animals, the inner ear. This system consists of three semi-circular canals called the anterior, posterior and horizontal canals (Platt & Popper, 1981). These canals surround a sac containing endolymphatic fluid called the utriculus (Platt & Popper, 1981). The second part of this system consists of two further sac structures attached to the utriculus, called the sacculus and the lagena, these are also filled with endolymphatic fluid (Platt & Popper, 1981; Popper & Platt, 1983). The three sac like structures also each contain an otolith or otolithian masses (Figure 2.5) (Platt & Popper, 1981) resting on a group of kinocilium that relay vibrations via nerve endings to the brain centres (Platt & Popper, 1981). Otoliths are bony disc-shaped structures, but they are not the only way that fish can detect acoustic vibrations in the water.

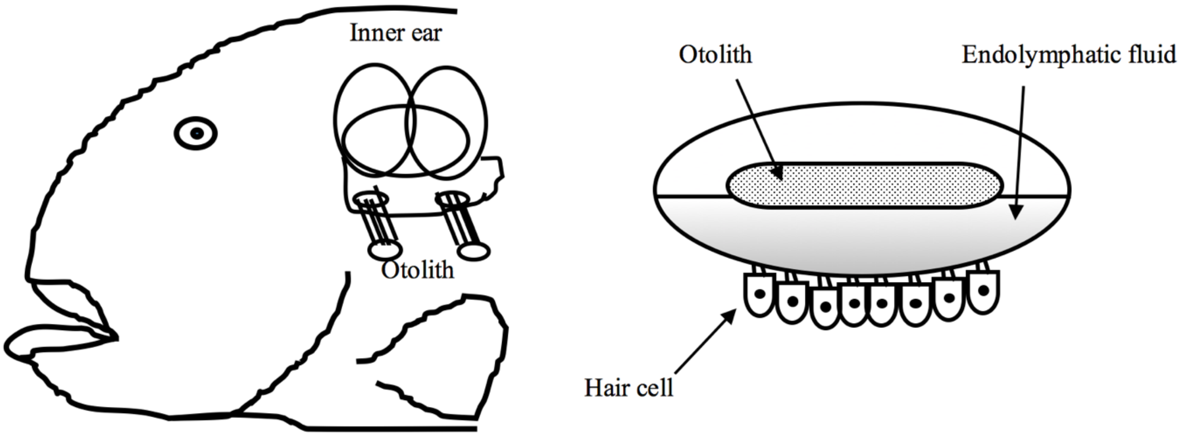


Figure 2.5 Placement of the Otolith bones within the head of a fish. Notice the endolymphatic fluid surrounding the otolithian bone.

Fish with advanced hearing have a more highly developed inner ear (Popper, 1971; Platt & Popper, 1981; Fay & Popper, 2000). Although the basic structure of the inner ear remains similar to that discussed above, there is the addition of structures that link the swim bladder to the inner ear (Popper, 1971; Fay & Popper, 2000). These structures, called Weberian ossicles, are key to the more advanced hearing that some fish possess. The linking of the swim bladder to the inner ear via these ancillary structures allows the fish to detect much less intense sounds than the inner ear alone allows, because the air in the swim bladder is far more compressible than the endolymphatic fluid contained in the inner ear. Compression and rarefaction caused by an acoustic wave produce more of a change in state in the swim bladder than in the endolymphatic fluid which has a similar bulk modulus to the surrounding water. This means the fish is more sensitive to changes in acoustic pressure than fish which rely on the inner ear alone.

Not all fish have both features and therefore some are not capable of hearing such a wide range of frequencies as others (Platt & Popper, 1981; Fay & Popper, 2000). Typically, these differences in fish hearing are described by dividing fish into two groups (Smith *et al.*, 2004; Popper & Fay, 2011). The first group, the hearing generalists, are capable of detecting changes in particle motion (section 2.2) and are therefore more capable of detecting lower frequency sound waves than the second group of fish, the hearing specialists (Smith *et al.*, 2004). These fish are capable of detecting changes in acoustic pressure in addition to changes in particle motion. This means that such fish are capable of detecting acoustic waves of much higher frequency than hearing generalists.

The terms hearing generalist and specialist have become less popular as the boundaries between the two groups have become more blurred (Popper & Fay, 2011). It is currently accepted that, despite having only two main methods of hearing, audiograms prove there is more of a spectrum of hearing abilities than two distinct groups. This spectrum of abilities includes the frequencies fish can hear and the intensities at which these frequencies become audible (Popper & Fay, 2011).

One way fisheries scientists measure the hearing ability of a fish is by using a technique called Auditory Brainstem Response (ABR). This technique involves placing electrodes on the head of a fish, playing sounds of various frequencies and intensities and then measuring any change in brain activity (Kenyon *et al.*, 1998). From these data an audiogram (a chart with frequency along the X axis and hearing threshold on the Y axis) is produced. This is useful for identifying which frequencies a fish can hear but it does not indicate how the fish reacts to these different frequencies.

2.5 Previous experimental work concerning fish and sound

2.5.1 Audiograms and behavioural audiograms in understanding fish behaviour

As described above, ABR can be used to detect what fish are capable of hearing (Kenyon *et al*. 1998) and involve a relatively simple experimental setup (Figure 2.6).

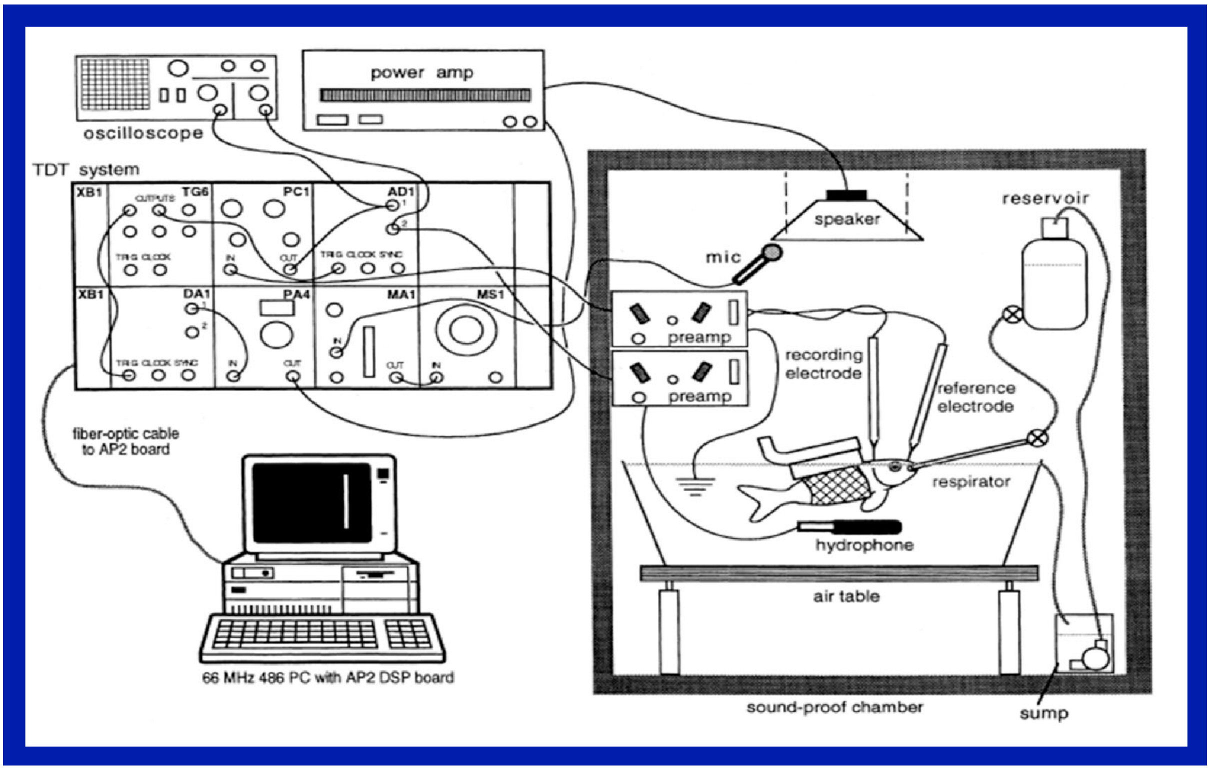


Figure 2.6 A diagram of an Acoustic Brainstem Response (ABR) experimental setup. (source: Kenyon *et al.* 1998).

The great advantage of conducting ABR experiments is that audiograms for numerous different species (aquatic and terrestrial) can be produced and compared to examine where intersectionality of hearing may occur (Figure 2.7). This is particularly relevant for fisheries managers who may to wish to use this data to design deterrents. However, consideration should be given to how various fish hearing sensitivities may overlap (Chapter 7). A disadvantage of this method of research is that, aversion to or behavioural response to the stimulus is not noted. Behavioural audiograms go some way toward showing how fish react to different sound frequencies (Ladich & Fay, 2013) but they can be limited in terms of the size of area that a fish is allowed to explore and in terms of the uniformity of both the acoustic field and the overall intensity of the sound. An experimental design is needed that attempts to quantify fish behavioural response to a more realistic acoustic environment. This same design could also be used to explore the difference in placement choice between schools and individual fish to see if there is a more complex behavioural system in a school other than the desire to avoid unwelcome acoustic stimuli. It is important to isolate these behaviours to understand how they influence the fundamental behaviours at work in their reactions. Once these fundamentals are understood, additional stimuli can be added until research can be conducted in-situ with an accurate focus on behavioural response to acoustic stimuli and not multiple cues; as in-situ, fish will always be reacting to multiple cues, but some cues will cause a stronger reaction than others.

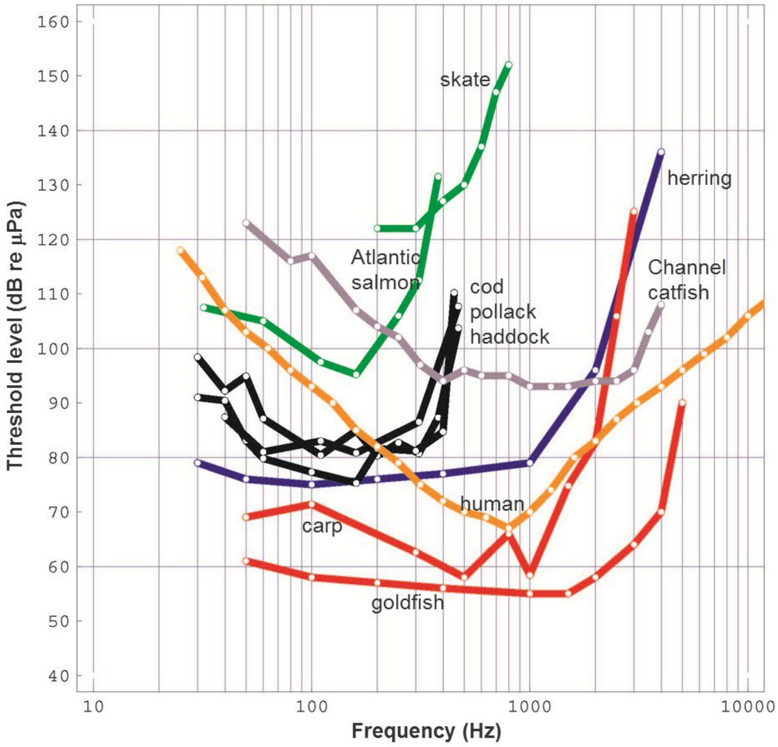


Figure 2.7 An audiogram plotting the hearing response of a range of fish species plus a human hearing response measurement. (source: http://www.geoexpro.com/articles/2011/03/marine-seismic-sources-part-viii- fish-hear-a-great-deal)

Audiograms and behavioural audiograms are also limited in terms of the number of replications (and therefore representatives from a particular species) that are needed to deem them reliable (Leighton *et al.* 2019). Although steps are being taken to increase the reliability of this method (Halvorsen *et al*. 2019) the limitations of existing literature should be acknowledged before important decisions or research design are based on their findings. To tackle this issue, audiograms can be used a foundation upon which broad understanding of fish hearing can be made before research heads in the direction of more applied and ecological work.

2.5.2 Wider work concerning underwater acoustics and fisheries science

Although using sound to control and understand fish behaviour is a relatively new field, there have been a number of pieces of work that seek to understand how fish, specifically their location within a river system, can be manipulated with sound (Noatch & Suski, 2012; Vetter *et al*., 2015; Vetter *et al*., 2017; Jesus *et al*., 2018). There has also been a focus on marine mammal deterrents, for example, in an attempt to mitigate for the negative impacts of becoming entrained in fishing gear (Stone *et al*., 1997; Werner *et al*., 2006; Kastelein *et al*., 2017a).

More recently, fish biologists have become interested in the finer scale behaviours that fish can display when exposed to acoustic stimuli (Kastelein *et al*., 2017b; Zielinski & Sorensen, 2017; Putland *et al*., 2018). This research covers a number of behavioural areas that have been identified to be influenced by sound. Vocalisations made by fish exposed to sound (Putland *et al*., 2018), swimming speed and cohesion of a group (Kastelein *et al*., 2017b) and orientation (Zielinski & Sorensen, 2017) are discussed in the literature. These pieces of research are however on a range of different species, conducted with different methodologies and with either groups of fish or individuals. A piece of research that examines the effects of a range of metrics on individuals and groups, using the same experimental methodology and focusing on one species would help to clarify wider understanding of the impact of underwater sound on the behaviour of fish.

2.6 Summary

Through conducting this literature review it has been established that: fish face threats from anthropogenic structures in the aquatic environment. Some fish approach these hazards in groups, and the collective behaviour of the group may affect how the fish pass the hazard or approach any fisheries management system. Water is a good medium for acoustic waves to travel through, and the review has also established that some fish have varied ability to detect acoustic stimuli. A lack of literature concerning group behaviour in the presence of acoustic treatment has also been discovered. The combination of group behaviour and fisheries acoustics is, therefore, fundamental to understanding how fish interact with mitigation technology and how the efficiency of these technologies can be increased.

Using the findings from this literature review, the research in this thesis will focus on group behaviour, specifically fish, under acoustic treatment. The lack of current literature in this area will make the research novel and help this PhD to contribute to addressing gaps in collective knowledge of group behaviour and fisheries science.

3 Research methodology

In conducting the experiments below, a range of equipment, materials and methods have been used. They are identified in this chapter and the justification behind their usage explained. This chapter will provide a background to the evolution of the materials and methods selected, previous work that was conducted as part of this PhD to lead up to the work presented in this thesis and an explanation of the basis for the experiments described in chapters 4, 5 and 6. To avoid as much repetition as possible, an overview of all common elements to those three chapters is presented here and more specific methodologies are provided in each of the results chapters 4, 5 and 6, where applicable to each section.

3.1 Experimental structures

3.1.1 Evolution of the structural design

Previous to the beginning of the experimental work described in chapters 4, 5 & 6, I undertook a variety of pilot experiments. All of this work involved creating a controlled, underwater acoustic field within a flume tank however it also involved working with a variety of species. Three experiments involved working with Eurasian minnow, one experiment involved working with various Salmonids, one experiment involved working with European Eel and a final experiment involved working with Pacific Lamprey. I will now outline below the lessons I learnt that contributed to my final experimental design.

The idea of working with underwater acoustics and fish behaviour was driven by the research funding and original, higher level, objective of my PhD studies. The focus on Eurasian Minnow was driven by the possible relevance of any research findings as they are a Cypriniform fish and my interests and identified literature gaps in understanding group behaviour. However, it was the idea of working in flume tanks in a laboratory that was inspired by previous work a colleague of mine Jasper (De Bie et al. 2017 & 2017) was leading. The work of De Bie et al. involved recirculating flume tanks however I wished to use still water as this removed additional (hydrodynamic) cues that had potential to confound behavioural analysis.

My first experiments involved the use of a steel framed, glass sided, metal-based flume tank of 45 cm x 45 cm x 1200 cm. The tank was originally designed to be a recirculating flume and so therefore I created baffles to retain the water (Figure 3.1). These baffles had the additional benefit of limiting reflected acoustic waves up and down the tank. However, the acoustic environment that resulted from using either a broadband stimulus similar to that used in the majority of this thesis, or using puretones was chaotic, full of features such as nodes and had a large range of SPLs (Figure 3.2). Even with assistance from Active Noise Cancelation (ANC) researchers, I was unable to produce an environment that was satisfactory for what I wanted for my research. The lessons learnt here were that the final flume tank had to be capable of producing and containing a far more uniform acoustic field and I believed that the problems with this flume tank was that the glass retained by the steel frame was being influenced far to much by the stimulus, causing vibrations that were feeding back into the tank itself. The other lesson was that mapping the acoustic field to such a fine scale (Figure 3.2) was important as this allowed me to understand specifically what I was exposing my fish to.

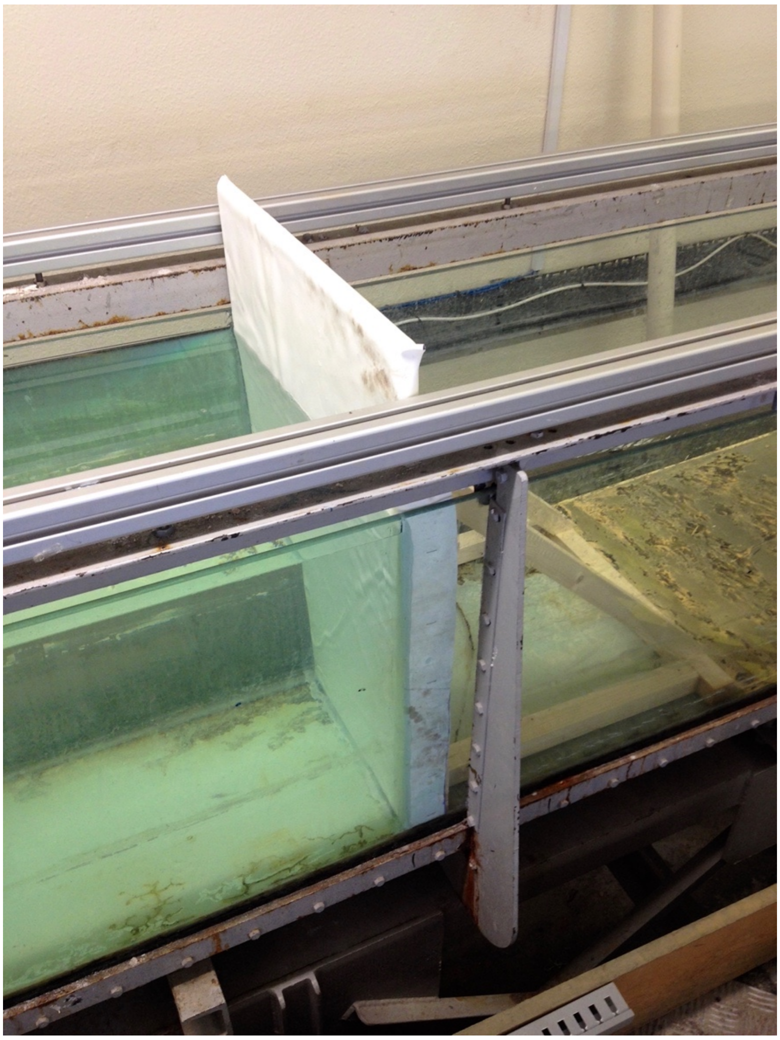


Figure 3.1 A photograph of one end of the original flume tank. Note the steel frame, glass walls and painted metal base. Also note the wooden baffle that was placed to retain the water to make the aquatic section of the flume to the desired length of 850cm.

Using the first flume was not all bad news however. The structure that I built around the flume did remove visual cues to the fish and reduced surface reflections on the water which made subsequent tracking much easier. However, the night-vision camera that

I used due to the low light environment and the inability to effectively light the tank without overwhelming the fish with visual cues was a limitation of this design. This limitation made automatic tracking of the fish impossible as the contrast was insufficient (Figure 3.3). There was another, somewhat less obvious, limitation to this tank design too. The width of the tank was 45 cm (Figure 3.4) however as can be seen in figure 3.3, this made placing the fish accurately within the flume difficult due to distortion from the camera. A slightly narrower flume would provide a better perspective from which to monitor the fish.

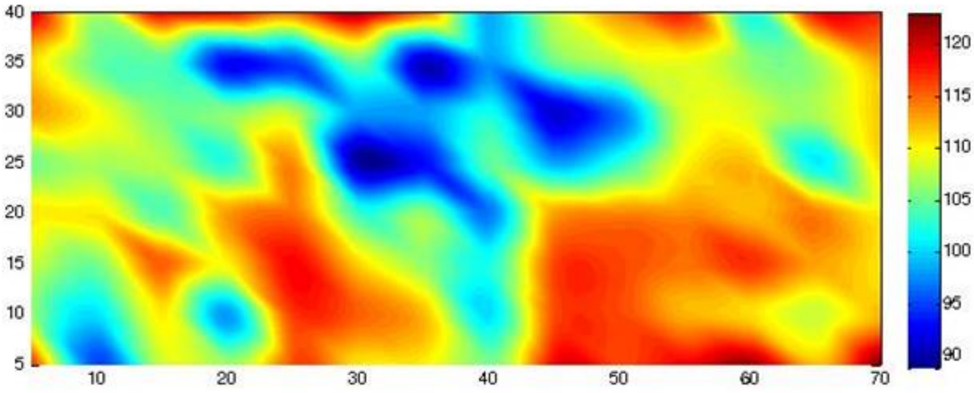


Figure 3.2 A colourmap plot of the sound pressure levels within the experimental area noted in figure 3.2. The scale is displaying SPL, expressed in dB re 1 μPa and the X and Y axis are measured in cm. Note the complex, non-uniform field with multiple features and a range of over 30 db.

The setup described above was used in pilot experiments of similar study-design to those presented later in this thesis however it wasn't until I conducted a study in a fiberglass tank in the USA with Pacific Lamprey that I realised how much more uniform it was possible to make an acoustic field. Whilst out in Oregon working with the United States Geological Survey (USGS) and the United Tribes of the Umatilla as part of my PhD funded work that I was investigating behavioural response of Pacific Lamprey to acoustic stimuli in a similar manner to that described above and in the studies detailed in full in this thesis. When mapping the acoustic field produced by the stimulus (a broadband signal from an Electro Voice UW-30) I noted how feature-less and uniform the acoustic field was. I also noted how much a speaker in one tank could influence the field in the tank next to it. Sadly, due to a mechanical hard-drive failure I have neither the mapping data to present here or the behavioural data to analyse. However, when I returned from Oregon, back to Southampton, I was keen to deploy the final two key lessons that I had learnt. Firstly, that I should be using a tank constructed of more rigid materials such as re-enforced fibreglass or a similar plastic. Secondly, that I needed to better consider how to shield my experimental tank from external noise.

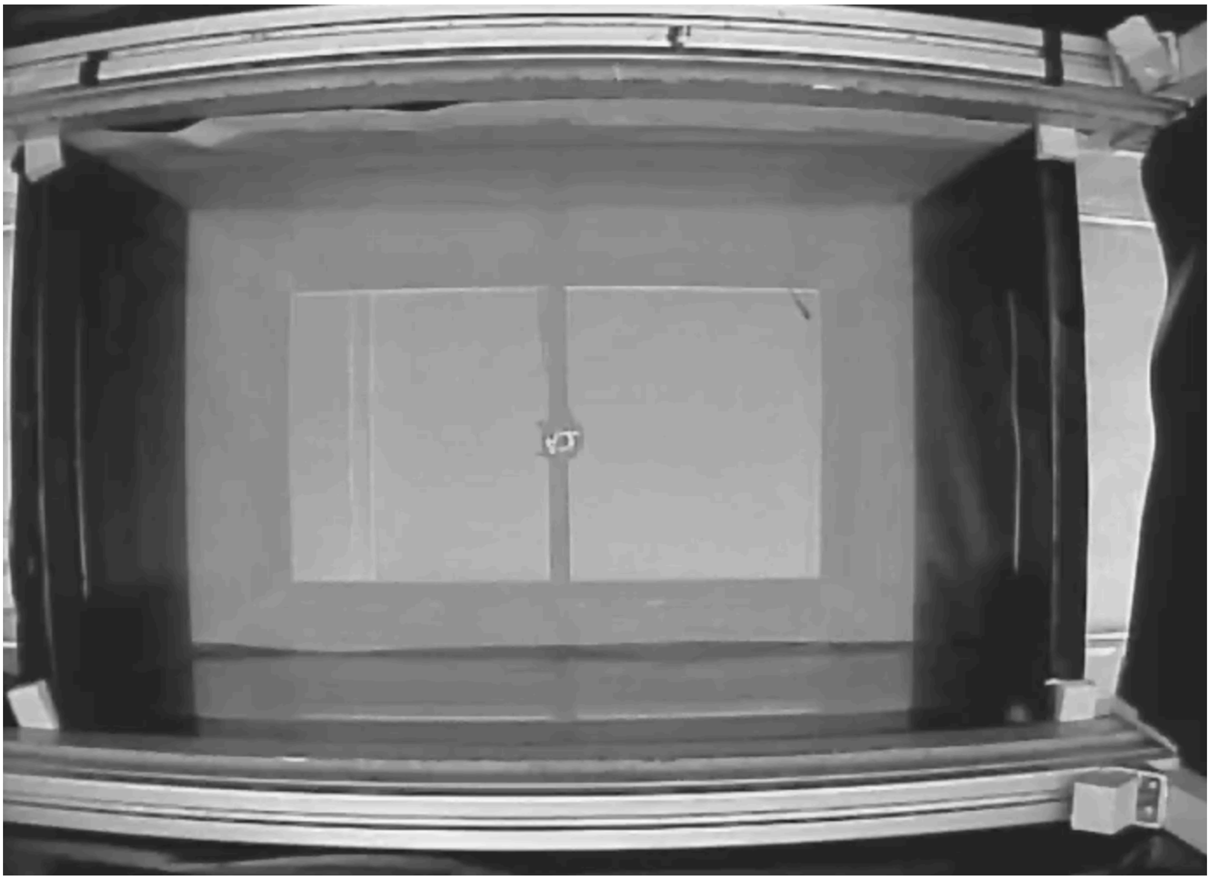


Figure 3.3 A still from the night-vision camera used to record trial footage in a pilot experiment. Not the reflections from the surface and the difficulty in identifying the five (!) fish present in the experimental area of the flume tank.

Upon returning to Southampton I used the lessons learnt regarding the tank materials and dimensions and sought out an existing tank that may satisfy these requirements. In addition to the acoustic properties of the tank, I was also keen to source a flume tank which would allow better contrast of and more visible fish during trials. I identified a transparent acrylic tank to test the acoustic stimulus within and due to its transparency, I decided to light the tank in a manner which provided excellent contrast. This contrast was tested prior to the acquisition of any subject fish and proved capable of enabling automatic tracking of the fish positions. In addition to this, the acoustic field was also stable and uniform.

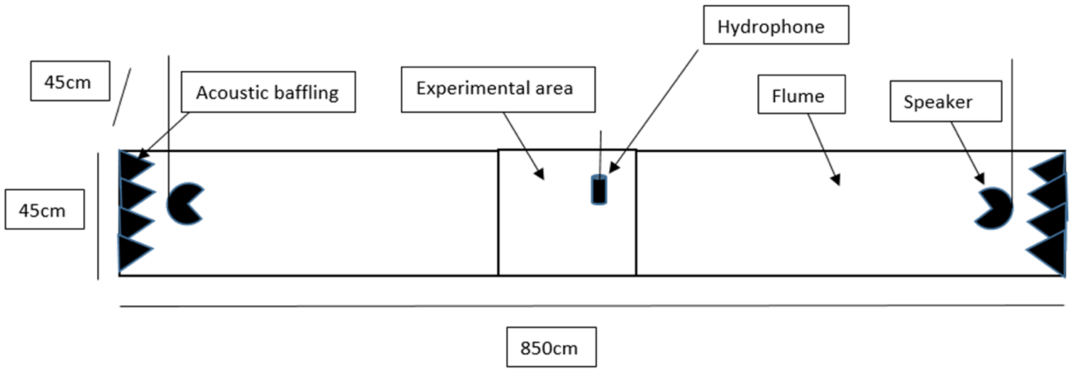


Figure 3.4 A diagram displaying the experimental flume tank design from an earlier pilot experiment.

