UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING AND THE ENVIRONMENT

&

INSTITUTE OF SOUND AND VIBRATIONS

Impacts of Anthropogenic Sound on Fish Behaviour

by

Mathias Deleau

Thesis for the degree of Doctor of Philosophy

December 2018

Success is not final, failure is not fatal: it is the courage to continue that counts.

Winston Churchill

Be always like the sea, than breaking up against cliffs it finds always the force to try again.

Jim Morrison

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF ENGINEERING AND THE ENVIRONMENT & INSTITUTE OF SOUND AND VIBRATIONS

Thesis for the degree of Doctor of Philosophy

Impacts of Anthropogenic Sound on Fish Behaviour

Mathias Deleau

Species like the European eel (*Anguilla anguilla*) and the river lamprey (*Lampetra fluviatilis*), as a consequence of their migratory life cycle, are more likely to experience several of anthropogenic pressures (e.g. infrastructures, pollution, barriers to migration). Both species populations have been severely declining for several decades, leading international organizations to institute some strict protective regulations. To meet the expectations of these regulations, there is a current need to investigate further the anthropogenic impacts responsible for this decline and to develop new methods to ensure the protection of these species.

In addition, there is scientific concern about the rising underwater sound levels due to human activity and its consequences on marine and freshwater life. This thesis investigated the effects of basic anthropogenic sounds on the behaviours of European eel and river lamprey. Furthermore, this research also looked into re-using the previous findings to improve current knowledge in behavioural mitigation techniques for fish passage at infrastructures.

Hearing capabilities of both species and their responses to acoustics were assessed using two approaches: (1) a confined experiment using specific test frequencies and allowing the establishment of a detailed panel of behaviour, (2) a novel approach involving an acoustic maze set in a large flume, to observe sounds impacts on fish movements. Finally, a third experiment involving a traditional mitigation system (bar-screen), tested the efficiency of this system in combination to two acoustic stimuli.

In terms of hearing capabilities, both species appeared to be more sensitive to low-frequency sounds. Fish swimming trajectories were poorly affected by sound. Nevertheless, the time taken by fish to pass the acoustic area was modified by the presence of the acoustic stimuli compared to control conditions. A rejection behaviour was observed with several fish in presence of sound. Furthermore, our results indicate that the response is very dependent of the test subject.

This research brought new material and methodology for the study of behavioural response of fish to sound. Nevertheless, more work is needed to develop a more effective acoustic signal to achieve better guidance of anguilliform species at infrastructure passage and also to determine long term effect of sound on fish.

Table of Contents

List of Tables

List of Figures

List of Figures

Declaration of Authorship

I, Mathias Deleau, 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.

IMPACTS OF ANTHROPOGENIC SOUND ON FISH BEHAVIOUR

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. Parts of this work have been published as:

Signed: .....

Date: .....06/12/2018.....

Acknowledgements

I wish to thanks the many people who have helped and supported during the course of completing this thesis. Firstly, I would like to thank my main supervisor Professor Paul Kemp for his unwavering patience and the time he spent correcting my “bad English” through this endless trek. Paul if you ever read these lines, believe me I would have rather spent more time degusting bottles of wine and chatting about birds. I am also deeply grateful to both Professors Paul White and Tim Leighton who always find time to help me out for experimental work or writing corrections even with their tight schedules.

I would like to say a massive thanks to all the members of “Team fish”, especially the one who helped me during long hours of night trials in the cold winter. Jim, Andy, Lynn, Gill, Matt, Jasper, Paolo, Sarah, Helen, Nick and Hannah, a huge thank you to all of you. Special thank goes to Jasper, we almost started this adventure together and we “almost” finished it together. A big thank for your help, moral support (“Don’t be like Laurence and Marny !”) and also for visiting me in South of France. I am hoping for another bird watching or diving session any soon. Also to Matt, I will always remember those epics rides in your car and the “Asian butterfly net fishing minnows technique”.

A huge thank to Toru Tsuzaki, who helped me sorting out all the experimental design, made me enjoyed Chilworth a bit more and has become a true friend over my time in Southampton.

I’d like also to thank Sian Leighton and Phil Newland who share with me their time and expertise on measuring physiological processes and helped me out on the AEP/ABR experiment. You two gave me motivation to carry on on this work as long as I could, even if it did not turn out the way we hoped.

This thesis and its statistical analysis would have not be whole without the help of the amazing people from Institut de Mathématiques de Toulouse (IMT) who generously took me as intern for 3 months. A big thank goes especially to Sebastien Dejean who shared with me all his available time and precious knowledge to complete my “Modèle Mixte”. Amazing to meet people that are so dedicated to their work and freely giving and sharing their experience to others.

I think I should also thank my family and friends who never had any clue of what I was doing or going through for the last years. My parents, especially my mom who gently “took me back” after my funding ran out and who could not wait for me to leave again. I will always remember her asking me if I passed every time I had a skype meeting with my supervisors and if my PhD was

finally over. My Dad, even if we have not really spoken to each other for a long time, I always had a thought for you and the hope that you will be proud of me.

I have also a very special thought for Roger Castle and his family. Roger was our silver eel "provider" and sadly passed out in 2017. I really enjoyed our brief encounters discussing about eel. Thank you for these brief enjoyable moments. You reminded me a lot of my grandfather who I would have loved to share this with.

Finally to Gaelle, you have always been there in the worth moments, when doubts and exhaustion were taking over motivation. Thank you for your patience, your support in all the moments of this very long and challenging experience. Quite a lot of memories at Chilworth with you but I will always remember that very poor day when we did the measurements of the acoustic sound field in the maze under a pouring rain. You were outside moving the hydrophone, I was in the little shed taking the measurements, and after an entire day without a complain under that miserable weather you kindly said: "Désolé, mais est-ce qu'on peut finir demain ? J'ai froid et même ma culotte est trempée ?" – "I'm so sorry, but could we finish tomorrow, I'm freezing, and even my underwears are soaking wet". I rewarded you with a Burger King. "If this isn't love ?"