The considerations, lessons learnt through testing and experience and the resources provided to me through my PhD funding and the laboratory environment which I was working all lead to the choice to use a 30 cm x 30 cm x 300 cm transparent flume tank located within a disused walk-in fridge at the Chilworth laboratory in Southampton. The details of how I set this tank up in are the subject of the next section.

3.1.2 Final structural design

All experimental work to test the behavioural response of individuals and groups of European minnow (*Phoxinus phoxinus*) to sound was conducted in a tank holding standing water, based at the International Centre for Ecohydraulics Research (ICER), University of Southampton, UK. The tank (total dimensions 30 cm wide, 30 cm deep, 300 cm long) was constructed of transparent acrylic with an experimental area in the centre measuring 30 cm wide, 30 cm deep and 85 cm long (Figure 3.5). The central area was created by two wooden frames with white plastic sheeting stretched over them at either end. For the remainder of this thesis, the phrase “experimental area” refers to the working area of the flume that fish were constrained to during any trial period. White sheeting was also placed around the tank to aid lighting, in particular increasing the contrast of the fish against the tank background whilst retaining an open-top. Lighting was provided by two photographic diffusers placed below the tank that projected onto the white sheets surrounding the experimental area to ensure uniform illumination. For all experiments, a wooden structure made from 25 mm x 25 mm pine was erected over the experimental flume section (Figures 3.6 & 3.7) to provide shielding from external visual cues for the fish.

Figure 3.5 A photograph of the experimental flume tank used in the research detailed in this thesis. Note the transparent acrylic construction and the concrete-block plinths the tank is mounted on.

All work used non-recirculating flumes in an area with controlled lighting. This was to isolate the response of the fish to the acoustic stimulus and not hydraulic or visual cues (Figure 3.6). Flumes have been used for all experimental research, as opposed to a field setting, as they can provide considerable benefit in terms of controlling confounding variables such as light, flow and temperature (Lee *et al*., 2003; Russen *et al*., 2011; Kemp *et al*., 2012). Experimental work allows the researcher to control for confounding variables while manipulating factors of interest. Therefore, the flume facilities provided the optimal environment for this work.

Figure 3.6 A photograph of the experimental area of the acrylic flume tank. Note the blackout structure encompassing this area and the white sheet material used to remove visual cues for fish and provide high contrast for video tracking. The space underneath the tank was used to place photographic lamps with large diffuser plates on.

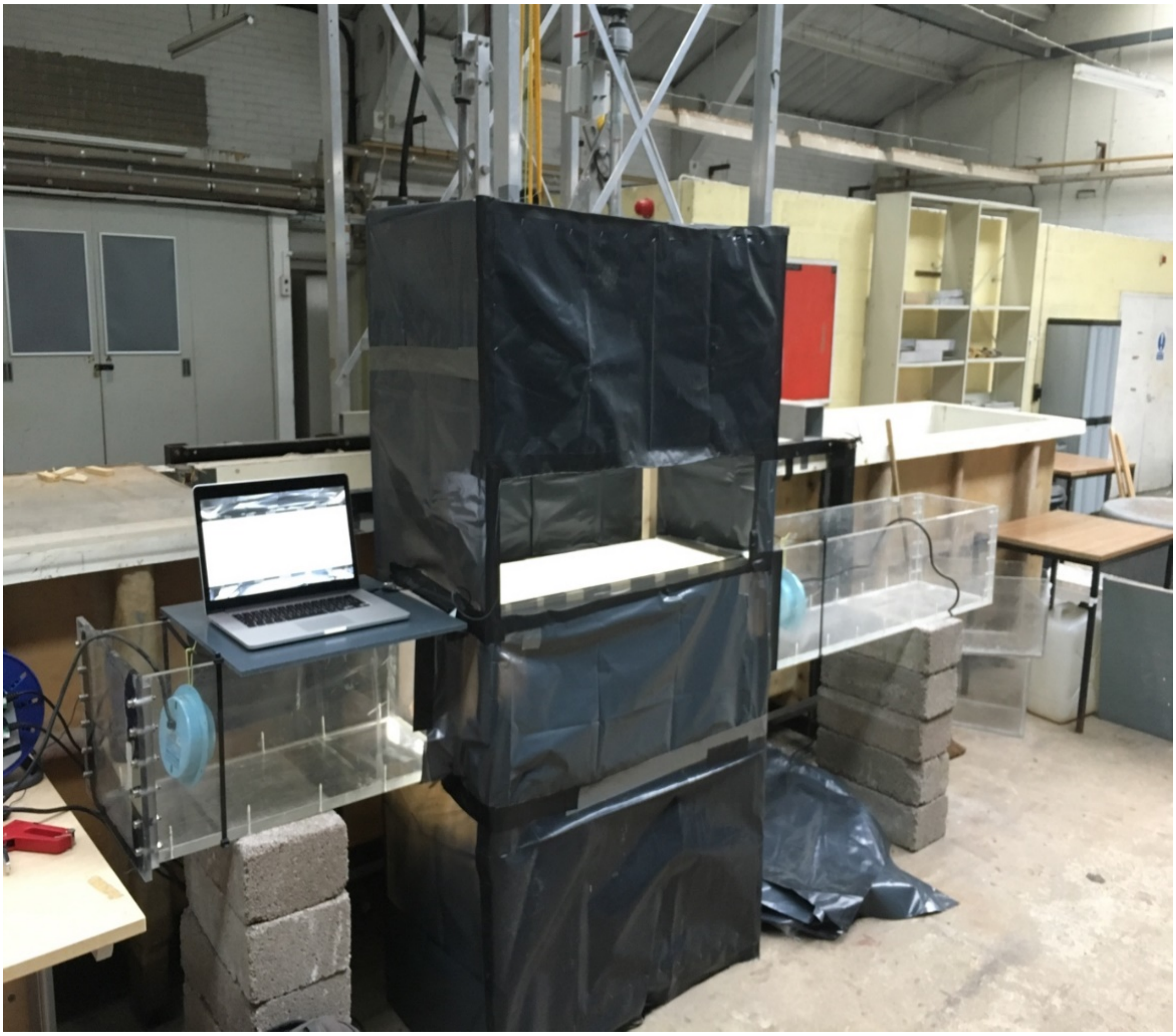


Figure 3.7 Experimental flume and black-out structure. The speakers (in blue) are also clearly visible outside of the experimental area. When trials are in progress there is an extra piece of black plastic which is secured over the visible gap.

The flume tank itself, whilst not designed specifically for the experiments described within this thesis, provided the optimum compromise between visibility of fish for tracking, space for fish to move and display natural behaviours such as shoaling and uniformity of the acoustic environment upon commencement of the stimulus. The flume is pictured in its pilot position at Chilworth Laboratory in figure 3.7, however it was moved to a decommissioned walk-in fridge for the experiments in chapters 4, 5 & 6 as this further reduced the impact of ambient noise on the experimental procedures. The difficulties of creating a uniform acoustic environment were discussed in section 3.1.1 and therefore, when this flume tank was finally chosen, I mapped the frequency response of the speakers, the Sound Pressure Level (SPL) and the Particle Acceleration (PA) at a number of depths, with the speakers in their final position (Figure 3.8). The depth of the water the fish predominantly occupied had been identified from previous pilot work outlined also in section 3.1.1 and so therefore this was the chosen depth for fine-scale mapping as detailed in chapters 4 and 5.

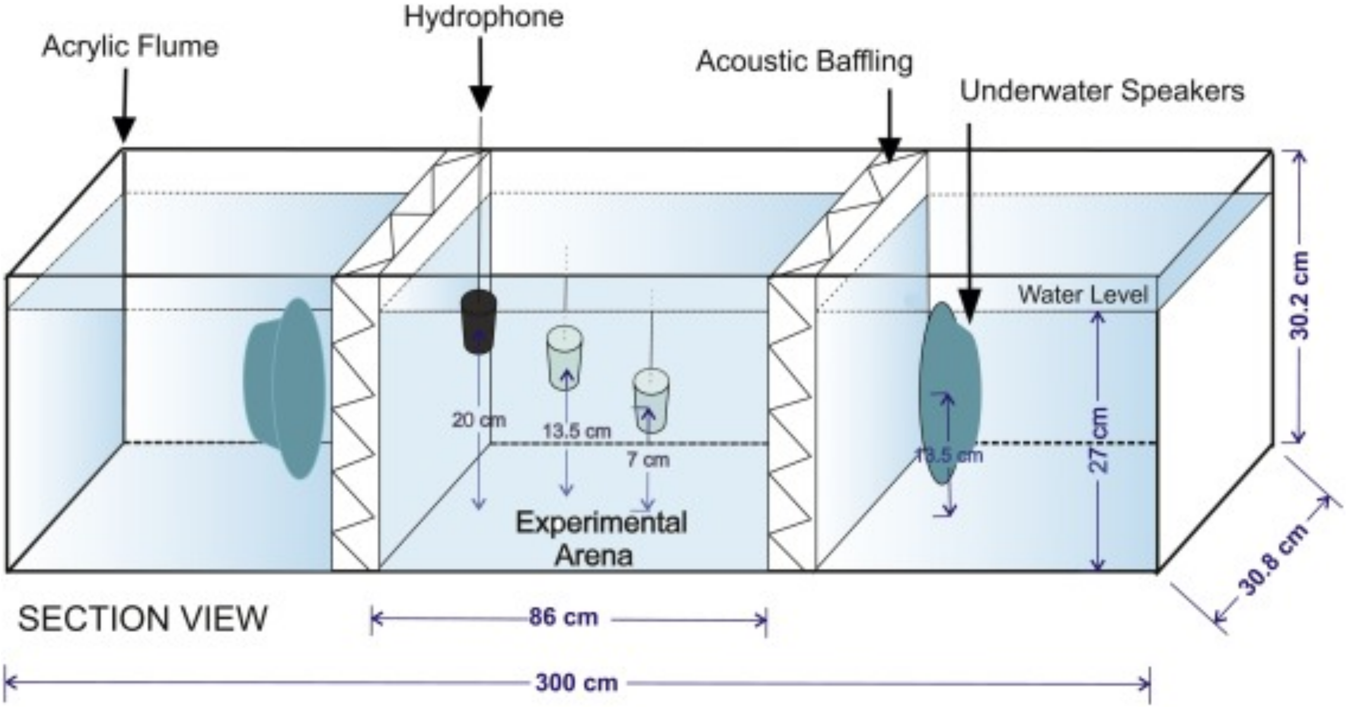


Figure 3.8 A diagram showing the layout of the experimental area within the flume tank and the layout of the speakers and baffling. Note the depths of the hydrophones that were used during calibration and mapping of the experimental area. (Source: Diagram adapted from Currie et al. 2021).

3.2 Acoustic signal generation, measuring equipment and software

For the generation and measuring of acoustic stimuli all experiments used a Windows PC running MATLAB 2016b. MATLAB was used for the ability to finely control the signal driving the speakers and measuring the signals from the hydrophone. In all cases, the signal was then passed through a Skytronic 103.100 Mini AV Digital Surround Amplifier to Electro-Voice UW-30 underwater speakers. The stimulus was created, in MATLAB, by filtering pseudo-random Gaussian noise using a 6th order band-pass Butterworth filter, with the pass band limits being 60 Hz and 2 kHz.

In all cases the sound field was mapped by measuring the sound pressure level in a 5 x 5 cm grid pattern, measured at three depths (7 cm, 13.5 cm and 20 cm above tank floor (ATF)). An example of this mapping data can be seen on the colour plot by sound pressure level (SPL) in figure 4.1 and onwards. This mapping was conducted using a Bruel & Kjaer 8103 hydrophone connected to a Bruel & Kjaer Charge Amplifier Type 2635. The transfer of information between MATLAB on the PC and the amplifier and charge amplifier was handled by a data acquisition system, namely a National Instruments USB-6341.

The mapping was conducted by delivering five second bursts of each pure-tone frequencies of 200, 400, 800 1000, 2000 Hz and the broadband white noise stimulus and measuring the SPL at each location as outlined above. The mapping began at 2.5 cm away from each tank wall to avoid vibration of the hydrophone against the boundary causing interference with the readings. The hydrophone was attached to a 3 mm steel bar via its own cabling and then fixed to a square-profile pole. This was screwed into place at each of the 5 x 5 cm locations to ensure that the location of the hydrophone did not change during measurements. A measurement of the frequency profile of the stimulus was taken for each experimental condition (both speakers, only the left and only the right speaker) and these are presented as intensity – frequency plots below (Figure 3.2). These recordings were taken with the hydrophone 15 cm ATF and 15 cm from each side wall. The hydrophone was then moved between three positions: 5 cm from the left end wall, 42.5 cm between each end wall and 5 cm from the right end wall. This gave a total of 9 measurements however, there was very little difference in terms of frequency response between the left, middle and right locations. Therefore, the middle position is displayed below with the left speaker, both speakers and right speaker displaying the frequency response of the stimulus (Figure 3.9).

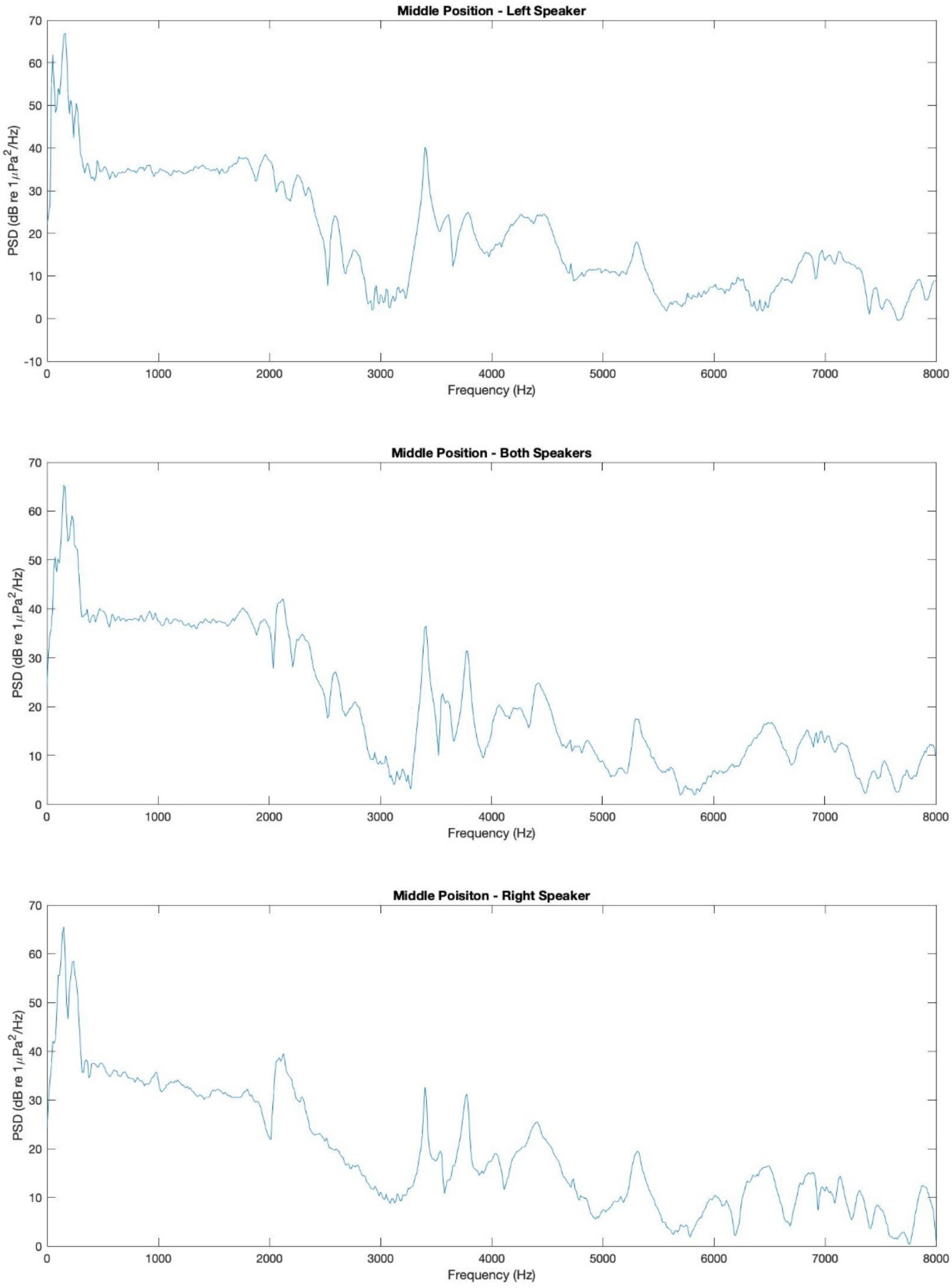


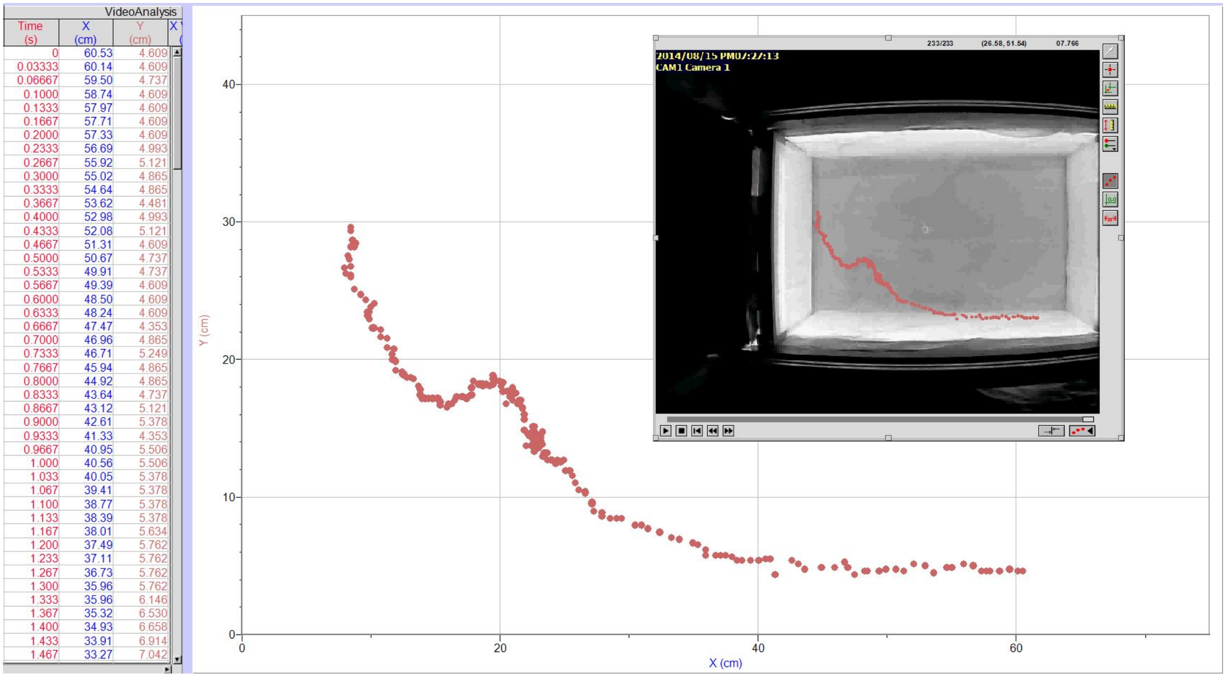
Figure 3.9 Power Spectral Density (PSD) frequency response for three different speaker configurations as measured from the centre of the tank with the stimulus playing.

3.3 Quantifying behaviour

All experimental work was recorded via an overhead video camera (Logitech c920 Webcam) mounted to the wooden frame, directly above the centre of the experimental area. The camera was connected via USB to a Macintosh laptop running QuickTime to capture the video data and the programme Webcam Settings was used to control the contrast settings of the camera. The contrast that this process provided, alongside the prepatory work of selecting and screening off the acrylic flume, was impressive and is demonstrated in figure 3.10. The raw video data were then prepared for analysis using Prism by NCH Software. The illumination created with the photographic lamps and diffusers mentioned in section 3.1 aided in creating good contrast of the fish against the tank background. This was important for the next step, the video analysis.

Video data were initially analysed using Vernier Logger Pro 3, by manually selecting fish positions every frame (Figure 3.11). Logger Pro 3 allows the user to import raw video, set a spatial scale, and click on the location of each fish to produce a table of time/co-ordinate data. Footage was initially analysed at a resolution of 0.6 frames per second (FPS). This was a prohibitively time-consuming process and was eventually replaced by a bespoke MATLAB script which automatically tracked the fish positions and fed-back time, 2D co-ordinate and orientation data. This process provided more information, was more efficient and was confirmed to offer the same information. The MATLAB process was therefore used to quantify all of the video data for the experiments described in chapters 4, 5 & 6. Metrics specific to each experiment were then calculated from these data.

Figure 3.10 A still from the video recording of a trial presented in chapter 6. Note the high contrast and the ease with which 5 individual fish, forming a group, are distinguished.



VideoAnalysis

Time (s)	X (cm)	Y (cm)
0	60.53	4.609
0.03333	60.14	4.609
0.06667	59.50	4.737
0.1000	58.74	4.609
0.1333	57.97	4.609
0.1667	57.71	4.609
0.2000	57.33	4.609
0.2333	56.69	4.993
0.2667	55.92	5.121
0.3000	55.02	4.865
0.3333	54.64	4.865
0.3667	53.62	4.481
0.4000	52.98	4.993
0.4333	52.08	5.121
0.4667	51.31	4.609
0.5000	50.67	4.737
0.5333	49.91	4.737
0.5667	49.39	4.609
0.6000	48.50	4.609
0.6333	48.24	4.609
0.6667	47.47	4.353
0.7000	46.96	4.865
0.7333	46.71	5.249
0.7667	45.94	4.865
0.8000	44.92	4.865
0.8333	43.64	4.737
0.8667	43.12	5.121
0.9000	42.61	5.378
0.9333	41.33	4.353
0.9667	40.95	5.506
1.000	40.56	5.506
1.033	40.05	5.378
1.067	39.41	5.378
1.100	38.77	5.378
1.133	38.39	5.378
1.167	38.01	5.634
1.200	37.49	5.762
1.233	37.11	5.762
1.267	36.73	5.762
1.300	35.96	5.762
1.333	35.96	6.146
1.367	35.32	6.530
1.400	34.93	6.658
1.433	33.91	6.914
1.467	33.27	7.042

Figure 3.11 Logger Pro 3 being used to track a fish. The time/location data can be seen in a table to the left of the picture with the plotted data shown to the centre. On the right is the original video footage which is being quantified.

For the automated tracking through MATLAB, the original footage was loaded into MATLAB at its native resolution of 30 frames per second (FPS). This higher, native resolution could be made advantage of due to the automated tracking that was implemented. The programme then broke the image of the tank down into a background image with no fish present and located the starting point of the x / y axis, in the bottom left corner of the tank. The number of fish to be found in each image was then entered as either one for an individual trial or five for a group. Once the location of each fish in each frame was identified and assigned a time stamp, various other metrics were calculated. For the remainder of this thesis, the influence of sound on collective behaviour was quantified using the: (i) speed at which fish moved, (ii) the persistence of their swimming paths, (iii) the cohesion of the shoal, and (iv) the orientation of members of the group. These were calculated using the formulae given below and are referred to in chapters 4, 5 & 6 using letters (eg. iii) and numbers of the equations.

The position of the i^{th} fish in the n^{th} video frame was represented as the vector $\underline{X}_i(n)$ which is defined as:

$$\underline{X}_i(n)=(x_i(n),y_i(n))^t \tag{1}$$

$x_i(n)$ represents distance along the length of the tank of the i^{th} fish in frame n and $y_i(n)$ corresponds to distance across the breadth of the tank. During video processing, the position of an individual fish was defined as the centre of mass of those pixels associated with it.

When analysing the movement of the groups of fish, location was based on the shoal's centroid, $\underline{X}_c(n)$. The shoal's centroid position was calculated using:

$$\underline{X}_c(n)=(x_c(n),y_c(n))^t=(\underline{X}_1(n) + \underline{X}_2(n) + \underline{X}_3(n) + \underline{X}_4(n) + \underline{X}_5(n))/5 \tag{2}$$

(i) Swimming speed was calculated for both shoals and individuals, $v_c(n)$, based on the motion of the centroid of the group or individual. It was evaluated by first computing the change in position between two frames. For shoals this was $\underline{dX}(n)=\underline{X}_c(n)-\underline{X}_c(n-1)$, whereas for individuals it was $\underline{dX}(n)=\underline{X}(n)-\underline{X}(n-1)$. The speed was the length of this vector divided by the time interval between two frames (in this case $\delta=0.033$, corresponding to 30 frames per second):

$$v\,(n)= dX\,(n)/\delta \tag{3}$$

(ii) Persistence of the swim path provided a measure of its predictability with low persistence corresponding to more erratic movements. Persistence described the difference between expected, based on the trajectory of prior positions, and observed location recorded for individuals or groups at each time step. Specifically, using the locations of the fish/group in two preceding frames, n-2 and n-1, then assuming the fish was swimming at constant speed along a straight line, the location in the n^{th} frame was predicted. The prediction denoted as $\underline{P}_c(n)$ was defined as:

$$\underline{P}_c\,(n)= \underline{X}_c\,(n-1)+ \underline{X}_c\,(n-1)-\underline{X}_c\,(n-2) =2\underline{X}_c\,(n-1)-\underline{X}_c\,(n-2) \tag{4}$$

Persistence was based on the distance between the predicted (4) and observed location, so that:

$$\underline{E}(n)=\underline{X}_c(n)-\underline{P}_c(n) \tag{5}$$

The persistence is quantified by the norm (length) of the vector $\underline{E}(n)$.

(iii) Cohesion of the group was quantified by measuring the standard deviations of the locations on the *x* and *y* axis and combining them. This was defined as:

$$\sqrt{\frac{1}{5}\sum_{i=1}^{5}\left(x_i(n)-x_c(n)\right)^2}+\sqrt{\frac{1}{5}\sum_{i=1}^{5}\left(y_i(n)-y_c(n)\right)^2} \qquad (6)$$

(iv) Orientation of each individual fish, ($\theta_i(n)$) was represented by an angle in the range -90° to 90° and represented the direction in which the body of the fish was aligned. This orientation does not account for the heading of the fish due to an inability of the analysis software to determine heading. For example, the software does not recognise the difference between a fish swimming horizontally to the right or left. Mean orientation for the group was defined as:

$$\theta_c(n)=(\theta_1(n)+\theta_2(n)+\theta_3(n)+\theta_4(n)+\theta_5(n))/5 \qquad (7)$$

How dissimilarly oriented individual fish were in relation to each other was defined as the standard deviation of the orientations:

$$\theta(n)_{sd}=\sqrt{\frac{1}{5}\sum\left(\theta_i(n)-\theta_c(n)\right)^2} \qquad (8)$$

The standard deviation of the orientation is a measure of how aligned the fish are relative to each other (a low value of $\theta(n)_{sd}$ corresponding to a high degree of alignment). Note that the absence of flow and use of optical screens worked to reduce anisotropic external stimulus.

The four metrics were calculated for every frame in the image. The data were averaged for over one second (30 frames). To reduce noise and mitigate against tracking artefacts a median value was calculated for every 20 second (block) for each trial.

For qualitative comparison between individual fish and groups, a table of initial reaction types was created and used in chapters 5 & 6. This was conducted in an attempt to capture any additional or anecdotal evidence that may be used to direct future research or current data analysis.

3.4 Subject fish and husbandry equipment

Fish used in these experiments were captured from the River Itchen Navigation, (St. Catherine’s Hill, Winchester, UK, 51.049783 -1.311416) using a 10 x 1 m seine net with 3 mm holes. Chest waders were worn by two researchers, one stayed at the bankside and one fished into the deeper water. The deeper operative made a loop of the seine net into the deeper water while the 1st researcher held one end of the net at the bank. The loop was closed and pulled on to the bank and the fish examined and counted. Any fish caught that were not to be used were carefully returned to the water immediately. They were transported to the International Centre for Ecohydraulics Research’s (ICER) holding facilities at Chilworth Science Park near Southampton, UK, using a 100 L, 30 cm diameter tank with an air pump and diffuser. The fish were maintained in a tank (150 cm wide, 150 long and 100 cm deep with a water depth of 70 cm) prior to conducting trials. The tanks were filtered with external pond and Ultra-Violet (UV) filters. Aeration was provided by the return water plunging into the tank and an external air pump with diffuser. Daily water testing with an API test kit and subsequent changes (approximately 25%) ensured high water quality was maintained (nitrite < 1 mg L^{-1} and nitrate < 50 mg L^{-1}). Physical inspections of the fish were also carried out daily in accordance with UK Home Office guidelines All water used was mains city water treated with a dechlorinating solution. Small aquarium nets and plastic containers measuring 15 x 8 x 8 cm were used for handling and transporting fish between trials. A measuring board and set of scales were used for all fish measurements. At the end of all experimental work fish were euthanised according to Home Office Schedule 1 procedures.

When deciding on an animal or a behaviour to focus on, a number of factors must be considered, and terms and methods defined (Carter *et al.*, 2013). The ecological importance of the animal, the economic value of that animal or the ecosystem in which it is found, the conservation status of an animal, the importance of the behaviour and its applicability to other animals and studies, and the contribution that understanding this particular behaviour or animal could make to the wider scientific community. The fish used in this study have been chosen for their advanced hearing capabilities and schooling behaviour. The European minnow is an abundant species in rivers throughout Europe, including the United Kingdom and the fish used in this research have been wild caught from the River Itchen in Winchester, local to the University of Southampton. These fish, once caught, acclimatise well to a laboratory environment. Wild fish were used for these experiments as they were available in sufficient quantity to provide statistical power and are the best example of the fish species they represent as farmed fish can often provide weaker responses to stimuli (Jackson & Brown 2011).

3.4.1 European minnow ecology

Minnows are found in a diverse range of habitats throughout the UK. They are an ideal fish species for these studies as they have a strong schooling instinct and well-developed hearing (Popper & Coombs, 1980). The European Minnow is a small cypriniform fish (Figure 3.12). They have good burst and prolonged swimming fitness and are found in a range of aquatic habitats from fast flowing upland streams to oligotrophic lakes, slow moving navigations and lowland rivers throughout Europe and Russia (Freyhof & Kottelat, 2008).

Figure 3.12 The European minnow.

The hearing range of the European minnow has not been quantified using an ABR test, although the closely related species fathead minnow (*Pimephales promelas*) has been (Scholik & Yan, 2001; Scholik & Yan, 2002). It is agreed that these fish have physiologically enhanced hearing, and this research has utilised that fact when designing experiments to answer the research objectives. This species is not of high economic value, or of high conservation concern and their only real threat seems to be from the overstocking of Salmonids in some fisheries (Freyhof & Kottelat, 2008).

It is also relevant to this thesis and the position of this research within the wider literature that the location all fish were taken from, the Itchen Navigation at St. Catherine's Hill, is a very heavily managed site. The riverine environment there is not natural; it is based upon a chalk stream, but it has been heavily modified over centuries. It has a solid, heavily compressed riverbed and a mix of retained earth and concrete banks at 90° to the river bed (Figure 2.3). It might therefore be considered that the, not uncommon, construction of this inland waterway draws some comparisons to a flume tank in a laboratory (Leighton *et al*. 2019). The acoustic properties of the river channel and the flume tank used are also, therefore, likely to be comparable.

3.5 Trial structure

For the results chapters of this thesis (4, 5 & 6), the structure of the trial varies. In all experiments, individuals and groups of five minnows are placed into a flume tank and provided with time to acclimatise. This time is set at a standard of 40 minutes before the trial begins. All the trials in the experiments described in this thesis include a pre-treatment period, prior to stimulus activation. This was scheduled between the acclimation and the commencement of the treatment via activation of the stimulus. The timings and duration of the treatment and pre-treatment periods varied between the experiments as detailed in chapters 4, 5 & 6. This was due to the analysis designed for each of the experiments.

4 Quantifying avoidance of high intensity sound by a shoaling Cypriniform

Controlling the behaviour of animals has been a goal for humans for millennia, such as managing agricultural flocks of animals with other animals, for example herds of sheep with sheep dogs. Much of this has targeted spatial manipulation of animals, from penning sheep to deterring birds with scarecrows. Much more recently, the advancement of technology in various areas has enabled agricultural and other animal managers to develop new ways to spatially manipulate animals and for biologists to investigate the mechanism by which this occurs.

In fisheries, using behavioural deterrents is a relatively new development which has primarily focused on commercially or ecologically important species. This research aimed to explore if the spatial preference, displayed by a shoaling cypriniform, changes upon the introduction of an acoustic stimulus. The experimental setup allowed multiple fish, in a group setting, and solitary individuals to be examined and their differences compared.

Individual fish displayed no significant avoidance of high-intensity sound, although groups of fish did. The groups also displayed a, non-significant, habituation to the sound field. To validate the findings, the relationship between the placement of fish and time was examined and a period of no-correlation was established. Finally, the cohesion of the fish and the intensity of the sound was analysed. The study found no significant relationship between these two metrics, although other studies have found this. Due to the directional nature of the sound field, it is suggested that further work should examine the fine-scale behaviour of these fish in a more uniform acoustic environment.

The findings of this study contribute to knowledge in two main ways. Firstly, the knowledge that whether fish encounter a deterrent as a group or not, may impact the efficacy of the device. Second, as an indication that further work needs to be conducted to explore the relationship between exposure to high-intensity sound and fine-scale behaviours.

4.1 Introduction

Anthropogenic-led modification of the aquatic environment has been occurring for millennia (Kreuzer, 1974; Welcomme, 2007) and is increasingly prevalent and complex (Kopf *et al.*, 2015). The impact that human activity has on watercourses is debatable (Kopf *et al.*, 2015), although there is currently significant energy being invested in assessing, and mitigating for, potential negative impacts of anthropogenic lead activity and structures e.g. Kemp and O'Hanley (2010). Diverting and restricting the movements of fish forms the basis for much of this mitigation, and traditional fish passage research is a well-studied field (Kemp & O'Hanley, 2010). However, traditional mitigation technology, such as screens at intake points, can cause damage to fish through impingement (Hadderingh *et al.*, 1983; Chen *et al.*, 2018), can reduce the throughput of a hydropower device or water extraction point, are expensive to install and maintain and are very complex to manage for legislators and enforcement agencies to manage (Schramm *et al.*, 2016). For these reasons, there is increasing demand for alternative methods for managing the passage of fish.

One such solution is the behavioural deterrent. Directing fish to a bypass or away from a turbine housing by manipulating the inherent behaviour of these animals. Behaviours that animals display and the circumstances in which they do so have fascinated biologists for millennia (Bolhuis & Giraldeau, 2005). Their behaviour is a direct response to the world around them as interpreting their surroundings and detecting threats and opportunities is key for all animals (Godin, 1997; Durant, 2000; Sand *et al.*, 2000; Childress & Lung, 2003). These behaviours range from the simple to the complex (Huntingford, 2012; Broom & Fraser, 2015) and can be influenced by a range of factors and environmental stimuli (Huntingford, 2013; Staub, 2013; Coghill, 2015).

Movement of a subject in response to a stimulus is a well-observed and reported phenomenon in the natural world and in laboratory experiments (Bowler & Benton, 2005; Bartumeus & Catalán, 2009; Iwasaki, 2015). These observations cover a range of flora and fauna. Observed phenomena include tropism (growth towards or away from a stimulus) in plants (Bastien *et al.*, 2013) and taxis in animals, such as dispersal strategies (Bowler & Benton, 2005). These movements can be triggered in response to a range of stimuli, such as light and touch in the golden mussel *Limnoperna fortunei* (Iwasaki, 2015), heat as in the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* (Garrity *et al*., 2010) and rheotaxis in chub *Squalius cephalus* and barbel *Barbus barbus* (De Bie, 2017). It is important to note that these behaviours are innate and typically not directed by any premediated planning. The directional response displayed by an animal encountering a stimulus allows behavioural biologists to better understand the preferences of their subjects. It is assumed that an animal moving away from a stimulus is displaying a negative preference for that stimulus. When an animal learns about an environment, they develop positive and negative preferences for zones within that area. These preferences can be identified through their spatial distribution over time. This distribution in the presence of a stimulus can help shape our knowledge of the impacts of anthropogenic interference in a natural system and the unnatural or enhanced stimuli that can result (Schick & Urban, 2000; Luo *et al*., 2015). These behaviours are not always consistent, as an animal's initial reaction may reduce over time with familiarity; for example, as a consequence of habituation (Blumstein, 2016).

Consistent or prolonged exposure to a stimulus, or stimuli, can result in the behaviour of an animal changing and adapting (Blumstein, 2016). The initial reaction to a stimulus stems from imbedded knowledge and lived experience (Fantz, 1957; Versace & Vallortigara, 2015; Sutherland & Mackintosh, 2016). Subsequent differences can be attributed to diminishing, or increasing, response to a repeated or prolonged stimulus (Blumstein 2016). In nature, this may be part of learning to identify threats. However, it should be noted that in a laboratory environment there is no actual threat of harm and so habituation may occur more quickly. When combined with a reduced number of available stimuli, i.e. a very simple environment with one tested variable, this may serve to accelerate the habituation process. When considering response to anthropogenic pollution, how rapidly an animal habituates to a stimulus may be of interest as this may be a measure of how much the subject is affected by this potentially negative stressor (Blumstein, 2014). Habituation is also of interest when using stimuli as management tools as their efficiency may be linked to the rate at which this happens.

Directional response and habituation are not the only behaviours that animals display. This is particularly the case when external and environmental factors are considered. For an animal that is solitary, they may be defensive or protective of a territory when confronted by a conspecific (Schradin, 2004). For an animal that cohabits with conspecifics, the interactions between these individuals may be of significant importance to the overall functioning of the group (De Bie, 2017). When considering group behaviours, the distance between members in an aggregation is often used as a metric (Couzin *et al*., 2002; Ballerini *et al*., 2008a). This group cohesion may vary in the presence of a stimulus, indicating whether a positive or a negative preference is displayed. Familiar examples of animals that group include flocks of birds and shoals of fish. In this study, a shoaling cypriniform fish is used as it is a typical example of an animal that displays grouping behaviour.

This study focuses on the auditory system as used by fish. In the aquatic environment acoustic signals cover greater distances in less time, and of less degraded quality, than in air (Stafford *et al*., 1998). This makes noise a highly suitable method for interacting with fish. The importance of sound to fish and its role in fish ecology has been the focus of several studies (Popper & Coombs, 1980; Popper & Carlson, 1998; Popper *et al*., 2003a; Popper & Fay, 2011). The study of this area allows better understanding of how fish perceive their surroundings which is important to recognise when considering a number of key issues in conservation and fish biology. These include group behaviour knowledge; how anthropogenic activities may affect fish and how management and passage technologies may be improved. In particular, the understanding of how fish respond to environmental stimuli in terms of their behaviour and placement within an environment are key to this.

The study of fish response to acoustic fields is not new (Scruton *et al*., 2003; Taylor *et al*., 2005; Zielinski & Sorensen, 2015). The ability to understand the behaviour of fish when exposed to a stimulus is key (Piper *et al*., 2012; Schakner & Blumstein, 2013; Poletto *et al*., 2014a; Poletto *et al*., 2014b). However, the influence that an acoustic field has on the behaviour of a particular fish is something that has not been quantified to any great extent (Zielinski *et al*., 2014). Understanding the fine-scale behaviour of fish under acoustic treatment will provide information which could potentially lead to enhanced technologies.

Understanding the differences between these behaviours in different group settings is also a major part of this study. An individual's reaction to a stimulus may be different when in the presence of conspecifics (Schradin, 2004). This may be due to a number of causes, varying from specific group behaviours such as vigilance (Pulliam, 1973) to more complex and inter-related factors. The behaviour of the group, if considered to be an entity in itself, may also differ to that of one individual and to a member of a group. It is therefore important to explore any changes in directionality, habituation and more complex behaviours, in these conditions of varying group descriptors.

This experiment aims to explore the differences in spatial distribution and behaviour of cypriniform fish in a tank when exposed to a directional acoustic stimulus in groups and individually. This aim will be met by answering the following objectives:

- Objective 1. To explore if fish display a preference for spatial distribution before and during treatment from a directional acoustic stimulus individually and as a group. This objective will use spatial distribution to explore any directional response to the stimulus.

- Objective 2. To explore if fish habituate to a directional acoustic stimulus, by returning to a normal spatial distribution individually and as a group.

- Objective 3. To explore the correlation between the distribution of a group of five fish and their overall cohesion during treatment from a directional acoustic stimulus.

4.2 Methodology

4.2.1 Experimental set-up

Using the experimental setup as described in section 3.2, the reaction of individuals and groups of European minnow to a continuous acoustic signal was measured in this experiment. In this experiment, two speakers were employed however only 1 was used for playback per trial. The amplifier was switched between treatments from the left to the right. The speakers were placed behind the barriers that bordered the experimental area. This ensured no visual cues as to the location of the speakers were available to the subjects. The stimulus played through the speakers was filtered pseudo-random Gaussian noise (60 Hz – 2 kHz). The stimulus was played continuously for 5 minutes after the subjects had been present in the flume for 45 minutes. The stimulus was intended to provide a clear and directional sound field covering a range of frequencies where minnow hearing is believed to be most sensitive (Scholik & Yan, 2001; Scholik & Yan, 2002). Between treatment trials the speaker was moved from one end of the flume to the other.

As part of setting the novel acoustic environment for these trials, measurements were made to quantify and map the sound field. This was conducted in the manner as described in section 3.2 and examples can be seen in figure 4.1 with the speaker placed on the left and on the right.

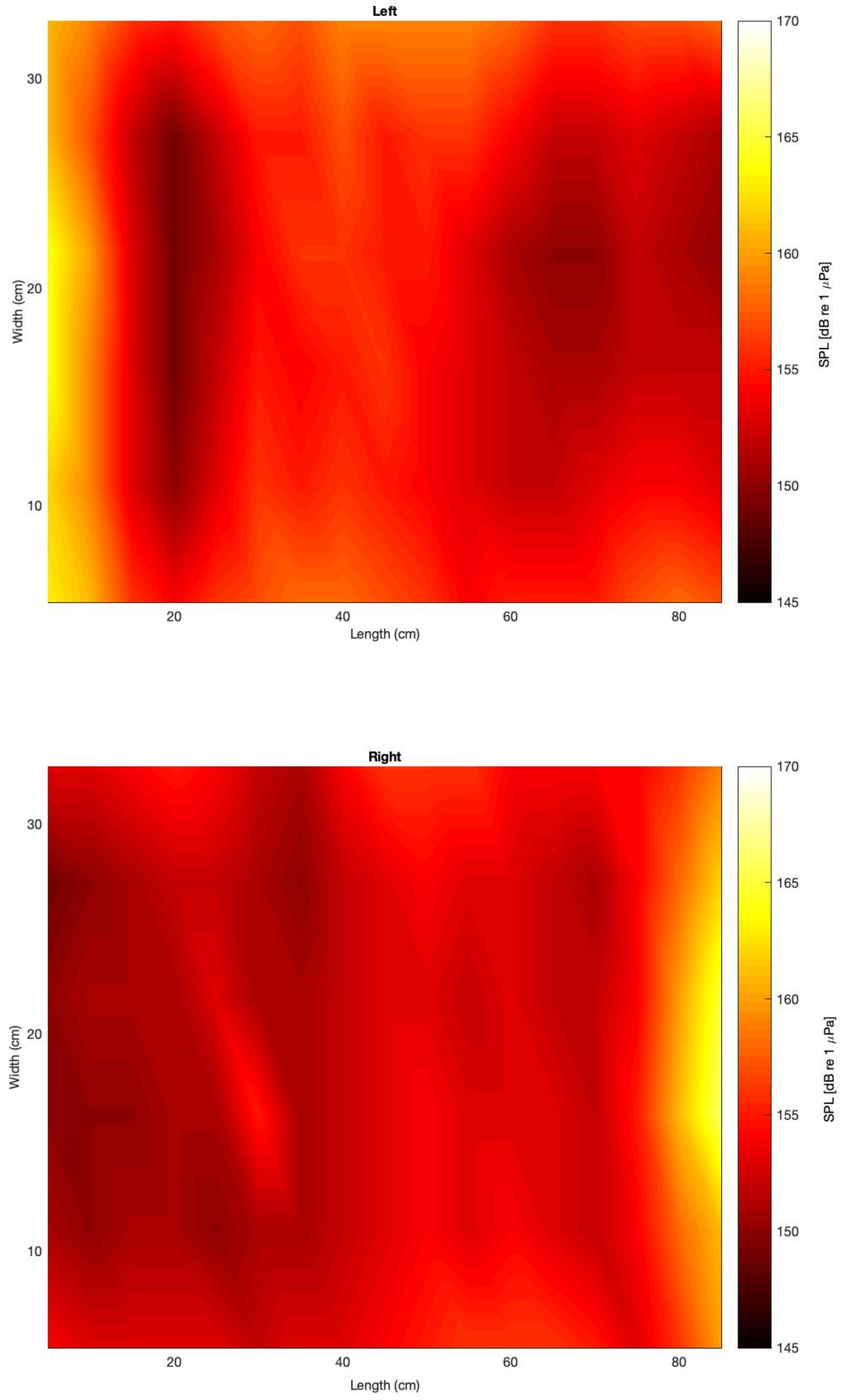


Figure 4.1 SPL Heatmap displaying the sound pressure levels of the broadband noise stimulus when directed from the left (top) and the right (bottom). The colour scale

represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades.

4.2.2 Fish collection and maintenance

European minnow (n = 120, mean total length and mass ± SD = 54 ± 5.65 mm, 1.41 ± 0.53 g) were collected as described in section 3.4 and were transported to the University of Southampton hydraulics laboratories at Chilworth Science Park, Southampton, UK. They were moved in a 100-litre container with forced aeration and maintained in a water tank as described in section 3.4. Water parameters remained within those described in section 3.4. The water temperature in the holding tank remained stable throughout the experiment (mean ± SD = 17.34 ± 1.41 °C).

4.2.3 Experimental trials

The fish were taken from the holding tanks and transferred to the flume individually and in groups of five for an acclimation period of 40 minutes. A pre-treatment period of five minutes was then used to establish baseline positions and behaviour. Following this, the stimulus was switched on for a five-minute period. The flume was lit by photographic diffuser lighting from below and filmed from overhead by a Logitech c920 Webcam. The resulting footage was saved for later analysis. Between trials, the speaker that was to be powered was changed from the left to the right. This was achieved by switching the output of the amplifier from left to right and not by removing the speaker. This was to ensure that the position of the speakers never changed and that the sound field remained consistent. After the five-minute treatment had elapsed, the fish were removed from the flume, weighed measured and euthanised as described in section 3.4. In total, 10 trials were conducted for each condition. These were individuals with the sound from the left, individuals with sound from the right, groups with sound from the left and groups with sound from the right which resulted in a total of 40 trials.

4.2.4 Analysis

The subsequent video data were quantified to coordinates using MATLAB to provide location over time and orientation data from which other metrics are calculated. The video analysis provides a location and an orientation at each time point for each fish. The position of the i^{th} fish in the n^{th} frame is represented as the vector $\underline{X}_i(n)$ which is defined as $(x_i(n),y_i(n))^t$, x representing distance along the longer dimension of the tank, i.e. the longitudinal dimension, and y distance across the shorter tank dimension, i.e. the transverse direction.

When analysing the data from the groups, this is based on the location of the centre of gravity for the shoal, denoted $\underline{X}_c$. $\underline{X}_c$ is a vector and is calculated using $\underline{X}_c(n)=(x_c(n),y_c(n))^t=(\underline{X}_1(n) + \underline{X}_2(n) + \underline{X}_3(n) + \underline{X}_4(n) + \underline{X}_5(n))/5$.

When examining the data to answer objectives 1-3, the correlation between spatial points of the fish was considered. The covariance between the longitudinal co-ordinates as a function of time and the time at which the covariance becomes zero was identified. This was calculated for individuals and for groups. The value was also calculated for pre-treatment and treatment periods to see if this varied during the treatment period. These values were calculated as covariances over time based on the video frames as a reference. The footage was filmed at 30 FPS.

To determine if fish displayed a spatial preference during treatment that differed to placement before the stimulus, the proximity of fish to the area of high intensity sound (Figure 4.1) was calculated before and during treatment. The study considered only the longitudinal position of the fish. A zone 15 cm in front of the active speaker was defined. This distance was selected based on the map in figure 4.1, where it is evident that the SPL in this region tends to be greater than elsewhere in the tank. The percentage of time the fish spent in this zone close to the active speaker was computed for both the 5-minute pre-treatment and treatment periods. The proportion of trials where the fish spent more time close to the speaker in the treatment period were computed. Trials where, during the pre-treatment period, the fish did not approach the speaker were discarded. The proportions thereby computed, were tested using Fisher's exact test under a null hypothesis that the probability of approaching the speaker in each trial is equal. A significant positive result was calculated by taking the number of trials where the amount of time spent in the pre-treatment period in the 15 cm area was normally distributed and comparing to the number of trials where the time spent in this area was greater than 1 % of the total treatment time. The result of this analysis will meet objective 1.

Assuming there was a change in spatial distribution of the fish when treated with underwater sound, the next objective looked at habituation to the stimulus. This was computed by dividing the treatment period in half (i.e. two segments of 2.5 minutes) and comparing each half to the pre-treatment value as calculated for objective 1 (Table 4.2). The difference between the two treatment period halves was then compared. This was also conducted for individuals and groups. The result of this analysis will meet objective 2.

Finally, to meet objective 3, the cohesion of the group was compared to the experienced SPL at a given location. This was conducted every 56 seconds (6 data points per trial) due to the correlation gap identified in step 1 of the results section. The cohesion of the group is assessed by measuring the spread of each fish on the x and y axis about $\underline{X}_c$ and combining them. This is defined as:

$$\sqrt{\frac{1}{5}\sum_{i=1}^{5} \left(x_i(n)-x_c(n)\right)^2}+\sqrt{\frac{1}{5}\sum_{i=1}^{5} \left(y_i(n)-y_c(n)\right)^2}$$

The SPL data were taken from the map taken as highlighted in section 4.2.1 and was used to calculate the experienced SPL at a point in time for the centre of a group of fish.

4.3 Results

When the covariance between the longitudinal co-ordinates was explored, individuals were observed to display a zero-covariance point (the point at which X-values are not correlated) of 56.4 seconds during pre-treatment (Figure 4.2) and 77.3 seconds during treatment (Figure 4.3). Groups were observed to display a zero-covariance point of 55.6 seconds for the pre-treatment period (Figure 4.4) and 6.1 seconds for the treatment (Figure 4.5).

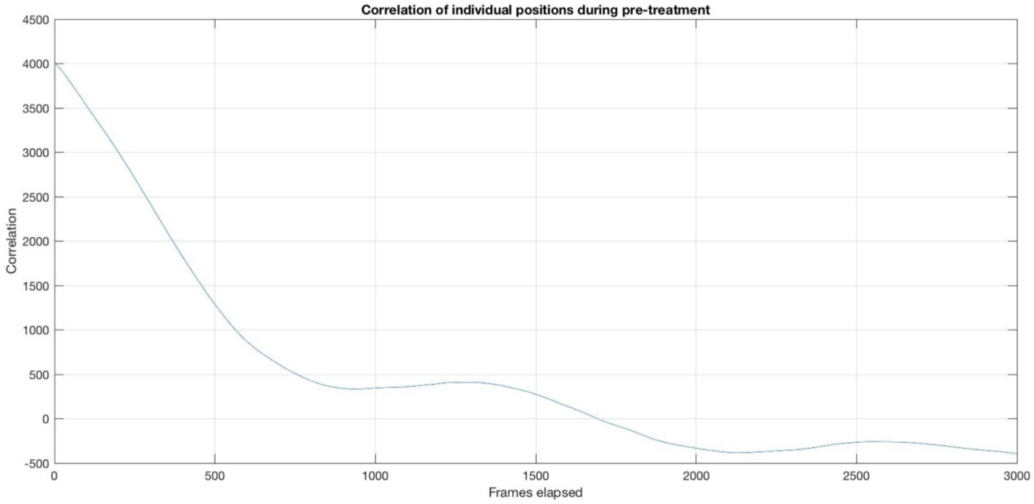


Figure 4.2 Covariance of individual fish positions over time, during the pre-treatment period.

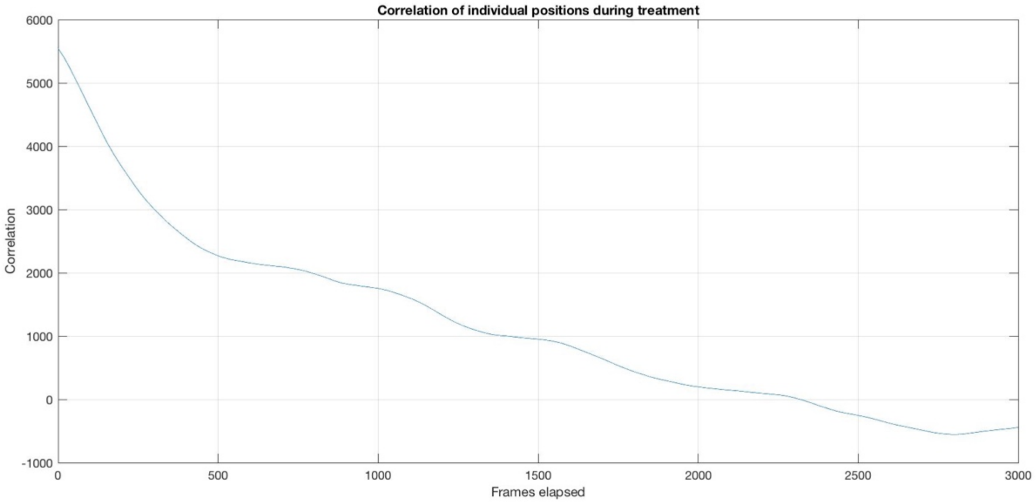


Figure 4.3 Covariance of individual fish positions over time, during the treatment period.

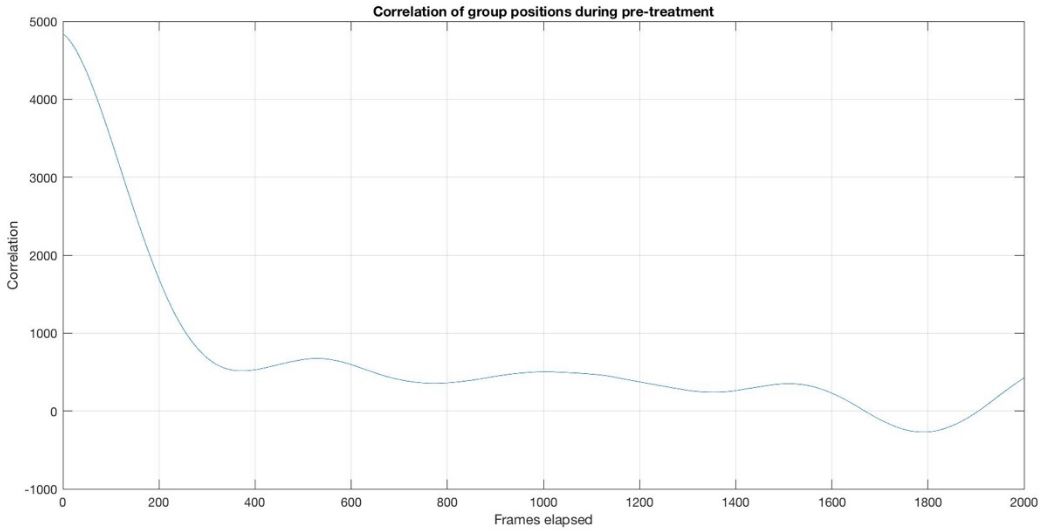


Figure 4.4 Covariance of groups of fish positions over time, during the pre-treatment period.