Funding for this research were provided by the Environment Agency and Cefas who I greatly thank too.

Notation and Glossary

A. Organisms referred to in this research:

A.1. Class and subclass

Cephalaspidomorphi: class or clade that regroups the jawless fish (lampreys, hagfish). Nevertheless, this denomination is very debated by the scientific community.

Chondrychthyes: class that contains the cartilaginous fish with jaws. The class includes the subclass Elasmobranchii that regroups the sharks, rays, skates and sawfish.

Elasmobranchii: subclass of the Chondrychthyes, regrouping the sharks, rays, skates and sawfish. Members are characterised by five to seven pairs of gill (without operculum).

Sarcopterygii: class that designates the lobe-finned fish. Two species of coelacanths and six species of lungfish constitute this class. Tetrapods and humans are descendants from that class.

A.2. Order

Ostariophysi: second largest superorder of fish. It regroups almost 8000 species including 68% of the world freshwater species. All the fish from this superorder share two common features, an alarm substance and a Weberian apparatus (Weberian ossicles).

A.3. Family & Subfamily

Alosinae: subfamily of the Clupeidae (herring family) often referred as the “shads”. Shads are pelagic, schooling fish with some species being anadromous.

Anguillidae: family of ray-finned fish with elongated body, shaped like snakes. All species are catadromous.

Batrachoididae: family of ray-finned fish which members are often designated as toadfish or frogfish. Individuals from this family are known for the ability of males to “sing”.

Centrarchidae: family of fish from the Perciformes order. Species are also known as freshwater sunfish.

Cichlidae: family of fish from the Perciformes order. Currently, about 1,650 species have been described and the number of species is estimated up to 2,500. Many species like the tilapia genus are important food fishes.

Clupeidae: family of ray-finned fish, mostly marine species, with a very important commercial value. For instance, this family includes species like shads, sardines, herrings.

Gobiidae: family that includes more than 2,000 species of fish. Generally, their size does not exceed 10 cm. They are an important prey for larger commercial fish species like haddock, cod and flatfish.

Molidae: family that includes the largest ray-finned bony fish, the ocean sunfish. Species are marine, lack a swim bladder and able to produce sound.

Pomacentridae: family from the Perciformes order, that includes the damselfishes and the clownfishes. Most species are marine with some exception inhabiting freshwater and brackish environments. They are well known for their territoriality.

A.4. Species

Common name	*Latin name*
American eel	*Anguilla rostrate*
American shad	*Alosa sapidissima*
Atlantic herring	*Clupea harengus*
Atlantic salmon	*Salmo salar*
Brown trout	*Salmo trutta*
Burbot	*Lota lota*
Chinook salmon	*Oncorhynchus tshawytscha*
European eel	*Anguilla Anguilla*
European flounder	*Platichthys flesus*
European sea bass	*Dicentrarchus labrax*
European sprat	*Sprattus sprattus*
Goldfish	*Carassius auratus*
Mottled sculpin	*Cottus bairdii*

Mozambique tilapia	*Oreochromis mossambicus*
Northern pikeminnow	*Ptychocheilus oregonensis*
River lamprey	*Lampetra fluviatilis*
Three spot gourami	*Trichogaster trichopterus*

A.5. Non-fish species

Ardeidea: family of birds, generally long-legged, that includes heron, egrets and cranes.

Common name	*Latin name*
Common merganser	*Mergus merganser*
Grey heron	*Ardea cinerea*
Great egret	*Ardea alba*

B. Acronyms

ADV	Acoustic Doppler Velocimeter
ABR	Auditory Brainstem Response
AEP	Auditory Evoked Potential
ANOVA	Analysis of Variance
CBS	Continuous Broadband Sound
CCTV	Closed-circuit television
CITES	Convention on International Trade in Endangered Species
GAS	General Adaptation Syndrome
HELCOM	Helsinki Commission
HPI	Hypothalamic Pituitary Interrenal (axis)
ICER	International Centre for Ecohydraulics Research

IQR	Inter-Quartile-Range
IUCN	International Union for Conservation of Nature
JNCC	Joint Nature Conservation Committee
MEM	Mixed Effect Model
ML	Maximum Likelihood
MSFD	Marine Strategy Framework Directive
MWL	Maximum Water Level
OSPAR	Oslo and Paris convention
PIT	Passive Integrated Transponder
PTS	Permanent Threshold Shift
REML	Restricted Maximum Likelihood
SAC	Special Area of Conservation
TTS	Temporary Threshold Shift
WFD	Water Framework Directive

C. Notation

C.1. General notation

Notation	Unit	Description
u	m s^{-1}	Longitudinal velocity component
v	m s^{-1}	Lateral velocity component
w	m s^{-1}	Vertical velocity component
df		Degrees of freedom (statistical value)
N	Count	Number of fish
p		p-value, probability value or asymptotic

		significance.
S.D.		Standard deviation
SPL	dB 20 µPa (air) or dB re 1µPa (water)	Sound Pressure level. Intensity of an acoustic signal.
Tmax	s	Maximal duration of a trial

C.2. Axis definitions as used in the laboratory:

Axis	*Position*
X	Longitudinal axis
y	Lateral axis
z	Vertical axis

D. Glossary

Acclimation: The physiological adjustment of an organism to environmental conditions (e.g. osmotic balance).

Ammocoete: larva stage of the lamprey.

Anadromous: Diadromous fish for which the majority of feeding and growth is undertaken in marine environments before the adults migrate to freshwater to spawn.

Anguilliform: Shaped like or resembling an eel. Also a swimming mode (see anguilliform locomotion).

Anguilliform locomotion: A swimming mode where the whole body participates in large amplitude undulations. Since at least one complete wavelength of the propulsive wave is present along the body, lateral forces are adequately cancelled out, minimizing any tendencies for the body to recoil.

Anthropogenic: Relates to an effect or object resulting from or induced by human activity. Baffle: A device used to restrain the flow of a fluid.