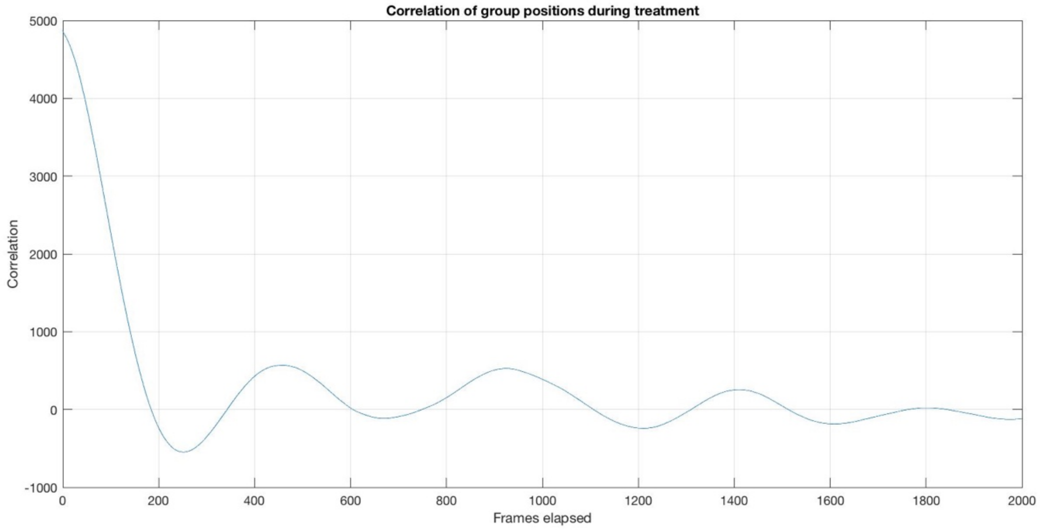


Figure 4.5 Covariance of groups of fish positions over time, during the treatment period.

When investigating objective 1, the binomial test of the times spent close to the speaker in the treatment and pre-treatment period just failed to reach significance (p=0.0537) for individual fish. For groups, this test did reveal a significant result. Groups were observed to display significant avoidance of the area of high intensity sound (p=0.0278) as the number of trials with a larger pre-treatment number were higher than treatment (Table 4.1).

Table 4.3 Results of comparison of changes in spatial positioning of European minnow. Larger pre-treatment values than number of significant values indicates a positive result.

Number of fish in trial	Number of valid trials	Number of trials where more time was spent in the area of high intensity sound in the pre-treatment than treatment	p-value of T
1	12	10	0.0537
5	16	13	0.0278

Following prolonged (five minute) exposure to the sound field the individual fish showed no significant reaction in the first or second halves (p=0.0873). However, the difference between the two halves was significant (p=0.0349). Groups of fish displayed a significant avoidance of the high-intensity area in the first half (p=0.000244) of the treatment period and in the second (p=0.0278), although there was a difference in the number of trials where this was observed. This difference was not significant (p=0.0667), (Table 4.2).

Table 4.4 Results of habituation comparison between individual and groups of five fish

Number of fish in trial	Number of valid trials	Number of trials that display a difference in the first period	p-value of first period	Number of trials that display a difference in the second period	p-value of second period	Number of trials with a difference between first and second periods	p-value of difference between periods
1	12	10	0.0873	10	0.0873	4	0.0349
5	17	16	0.000244	13	0.0278	12	0.0667

When the relationship between cohesion and SPL was examined, there was no positive relationship identified concerning the 2 metrics. The correlation value calculated was -0.0933 where p=0.3108 (Figure 4.6).

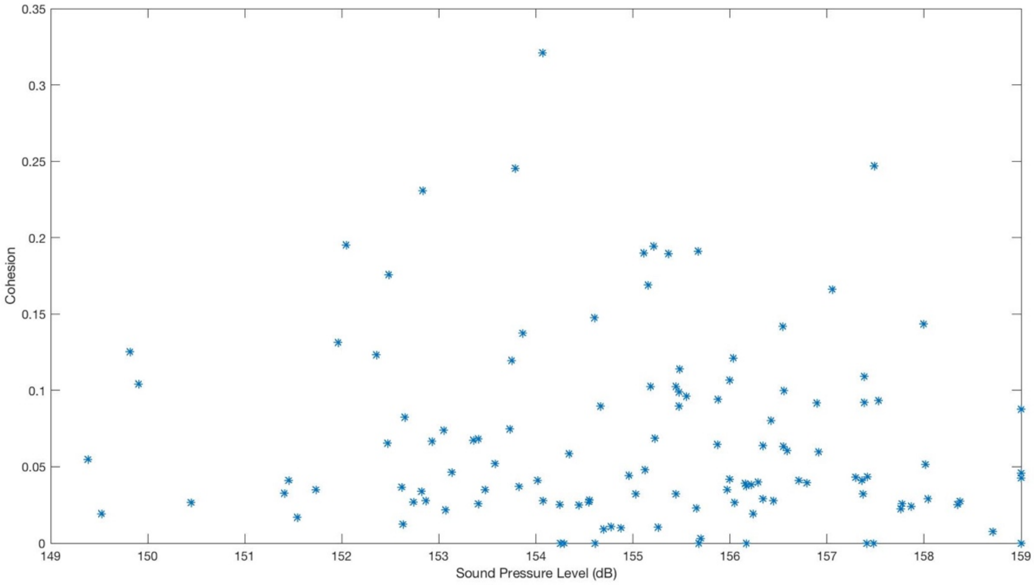


Figure 4.6 Relationship between cohesion and experienced SPL for groups of five European minnow during treatment from high-intensity underwater sound.

To display a more contextually enhanced view of the response to objectives 1 & 2, the position of the individual fish or of the centre mass of the shoal are plotted below (Figures 4.7 – 4.20). These display the placement of the fish throughout the various stages of the trials overlayed onto the SPL colormaps from figure 4.1. To better understand how these placements changed over the course of the trials the pre-treatment positions of the fish were plotted (Figures 4.7 & 4.14). Then, the treatment placement of the fish were plotted (Figures 4.8 & 4.15). These do not provide a fine scale enough view of the behaviour of the fish however, so the treatment is subsequently broken down into the first 2.5 minutes (Figures 4.10 & 4.17) and the second 2.5 minutes (Figures 4.11 & 4.18) of treatment (as per table 4.2). Finally, these conditions are compared with pre-treatment and treatment (Figures 4.9 & 4.16), the first half of treatment and the second half (Figures 4.12 & 4.19) and, importantly, the pre-treatment and the first half (Figures 4.13 & 4.20). This last set of figures are included due to the findings presented in table 4.2, indicating that there is a significant difference between the behaviour demonstrated in these conditions. These comparisons are plotted for left and right speaker configurations. It should be noted that, although explained in the figures, where placements are compared in one figure, the first set of placements is in black and the second in white (i.e., pre-treatment in black and treatment in white).

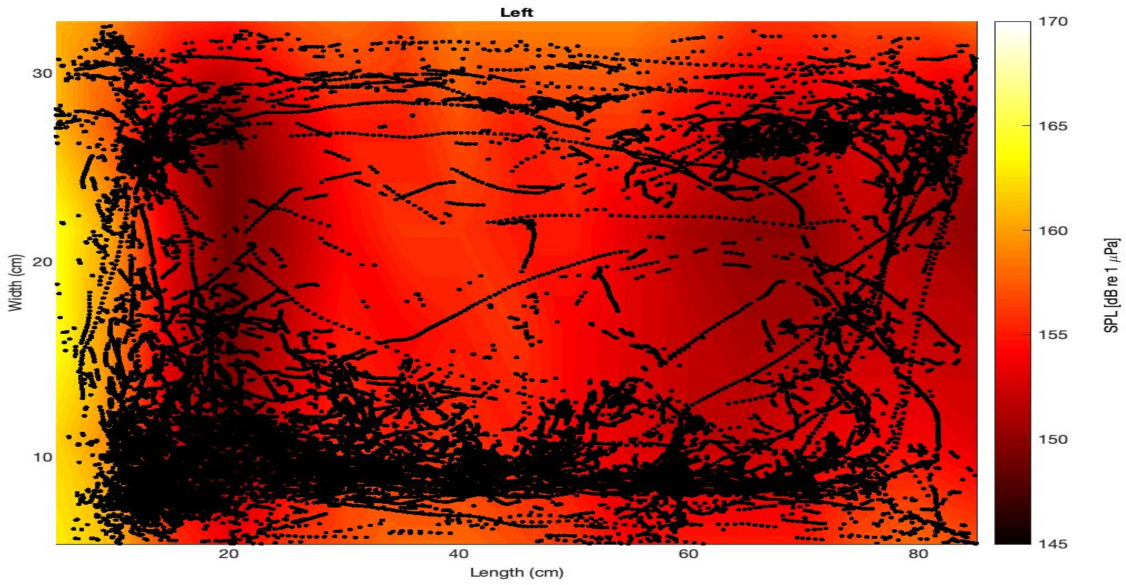


Figure 4.7 Plot displaying fish placement during the pre-treatment section of the left-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the stimulus is not turned on at this point.

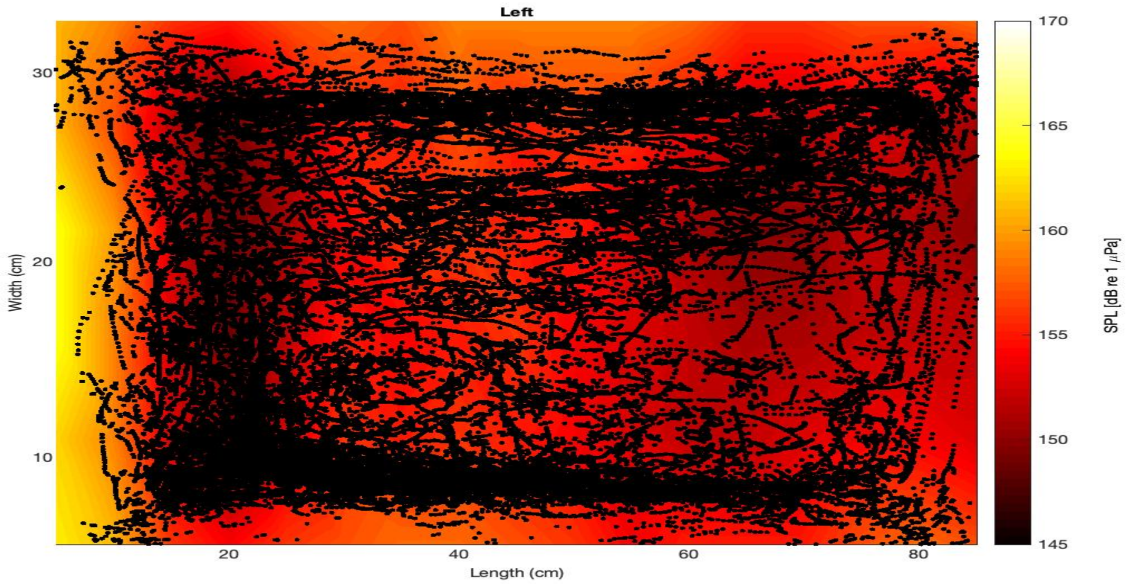


Figure 4.8 Plot displaying fish placement during the treatment section of the left-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated.

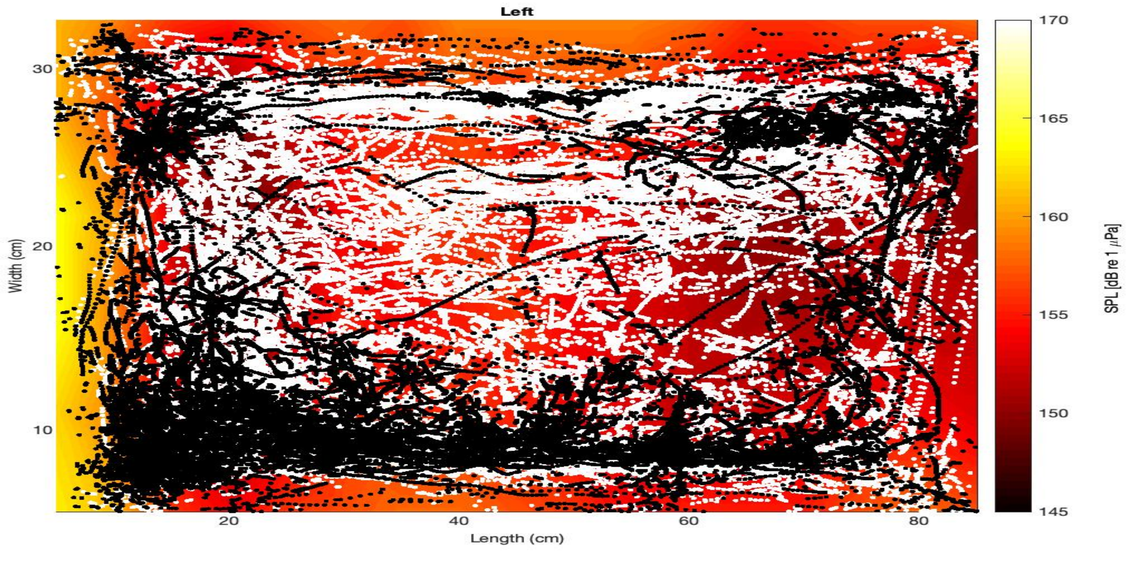


Figure 4.9 Plot displaying fish placement comparing the pre-treatment placement to the treatment placement of the fish. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the pre-treatment is in black and the treatment is displayed in white and that this is for the left treatment.

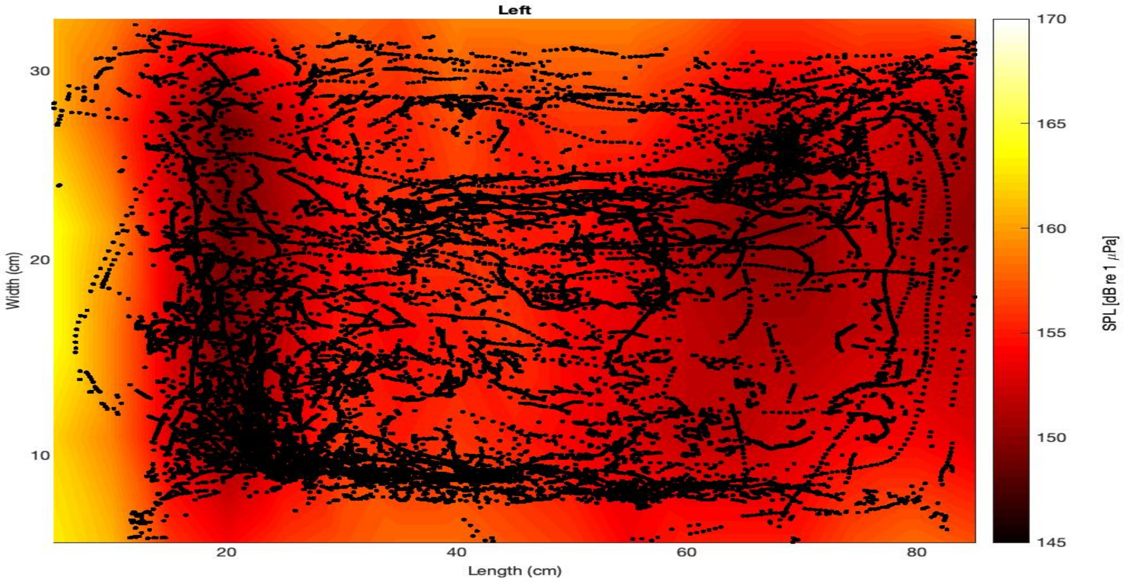


Figure 4.10 Plot displaying fish placement during the first half of the treatment part of the trial of the left-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated.

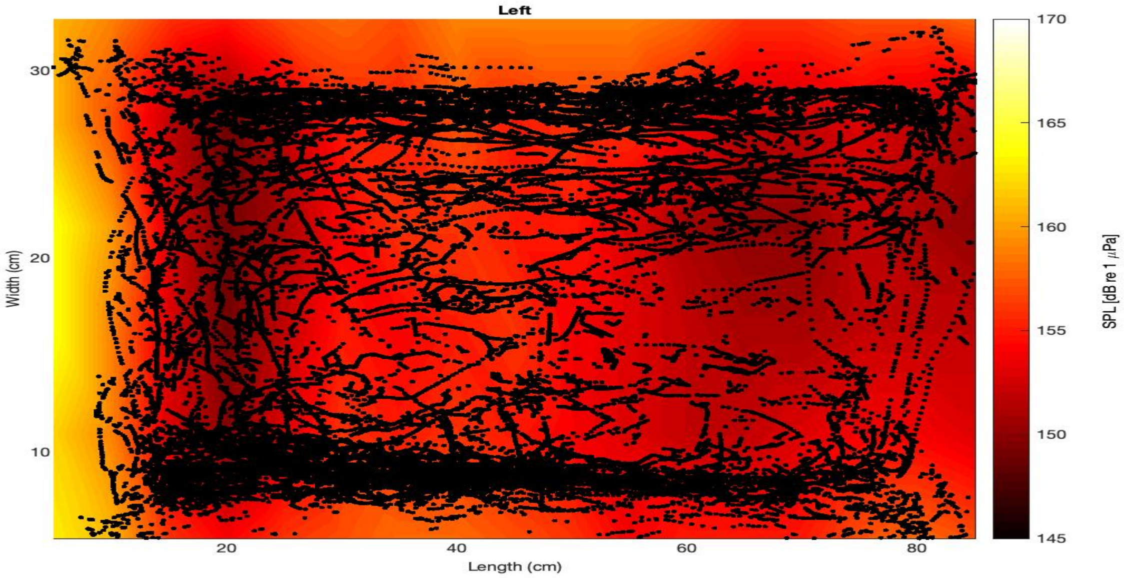


Figure 4.11 Plot displaying fish placement during the second half of the treatment part of the trial of the left-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated.

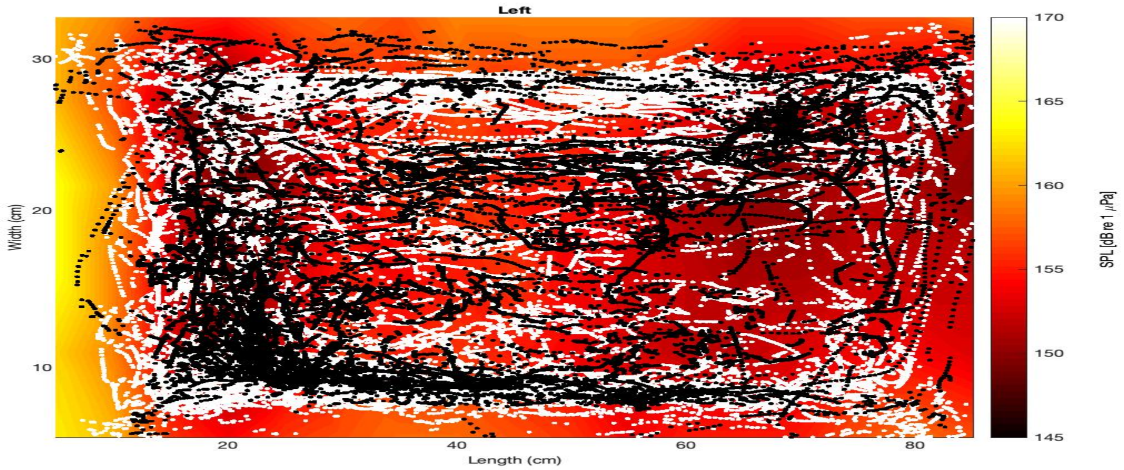


Figure 4.12 Plot displaying fish placement comparing the first half placement to the second half placement of the fish during the treatment period. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the first half is in black and the second half is displayed in white and that this is for the left treatment.

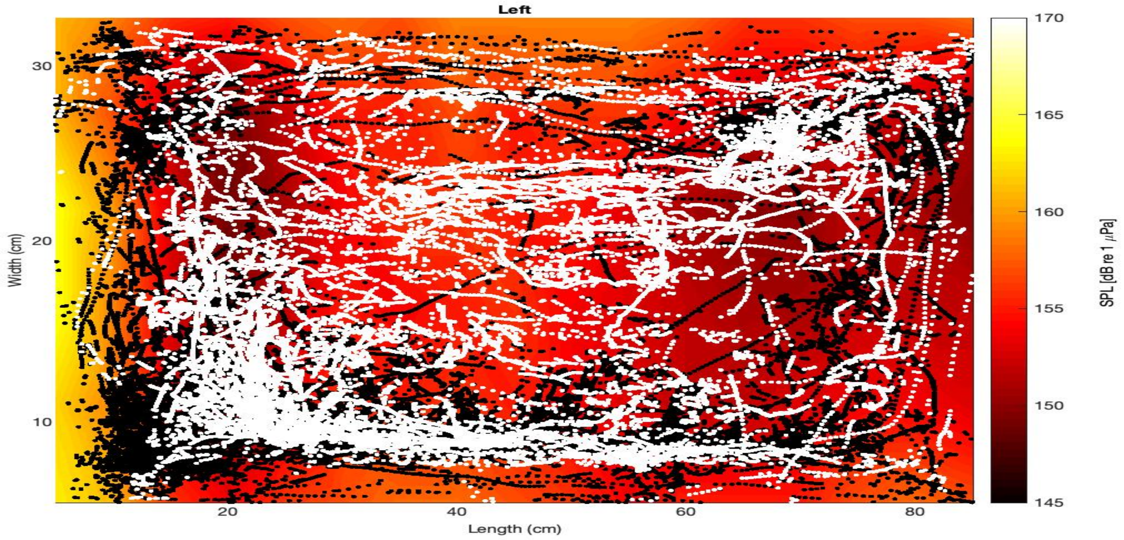


Figure 4.13 Plot displaying fish placement comparing the pre-treatment placement to the first half of the treatment placement of the fish. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the pre-treatment is in black and the first half of the treatment is displayed in white and that this is for the left treatment.

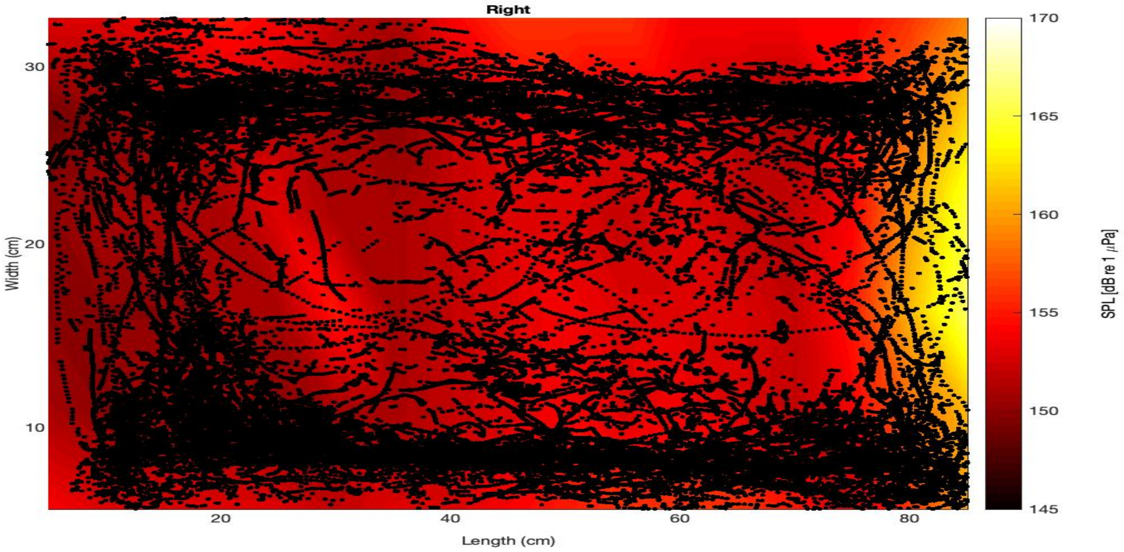


Figure 4.14 Plot displaying fish placement during the pre-treatment section of the right-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the stimulus is not turned on at this point.

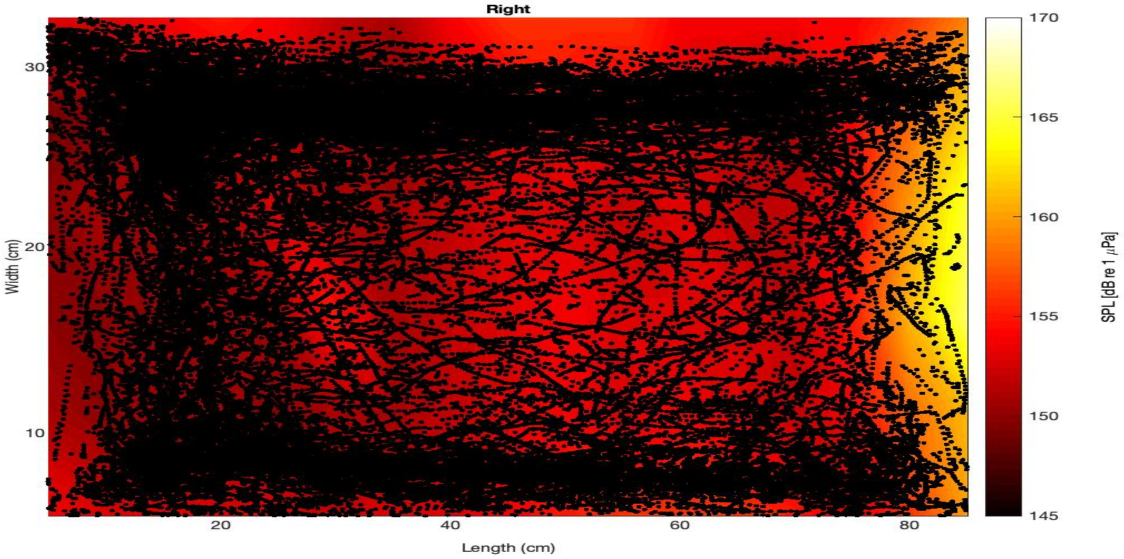


Figure 4.15 Plot displaying fish placement during the treatment section of the right-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated.

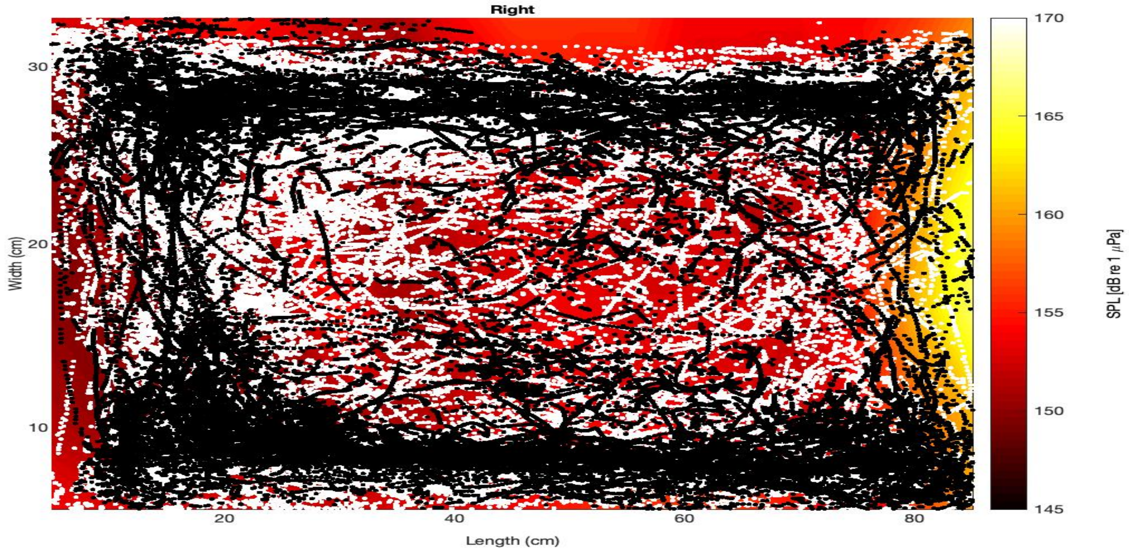


Figure 4.16 Plot displaying fish placement comparing the pre-treatment placement to the treatment placement of the fish. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the pre-treatment is in black and the treatment is displayed in white and that this is for the right treatment.

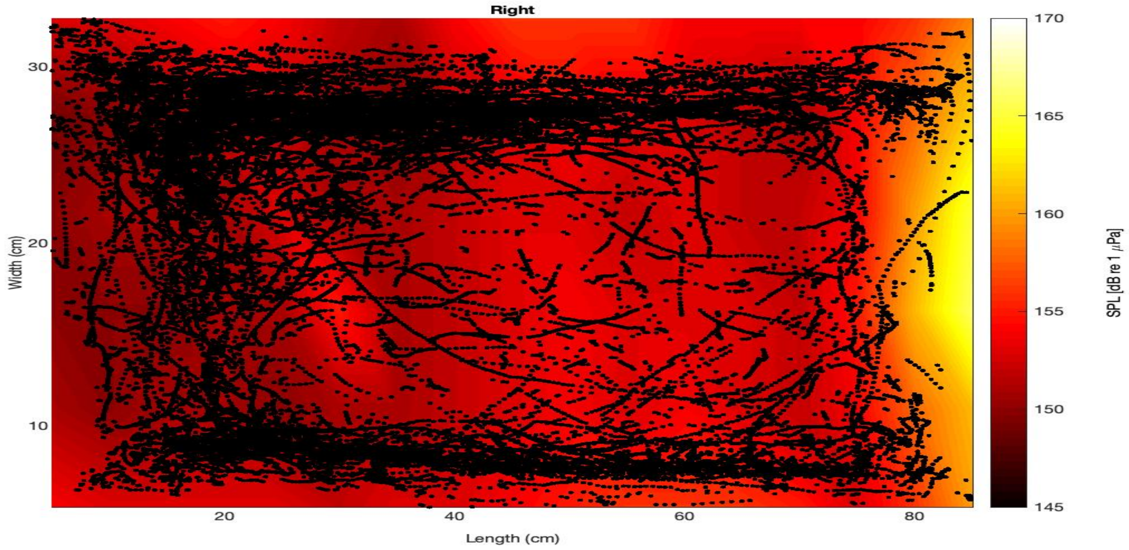


Figure 4.17 Plot displaying fish placement during the first half of the treatment part of the trial of the right-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated.

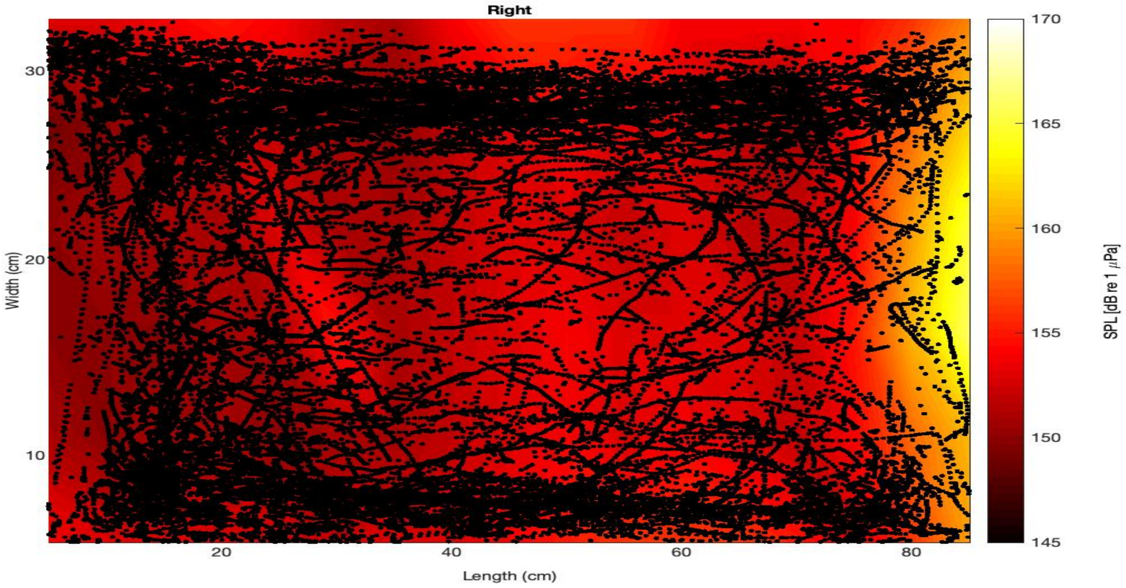


Figure 4.18 Plot displaying fish placement during the second half of the treatment part of the trial of the right-treatment trials. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated.

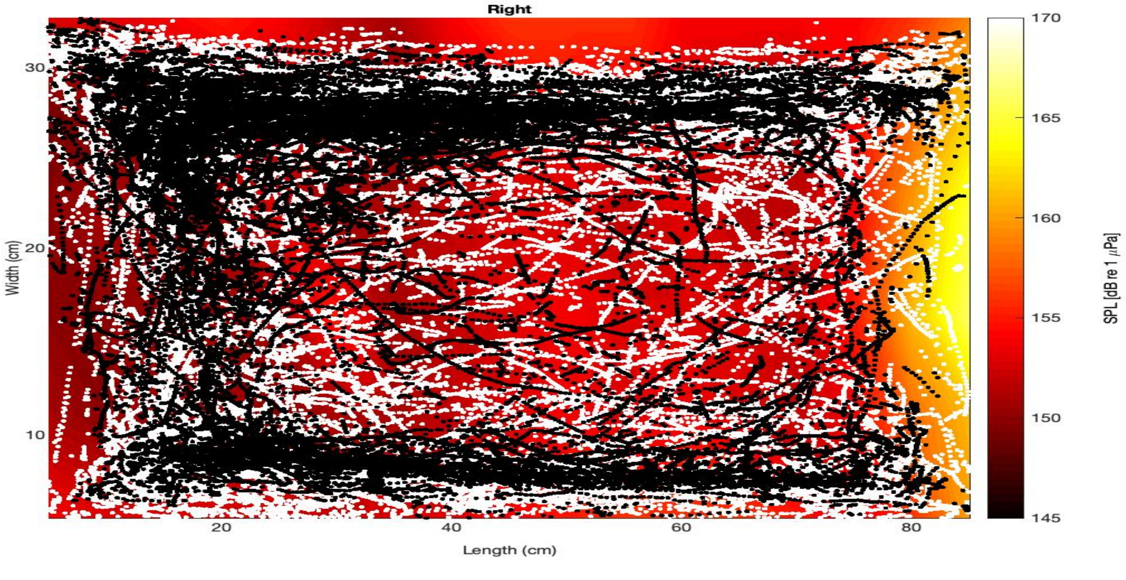


Figure 4.19 Plot displaying fish placement comparing the first half placement to the second half placement of the fish during the treatment period. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the first half is in black and the second half is displayed in white and that this is for the right treatment.

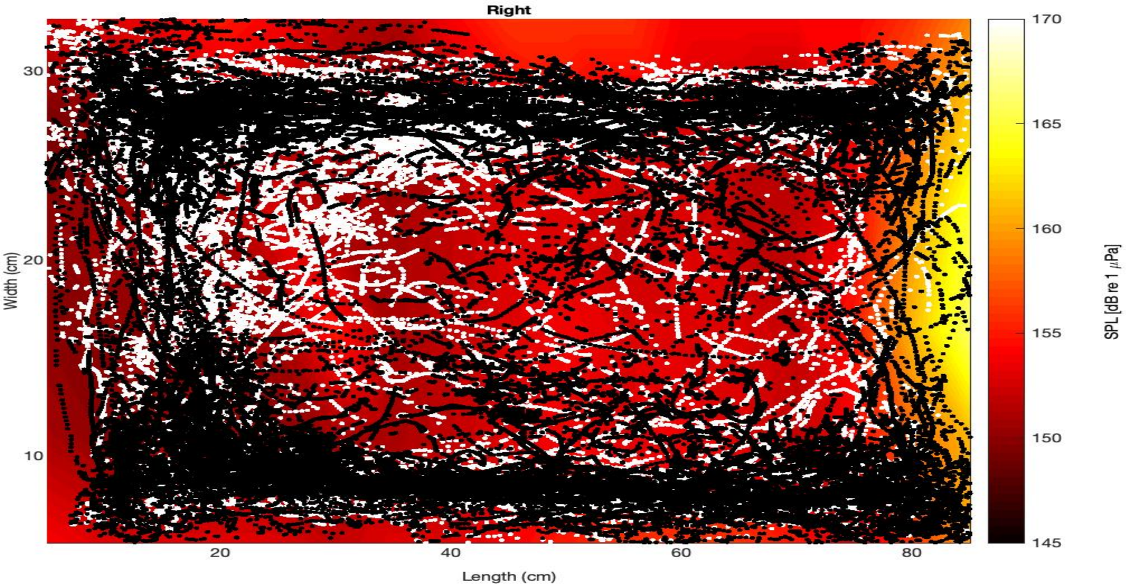


Figure 4.20 Plot displaying fish placement comparing the pre-treatment placement to the first half of the treatment placement of the fish. Fish positions have been overlayed onto a SPL colormap of the sound field when the stimulus is activated. Note the pre-treatment is in black and the first half of the treatment is displayed in white and that this is for the right treatment.

4.4 Discussion

This chapter aimed to explore differences in spatial distribution of fish treated with directional, high intensity sound. In the introduction to this chapter, three objectives were established which will now be discussed in relation to the findings of this research and the wider literature.

To establish the validity of using time-based analysis for avoidance of sound, the relationship between frames elapsed and position along the X axis was calculated and a relationship established for the pre-treatment and treatment periods. The time at which no relationship was noted (the period of no correlation) was suitably less than the time period used to measure a difference in behaviour (Figures 4.2 – 4.5). This means that the chosen time frames for experimental work are valid and that the results so far and those presented in the remainder of this are not impacted by the problems associated with auto correlation and animal movement (Rooney *et al.* 1998). It is worth noting that the data displays a difference between the pre-treatment and treatment values. However, the time period increases between pre-treatment and treatment for individuals and decreases by a large percentage for groups. This indicates that perhaps groups are more affected by this treatment than individuals. In future research, it would be interesting to compare these group effects and differences with the work of Dolder (2014). Specifically investigating if these group effects vary with shoal size and, if so, if this is attributable to changes in animal group organisation and behaviour or changes in the acoustic field due to energy absorption from shoal members. This would further complement previous work in this field such as Hoare et al. (2004) , Saxby et al. (2010) and Schaerf et al. (2017) that explored the differences in behavioural response of groups of fish to various external stimuli.

The distribution of groups changed significantly following treatment by directional, broadband acoustic stimulus. A clear avoidance is displayed by these fish to the area of highest intensity sound at a significant level. The stimulus employed is similar to other studies which have had success in spatially manipulating cypriniform fish (Vetter *et al.*, 2017) in that it was a continuous, broadband sound and not a pure-tone or pulsed signal similar to that used by Neo et al. (2015) on zebrafish (*Danio rerio*). This also aligns with findings from Vetter et al. (2015) that a broadband stimulus (in their case 0 – 10 kHz) was more effective as a deterrent on silver carp (*Hypophthalmichthys molitrix*) than pure tones of specific frequencies between 500 and 2000 Hz. This also aligns with my findings from previous pilot experiments that are discussed in chapter 3. A continuous stimulus was used to ensure uniform experience of the spatial environment such that fish do not enter zones of high-intensity sound without being subjected to the treatment. This is important to consider across small spatial areas as the research presented in this chapter focused on the spatial distribution of fish in an area for the purposes of quantifying avoidance behaviour. With larger experimental areas and more gradual gradients, a directional experiment using a pulsed stimulus becomes more viable. Experiments of this nature include Herbet Read et al. (2017) work on shoaling juvenile sea bass (*Dicentrarchus labrax*) which is discussed in relation to the findings from this chapter, and also chapters 5 & 6 in chapter 7. The smaller size of the water tank was used to ensure positions of the small fish used were captured accurately. By not pulsing the stimulus, the study has attempted to overcome one of the known limitations of conducting experimental work with acoustics in water tanks. Specifically, using tanks and stimuli where the relationship between the tank materials and the observed SPL and particle acceleration field vary over time and are not representative of the mapping exercise as undertaken by the researcher (Duncan *et al.* 2016).

When observing the physical placement of the fish in the experimental area before and during treatment (Figures 5.7 – 4.20) it is not immediately obvious what, if any difference there is in placement. For this reason, there placement has been broken down into several key temporal stages and overlayed onto the SPL colour map plots created through the mapping process described in sections 3.2 and 4.2. Initial examination of placement before (Figures 4.7 & 4.14) and during treatment (Figures 4.8 & 4.15) shows a subtle movement away from the 15 – cm zone of higher intensity sound for the left treatment, but no discernible movement for the right treatment. Figures 4.8 and 4.16 emphasise this point by overlaying this data. To better examine any differences, the plotted data was then broken down into the first half (2.5 minutes), (Figures 4.10 & 4.17) and the second half (2.5 minutes), (Figures 4.11 & 4.18) of the treatment. This decision to split the treatment period in half in this part of the analysis was driven by the fact that the analysis presented in table 4.2 displayed a significant difference in placement between the first and second half of the treatment period. When exploring these differences visually (Figures 4.12 & 4.19) there is a clear difference in the placement of the fish between these two halves of the treatment period, with more white dots than black in the area of highest intensity sound and also more black dots in the middle. This means that fish tended to avoid the areas of highest intensity sound in the first half of the trial. It is also interesting that there were more black dots in the middle sections for both the left and right treatment. As a general rule, the fish tended to remain against the sides of the tank at all times during the pre-treatment periods. This could indicate a disruption to their foraging or anti-predator behaviours when treated in this way with this stimulus (Neo *et al.* 2014). Finally, plotting the placement of the fish in the pre-treatment period against the placement of the fish in the first half of the treatment (Figures 4.13 & 4.20) revealed a clear movement away from the area of highest intensity sound (Figure 4.13) and a much greater density of fish positions at the opposite end of the experimental area (Figure 4.20). These findings further re-enforce the claim that these fish displayed avoidance behaviour in the presence of a high-intensity acoustic stimulus.

Habituation by return to normal distribution is an important measure when considering the implications for those interested in behavioural deterrents and the impacts of noise pollution. When considering that the stimulus was not pulsed it may be assumed that an animal may habituate to the stimulus more quickly (Blumstein, 2014). There was however no significant acclimation to the sound field noted in this experiment. This may be due to the ability of the fish to avoid the area of high-intensity sound. The minnows displayed a negative preference for this area at the beginning and this remained until the end of the treatment. Because the option of an area of lower intensity sound was available and the fish displayed initial avoidance of the louder zone, it is concluded that the lack of habituation by entering the area of high intensity is due to a continued preference for the quieter area. When considering more complex metrics, further research should expose fish to a non-directional stimulus to measure habituation by fish to a sound field which cannot be avoided.

This research also aimed to explore any correlation in the distribution of the group of fish and their overall cohesion during treatment with a directional acoustic stimulus. This was measured by calculating the cohesion of the group of fish at the point of no correlation

(Figure 4.5, every 56 seconds during the treatment period) for every trial. This cohesion was then compared to the location of the centroid of the group of fish (as calculated in equation 2, section 3.3) and the sound intensity as measured at that location. The results plotted in figure 4.6 and the subsequent data analysis reveals no positive relationship between the cohesion of the group and the intensity of the acoustic field. This may be because the areas of highest intensity were avoidable, and the fish displayed a tendency to avoid them. For this reason, subsequent research presented within this thesis used more uniform acoustic fields to ensure changes in fine-scale behaviours were captured and reported on.

Through addressing and satisfying the aim stated in section 4.1, that is to explore the differences in spatial distribution and behaviour of cypriniform fish in a tank when exposed to a directional acoustic stimulus in groups and individually, this chapter has contributed to knowledge. Firstly, through outlining the avoidance of a small, shoaling cypriniform to sound. Second, by demonstrating a difference in reaction between groups and individuals. Third, in demonstrating a difference in habituation that needs to be explored at a finer resolution and fourth, by demonstrating that there is further need to explore complex behaviours displayed by fish under treatment from acoustic stimuli, both in groups and individually.

Studies that focus on changing the spatial position of a fish by using acoustic stimuli have been conducted as part of a managerial approach, but they risk missing other important information regarding how the fish are reacting at a more-subtle level (Zielinski *et al*., 2014). Spatial data is particularly useful for fisheries managers and designers of behavioural deterrents in the most direct sense as the movement of fish in a waterway away from a target site is the goal of a behavioural deterrent (Patrick *et al*., 1985; Popper & Carlson, 1998; Taylor *et al*., 2005; Zielinski & Sorensen, 2015). As behavioural deterrents are inherently less than 100% effective (Taylor *et al*., 2005; Mussen *et al*., 2014; Zielinski & Sorensen, 2015), understanding the influence of the stimuli they provide to fish on the behaviours of the animals themselves will contribute to increased passage efficiency. Exploring these additional behaviours and interactions between individuals in a group is vital to enhanced passage technologies but does not drive the direction of this research. The importance of greater understanding of how fish respond to sound and how group behaviour may play a key role in this response is given priority in the next chapter of this thesis. A formal discussion that brings together the findings from this chapter and the following two chapters will be presented in chapter 7.

5 The behavioural influence of a non-directional acoustic field on individual European minnows

Understanding collective behaviour of animals and how it might be influenced by anthropogenic activity is vital to their protection in an increasingly urbanised world. Transport networks and other logistical operations in and around the aquatic environment have the potential to produce damaging acoustic noise. The reduced attenuation of sound in water, coupled with the highly evolved hearing ability of many fish, exposes these animals to sound produced from a wide spatial area and has the potential to put them and their behavioural bonds at risk. Many studies have, to date, covered avoidance of stimuli in laboratory and field settings however, frequently the scale of these experiments prevents finer-scale behaviours being observed.

Following on from the results of chapter 4, this piece of research aimed to measure the behavioural impact of a non-directional acoustic field on the behaviour of individual European minnows. When treated with this intense, non-directional acoustic field individual minnows were discovered to swim faster and less persistently. Individual minnow also displayed a range of initial reactions: startle, holding station and no discernible response. Individual minnow also displayed changing behaviours across the treatment period with no significant acclimation to the stimulus.

This chapter contributes to knowledge by demonstrating that these fish display finer scale behaviours and that, as individual members of a shoaling fish species, their initial reaction varies but they did not acclimate during the experimental period provided. These findings have wider impacts for scientists working in the field of behavioural deterrents and barrier management.

5.1 Introduction

Management of fisheries and waterways has driven research for many years (Beveridge & Little, 2002) and has led to a balance of papers that focus on the large-scale behaviour and avoidance (or not) of potential acoustic and other behavioural deterrents (Scruton *et al.*, 2003; Piper *et al.*, 2012; Zielinski *et al.*, 2014; Vetter *et al.*, 2017). There is another part of the aquatic noise community that is interested in the impacts of noise pollution, whether in the marine (pelagic or in-shore), (Myberg, 1990; Parks *et al.*, 2011) or freshwater environments (Popper *et al.*, 2003b; Herbert-Read *et al.*, 2017). These studies typically focus on ABR measurement or, similar to the management driven research, avoidance behaviours. One common theme is behavioural change, that is induced by an external stimulus, which is at the core of the majority of fisheries biology research (Popper, 2003).

Fine-scale behaviours of fish exposed to sound are particularly important as they are comparatively little-studied and may provide greater insight to the manner in which fish elect to avoid (or not) high intensity sound sources, or how they are adversely affected by them. Fish, as organic animals, are capable of more complex behaviours than simple avoidance-based, taxis responses. The multiplicity that the behaviour of these fish represents is influenced by inherited and learnt factors and it is too simple an understanding to suggest that an animal either avoids a noise or it does not. The reasons for an avoidance behaviour are important to understand as they may impact the design of a deterrent to increase efficiency or the mitigation approach for an activity that produces noise pollution. However, a focus on avoidance and simple, phono-taxis potentially misses much detail. Measuring a number of different metrics in this study will aid greater understanding of the influence of underwater sound on aquatic life.

The stimulus used in the experiment presented in this chapter is a pulsed stimulus because pulsing is a frequently used characteristic of deterrents (Culik *et al.*, 2001; Götz & Janik, 2015; Romine *et al.*, 2015) and also more accurately represents many forms of noise pollution that are not continuous, for example pile-driving (Herbert-Read *et al.*, 2017). Due to the non-directional and more uniform nature of the sound field in comparison to that used in chapter 4, habituation may be measured when treated by aquatic noise which is less predictable (for example, pulsed) and inescapable. Habituation is a useful metric when considering the temporal effects of aquatic noise pollution on underwater life and also the potential impact of sound on an individual, separated from a group setting. The rate of return to normal behaviour can be used by those designing deterrents and other fisheries management technologies but is also important to understand from the point of view of environmental impact assessment and mitigation. The effective period of a noise on the behaviour of an animal can be used as information to design and restrict activities with potentially damaging consequences for these fish.

With a species that displays group behaviour, the picture and driver behind the individual's behaviour is even more complex. Isolating an individual may provide significant insight into how group settings influence behaviour. Focusing on an individual's behaviour, without influence from additional stimuli, will also provide further information on fine-scale behaviours. These behaviours may not be naturally displayed in a group setting and therefore have the potential to be missed in studies that focus on group behaviour. As an individual has the potential to be isolated from conspecifics, understanding the behaviour of fish in these circumstances is important and, often, not considered.

When considering the impacts of acoustic stimuli on the behaviour of fish there has also traditionally been a focus on either groups of fish or individuals (Slabekoorn *et al*., 2010; Popper & Fay, 2011). By comparison, little work has been done linking group behaviour and that displayed by solitary fish. This study aims to build on the previous work described in chapter 4 to explore the differences in behaviour exhibited by individuals under acoustic treatment. The links between these two conditions (solitary and grouped fish) that are explored in this work may prove highly valuable in gaining a greater understanding of the mechanism by which all fish change their behaviour when stimulated by a sound source.

Focusing on swimming speed and persistence also provides insight regarding the extent to which an individual fish may attempt to explore and potentially avoid the stimulus. Understanding these behaviours can be beneficial to a fisheries manager deploying a behavioural deterrent, although they have the potential to come at a higher cost through additional expended energy. These energetic costs can be better accounted and mitigated for if they are understood. The additional exploration that an individual may undertake if an increase in swimming speed and a change in persistence is observed may also contribute to the design of fish passage technology as an individual fish is more likely to encounter a pass if their exploration is greater.

Individuals of a shoaling species were chosen to investigate as individuals of a shoaling species may encounter noise pollution or acoustic deterrents in a solitary condition and there is currently no understanding for this species as to what reaction they might display in response to sound.

The experiment aimed to quantify the initial reaction and fine-scale behaviours of individual fish to a non-directional sound field by meeting the following objectives:

1) To create and measure a non-directional acoustic field in a water tank.
2) To explore if individual fish change their swimming speed or persistence of swim paths during treatment from an acoustic signal.
3) To examine if individual fish habituate to an acoustic stimulus within a water tank.

A following piece of research should look at these same chosen metrics and quantify the reaction of a small group for comparison with the findings of this chapter.

5.2 Methodology

5.2.1 Experimental set-up

This piece of research used the experimental setup as described in chapter 3. An experiment to test the behavioural response of individual minnow to sound was conducted in a tank holding standing water, based at the International Centre for Ecohydraulics Research (ICER), University of Southampton, UK. The tank (total dimensions 30 cm wide, 30 cm deep and 300 cm long) was constructed of transparent acrylic with an experimental area in the centre measuring 30 cm wide, 30 cm deep and 85 cm long. The central area was created by two wooden frames with white plastic sheeting stretched over them at either end. One speaker (Electro-Voice UW-30) was placed 10 cm behind each frame in mid-water. An overhead camera (Logitech c920 Webcam) was installed 1.5 m above the tank floor. White sheeting was also placed around the tank to aid lighting, in particular increasing the contrast whilst retaining an open-top. Lighting was provided by two photographic diffusers placed below the tank that projected onto the white sheets surrounding the experimental area to ensure uniform illumination. The camera was connected to a laptop running QuickTime to capture the video data at a 30 Hz frame rate. Water temperature remained measured over the experimental period (mean $\pm$ SD = 16.98 $\pm$ 1.55 °C).

The acoustic stimulus was generated from MATLAB via a National Instruments USB-6341 data acquisition system and powered by a Skytronic 103.100 Mini AV digital amplifier. The stimulus comprised a broadband signal of filtered pseudo-random Gaussian noise between 60 - 2000 Hz. This stimulus was pulsed on for 2 seconds and off for 1 second which was repeated for 10 minutes. The experimental area was subsequently mapped using a hydrophone (Bruel & Kjaer 8103) connected to a charge amplifier (Bruel & Kjaer 2635). The sound pressure level was measured over a 5 x 5 cm grid pattern, measured at three depths (2 cm, 13 cm and 24 cm above tank floor (ATF)) to quantify the noise field within the tank. Figure 5.1 illustrates one such map of the sound field in the tank measured as a broadband stimulus, where the acoustic field is greatest close to the speakers at either end of the experimental area. This is consistent with the fact that the water is very much shallower than the wavelength of the majority of the sound signal, which is approximately 3.75 metres.

Comparing the colourmap plots produced from the hydrophone mapping exercise allows the reader to compare differences between Sound Pressure Levels (SPL) and Particle Motion (PM) at specific spatial points. The plots below provide both these measurements across the experimental area at a depth of 13.5cm above tank floor for the broadband stimulus and also at frequencies of 200 Hz, 400 Hz, 600 Hz, 800 Hz, 1 kHz and 2 kHz. These plots display particular nodes and other specific features at discrete frequencies that are not present in the broadband stimulus as mapped in Figure 1 below. The broadly consistent nature of the sound field is a result of the previous work as noted in section 3.1.1. Note that there are also differences in the colourmap plots for SPL and PM at the same frequency.

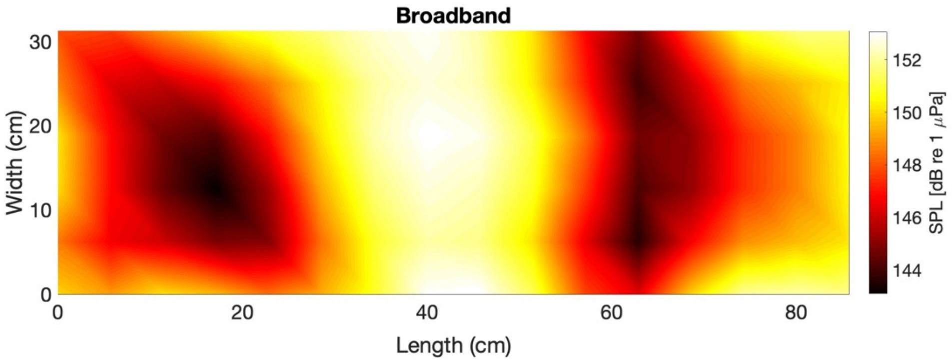


Figure *5*.1 Sound pressure level colourmap plot of the broadband stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades. Note the higher sound levels at either end of the tank due to the speakers placed in mid-water, 10 cm from either end.

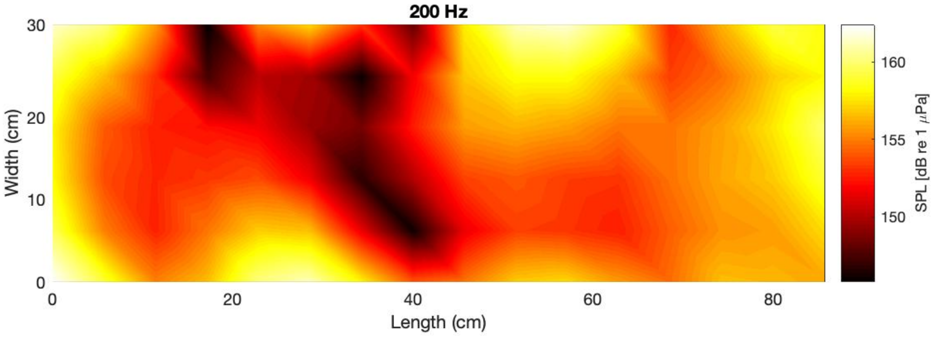


Figure 5.2 Sound pressure level colourmap plot of the 200 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades. Note the higher sound levels at either end of the tank due to the speakers placed in mid-water, 10 cm from either end.