Bypass: An alternative route for downstream moving fish, allowing them to bypass anthropogenic river barriers. Designed to be a safer and more benign route that that of the bulk flow of the river

(e.g. where the majority of the water may pass through a hydropower turbine). Catadromous: Diadromous fish for which the majority of feeding and growth takes place within freshwater prior to adults migrating to the sea to spawn.

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

Diadromous: Fish that migrate between freshwater and marine environments.

Degrees of freedom (df): number obtained in the final calculation of a statistic test representing the number of values that are free to vary.

Ecosystem services: The benefits humankind derives from the workings of the natural world.

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 lifestage of an eel between glass and yellow eel. Individuals are larger than glass eel and pigmented.

Endolymph: liquid contained in the inner ear.

Entrain: To pull or draw along after itself.

Epithelium: animal tissue composed of a single or several layers of cells.

Fitness: The contribution an individual makes to the gene pool of the next generation, relative to the contribution made by others in its present population.

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.

Glass eel: The lifestage of an eel between the leptocephali larvae and elver stage. Individuals conform to the elongated eel morphology but are unpigmented.

Habitat: An area that provides the resources (e.g. food, space) necessary for the existence of an organism or particular life-stage.

Habitat connectivity: The size and distribution of suitable habitat patches and the ease with which a species can move through the space between patches.

Habitat fragmentation: The subdivision of a specific habitat into smaller and more isolated fragments or patches, through either natural or anthropogenic activities, resulting in changes to the space composition, structure and function.

Homeostasis: optimal stable stage of functioning for an organism.

Hydrodynamic: Pertaining to forces in or motions of liquids.

Impingement: The non-volitional entrapment of a fish against a structure.

Interspecific: In reference to between different species and to inter-subject variability.

Intraspecific: In reference to within the same species.

Lagena (fish): is a part of the vestibular system and contains the otoliths. The lagena is involved in hearing and registering the vertical acceleration.

Leptocephali: A colourless, transparent, flattened larva, especially of certain eels and ocean fishes.

Macula neglecta: thick epithelium located in the inner ear and formed of arrays of hair cells.

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

Model (statistical model): mathematical model, used to describe the distribution of a dataset. (see McCullagh 2002).

Neuromast: mechanoreceptive organ used by fish to sense the mechanicals changes in water.

Octavolateralis system: it includes the vestibular, auditory and lateral line of fish. (Braun, 2015)

Otolith: small calcium carbonate structure present in the saccule and utricle of fish. It can also be called: statoconium, otoconium, statolith.

Potamodromous / potamodromy: fish displaying small/local scale migratory patterns, e.g. between lakes and rivers or from one area of a river to another.

Saccule: otolith organ located in the inner ear.

Silver eel: Eels at the end of the growth phase which have undergone reproductive and osmoregulatory changes in preparation for migration to spawning grounds. During these changes individual takes on a silver hue.

Smolt: The juvenile life-stage of an anadromous salmonid that has undergone physiological**.**

Stridulation: act of producing sound by rubbing together certain body parts.

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.

Vestibular system: mechanical sense system. It is responsible for the position of the body and its static and dynamic equilibrium. (Kasumyan A., 2004)

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.

Yellow eel: Growth stage of catadromous eels. Individuals are usually dark in colour with yellow hues.

CHAPTER 1

1 Introduction

1.1 The value and decline of fish stocks

About 70.8% of the planet Earth is covered by water (Pidwirny 2006). The greatest part (97.25%) is contained in the world oceans (marine ecosystems) and an extra 1.1% constitute the freshwater ecosystems (lakes, rivers and wetlands). As a whole, these two ecosystems contribute to an estimated US$27.5 trillion in ecosystem services per year (terrestrial accounts for 12.3) (Costanza et al. 1997). Within both ecosystems, fish play a preponderant role by acting in the food chain (Rice 1995; Dodds & Whiles 2010), influencing the nutrient cycles (Deegan 1993; Janetski et al. 2009) and even modifying the physical landscape (Bythell et al. 2000; Moore 2006). Fish represent an important direct and indirect source of food for many non-aquatic species (Polis and Hurd 1995 and 1996; Cederholm et al. 1999) including humans (Sargent 1997).

Migration is very common process in marine and freshwater fish lifecycles, being either local (small scale) or across extensive distances. Migrations take place seasonally or on a daily base (Mazeroll & Montgomery 1998) and generally respond to feeding, reproductive or survival (avoiding predators) needs (Lucas & Baras 2001; Brönmark et al. 2008). More relevant to this thesis and generally well known, are the migrations of diadromous fish like the European eel, *Anguilla anguilla* and the River lamprey, *Lampetra fluviatilis.* European eel are categorised as catadromous fish as adults spawn at sea (Sargasso sea) whereas the adults of River lamprey spawn in freshwater and are categorised as anadromous fish.

Nevertheless, freshwater and marine ecosystems, key environments of the lifecycle of these two species, have been for millennia shared with humans that have intensively increased their activity and control over them. Humans have fed on fish for thousands of years with the first archaeological evidence of fishing dated from 40 000 years ago (Henshilwood et al. 2002). Since these times, fishing techniques have evolved tremendously with, for instance, the apparition of new technologies starting with motorised boats, frigorific container and new fish tracking techniques like sonars. In 2014 the world fishing fleet was estimated to be around 4.6 million fishing vessels (FAO 2016), an increase of 300 000 fishing vessels compared to 2012 (FAO 2012). In

addition to the direct impact that such activity has on the world fish populations stocks (fish stocks are in crisis see; Pauly & Zeller 2017), fisheries introduce other threats to the marine environment.

This topic looks at the response of eels and lampreys to manmade sounds, for two reasons. First, the increase in manmade sound in marine and freshwater is seen as a potential threat to the health and wellbeing of fish species (developed in the next section). Second, effective deterrents might be developed which would protect endangered species from water extraction points, or guide them to fish passes etc.