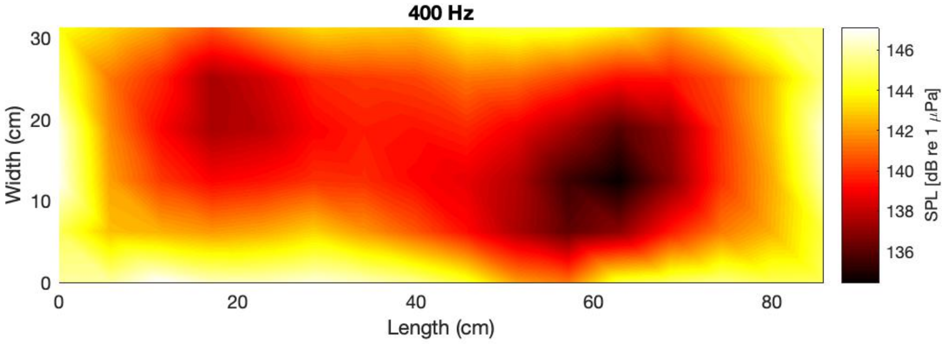


Figure 5.3 Sound pressure level colourmap plot of the 400 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades. Note the higher sound levels at either end of the tank due to the speakers placed in mid-water, 10 cm from either end.

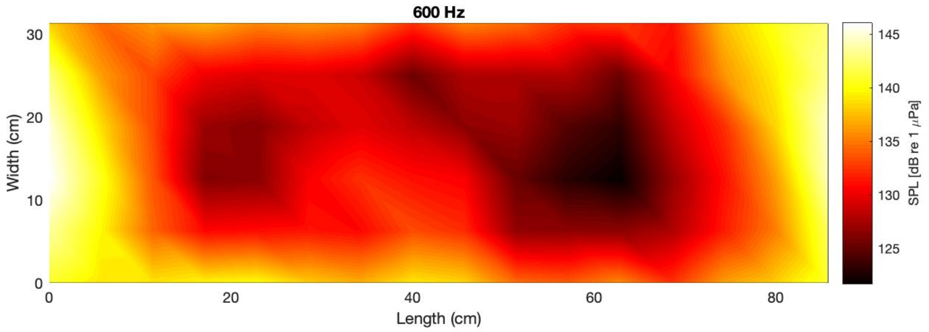


Figure *5*.4 Sound pressure level colourmap plot of the 600 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades. Note the higher sound levels at either end of the tank due to the speakers placed in mid-water, 10 cm from either end.

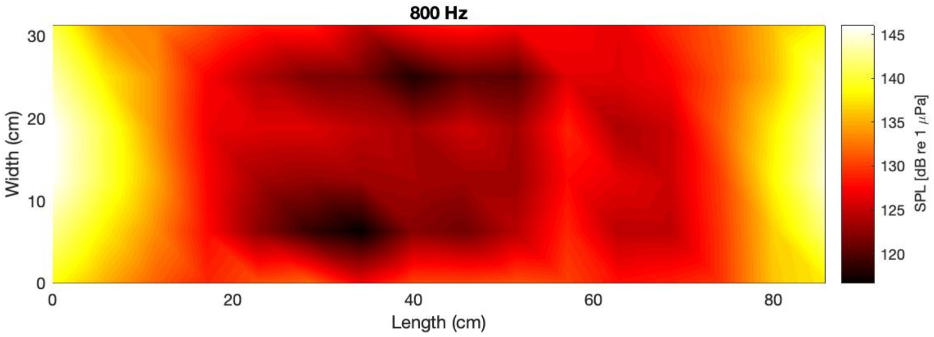


Figure *5*.5 Sound pressure level colourmap plot of the 800 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades. Note the higher sound levels at either end of the tank due to the speakers placed in mid-water, 10 cm from either end.

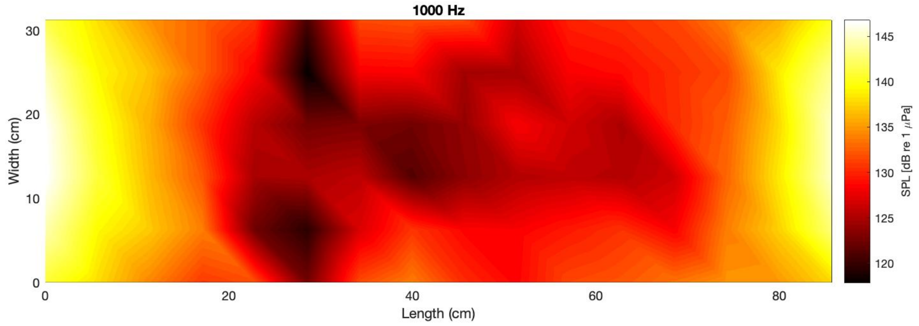


Figure *5.6* Sound pressure level colourmap plot of the 1000 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades. Note the higher sound levels at either end of the tank due to the speakers placed in mid-water, 10 cm from either end.

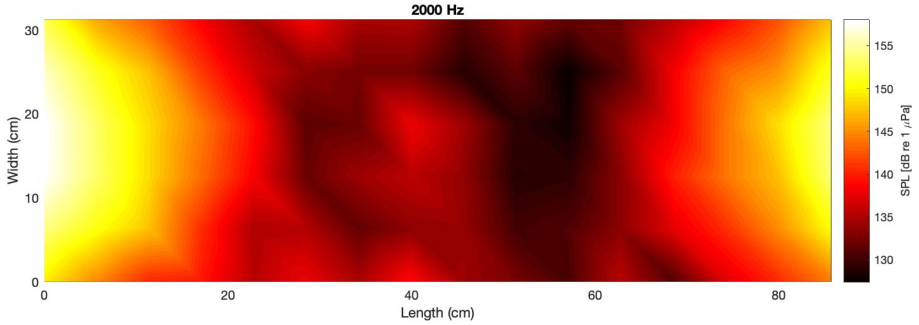


Figure *5.7* Sound pressure level colourmap plot of the 2000 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re 1 μPa, with dark shades corresponding to lower intensities than light shades. Note the higher sound levels at either end of the tank due to the speakers placed in mid-water, 10 cm from either end.

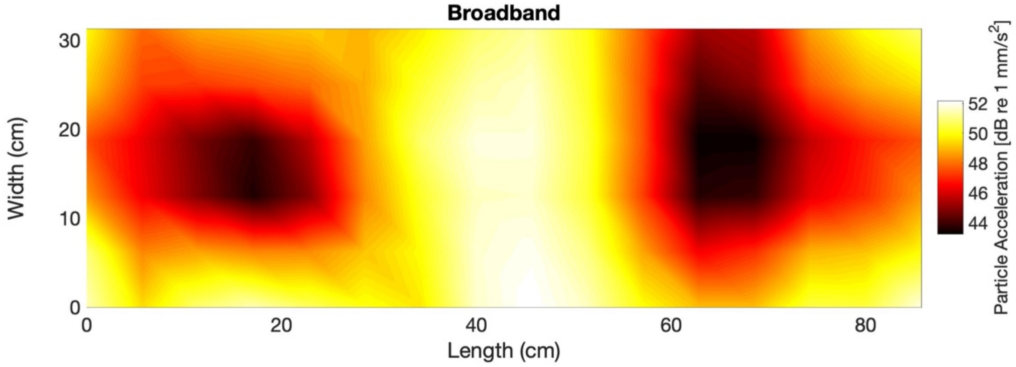


Figure *5.8* Particle acceleration colourmap plot of the broadband stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the Particle Acceleration at a point, expressed in dB re 1 mm/s^2, with dark shades corresponding to lower acceleration than light shades.

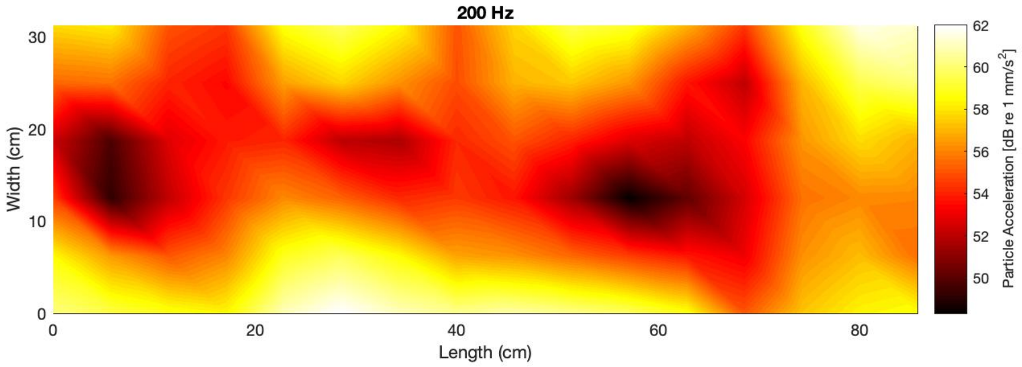


Figure *5.9* Particle acceleration colourmap plot of the 200 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the Particle Acceleration at a point, expressed in dB re 1 mm/s^2, with dark shades corresponding to lower acceleration than light shades.

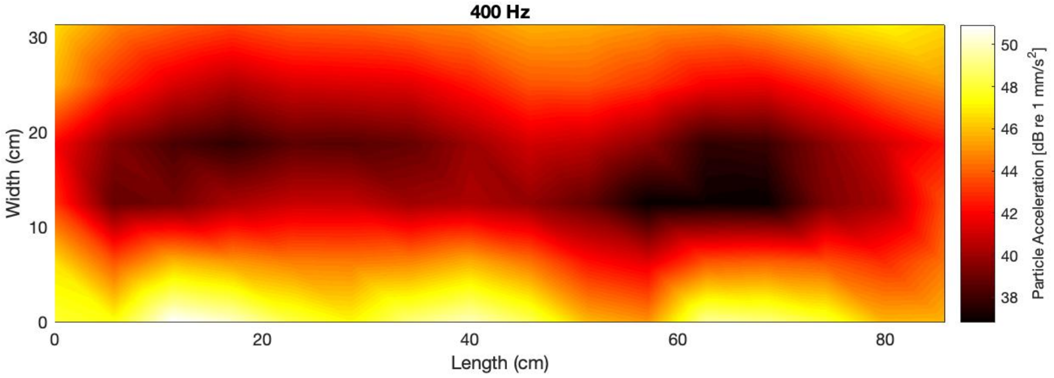


Figure *5*.10 Particle acceleration colourmap plot of the 400 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the Particle Acceleration at a point, expressed in dB re 1 mm/s^2, with dark shades corresponding to lower acceleration than light shades.

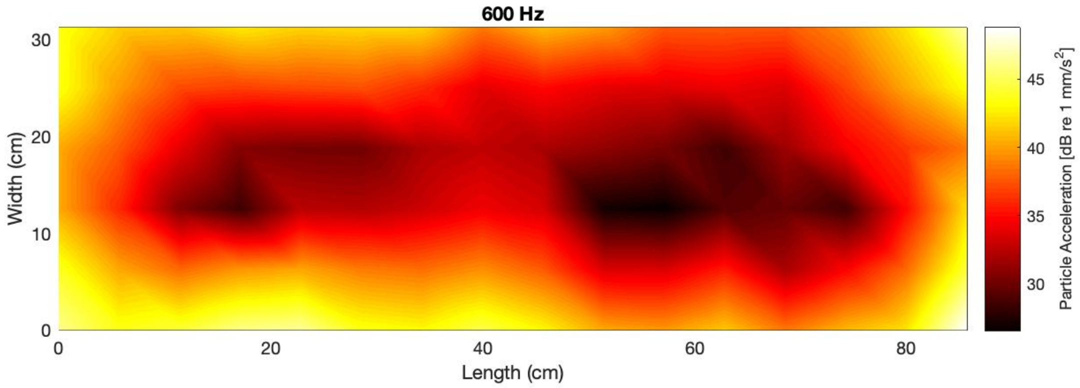


Figure *5*.11 Particle acceleration colourmap plot of the 600 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the Particle Acceleration at a point, expressed in dB re 1 mm/s^2, with dark shades corresponding to lower acceleration than light shades.

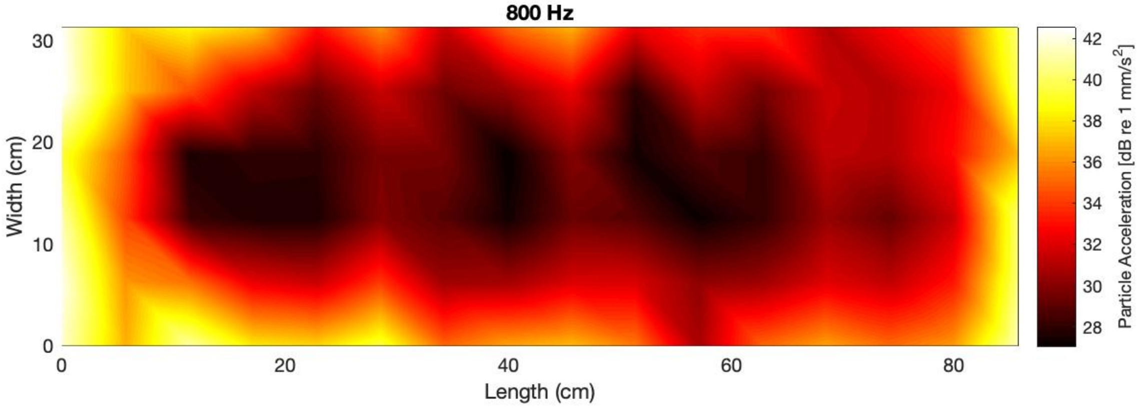


Figure *5*.12 Particle acceleration colourmap plot of the 800 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the Particle Acceleration at a point, expressed in dB re 1 mm/s^2, with dark shades corresponding to lower acceleration than light shades.

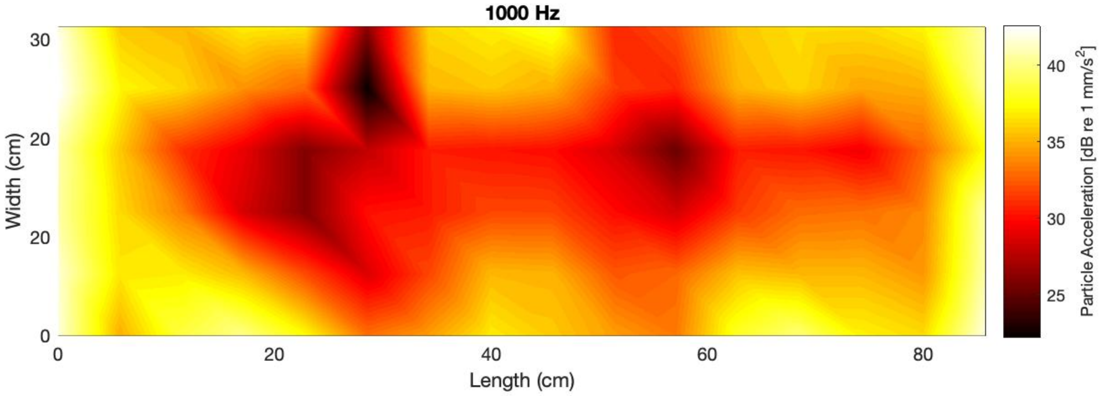


Figure *5*.13 Particle acceleration colourmap plot of the 1000 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*).

The colour scale represents the Particle Acceleration at a point, expressed in dB re 1 mm/s^2, with dark shades corresponding to lower acceleration than light shades.

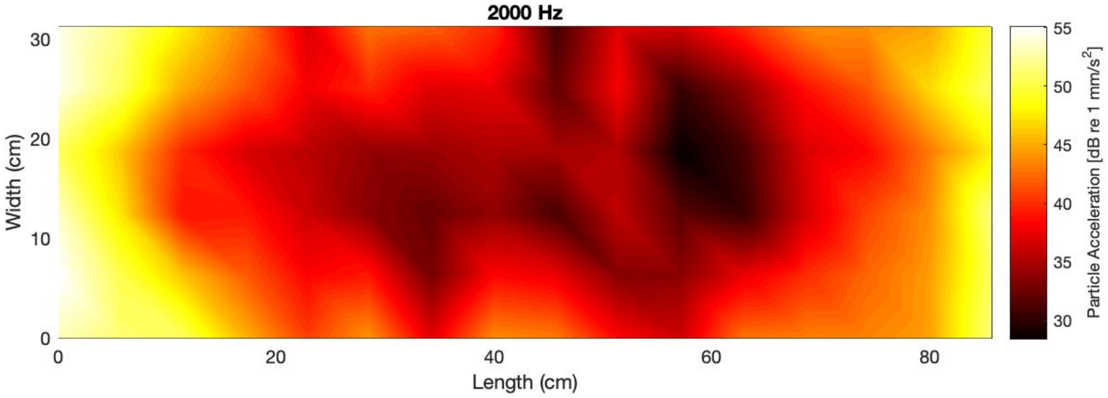


Figure *5.*14 Particle acceleration colourmap plot of the 2000 Hz frequency band as part of the stimulus across the experimental water tank, measured over a 5 x 5 cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of acoustic stimulation on shoaling behaviour of the European minnow (*Phoxinus phoxinus*). The colour scale represents the Particle Acceleration at a point, expressed in dB re 1 mm/s^2, with dark shades corresponding to lower acceleration than light shades.

5.2.2 Fish collection and maintenance

European minnow (n = 20, mean total length and mass ± SD = 54.30 ± 4.23 mm, 1.71 ± 0.49 g) were collected from the River Itchen Navigation, (St. Catherine's Hill, Winchester, UK, 51.049783 -1.311416) using a 10 m seine net and transported in a 100 L aerated container to the ICER holding facility. The fish were maintained in a tank (150 cm wide, 150 long, 100 cm deep with a water depth of 70 cm) prior to conducting trials. The holding tank was filtered and aerated and daily water testing and subsequent changes (approximately 25%) ensured high water quality was maintained (nitrite < 1 mg L−1 and nitrate < 50 mg L−1). The water temperature in the holding tank remained stable throughout the experiment (mean ± SD = 16.98 ± 1.55 °C).

5.2.3 Experimental trials

A total of twenty 50-minute trials, using individual fish were conducted between 29th and 31st May 2016. Trials were conducted throughout the day and night. At the start of each trial the fish were placed into the experimental area and a 20-minute acclimation period commenced. A further 20-minute pre-treatment in which no acoustic stimulus was present followed. In this period, the analysis begins with the final 2 minutes prior to the 10-minute exposure to the stimulus (treatment). After this, the trial was ended, and the fish were removed from the experimental area and weighed and measured. No fish were used in more than one trial. Permission to remove European minnow from the Itchen was provided by the Environment Agency (permit reference EP/EW083-L-263/5065/02). Ethical approval for this study was provided by the University of Southampton Animal Welfare and Ethical Review Board.

5.2.4 Analysis

Qualitative observations

Qualitative descriptions of fish behaviour were categorised as:

(1) Startle – where the fish reacted immediately to the treatment in a manner different to control behaviour.
(2) Holding station– where the fish stopped moving momentarily.
(3) No discernible response– where no change in behaviour was apparent.

Quantitative analysis

Analysis was then divided into two key metrics, swimming speed and persistence of swimming path, to demonstrate any differences under treatment. To quantify these reactions, video data were analysed and 2D co-ordinates of fish position relative to the walls of the tank recorded using MATLAB to provide location over time data. The method for this is as described in section 3.3 for persistence of swim path and swimming speed of the fish.

Once these metrics were computed for every frame in the image, the data were averaged for each second, i.e., over 30 frames, to reduce noise and mitigate against tracking artefacts. The resulting data were then analysed using a Wilcoxon Rank Sum Test (WRST) to explore any differences in behaviour. The data compared using the WRST were the 1 second averaged data from 10 minutes prior to the treatment period (the control period) and the 10 minutes of noise (the treatment period). Due to the manner in which the data is tested with a moving average there is a merging effect on the data displayed (Figures. 5.15 & 5.16).

5.3 Results

Qualitative observations

Upon activation of the stimulus, 45% of the individuals displayed a startle reaction, 30% of the individuals held station on initiating the treatment, whereas 25% displayed no perceivable reaction (table 5.1).

Table 5.1 List of behaviours initially displayed by fish treated with an acoustic stimulus. One reaction type is noted for each trial.

	Startle	Holding station	No discernible reaction
Individual	9	6	5

Swimming speed

Individual fish initially swam more rapidly during the treatment period than under the control. For 20 seconds immediately after the stimulus was turned on the difference was highly significant ($W = 5.11$, $p < 10^{-7}$) (Figure 5.15), and remained significant during the first 40 seconds of the test after which the significance diminished. A difference between treatment and control remained apparent from 1 minute 40 seconds until the end of the trial, except for at approximately 6 minutes. This was due to a lower swimming speed of the treatment fish in comparison to the control.

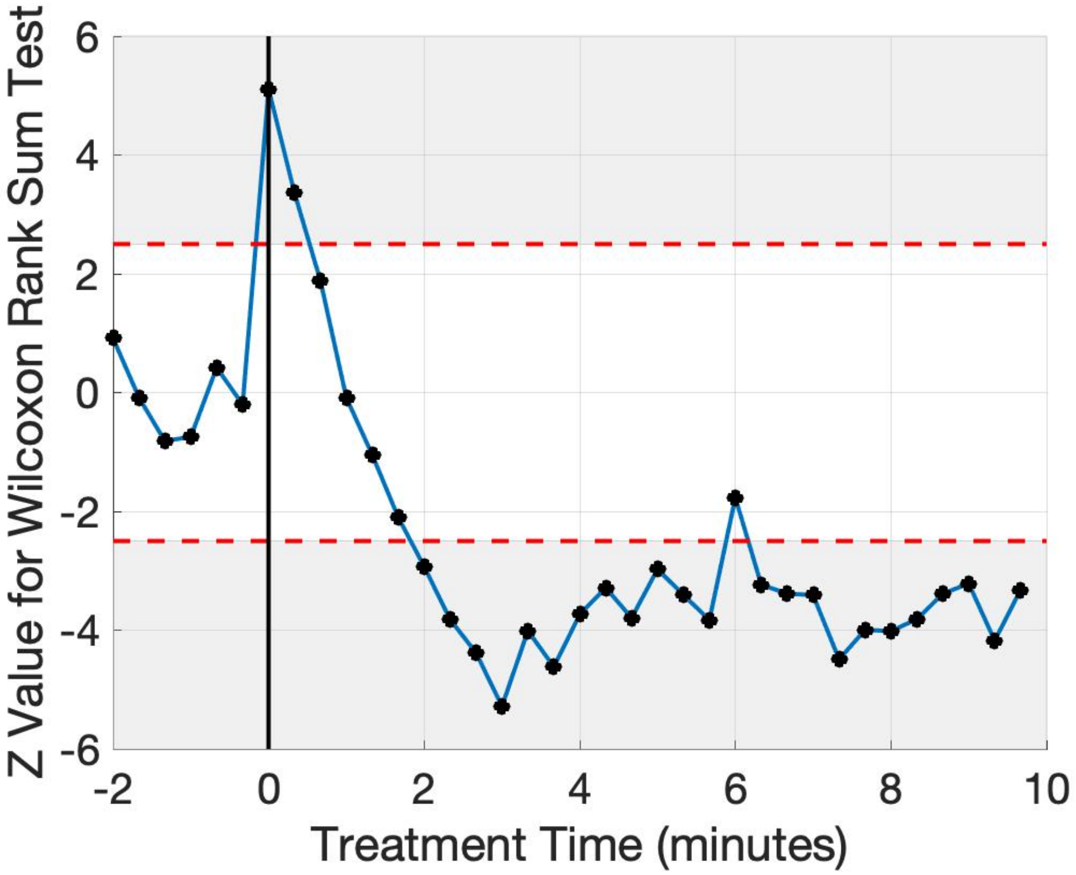


Figure 5.15 *Z*-values calculated using the Wilcoxon rank sum test (WRST) of swimming speed behaviour exhibited by fish under experimental conditions. The figure displays 2 min of pre-treatment followed by 10 min of treatment time. The black line at 0 indicates the start of the treatment. The circles indicate the data points with lines connecting them to show behavioural trends over time. The white area in the middle is an area where values display no significant difference from previously measured behaviour. The grey areas, marked by the dashed red line, at the top and bottom of the plots indicate where significant differences were observed. The horizontal dashed line indicates the significance boundary of p = 0.0125. The Y axis contains the *Z*-values (test statistic) from the WRST where a stronger behaviour results in a more positive number.

Persistence

For individuals the swim paths were less persistent, i.e., more erratic, during the treatment compared to the control. Immediately after the onset of the stimulus the differences between control and treatment were highly significant ($W = 3.27$, $p < 0.0011$) and remained so for a further 20 seconds until the effect was lost (Figure 5.16). From 3 minutes onward, a difference was once again apparent, and remained so for the majority of the remainder of the trials.

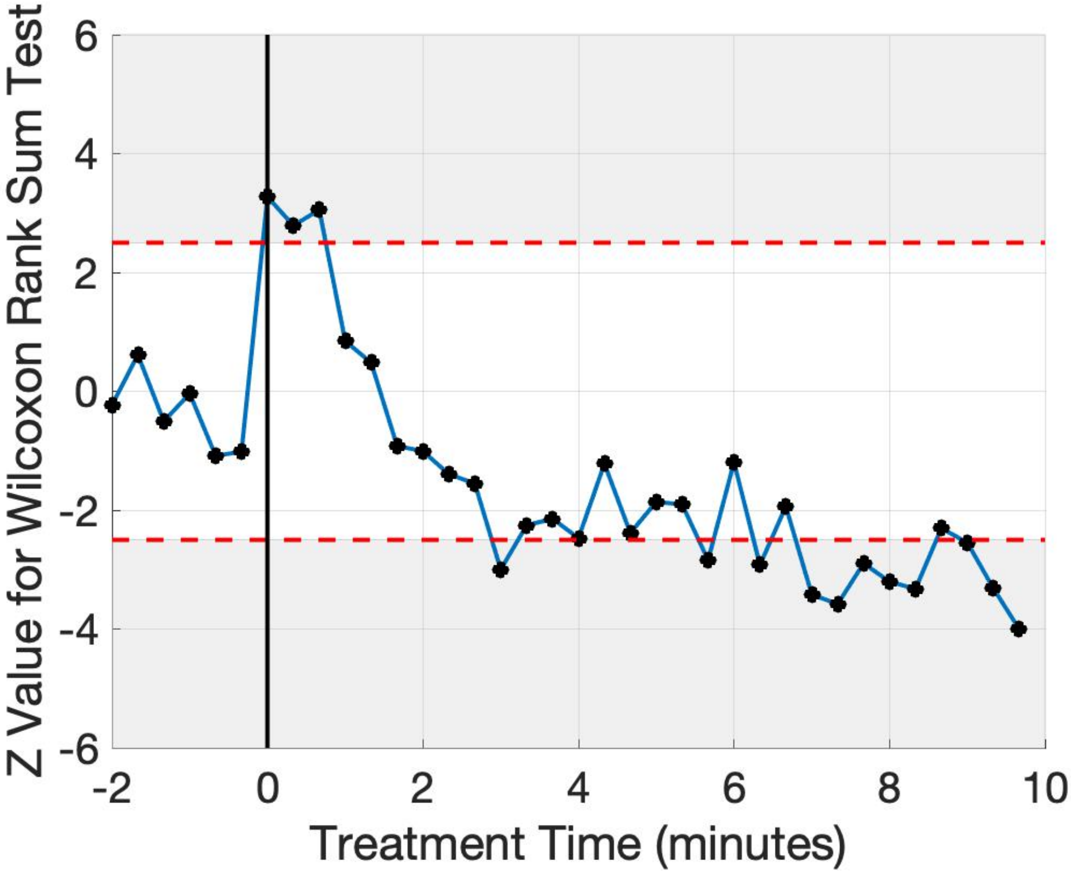


Figure 5.16 *Z*-values calculated using the Wilcoxon rank sum test (WRST) of persistence behaviour exhibited by fish under experimental conditions. The figure displays 2 min of pre-treatment followed by 10 min of treatment time. The black line at 0 indicates the start of the treatment. The circles indicate the data points with lines connecting them to show behavioural trends over time. The white area in the middle is an area where values display no significant difference from previously measured behaviour. The grey areas, marked by the dashed red line, at the top and bottom of the plots indicate where significant differences were observed. The horizontal dashed line indicates the significance boundary of $p = 0.0125$. The Y axis contains the *Z*-values (test statistic) from the WRST where a stronger behaviour results in a less positive number.

5.4 Discussion

Upon immediate activation of the stimulus, the individual fish displayed varying initial reactions. This may be due to a degree of uncertainty brought on by being isolated from their conspecifics. The main initial reaction type displayed was that of a startle response (45% of all initial reactions). This links in with the findings of the quantitative analysis of swimming speed and persistence and is also related to a flight-type response as there is greater initial activity. Research that supports this hypothesis includes the findings of Domenici and Blake (1997) that this behaviour is analogous to an anti-predator reaction. The holding station and no observable reaction responses may be linked to the featureless nature of the tank as there is no sheltered space provided. It is possible that future research that involves groups of fish or physical structures in which to hide may give a different result. However, in this research it was important to separate out as many confounding variables as possible and therefore no such structures were present in the experimental area of the flume tank.

Individual fish were highly responsive to the treatment in terms of their swimming speeds. It is likely this is due to a form of anti-predator reaction or hyperactivity, as has been noted in previous work (Rehnberg & Smith, 1988; Neo *et al.*, 2015). Initially, the fish swam significantly faster than was measured during the pre-treatment period, although this did not last long. One minute into the treatment, the individuals had returned to a short period of normal behaviour and then immediately moved to a level of significantly different behaviour. This second significantly different behaviour was a very slow swimming speed with some fish not moving at all. These two ends of the swimming speed spectrum explain the return through normal displayed at time point 3, which was 1 minute into the treatment period (Figure 5.15), as the swimming speed of the fish moved from faster, through normal to slower. This is re-inforced by the work of Neo et al. (2014) where these changes in observed reactions from one extreme to the other are considered to be a form of hyper-attention related to anti-predator response behaviour.

When measuring persistence, as was observed for swimming speed, the individual fish were noted to react to the treatment immediately and showed very limited acclimation.

This behaviour also returns through normal behaviour (that which was observed before the treatment began) from a significantly different low value to a significantly different high value. As with the swimming speed of the fish, this was due to an initial period of swimming with a very low persistence (swimming with an increased number of more volatile turns) to a change after 2 minutes of treatment. After this point, 3 minutes into the stimulus being active, the persistence of the fish became much greater. When combined with the swimming speed data, this increased persistence may be explained by the fish not moving a great distance. A relatively stationary fish would display strong persistence as they are not turning and changing direction to a large degree or frequently.

It is possible that these behaviours that have the potential to reduce the fitness of an individual fish are diluted when the fish is in a group setting. Unlike fish that are designed to swim significant distances, such as European eel (van Ginneken *et al*., 2005), freshwater fish that spend their entire life cycle in a small spatial range may be more negatively affected by the additional energetic cost. Change in behaviours that cost groups effort, such as through additional hydrodynamic strain (Abrahams & Colgan, 1985), may be exaggerated in an individual setting. Further experimental work should seek to understand the influence that sound has on a group of fish, for these two behaviours. Specifically, in relation to these two metrics, it is possible that the influence of other members of the group would cause the fish to continue swimming and not to slow or settle as they are given more confidence by the presence of their conspecifics.

It is also interesting to note that there was no overall habituation (by return to normal behaviour) for the swimming speeds or persistence of the individual fish. It is possible that this is due to the pulsed nature of the stimulus; this hypothesis is supported by other work (Neo *et al*., 2014; 2015). It is also possible that this is a result of isolating the fish individually, as suggested in the paragraphs above. Although there was no habituation displayed, there was a rapid change after a short period of treatment from one end of the behavioural spectrum to the other. Further research may wish to focus on this initial period of treatment to identify if there are other behavioural cues exhibited by the fish in this time. Certainly, the lack of habituation overall (by means of return to and remaining within baseline parameters) is counter to the findings of a number of pieces of research over the last twenty-five years, including Domenici and Blake (1997), Kastelein et al. (2008 & 2017b) and Currie et al. (2021). A recent paper from Vetter et al (2017) investigating the impact of acoustic stimuli on silver carp noticed continued reaction (i.e., no habituation) over the same time period of 10 minutes. Possible explanation for this include the specific species and the specific stimulus used, however further research into this conflicting space on habituation is welcomed to understand specifically what is working and why.

Anthropogenic noise pollution has been observed to impact the success of communication amongst vocalizing freshwater fish (Luigi, 2010; Holt & Johnston, 2015). This study demonstrates the impact that sound, and by extension anthropogenic noise pollution, can have on the finer behaviours that these animals demonstrate. Additional research that looks at the impact that this same signal has on the behaviour of European minnow in a more natural occurrence (a group) in relation to these two metrics (swimming speed and persistence of swim paths) and additional measures (initial reaction) is needed and will now be presented in chapter 6. A final, fuller discussion the incorporates the findings from this chapter and chapters 4 and 6 is presented at the start of chapter 7.

6 Effects of anthropogenic noise disturbance on the collective behaviour of a shoaling Cypriniform fish

Understanding collective behaviour of animals and how it might be influenced by anthropogenic activity is vital to their protection in an increasingly urbanised world. Transport networks and other logistical operations in and around the aquatic environment have the potential to produce damaging acoustic noise. The reduced attenuation of sound in water, coupled with the highly evolved hearing ability of many fish, exposes these animals to sound produced from a wide spatial area and has the potential to put them and their behavioural bonds at risk. The behavioural response to an acoustic stimulus of a shoaling cypriniform (*Phoxinus phoxinus*) was measured in a tank containing still water. Four metrics were considered: swimming speed, persistence of swim paths, cohesion of the group and the dissimilarity of orientation of members of the group. Differences in behaviours exhibited by groups of fish prior to, and during, presentation of a broadband acoustic stimulus were compared. Statistically significant differences were identified for cohesion and orientation, but not for swimming speed or persistence. Under treatment, groups became more cohesive and more similarly orientated. It was also demonstrated that groups behaved in a more uniform manner immediately following the onset of the treatment. These results provide evidence for the extent to which fine scale behaviours of a shoaling fish are interrupted by sound

6.1 Introduction

Collective behaviour, in which coordinated groups are formed as a result of local interactions among individuals in the absence of centralised control, is widely observed in nature, e.g., in animals that form flocks, schools and swarms. The potential benefits for individual fitness of group membership are well described, and include enhanced antipredator defence (Uetz *et al*., 2002) for the Mexican colonial web-building spider Metepeira incrassata, foraging efficiency (Sullivan, 1984) for the downy woodpecker Picoides pubescens, thermoregulation (Gilbert *et al*., 2010) for a review of huddling in endotherms, and information transfer (Laland & Williams, 1997) for foraging guppies *Poecilia reticulata*. Although understanding the mechanisms and significance of collective behaviour has been of great interest in the fields of ecology (Couzin *et al*., 2005), ethology (Ballerini *et al*., 2008b) and evolution (Couzin *et al*., 2002), there has been little consideration of how it may be disrupted by anthropogenic activity, and the resulting ecological consequences.

Global resource development, transportation, the expansion of the built environment, and the construction and operation of associated infrastructure, alters the acoustic environment when compared with reference conditions that existed prior to anthropogenic influence. The impacts of acoustic disturbance on animal behaviour are relatively well studied for terrestrial systems (Barber *et al*., 2010), while concerns over the potential effects of underwater noise on marine wildlife stimulated an increase in research in this area over recent decades. In particular, considerable attention has been directed towards understanding the response of marine mammals (usually cetaceans) to noise associated with shipping, seismic exploration, construction and naval sonar (Weilgart, 2007; Shannon *et al*., 2016). Interest in the impact of underwater noise on marine fish is also increasing (Popper *et al*., 2003; Hawkins & Popper, 2017), largely due to the commercial significance of many species. In comparison, the effects of human-generated noise on freshwater species have been studied to a lesser extent (Payne *et al*., 2015).

The sources and characteristics of anthropogenic underwater noise pollution range from activities as diverse as pile driving during construction, which tends to produce high-intensity short-duration pulses (and may have acute consequences) to shipping, which generates moderate intensity levels over long durations and may lead to chronic effects (Slabbekoorn *et al*., 2010). The properties of sound propagation differ in water from those in air; in water it travels farther and faster and attenuates less. In the deep-water marine environment, sound can propagate many hundreds of kilometres (Stafford *et al*., 1998). Conversely, in freshwater environments the intimate and close proximity of lake and riverbanks and bed, which often are well-coupled acoustically to the water (Leighton & Evans, 2008), mean that sound can be contaminated by ground-borne acoustical noise generated a considerable distance away (e.g., by roads, factories, urban construction etc.). As a result, the scale of the spatial impacts of anthropogenic sound on aquatic ecosystems is likely to be greater than in many marine environments.

It is assumed that fish perceive and respond to anthropogenic disturbances, such as those caused by underwater noise, in a way that is analogous to antipredator responses (Frid & Dill, 2002; Leighton *et al*., 2007). These include increased vigilance, fleeing, and hiding, all of which divert time and energy from other fitness-enhancing activities such as feeding and reproduction. However, the results of both experimental and field-based studies have been contradictory. For example, under laboratory conditions, three-spined stickleback (*Gasterosteus aculeatus*) and European minnow are more frequently startled, and exhibit lower feeding rates, when field recordings of ships passing through harbours are played back (Voellmy *et al*., 2014a). Furthermore, in a similar experiment, the sticklebacks respond more quickly to a visual predatory stimulus in the presence of noise than during control conditions, while minnows exhibit no difference in response (Voellmy *et al*., 2104b). Conversely, juvenile European eel are slower and less likely to exhibit a startle response to a simulated predator, thereby increasing the probability of capture, again under treatments employing the playback of shipping noise (Simpson *et al*., 2015). In the field, interspecific variation in response to an acoustic deterrent system designed to repel estuarine fish from a power station cooling water intake has been observed (Maes *et al*., 2004), with clupeoid species being the most easily deflected and *Lampetra fluviatilis* and *Pleuronectiformes* the least. Brown trout (*Salmo trutta*) held in cages in an estuary did not appear to startle or become more active when exposed to underwater piling (Nedwell *et al*., 2006). A common tendency in these types of study is to focus on individual, rather than collective, behaviour in response to a manipulated acoustic environment. This is interesting considering the potential value of group membership for the many commercial species of fish that form schools or shoals. The observations that exist are often anecdotal, and not specifically focused on the impacts on collective behaviour per se, for example Gerlotto and Fréon (1992) reported evasive diving by fish schools in response to approaching motorized vessels. Thus, there is a need to investigate how human-generated sound can influence the coordinated behaviours of groups of fish to better understand implications in terms of potential negative impacts before considering options for mitigation.

Using European minnow as the model species, this study adopted a reductionist experimental approach to compare the influence of underwater sound on the behaviour of groups and individuals. Rather than use play back of recordings of specific noise generating activities, such as shipping, which have been frequently employed to create the treatment conditions described in earlier experiments, the fish were exposed to a broadband frequency range (60 – 2000 Hz). The broadband frequency used was selected to cover the hearing range expected of the European minnow based on current understanding for other similar species, for example fathead minnow (Scholik & Yan, 2001; 2002), and to replicate anthropogenic-driven sound commonly encountered in aquatic environments (Slabbekoorn *et al.*, 2010). Behaviours observed for the acoustic treatment were compared with those recorded prior to treatment. The influence of sound on collective behaviour was quantified as: the speed fish moved at, the persistence of the swimming paths, cohesion of the group, and orientation of individual fish relative to other members of the group. Qualitative descriptions of initial behaviour were also noted and were defined as: the fish showing a startle, a momentarily holding station and no discernible change in behaviour. This study will inform theoretical models commonly advanced to describe, explain and predict collective behaviour (Giardina, 2008). It will also provide a foundation on which to consider the potential impacts of anthropogenic activity on fish shoaling, which is potentially applicable to a wider range of species.

6.2 Methodology

6.2.1 Experimental set-up

An experiment to test the behavioural response of groups of Minnow to sound was conducted in a tank holding standing water, based at the International Centre for Ecohydraulics Research (ICER), University of Southampton, UK. The experimental structures and materials are the same as described in the methods section (chapter 3) and the previous results chapter (chapter 5). Water temperature remained measured over the experimental period (mean ± SD = 16.98 ± 1.55 °C).

The acoustic stimulus was generated from MATLAB via a National Instruments USB-6341 data acquisition system and powered by a Skytronic 103.100 Mini AV digital amplifier. The stimulus comprised a broadband signal of filtered pseudo-random Gaussian noise between 60 - 2000 Hz pulsed on for 2 seconds and off for 1 second which was repeated for 10 minutes. The experimental area was subsequently mapped using a hydrophone (Bruel & Kjaer 8103) connected to a charge amplifier (Bruel & Kjaer 2635); the sound pressure level was measured over a 5 x 5 cm grid pattern, measured at three depths (2 cm, 13 cm and 24 cm above tank floor (ATF)) to quantify the noise field within the tank. The mapping was conducted at the same time as for the research presented in the previous chapter and identifies a non-directional acoustic field as represented in figure 5.1.

6.2.2 Fish collection and maintenance

European minnow (*Phoxinus phoxinus*), (n = 100, mean total length and mass ± SD = 56.90 ± 4.55 mm, 1.66 ± 0.47 g) were collected from the River Itchen Navigation (St. Catherine's Hill, Winchester, UK, 51.049783 -1.311416) using a 10 m seine net and transported in a 100 L aerated container to the ICER holding facility, as described in chapter 3. The fish were maintained in a tank (150 cm wide, 150 long and 100 cm deep with a water depth of 70 cm) prior to conducting trials. The holding tank was filtered and aerated and daily water testing and subsequent changes (approximately 25%) ensured high water quality was maintained (nitrite < 1 mg L−1 and nitrate < 50 mg L−1). The water temperature in the holding tank remained stable throughout the experiment (mean ± SD = 16.35 ± 1.35 °C).

6.2.3 Experimental trials

A total of twenty 50-minute trials, using groups of five fish were conducted between 29th and 31st May 2016. Trials were conducted throughout the day and night and alternated between treatments (individuals and groups). At the start of each trial the fish were placed into the experimental area and a 20-minute acclimation period commenced. A further 20-minute pre-treatment (control) in which no acoustic stimulus was present was followed by a 10 minute exposure to the stimulus (treatment). After this, the trial was ended, and the fish were removed from the experimental area and weighed and measured. No fish were used in more than one trial. Permission to remove European minnow from the Itchen was provided by the Environment Agency (permit reference EP/EW083-L-263/5065/02). Ethical approval for this study was provided by the University of Southampton Animal Welfare and Ethical Review Board (ERGO ethics submission no. 19783).

6.2.4 Analysis

Qualitative observations

Qualitative descriptions of fish behaviour were recorded based on visual analysis of the video. These behaviours were categorised as: (1) startle – where the fish reacted immediately to the treatment in a manner different to control behaviour; (2) holding station– where the fish stopped moving momentarily; and (3) no discernible response– where no change in behaviour was apparent. These results are summarised in table 6.1.

Quantitative analysis

Analysis was then divided into four key metrics to demonstrate any differences under treatment. To quantify these reactions, video data were analysed and 2D co-ordinates of fish position relative to the walls of the tank recorded using MATLAB to provide location and orientation over time, data on which other metrics were based. This is described in section 3.3 in detail for the methods of identifying fish and calculating the four metrics.

As described in chapter 5, the resulting data were then analysed using a Wilcoxon Rank Sum Test (WRST) to explore any differences in behaviour. The data compared using the WRST was the 1 second averaged data from 10 minutes prior to the treatment period (the control period) and the 10 minutes of noise (the treatment period). Due to the manner in which the data is tested with a moving average there is a merging effect on the data displayed (Figures 6.1 & 6.2).

6.3 Results

Qualitative observations

All groups (100%) of five fish were observed to exhibit a startle response on activation of the treatment (table 6.1). This is different to the findings of the same analysis in chapter 5 (section 5.3) where fish treated individually displayed a spectrum of behaviours.

Table 6.1 List of behaviours initially displayed by groups of fish treated with an acoustic stimulus. One reaction type is noted for each trial.

	Startle	Holding station	No discernible reaction
Group	20	0	0

Swimming speed

Following analysis, no difference in swimming speed of the groups was noted, (WRST average Z=-0.096, p <0.525 overall). There was only one time block (2 minutes after treatment onset) when swimming speed differed between control and treatment for shoals (W = -2.54, p < 0.01). This is in contrast to the findings of chapter 5 where there was a significant difference in the swimming speed of individual fish following treatment.

Persistence

Groups of fish did not demonstrate any difference in persistence after the start of the treatment, (WRST average Z=0.34, p=0.488 overall). There was only one instance when this was not the case (W = 2.20, p = 0.03). This is, again, different to the behaviours displayed by individual fish in chapter 5.

Cohesion

Groups were more closely grouped together immediately following activation of the acoustic stimulus and remained so for 2 minutes 40 seconds, with the difference between the treatment and control being highly significant ($W = 3.57$, $p < 10^{-4}$) during the first 20 seconds (Figure 6.1). The cohesion of the group was also shown to demonstrate a difference in collective behaviour under treatment over time (WRST average Z=3.14, p <0.002 over the first 3 minutes), with the fish becoming more closely grouped immediately following the activation of the treatment. This effect begins to degrade after the first 3 minutes (Figure 6.1), suggesting the fish were habituating to the stimulus.

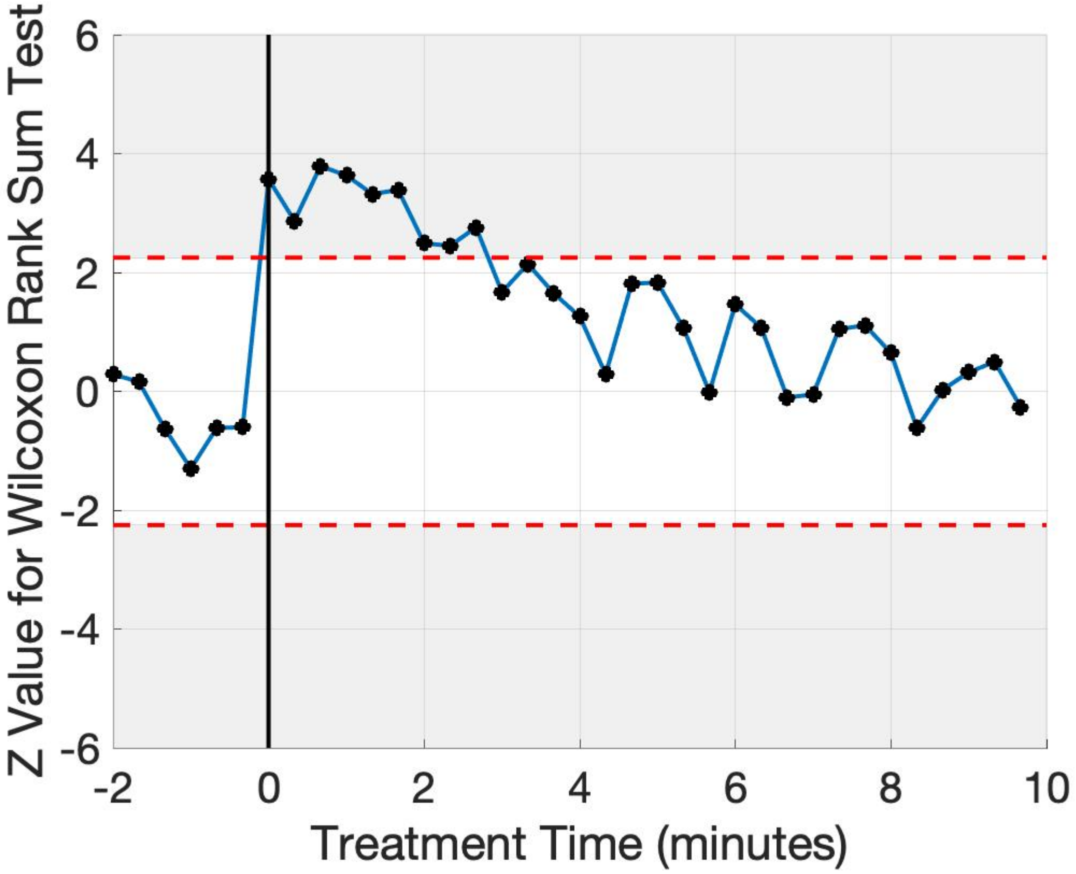


Figure 6.1 *Z*-values calculated using the Wilcoxon rank sum test (WRST) of cohesion behaviour exhibited by fish under experimental conditions. The figure displays 2 min of pre-treatment followed by 10 min of treatment time. The black line at 0 indicates the start of the treatment. The circles indicate the data points with lines connecting them to show behavioural trends over time. The white area in the middle is an area where values display no significant difference from previously measured behaviour. The grey areas, marked by the dashed red line, at the top and bottom of the plots indicate where significant

differences were observed. The horizontal dashed line indicates the significance boundary of p = 0.025. The Y axis contains the *Z*-values (test statistic) from the WRST where a stronger behaviour results in a more positive number.

Orientation

A similar trend in behavioural difference was noted for the dissimilarity of orientation of the fish within the group, (Figure 6.2). When exposed to the treatment there was a change that reduced after 3 minutes (WRST average Z=3.326, p <0.0003 over the first 3 minutes), demonstrating that initially the fish in the group share a more common orientation when first exposed to the sound field. The difference between treatment and control was most stark during the first 20 seconds (*W* = 3.61, *p* < 0.0003). Towards the end of the 10-minute treatment period the behaviour of the fish returned to previously measured levels.

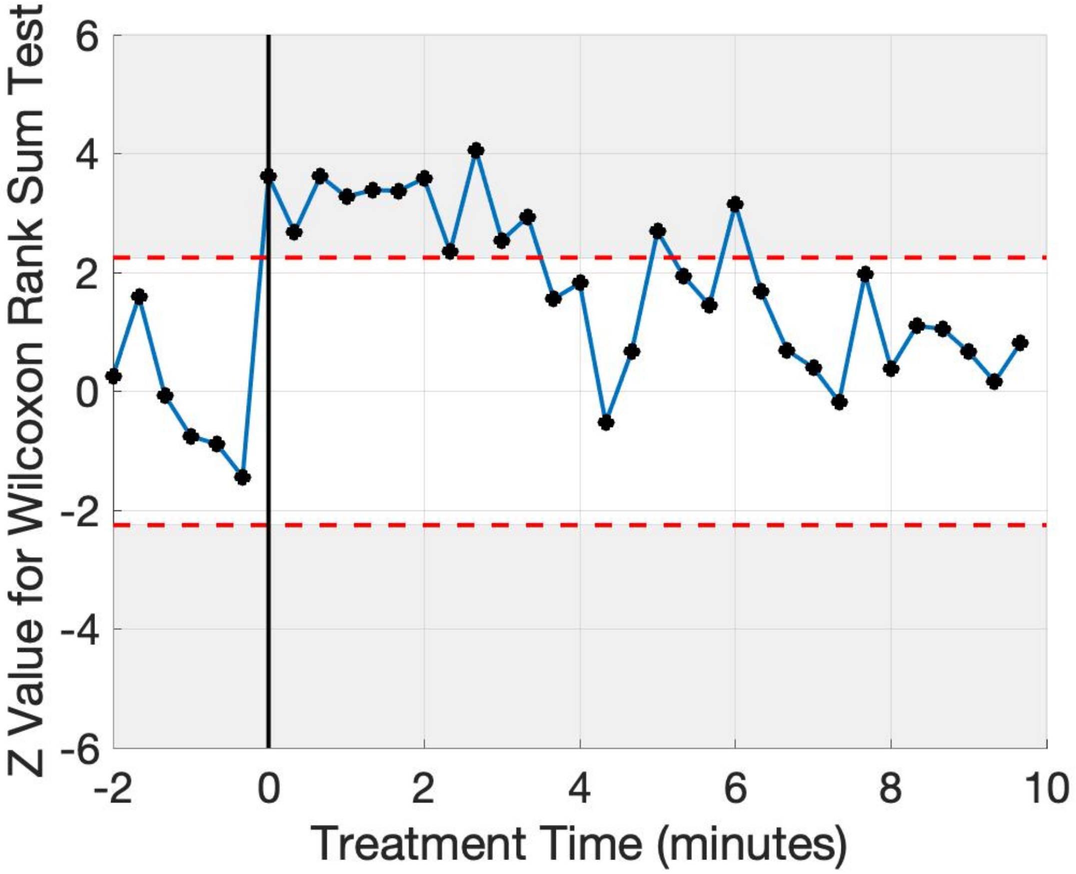


Figure 6.2 *Z*-values calculated using the Wilcoxon rank sum test (WRST) of orientation behaviour exhibited by fish under experimental conditions. The figure displays 2 min of pre-treatment followed by 10 min of treatment time. The black line at 0 indicates the start of the treatment. The circles indicate the data points with lines connecting them to show behavioural trends over time. The white area in the middle is an area where values display no significant difference from previously measured behaviour. The grey areas, marked by the dashed red line, at the top and bottom of the plots indicate where significant differences were observed. The horizontal dashed line indicates the significance boundary of p = 0.025. The Y axis contains the *Z*-values (test statistic) from the WRST where a stronger behaviour results in a more positive number.

6.4 Discussion

Measuring the behavioural impact anthropogenic noise has on collective behaviour has involved exploring the reactions of groups and individual European minnow exposed to an acoustic stimulus and quantifying the behaviours demonstrated. The data gathered supports some of the assumptions that there would be an increase in anti-predator behaviour (Domenici & Blake, 1997; Frid & Dill, 2002; Leighton *et al*., 2007), such as increased swimming speeds, more tortuous paths and an increase in vigilance through grouping and orientation. It is noted in table 6.1 that the nature of initial response is that of a startle response, where all groups were observed to react in a similar manner under treatment. The metrics that were used to analyse fish behaviour demonstrate that groups of fish react to this non-directional acoustic field in a complex manner.

Groups of fish did not show a difference in their swimming speed when exposed to the treatment which suggests that the presence of conspecifics has a moderating effect on their reaction. This observation is counter to the findings of Neo *et al*. (2014) who noted increased swimming speeds for groups of European Sea Bass exposed to noise. It is possible that this is due to strong individual traits overruling commonly displayed behaviour (Dussutour *et al*., 2008; Jolles *et al*., 2017). Suboski *et al*. (1990) observed one treated individual Zebrafish passing on an alarm reaction to all conspecifics and causing a group reaction. This supports the idea that individual fish can affect the behaviour of a whole group. In this instance, this research would support these findings if it was assumed that a strong individual not reacting to a stimulus would cause the whole group to follow. Other research further contributes to the idea that the behaviour of a group is the result of the actions of its members (Bleakley *et al*., 2007). This could mean that reactions vary from group to group but are more likely to vary from individual to individual. This is further supported by the variance of behaviour as displayed, initially, by individual fish as outlined in table 5.1. Independently to this research, this idea is supported by previously published works that examined the roles that localised interactions play in the overall structure of the shoal (Ward *et al.* 2017). This research identified the importance of metrics such as orientation and cohesion in the overall dynamics of the shoal and found that group size was less important to the global behaviour of the shoal. Perhaps contrary to the findings of this research however was the conclusion that individual swimming speed was highly important in terms of the overall group behaviour. This study described that variance amongst individuals was the most influential metric related to swimming speed. How these behaviours relate to each other in an anti-predator context is unclear, however this is an area that is currently developing where interesting new research is examining parts of this, including how shoals of fish behave across three-dimensions (Romenskyy *et al.* 2020).