1.1.1 Sound pollution and fish

Motorised crafts (recreational or professional) generate underwater sound; this sound can be loud enough to disturb important behaviour of fish (Slakebborn et al. 2010). Simpson et al. (2014) have for instance shown that acute exposure to boat noise was generating a stress response (hyperventilation) in European eel. Other behaviours like communication (Codarin et al. 2009), foraging (Voellmy et al. 2014), mating (Vasconcelos et al., 2007) have been reported to be impaired by ship noise. However, even if ship noise is one of the predominant source of anthropogenic noise in the freshwater and marine ecosystems, it does not outcompete, in terms of direct impacts, with noise emanating from pile driving (Williams 2015). With the increasing global needs in energy and the general concern to reduce the use of fossil energy sources, humans have deployed new infrastructure to produce sustainable energy, using for instance wind (offshore windfarms) and stream power (dams). The construction of such infrastructure often involves the use of pile driving (Bailey 2010) in order to establish the foundations for the future turbines or dam structure. The hammering of a pile produces a very loud noise that propagates large distances underwater and can affect fish's previously enunciated behaviours (Thomsen et al. 2006; Andersson 2011) and can also cause some moderate (Temporary Threshold Shift – TTS) to irreversible damage (Permanent Threshold Shift – PTS and internal injuries) as reported by Thomsen et al. (2006) and Casper et al. (2012).

The noise produced during the construction of these structures is not the only element affecting fish. Dams (weirs and barrages) and hydropower stations constitute physical barriers for fish, especially the species undertaking important migrations. According to the World Commission on Dams (2000), there are currently over 45,000 dams greater than 15 m high deployed in the world. The impairment of such structures on fish movements is very dependent of the hydrology of the river and also on the fish species specificities like behaviour, swimming capabilities, morphology and migration timing (Northcote 1998). Small barriers (< 2 m high) can limit (Lucas & Frear 1997;

Lucas and Barras 2008) or entirely block (Ovidio & Phillippart 2002; Russon et al. 2011) fish movements, while higher barriers (> 5 m high) are reported to usually block upstream fish movements (Xie 2003; Wu et al. 2004). The consequences of such restrictions on fish movements directly affect their fitness (survival and genes exchanges) but also reduce access to essential habitats to fulfil their lifecycle (Esguicero & Arcifa 2010). Finally, during the downstream migration, hydropower facilities increase severely the risk of injuries and mortality with fish taking hazardous paths through the turbines and the overspills (Winter et al. 2006; Teague & Clough 2014; Pracheil & DeRolph 2016).

As reported by multiple studies, several fish populations around the world are in decline, both marine (Hutchings 2000; Hutchings & Reynolds 2004; Limburg & Waldman 2009; Nieto et al. 2015) and freshwater (Aparicio et al. 2000; Dudgeon et al. 2006; Allibone et al. 2009; Freyhof & Brooks 2011). For both marine and freshwater environments, most of this decline is associated with human activities including industrial fishing, pollution, modifications of the coast, estuaries and rivers, energy production and mining (Nieto et al. 2015; Freyhof & Brooks 2011). Due to the severity of their population decline (Aalto et al. 2015), the European eel has been categorised as a critically endangered species (Jacoby & Gollock 2014). River lamprey are not classified as an endangered species, their status was of “near endangered” which has been updated to “least concern” in 2008 on the IUCN list (Freyhof 2013). Nevertheless, this information has to be specifically considered in particular regions of Europe, like Portugal and Spain, where river lamprey are respectively listed as “critically endangered” and “regionally extinct” (Mateus et al. 2012; Kujawa et al. 2017). Barriers to migrations and habitat fragmentation have been identified as one of the main causes for the decline of both European eel (Dekker 2007; Verhelst et al. 2018) and River lamprey (Mateus et al. 2012; Tummers et al. 2016).

Awareness to these threats has been raised for decades and several protective legislative frameworks have emerged to protect fish species. The Bern Convention (1982) focused on the conservation of wild fauna and flora in Europe. The 1982 Bern Convention, was later reinforced by the Habitats Directive (1992), which also aims to protect natural habitats and wildlife across Europe but also establishes the Natura 2000 network of protected areas. Since 2008 and the formalization of the Marine Strategy Framework Directive (MSFD), marine species in Europe have received specific protection and conservation measures. Similarly, the Water Framework Directive (WFD) was also adopted in 2000 with a focus on the protection of freshwater species and insists on the need to ensure fish migrations and habitat connectivity. As regards of sound pollution, several regulations are already in place with some included in the MSFD, mainly focusing on the

tracking of vessels equipped with sonars, the registration of low- and mid-frequency impulsive sounds and the monitoring of continuous low frequency sounds (Erbe 2013).

In order to fulfil legislative requirements and improve conservation status of fish populations, guidelines and mitigation strategies have been established and made compulsory in specific cases. As reported by Erbe (2013) these mitigation measures vary between countries. One of the main mitigation in place around Europe is the use of a bubble curtain to absorb and scatter some of the noise from impact pile driving (Erbe 2013). In the UK, the Joint Nature Conservation Committee (JNCC) has produced guidelines for seismic surveys (House & Street 2017) and pile driving (JNCC 2010) to limit the risk of injuries of marine mammals and can also be applied to fish. Nevertheless, there is still a need to better understand how fish interact with anthropogenic sounds in order to produce appropriate mitigation measures and improve current legislation.

1.1.2 **The use of sound as a deterrent and guide**

Given the threat presented to fish by dams, water extraction points and other manmade infrastructure, the use of sound as a mitigation measure has been considered for a long time. The idea is to use sound to trigger a specific behavioural response to encourage fish movements towards a safer route. The first patents for acoustic guiding and/or deterring devices can be found as early as 1987 (see Kowalewski et al. 1987). This preliminary invention called an “acoustic generator” consisted of a water tight enclosure filled with air and in which a radiating surface was responsible for producing a soundwave after being “hammered” by a mass. This system offered the possibility to modulate the acoustic signal by adding some plates between the mass and the radiating surface (see. Kowaleski et al. 1987). Following the specifications provided by the authors, this apparatus was able to produce sounds ranging between 20 to 1000 Hz with a peak intensity reached within 3 meters of the radiating surface. Nevertheless, the scientific literature does not suggest that any further testing has been done with this device, especially experimental testing on fish.