When examined, the groups of fish observed in chapter 6 displayed no difference in behaviour under acoustic treatment in terms of the persistence of their swim paths. A less persistent swim path has been associated with antipredator behaviour in other work (Ioannou *et al*., 2012). It is, therefore, interesting that these groups did not display this behaviour. This could be because the groups of fish have other antipredator behaviours that they can exhibit. Lingle (2001) identifies that individual mule deer formed small groups when faced with the threat of predation from coyotes. It is possible that if the fish in chapter 5 had been given the option of forming groups they would have not increased their swimming speed or decreased their persistence. Instead, they may have formed groups and the other two metrics examined, cohesion and orientation, would have changed instead. A change in these two metrics is indeed what is noted in this chapter and is further re-enforced by findings from Ward et al. (2017).

Although these results are contrary to Neo *et al*. (2014) for some metrics, the increased group cohesion under treatment that was observed is noted in many other works (Pitcher, 1986; Gerlai, 2010; Neo *et al*., 2010). This is thought to be another example of anti-predator behaviour brought on by the treatment (Magurran & Higham, 1988). It should also be noted that there is habituation to the treatment displayed for this metric. After 3 minutes of treatment the behaviour of the fish tends towards normal with the relative distribution of the fish becoming as spread out as it had been prior to treatment. Although increase in cohesion following a potential threat is recorded in other literature, the time to return to normal behaviour varies with some studies recording re-acclimation periods of over one hour (Sogard & Olla, 1997) depending on the treatment. This difference in cohesion is also at odds with findings from more recent research (Herbert-Read et al. 2017; Currie et al. 2021) where a temporary, less cohesive group was observed following the immediate onset of an acoustic stimulus. Whilst the work of Herbert-Read et al. focused on a different species (juvenile sea bass) the work of Currie et al. (2021) used the same species of fish as that focused on in this thesis (eurasian minnow). In contrast to this, previous work from Currie et al. (2020) had found similar reaction, regarding cohesion, with eurasian minnow and acoustic stimuli in a similar study design. Whilst this is discussed in greater detail in chapter 7, it is reasonable to conclude that these fine-scale behaviours vary considerably and are very context sensitive. I would therefore urge any researcher or fisheries manager to consider this when using these and other's research findings to design methods or lead policies.

The orientation of members of the group relative to each other was found to be influenced by the treatment with an increase in commonality of direction when treated with a sound stimulus. It is a characteristic of schooling that animals are arranged closely with a common orientation (Pitcher, 1983). Therefore, combining the cohesion and orientation data allows us to conclude that the stimulus increases the schooling instinct of these fish. Behaviours associated with avoiding predators are key to the survival of many animals, whether or not they exist in aggregations or not (e.g., Uetz *et al*., 2002; Ward *et al*., 2011). However, false alarms can be energetically costly due to reduced foraging time (Lima, 1998). Change in group behaviours such as orientation and cohesion can also have a negative impact on anti-predator response (Ioannou *et al.* 2012), however this in itself may be due to changes in shoal structure and information transfer between individuals (De Bie *et al.* 2017; Romenskyy *et al.* 2020). Anthropogenic disturbance may therefore have a negative ecological impact on animals that regularly engage in collective behaviour. In a similar manner to the cohesion, there was an acclimation noted for the relative orientation after 3 minutes of exposure to the stimulus. In further studies, it would be useful to explore this acclimation by varying the temporal element of the acoustic pulse and indeed, there is currently work underway to explore modifying the acoustic stimulus within the experimental setup designed for this thesis research (Currie *et al.* 2020 & 2021).

The results presented here suggest that anthropogenic noise can significantly alter the behaviour of a freshwater fish species, both in the way the fish move but also in the way they position themselves within a group. The behaviours noted above indicate that these fish associate this acoustic disturbance with predators. The more compact nature of the group, measured as a function of cohesion, and their relative orientation following activation of the stimulus would appear to mimic behaviour observed in the marine environment when fish are hunted by cetaceans and pinnipeds (Simon *et al*., 2006). However, further work is required to investigate the impact of anthropogenic disturbance on collective behaviour. The disruption that this study has observed for a group of fish may negatively affect individual fitness and survival (Jolles *et al*., 2017).

7 Thesis discussion

7.1 Discussion of chapters 5 & 6

Both studies presented in chapters 5 and 6 found significant, but different, behaviours displayed by groups and individuals. Chapter 4 also demonstrated significant avoidance behaviour to an acoustic stimulus for groups of fish, but not individuals. These differences, their implications and their place within the wider literature are discussed below.

In recognition of the fact that the effects of anthropogenic noise on fish in fresh water has received less attention than that in the marine environment (Mickle & Higgs, 2017), this thesis focused on the impact of acoustic stimuli on the behaviour of a common freshwater species, the European minnow. In particular, the research focused on the difference in the effect on the collective behaviour of shoals when compared to that exhibited by solitary individuals. On exposure to sound the behaviour exhibited by the shoals and individuals differed. The acoustic signal evoked a consistent and clear startle behaviour for all groups, but only 45% of individuals exhibited such a response. During the acoustic treatment, the orientations of shoals become more aligned and more closely grouped, although this response declined with time. Solitary fish tended to swim more rapidly and follow less predictable trajectories. This thesis offers an interesting insight into similarities and differences between responses to acoustic disturbance and predators, information transfer, and habituation.

Within the sound field created for these studies (Figure 5.1) it is possible to identify zones with more intense sound pressure levels at either end of the tank. This presents an acoustic environment with sound pressure levels between of 110 dB and 140 dB. The intention of this study was to expose fish to a stimulus from which there was no directional escape route, and to explore their fine-scale behaviours when exposed to sound (not simply avoidance or attraction). Anthropogenic disturbance, such as that created by an acoustic signal, is thought likely to evoke behaviours that are akin to anti-predator responses (Frid & Dill, 2002). In this study, a startle, defined by a sudden rapid burst and brief acceleration of velocity (Andraso, 1997), was common on exposure to the sound stimulus; this is a behaviour similar to the fast start that occurs during the first few milliseconds of an escape from a predator (Domenici & Blake, 1997). Indeed, all shoals startled and grouped closer together while adopting a more polarised orientation. This differs from the observations of Herbert-Read *et al*. (2017) who found that shoals of juvenile sea bass became less cohesive and less directionally ordered during playback of pile-driving compared with ambient background noise. However, these results are similar to those previously described by other researchers. For instance, several species of marine fish maintained in cages more frequently exhibited alarm responses and formed tightly cohesive groups when exposed to increasing levels of air-gun noise (Fewtrell & McCauley, 2012). The inter-individual distance declined in shoals of five zebrafish (*Danio rerio*) during acoustic treatment in a small tank; this was a behaviour which suggested to indicate anxiety in response to potential danger (Neo *et al*., 2015). European sea bass enclosed in an outdoor basin where they were exposed to sound treatments exhibited startle responses and increased group cohesion (Neo *et al*., 2014). Regarding orientation, a study that compared the escape response of solitary individuals and schools of herring (*Clupea harengus*) indicated that members of the group tended to swim in the same direction as their neighbours, which was more frequently (90%) away from a sound stimulus (Domenici & Batty, 1997). This suggests that maintaining a common direction of orientation is likely to enhance the co-ordination of escape.

Interestingly, in chapter 6, the swimming speed of the shoals did not change between control and treatment conditions. Herbert-Read *et al*. (2017) observed a decrease in swimming speed of the juvenile sea bass during the acoustic treatments, which is contrary to previous observations of faster swimming in more cohesive (e.g. Fewtrell & McCauley, 2012, for several marine species) and polarized groups (e.g., giant danio, *Danio aequipinnatus* (Viscido *et al*., 2004)). Although a lack of a change in speed appears to contradict the concept of fleeing, slow speeds may bestow benefits related to the identification and greater monitoring of the risk, such as a chasing predator (Domenici, 2010). Indeed, allowing more time for sensory processing has been linked to longer escape latencies (slower responses), which are known to be more accurate than shorter ones (Chittka *et al*., 2009), and greater in schooling than solitary fish (Domenici & Batty, 1997). It is important to note that swimming speed and persistence of the shoals of fish are measured from the average position within the group. Therefore, changes to individual behaviour may not be captured by these methods.

In the absence of the opportunity to join a group, solitary individuals adopted alternative strategies, presumably in response to a perceived risk similar to that posed by a potential predator. During the acoustic treatment, solitary minnows moved more rapidly along less predictable paths, as evidenced by a lower persistence. In their study, Domenici and Batty (1997) also described a less predictable escape response for individual herring that more frequently turned toward the simulated threat than schools. However, after the immediate escape response, the benefit of continued rapid swimming over a longer period is not obvious, as a greater distance covered will likely increase probability of encounter should a real predator exist. Indeed, while some studies suggest increased swimming speed (e.g., for groups of zebrafish (Neo *et al*., 2015)) reflects hyperactivity in response to predator presence, others have observed decreased activity and potentially increased vigilance to a potential threat (e.g., black carp, *Mylopharyngodon piceus*, prey and snakehead, *Channa argus*, predator (Tang *et al*., 2017)), possibly highlighting a difference in response between a real predator-prey interaction and acoustic disturbance.

In addition to antipredator response, one of the most-often cited benefits of group behaviour is the potential for enhanced information transfer (Miller *et al*., 2013), including for the subject species used in this study (Magurran & Higham, 1988). As described above, it was observed that the startle behaviour exhibited by groups on exposure to the stimulus was universal, although for individuals it was variable. Within a group of five fish, there is a higher probability that one or more members would elicit a startle response, and that this might excite others to follow suit, even if they had not reacted initially to the sound being turned on (Ward *et al*., 2008). Indeed, propagation of a startle response has been previously described empirically (e.g., for banded killifish, *Fundulus diaphanous* (Godin & Morgan, 1985); and for herring (Marras *et al*., 2012)) and through simulation (Chicoli & Paley, 2016). Furthermore, in the experiment in chapter 6 the aligned polarity of the shoals increased on exposure to sound. In another study in which a probabilistic model of epidemics was adapted to investigate how information spreads through a simulated fish school, Chicoli and Paley (2016) predict a slower, but more accurate, response in groups that become more commonly oriented after detecting a threat, than for those that are already strongly aligned. Likewise, in the study presented in chapter 6, there was no increase in swimming speed for shoals during the treatment, after the initial startle response. These observations suggest that the fish were attempting to maximise information transfer and accuracy, rather than speed, of response; this is a logical strategy in the absence of visual evidence of an imminent directional threat.

Habituation, in which the response to a behavioural stimulus declines after prolonged exposure and in the absence of reinforcement, has been widely described for aquatic organisms (Atema *et al*., 1998). Several previous studies have investigated the long-term response to sound using both physiological indicators of stress, e.g., cortisol (e.g. for roach, *Rutilus rutilus*, and perch *Perca fluviatilis* (Johansson *et al*., 2016)), and behavioural surrogates, e.g. ventilation rates (e.g. for sea bass (Radford *et al*., 2016)). In this thesis there has been a focus on short-term behaviours exhibited immediately before and during the acoustic treatment. It was found that the increased shoal cohesion and polarised orientation observed on first exposure to the stimulus was relatively short lived, with a return to the pre-treatment levels only after approximately three minutes. The swimming speed of the solitary fish increased immediately after the onset of the stimulus and then decreased with time to a speed significantly slower than that observed during the pre-treatment period. The swimming speed had not returned to the pre-treatment values by the end of the 10-minute treatment. As the swim speed reduced after the initial increase, and remained close to the pre-treatment value, there was as a consequence a short period, 1 minute into the treatment, when the p-value indicated a non-significant effect. This is due to the behaviour of the fish moving from a level above, or below, that observed during the control period, to the opposite end of the same behavioural range; in doing so the behaviour temporarily appears similar to that in the control period. Similar observations can be made regarding the persistence of the paths of solitary fish. Immediately after the onset of the stimulus the paths become significantly less predictable and then settled to become more predictable than that during the pre-treatment period. It should be noted that one would expect some correlation between swimming speed and persistence, and for differences between the predicted and measured position of a fish to be less if the fish swam more slowly.

A diminishing anti-predator response, despite continued exposure to the alarm stimulus, makes sense if there is an energetic cost associated with maintenance of the behaviour. If this is the case, the findings in this thesis suggests the potential for higher short-term costs of being solitary as opposed to being a member of a group. This research leads to agreement with Mickle and Higgs (2017) that further research is needed to investigate physiological consequences of fish behaviour in response to acoustics across temporal scales to shed light on the implications of anthropogenic sound disturbance for fitness.

The impact of anthropogenic noise on the marine environment has been a subject of conservation concern for a number of years as evidenced by its consideration in international legislation such as the EU Marine Strategy Framework Directive (2008/56/EC). Anthropogenic noise in the freshwater environment, however, has been less often considered, despite the potential magnitude of influence which is likely to be higher due to the extent of human activities (e.g., urbanisation, industry, transportation, agriculture) that occur along the world’s rivers and lakes (over 50% of the global population lives closer than 3 kilometres to a surface freshwater body, (Kummu *et al*., 2011)) compared to the oceans. This thesis provides experimental evidence of the impact of sound on the short-term behaviours of shoals and solitary individuals of a model species. The change in behaviours exhibited by the shoals and individuals in response to an acoustic stimulus, although different, resemble those typically exhibited on detecting a predator. False alarms in the absence of a real immediate threat, e.g., in cases where the sound itself does not have a direct negative consequence, such as hearing loss, may be costly due to increased energy expenditure and lost opportunities associated with reduced time allocated to other activities, such as foraging and reproduction (Lima, 1998). These results also highlight that the response within a species is context dependent, and in this case varied depending on whether individuals were members of a group. Intra- and interspecific variation (note the contradictory results of Herbert-Read *et al*., 2017) was also apparent when considering these findings. This highlights a need to investigate the responses to a range of acoustic stimuli that differ, in a planned way, in their characteristics when measured at the position of the fish, in terms of frequency content, time history, acoustic pressure amplitude and pulsing characteristics (Neo *et al*., 2014). The variability in response, and potential for habituation, raises concerns when considering the use of acoustic stimuli as behavioural deterrents to protect desirable fish at hazardous infrastructures, such as power plant cooling water intakes (Maes *et al*., 2004), or vulnerable recipient ecosystems from the colonisation of undesirable invasive species (Vetter *et al*., 2015).

7.2 Future research

This work has highlighted the limited reaction of a schooling cypriniform through fine scale and multi-metric analysis of fish behaviour displayed under acoustic treatment. Further work could explore the exact impact acoustic stimuli can have on the reaction of these fish which will provide information to industry where acoustic deterrents are designed and implemented to reduce fish mortality at anthropogenic barriers. The reaction of the fish in chapters 4, 5 & 6 compared with behaviours discussed in the wider literature will help guide further research in this area and inform the direction that future analysis of existing data could take. The potential of acoustic deterrents, as outlined in chapter 1 and further explored in chapter 4, to protect fisheries and freshwater ecosystems is significant and the research presented in this thesis will help advance collective knowledge in pursuit of their development. Based on data gathered from the experiments conducted so far, and described within this thesis, future work could be conducted in the areas given below.

7.2.1 Breaking down the broadband signal

In the experiments in chapters 4, 5 and 6 the broadband signal induces a distinct reaction in the subject fish. As part of a programme of further research in this area fish should be exposed to different elements of the broadband signal and the difference in their reactions measured. Part of this research is already being conducted within ICER at the University of Southampton (Currie *et al.*, unpublished manuscript). Experiments conducted to date have utilised the same subject fish, the same experimental setup and a similar experimental protocol. In these experiments, discrete frequencies and octave bands based on the pseudo-randomly generated Gaussian noise have been used.

7.2.2 Additive stimuli – acoustics and hydraulics

In future experimental work, it is important to build on the work conducted so far and introduce more elements to the experimental design. This will allow for further development of knowledge of how multiple factors affect fish response to an acoustic stimulus and whether or not it is possible to overcome visual and rheotactic behavioural cues with an acoustic deterrent.

Knowledge of how acoustic stimuli will affect fish behaviour in-situ is the ultimate goal of a research project such as this; however, the aquatic environment is a complex one and has multiple and different types of behavioural cues for distinct fish species. To ensure that the impact of an acoustic field on fish behaviour is not lost, it is important to introduce these stimuli slowly, and, if possible, in isolation to each other. This will allow differentiation to establish if a change in a fish's behaviour is due to the acoustic stimuli or to additional cues that the experiment introduces. The first additional experiment should introduce a hydraulic stimulus. This is important because most aquatic environments to which this research has application to involve moving water, such as rivers and streams. Future work could involve either inducing an artificial hydraulic flow in an otherwise still environment or running a recirculating flume with a portioned area that retains a constant water level when the pumps are switched off to compare fish behaviour under acoustic treatment in still and flowing water.

7.2.3 Field studies

Implementing these research findings into a field-based setting is an important stage in future work as this thesis has been fundamentals and laboratory-based. Designing an acoustic deterrent and installing it in-situ will allow exploration of the application of this research to real-world problems. This, however, will only be possible if promising results are identified from earlier experiments.

Using data gathered from previous experiments, an acoustic deterrent could be installed at an abstraction point along with the release of a group of minnows, or other fish species, into the immediate area. Traps should be set for these fish to measure the success rate of catch against the operation of the acoustic deterrent. This would allow observation of how effective an effective acoustic deterrent could be deployed in-situ. This data could then be used to further develop the field of acoustic deterrents. It would be important to carry out this work as it is the application of the research findings that will have the most impact in terms of mitigating for anthropogenic activity.

7.3 Conclusion

This thesis has investigated the threats that fish face from anthropogenic activity and the resultant research question and objectives behind the thesis (chapter 1). Collective behaviour, physical acoustics and the hearing ability of fish have then been discussed in relation to scientific literature in chapter 2. Chapter 3 outlined the materials and methods that would be used to answer the research question and chapters 4, 5 and 6 explained in detail the experimental work that was conducted as part of this thesis. Chapters 4, 5 and 6 also critically analysed the results of the experimental work and how they answered objectives 1 to 4 were addressed. Finally, chapter 7 has discussed the future of this research and highlighted the impacts and implications of the work contained within this thesis.

Chapters 1 and 2 highlighted the need for a greater understanding of how fish react to acoustic fields and how this reaction might be used to influence the design and efficacy of behavioural deterrents. The research aim, to enhance scientific understanding of how fish react to acoustic fields, was therefore introduced alongside the research question ”To what extent is the behaviour of individual and shoals of fish different in response to acoustic stimuli?”. How this research met this aim is demonstrated in section 7.4.

7.4 Impacts and applications of research

Sharing the findings of work that has been conducted and publishing research is an important aspect of a PhD as sharing knowledge with the wider scientific community is the ultimate goal. The ways in which the objectives introduced in chapter 1 (and repeated below) were met by the research contained in this thesis are given below.

1. To quantify an acoustic field within a flume tank

    - All experiments conducted whilst completing this PhD involved mapping the experimental area within the water tank to quantify the acoustic field. This was an important step, the methodology for which is presented in chapter 3 and the results of each map are presented in chapter 4, 5 and 6.

2. To quantify reaction to the acoustic field of a shoaling fish, through video tracking within a flume tank, individually and as a group

    - Chapters 4, 5 and 6 present research that examines the reaction of a shoaling cypriniform fish to acoustic fields. Chapter 4 looks at directional reaction of fish individually and in groups. Chapter 5 looks at more fine-scale individual reactions and chapter 6 looks at fine-scale behaviour of groups of these fish. All chapters then use the data to quantify the reaction of the fish as defined by the metrics used.

3. To compare the fine-scale behaviours of individual and groups of fish to acoustic treatment

    - The fine-scale movements and behaviours of fish subjected to acoustic treatment have been compared at length in chapters 5, 6 and 7, and significant differences found both between individuals and groups and between behaviours.

4. To determine the effect the acoustic field has on the behaviour of fish

    - Analysing the experimental work conducted to meet aims 1 to 3 has provided novel information into the effect that acoustic fields have on fish behaviour. Significant changes in behaviour have been noted from fish under acoustic treatment.

The findings of this thesis are summarised by chapter below, including details of how this work has been shared with the wider scientific community.

- Chapters 1 & 2
    - The first two chapters of this thesis examined the current literature regarding the aquatic environment, underwater sound, behavioural deterrents and group behaviour. A gap in knowledge was identified surrounding the reaction of groups of fish, in comparison to individuals, exposed to an underwater, acoustic stimulus.
    - The literature examined and discussed in these chapters has been presented in 2, 1-hour lectures at the University of Southampton to 2 groups of ~25 second-year undergraduate students.

- Chapter 3
    - All experiments detailed in the results chapters 4, 5 and 6 had multiple SPL maps made of the experimental areas at multiple depths and of multiple frequencies to ensure the sound exposure the fish experienced was sufficiently quantified. The acoustic mapping techniques and equipment-use methodologies were shared with colleagues within ICER, and from the United States Geological Survey and the North American Oceanic and Atmospheric Administration, USA.

    - The techniques required and data produced in quantifying the acoustic fields has been shared at the international Aquatic Noise 2016 conference in Dublin, Ireland by means of a poster presentation. This included data from multiple experiments involving European minnow, European eel, brown trout and Pacific lamprey (*Entosphenus tridentatus*). These data and methodologies have also been resented at two international symposiums, held at the University of Southampton, by means of two thirty-minute presentations.
    - The novel video tracking method, as discussed in chapter 3, was used throughout this research to quantify the movement of fish from video data. This method is now being used by other researchers at the University of Southampton.

- Chapter 4
    - Chapter 4 focused on the spatial reaction to an acoustic field of individuals and groups of fish and proved, in a novel manner, significant avoidance of areas of high noise intensity by groups of fish, but not by individually tested fish.
    - This chapter also demonstrated that there was a change in habituation behaviour displayed by groups of fish and individuals.
    - When more complex metrics (group cohesion) there was no noted correlation between experienced SPL and a change in behaviour.
    - The findings presented here may have wider impact on the design and use of behavioural deterrents. Therefore, this chapter is in the process of being prepared for publication in “Ecological Engineering” under the title “Directional response of European Minnow (*Phoxinus phoxinus*) to acoustic treatment”.

- Chapter 5

  - In chapter 5, the reaction of individually treated fish to a non-directional acoustic field is measured.
  - Significant changes in the swimming speed and persistence of the fish is observed, with initial increases in speed and decreases in persistence.
  - Change in response was uniform and noted after 1 minute of treatment where the fish decrease their swimming speed and increase their persistence.
  - No habituation was displayed by the individual fish to the acoustic field.
  - A diversity of initial, instantaneous reactions were also noted for individuals.

- Chapter 6
  - Groups of fish were used in chapter 6 to examine the impacts of a non-directional acoustic field on shoals of fish.
  - No significant differences were noted in the swimming speed or persistence of the group under treatment.
  - Significant differences in the cohesion of the group and the relative orientation of group members to each other were measured. Under treatment, the groups became more cohesive, and members became more similarly oriented.
  - Habituation was noted to occur at a significant level after 3 minutes of treatment, with the behaviour of the fish returning to pre-treatment levels.
  - Observation of initial, instantaneous reactions of the groups of fish demonstrated a common reaction for all, a startle response.

- Chapter 7
  - This research, specifically chapters 5 and 6, was discussed and the notable differences between their findings were examined.
  - Individual fish were noted to react in different manners to groups (speed and persistence). Fish treated individually were also observed to change behaviour after a relatively short period.

  - Groups displayed a consistent habituation to the stimulus that individuals did not. These groups of fish also displayed much more consistent initial reactions than those that were individually treated.

A paper combining the novel work detailed in chapters 5 and 6 has been accepted for publication, following a round of minor revisions, in “Freshwater Biology” under the title “Influence of acoustics on the collective behaviour of a shoaling freshwater fish”. The analysis techniques developed during this research have also been shared with other members of the scientific community, specifically in Southampton, as have the fish capture, husbandry and experimental methods. Papers that credit this methodology have also now been published, including Currie et al. 2020 and Currie et al 2021. The work conducted in conducting this PhD and the resultant findings have been the subject of 60+, 1 hour outreach lectures that I have delivered to groups of 30-200+, 13–19-year-old students to encourage participation in higher education and STEM (Science Technology Engineering and Mathematics) subjects.

7.4.1 Real-world applications of research findings

In addition to describing how this thesis has met its aims and objectives and the wider research impact that this work has had, it is important to note that there are two main, real-world, applications for this research and its findings. One is the knowledge that fish react differently to stimuli depending on their situation (in a group, on their own etc.) and how this may impact Environmental Impact Assessment etc. The other is that this species of fish, *Phoxinus phoxinus*, avoid this particular stimulus in a tank setting and it may therefore be a usable deterrent for cypriniform fish in-situ.

Firstly, in this thesis a difference in behaviour is observed, quantified and placed in context for fish in two types of settings: individuals and groups. The differences in these behaviours discussed in chapters, 4 – 7 could be important when considering the impact that new or existing anthropogenic activity may have near waterbodies. This is because such a difference was observed, particularly in finer-scale behaviours such as swimming speed and persistence and differences in habituation time where also noted and discussed. If a waterbody contains a diversity of fish, then basing understanding of impact and behaviour off of research and studies that do not consider or measure these differences will mean a mitigation strategy that is not fit-for-purpose may be put in place. Additionally, if these statistically significant differences in behavioural response have been noted within different settings for the same species, it is reasonable to assume that these and further differences may occur between species. Therefore, it is my suggestion that the first real world application is for this research to acknowledge that fish respond differently to acoustic stimuli depending on their setting and that this may have wider than currently accepted considerations when conducting anthropogenic, noise-generating, activity in or near water bodies.

Secondly, there is evidence presented in chapter 4 of this thesis that Eurasian Minnow display avoidance behaviour when presented with a directional, acoustic stimulus. This has impact in the area of fisheries management as this species of fish, as a cypriniform, shares its hearing capabilities with a number of carp-like fish. It would be possible, through additional research, to turn this stimulus into an effective deterrent (an acoustic scarecrow) around areas where it may be important to restrict movement of fish. However, I would like to make clear that although this may be the case, with this species, in this laboratory setup, further understanding through research would be required before any deterrent should be deployed. The main reasons for this are the previously mentioned differences in response depending on the situation of this fish (individuals vs groups) and the possible difference in reaction between species. It would be irresponsible therefore to design and deploy a deterrent solution without understanding the various effects that it may have on different fish species, at different life-stages and in different situations. In combination with this, there is also the consideration of the environment in which this deterrent could be deployed. Figure 7.1 demonstrates the level of, at times discrete, engineering and proximity to anthropogenic structures that occur in many semi-natural river systems. These engineered banks, roads and river crossings that are prevalent throughout the UK and further afield are very well coupled, acoustically, to the riverine environment (chapter 2). This has the potential to reduce the directional nature of any deterrent used and also to change the finer scale behaviours exhibited by fish in these systems. This non-directional acoustic environment was the subject of chapters 5 & 6 and also discussed in chapter 3 where finding an appropriate laboratory setup was discussed. Because the materials surrounding an acoustic field have such potential to influence its nature, I therefore also suggest that the nature of the environment in which a deterrent is deployed in be tested before a deterrent be deployed.

Through sketching out these two real-world applications of the findings of this thesis I hope to provide narrative and direction to the areas that I think this research makes its greatest contribution and where and how I would recommend that others use this research.

Figure 7.1 Collection of photographs displaying anthropogenic structures and engineered banking around a semi-natural freshwater river system.

In summary, the analysis of the research described within this thesis, based on literature described in chapters 1 and 2, makes an important contribution to scientific knowledge. In fulfilling the four objectives above and answering both the aim and original research question, new knowledge has been sourced and new research methodologies created. The information contained within and generated by this thesis is of use in science, industry and politics and has already been shared at an international level through papers, presentations, conferences and lectures. It is hoped that the knowledge contributed by this thesis will contribute to providing a more effective way to mitigate for anthropogenic activity in aquatic environments and offer a more sustainable ecosystem to be enjoyed by society for future generations.

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