As a more common alternative, researchers on the field of acoustic deterrents have either preferred developing their own sound sources (Knudsen et al. 1992, 1994, 1997; Sand et al. 1999) or using more conventional acoustic equipment like underwater loud speakers projecting sounds directly underwater (Maes et al. 2004). The latter can range from basic, small and inexpensive speakers to, dedicated, larger and costly devices often referred to as Acoustic Fish Deterrent (AFD). While the first kind are easily available on the market and seem more appropriate to lab conditions (Bzonek 2017), the second are only produced by few companies as for example Fish Guidance Systems Ltd. (Southampton U.K.) and generally used in the field (Maes et al. 2004).

Maes et al. (2004) tested the efficiency of an array of 20 large AFDs developed by Fish Guidance Systems Ltd. The sound projectors were set along the water intake of a power plant, to deter local fish. The setup managed to produce a reduction in the total fish impingement of 60% and was significant for nine different species or taxa including: herring, sprat, white bream, bass, smelt, perch, sole, flounder and gobies. This is one of the fewstudies that did not focus on only one single species.

Moreover, as reported by Popper et al. (1998), there has been an extensive piece of literature focusing on clupeids and aiming to prevent them from entering hydropower stations and dam intakes. Results of these experiments were highly variable with both successful and unsuccessful outcomes. Sound has also been used on migratory species like the Atlantic salmon (*Salmo salar*). In a series of experiments, Knudsen et al. (1992, 1994) determined that Atlantic salmon were sensitive to infrasound and successfully managed to make fish swim away from an infrasound source. Knudsen et al. (1994) suggested that this system would potentially work in a river or a dam but will require a large number of sound projectors. Similarly, Sand et al. (2000), applied this technique on migrating European eel, and managed to decrease, by 57%, the catch rate of a trap placed close to the acoustic source. Nevertheless, this work and its application has not been further developed and this type of setup extended to other sites and suggest that further work is potentially needed especially with regard to the current conservations status of the European eel, recently listed as endangered (CITES).

Acoustic research on fish, as illustrated above, is quite a novel science and can lead to multiple research topics with the key ones being the impacts of anthropogenic sound on fish and how to use this knowledge to improve fish conservation via the development of new systems for modifying fish behaviour. Furthermore, it appears that the current scientific literature mainly focused on work that has been carried out on specific group of fish (e.g. clupeids). This also reveals that they are still many research gaps for potamodromous species (fish travelling on a small/local scale) or “real” migrants like for instance lamprey sp.; that are well known for their long migrations and high mortality rate at water intakes and dams but have been, to the author’s best knowledge, disregarded from any published research about acoustic guidance.

1.2 Research aim and objectives

The broad aims of this study are to:

1. Investigate the potential impacts of anthropogenic sounds on European eel and river lamprey behaviour.

2. Develop or improve mitigation techniques based on acoustics in order to improve the survival rates during the migrations of European eel and river lamprey.

To achieve these aims, the following objectives have been formulated:

1. Review the current literature to highlight research trends and biases, identify knowledge gaps regarding fish hearing capabilities and reaction to sound.

2. Assess the behavioural response of European eel and river lamprey to different sound frequencies in a confined and controlled (tanks) environments.

3. Assess the influence of a specific sound, on European eel and river lamprey migratory movements and behaviours in a controlled environment (flume).

4. Assess the effectiveness of the combination of an acoustic stimulus and a conventional vertical bar-screen as mitigation measures during downstream passage of European eel.

1.3 Thesis overview

Chapter 2 of this thesis includes a literature review to meet Objective 1. A series of experimental studies have been carried out and are presented in Chapters 4, 5 and 6 of the present thesis and respectively provide answers to Objectives 2,3 and 4. Each of the result chapters are voluntarily split into dedicated research sections, introducing, reporting and discussing the experimental work undertaken to meet the previous objectives. The general techniques and methods used during the experimental work of this research are presented and discussed in Chapter 3. Chapter 3 also introduces the two fish species involved in this research, describing their general ecology and the current bioacoustics knowledge around them. Chapter 7 discusses the outcomes of this

thesis, the gains in terms of knowledge and how these results can be used to improve current practices.

CHAPTER 2

2 Literature review

2.1 Fish and Sound

The detection of sound and its utilization by fish have been extensively studied during the last decades. Sound has been since recognised as a key factor involved in multiple ecological functions of fish. Appearance of disruptive sounds can result in an alteration of these essential functions and impact on fish welfare (Codarin et al. 2009). To prevent any damage on fish wellbeing and ecology, it is important to understand what the main functions involving sound in fish life are.

From an anthropocentric point of view, the first question that arises about the role of sound in our everyday life is its obvious implication in communication. A similar question applies to fish. One of the key purposes of sound is also communication with different levels of specificity. It is obvious that to be able to make use of sounds, fish need to be able to hear them and to possess anatomical features or organs dedicated to this function. Anatomical features involved in the hearing process, as well as their functioning, will be described in the section 2.2 of this chapter but more broadly it has been demonstrated that even the most primitive extant fish species are equipped of hearing structures (even if it has not been proven that they are functional) (Fay & Popper 2000).

As previously mentioned, communication can have different purposes or levels of specificity, as for instance, during courtship and mating events, during agonistic encounters and establishment of hierarchy, or to ensure the structures of a school. If sound has a deterrent effect on fish, it might not be because of physical harm, but because the fish is aware that it would be entering an acoustically noisier environment where inter-fish acoustic communication is less reliable, and this should be taken into account when designing deterrents.

One role of the type of sounds studied in this thesis, is that it can affect fish by interfering with their communications, effectively raising the noise level. This makes the use of sound by a fish (e.g. to trigger a given response in another fish) less reliable, and so it is important to take into

account in this thesis the extent to which responses to sound can be reliably induced in fish, even in a baseline noise-free environment. Some studies cast light on this, and on the possible reasons why fish might make sounds, are reviewed in the next subsection.

2.1.1 The possible uses of sound by fish

Sounds produced by fish during courtship and mating have been studied in several species of fish, and interestingly in some of them these sounds are only displayed during key times of the years and can also be specific to an individual. Gerald (1971) first reported time courtship sounds emitted by 86% of tested species of sunfish (Molidae). Bass & McKibben (2003) investigated acoustic communication in teleost fish and reported again specific sounds during courtship in Batrachoididae (midshipman and toadfish), but also Pomacentridae and Gobiidae (damselfish and gobies). Furthermore, in their discussion on patterns of fish calls in the nearshore of the Great Barrier Reef, McCauley & Cato (2000) suggested that some of the recorded calls emitted by fish at night were probably used "to maintain loose school structure" and allowed fishes to track planktonic prey aggregating. Finally, Amorim et al. (2003) looked also at courtship sounds in Mozambique tilapia (*Oreochromis mossambicus*) and noticed that during mating season and agonistic encounters, no sounds were displayed but after encounters only one male, the dominant one, was producing courtship sounds. In this species, sound can be a way for individuals to identify the dominant male within a group, and to achieve reproduction. The dominant male being the one producing courtship sound will have better chance to find a partner. In this case, sound plays an important role in the sexual selection and the fitness of the individuals.

Hearing is also a key factor for fish to locate themselves and investigate the surrounding environment. During the night, the efficiency of eyes is severely reduced due to the lack of light and even during daytime, oceans with deep waters and turbid rivers do not allow much light to penetrate through them, affecting also visual capabilities. It has been suggested by Bregman (1994), then by Fay & Popper (2000), that hearing in many vertebrates including fish could be used to get a representation of the surrounding environment and throw on this process named "auditory scene analysis". Sound might be used to identify the presence of potential prey and predators as suggested in a study by Platcha & Popper (2003) where American shad (*Alosa sapidissima*) showed detection for ultrasonic stimuli possibly similar to the one used by dolphins.

Even in their earlier stages of life it seems that sound plays a major role in several species of reef fish. In a first set of experiments, Tolimieri et al. (2002) tested the impact of reef noise on reef fish larvae. From their results, the authors deduced that fish larva were attracted by the reef noise and could actually identify the direction where the noise was coming from. Later on, scientists

from the same research team further developed this theory by testing if the invertebrate noise coming from the reef was a main component of the navigational cue for reef fish larva. Simpson et al. (2008) reported that higher-frequency invertebrate sound was enhancing directionality of fish larva toward the reef and was probably a fundamental cue used by pelagic larva to find an appropriate location to settle and proceed to the next stage of their life cycle.

2.2 Fish bioacoustics

Bioacoustics is a combination of two disciplines, Biology and Acoustics and is the study of sound production, propagation and receptions in animals. Bioacoustics implies investigations on anatomical features and physiological parameters of hearing and sound production but also of the relevant behaviours linked to hearing (Popper 2005).

From an anatomical point of view, fish (bony and cartilaginous) have two main structures implicated in the "hearing" process. When considered as a whole system, it is usually called the "octavolateralis system" and is composed of the inner ear, the lateral line but can also include additional supporting "Ancillary structures" (Fay & Popper 2000).

2.2.1 Inner ear

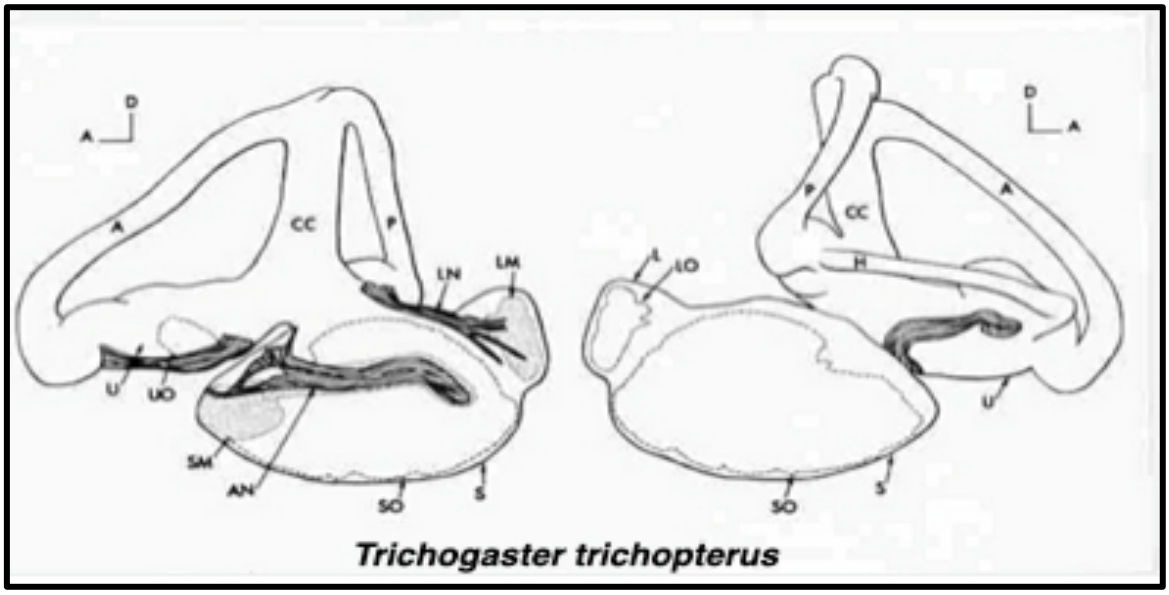


Figure 2.1 - Drawing of the ear of the blue gourami (*Trichogaster trichopterus*). A, H, P - anterior, horizontal, posterior semicircular canals; AN - auditory nerve to saccule; CC - crus commune; L - lagena; LM - lagenar macula; LN - eighth nerve to lagena; LO - lagenar otolith; S - saccule; SM - saccular macula; SO - saccular otolith; U - utricle; UO - utricular otolith. http://www.popperlab.umd.edu/background/anatomy.htm

The fish inner ear is located backward of the midbrain and is composed of a membraneous labyrinth that is divided in two parts, the pars superior and the pars inferior. The first one is composed of three main canals generally named semi-circular canals, all three ending in a common structure with a pouch like shape, the utricle. At the base of each semi-circular canals, there is a swelling named the ampulla which contains sensory hair cells on a transverse ridge called the crista ampullaris (Platt & Popper 1981; Popper et al. 2003.) The pars inferior is formed of the saccule and the lagena that are also two sac-like structures like the utricle. The three structures, utricle, saccule and lagena are filled with endolymphatic fluid, and are also fitted with a calcified matrix, the otolith. The otolith is overlying a macula that is a sensory epithelium containing hair cells (Popper et al. 2003). All these structures are illustrated on the Figure 2.1.

This anatomy is generally consistent among all fish species especially within the teleosts. The shape and size of the otolith can vary among species and are valuable clues in fish identification. Also in non-teleost fish like sharks and rays (Chondrichthyes), a huge macula neglecta (thick epithelium with hair cells arrays) can be found near the utricle (Corwin 1977) but it can also be found in smaller proportion in some species of teleosts like damselfish (Pomacentridae) (Maruska et al. 2007). In evolutionary "primitive" fish species like Chondrichthyes and Sarcopterygii, the otoliths are replaced by otoconial masses that are not always solid structures like the otolith (Popper et al. 2003; Casper & Mann, 2006). These two examples are just an indication of anatomical differencies present between fish species. However, in general, the main structure of the inner ear stays the same among fish species.

The inner ear insures two functions, the first one is called a "vestibular" sense and is responsible for determining the posture of the fish in the water column. The second function is the "auditory" sense involved in the hearing process (Popper et al. 2003). The otolith organs (saccule, lagena and utricle) are in charge of these two functions. It has been established that in teleost fish, the saccule has primarily auditory role while the utricle is involved in the vestibular function. The lagena might be involved in both functions. As reviewed by Popper et al. (2003), several experiments have been undertaken to identify any dedicated function for each of these three otolith organs. Nevertheless, it seems that, depending on the species it is still not completely possible to consider each of the otolith organs as playing only one function. It seems that the three organs have a multimodal capability with different influence on the various modes of sensing.

The epithelium (macula) covering the inner ear structures, contains hairs cells acting like sensory receptors. The distribution of hair cells varies between the different end organs (saccule, lagena, utricle) (Popper et al. 2003).

The hair cell structure is fairly simple. The cell is composed of an apical part topped by a bundle of cilia. Within this group of cilia there is only one true cilium that is called the kinocillium and responsible for generating the physiological response. The other cilia are called stereocillia or stereovilli (Siegelbaum & Hudspeth 2000). The stereovilli are disposed in an ascending way, with the tallest closer to the kinocillium. When the bundle is bent toward the kinocillium it causes the depolarization of the cell and gives a directional sensitivity for the excitatory nerve signal (Popper et al. 2003). The bundles are facing the endolymphatic fluid and the otolith in the lumen of the end organs, and the movements of either one, or both of them, leads to cilia movements. As previously mentioned, the inner ear structures are involved in vestibular and auditory senses. In the case of a gravitational stimulus (vestibular sense) a static bending provides a good explanation of the mechanism as referred in the Figure 2.2.

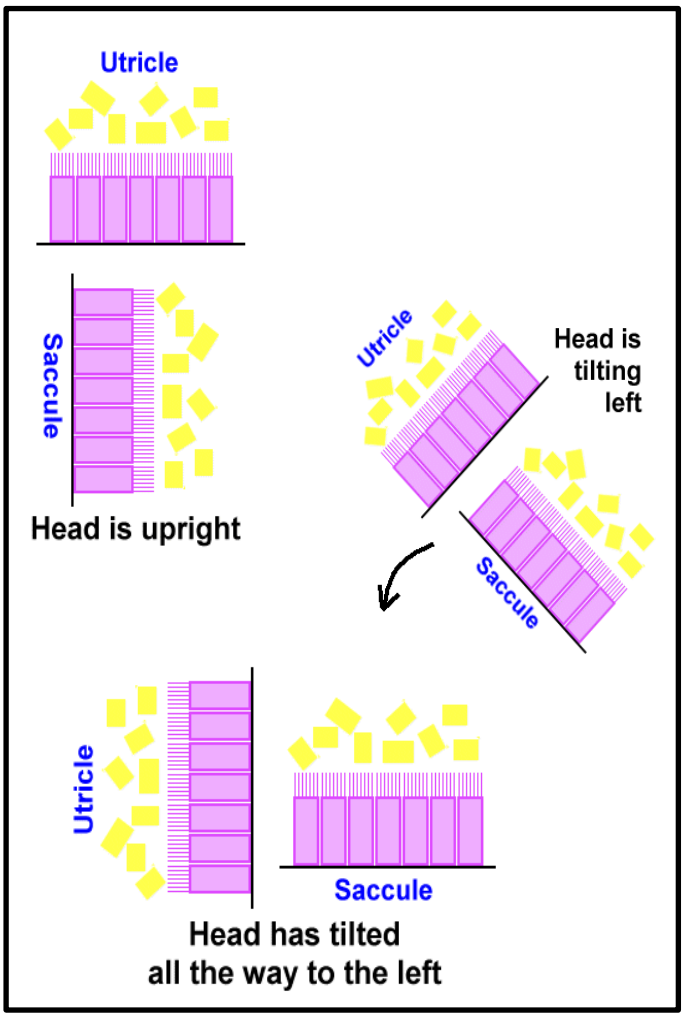


Figure 2.2 - Representation of a theoretical "static bending". According to head's orientation of kinocillium in the end organs (here saccule and utricle) are bent in one position or another by the endolymph or the otolith pressure informing then the Central Nervous System of the fish posture. (drawings from http://faculty.stcc.edu/AandP/AP/AP2pages/Units14to17/unit16/vestibul.htm)

On the other hand, for sound detection, instead of a constant stimulus, a dynamic stimulus covering a range of frequencies would be a possible interpretation. The body of fish has approximately the same density as water and consequently moves at the same speed as the sound waves. The otolith that are denser structures, due to their composition, are moved more slowly. This creates a relative motion between the fish and the otolith stimulating the hair cells which is sensed as a sound detection.

The hair cells do not have the same orientation, the orientation depends on the end organs. Currently, in the saccula of teleost fish, five typical patterns of hair cells positioning have been identified (diagrams of these patterns can be found in Popper et al. 2003).

It has been shown that these different patterns can have a strong influence on the sensitivity of hearing and can even enhance it (Popper & Fay, 1999). According to Popper et al. (2003), the variety of hair cell orientation that occurs in the ear allows fish to identify the direction of sound in their environment.

2.2.2 **Lateral line**

Fish are equipped of an additional system that is partially involved in the hearing process. If hearing is considered to its largest sense as “the detection by specialized mechanoreceptors of mechanical energy propagated through the environment” (Coombs & Montgomery 1999), then the lateral line and the inner ear are playing a common role in the hearing process.

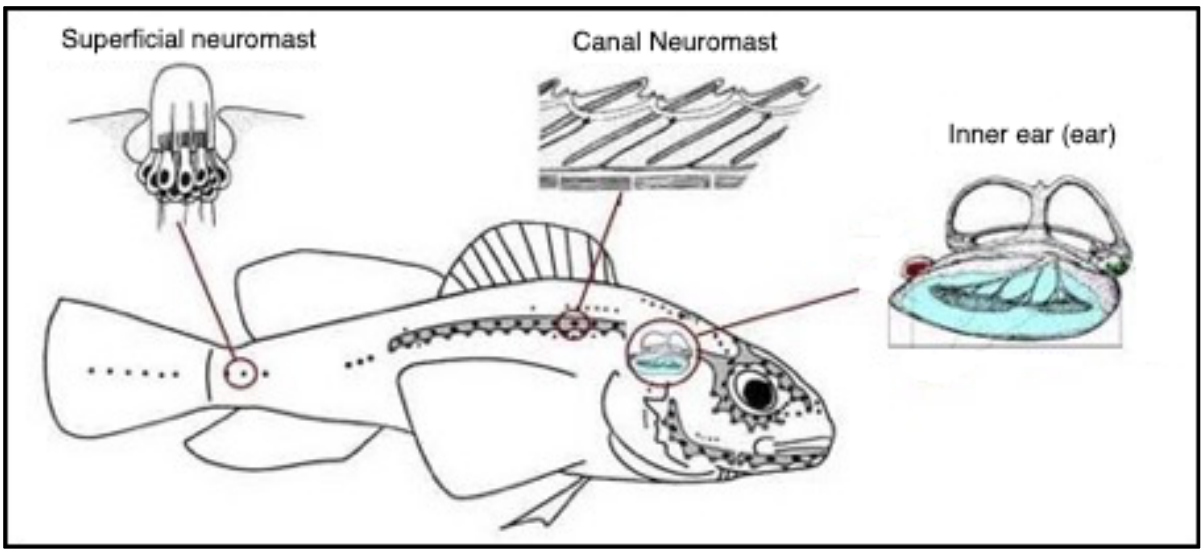


Figure 2.3 - Localization of the lateral line on a mottled sculpin drawing (*Cottus bairdii*) and details of the different structures of the octavolateralis system. Left: Superficial/Free Neuromast, Middle: Canal Neuromast, Right: Inner ear. (extracted from: http://www2.ulg.ac.be/morfonct/site-anglais/rech_lateral_line.html)

The lateral line (Figure 2.3) is composed of a succession of little structures that are called neuromasts and can be found in two regions, the canal system and the superficial neuromasts. One common point between the inner ear and the lateral line is that neuromasts are in fact structures essentially formed of hair cells. The canal neuromasts are evenly distributed within canals that can be found around the head and along the body of the fish, while superficial neuromasts are distributed over the body of the animal. The distribution and assemblage of canals and superficial neuromast varies between species. This variability is assumed to be linked to the specific needs and utilization of the lateral line for the species (Popper 2005).

The lateral line has to be included in our study for certain reasons. Indeed, the functions of the inner ear and the lateral line are overlapping. Both systems possess hair cells but with different receptivity to stimulus. The canal neuromasts are sensitive to the particle acceleration of the surrounding water and the superficial neuromasts are sensitive to the particle velocity. On the other hand, the inner ear structures are receptive to the acceleration of the whole animal body.

The lateral line has to be considered in this research due to its shared role with the inner ear and their joined sensitivity to a certain range of frequencies. Because some of this thesis experiments are carried out in confined environment it is likely that some of the generated signals will be stimulating the lateral line receptors. The main topic of this thesis is the impact of sound on fish behaviour and therefore, even though it is not considered to be part of the “hearing” process, the lateral line system is included in this introduction due to its capacity to detect sound waves. The lateral line is involved in, the detection of short distance movements, the regulation of the schooling behaviour and can also detect some sound frequencies. Due to these multifunctional properties, Coombs & Montgomery (1999) and Popper (2005) have suggested that it would be interesting to investigate on the impacts of sound on the schooling behaviour of fish.

2.2.3 **Air cavities**

The swim bladder is a gas-filled structure common to all the Actinopterygii fishes (fishes with rayed fins), excluding therefore the Elasmobranchii (sharks and rays) and the Cephalaspidomorphi (lampreys). The swim bladder’s primary role is as a regulatory organ for fish buoyancy which it achieves by controlling the bladder’s content.

The swim bladder is very often considered as an air-bubble in water. Air is more compressible and of different density than water and therefore the swim bladder can be set into motion by the pressure component of the sound field. This creates oscillations of the volume of the air-bubble (swim bladder) that reradiate the signal and generate a particle motion component that can travel to the inner ear and be sensed by the otolith (Popper & Fay 2011).

The inner ear is sensitive to particle motion (it is the motion of the otolith relative to the fish which is sensed), but with the presence of the swim bladder, fishes are also sensitive to sound pressure (Popper et al. 2003).

Fishes can be divided in three arbitrary groups based on the presence of swim bladder and connection with it. The first group or “hearing specialists” is generally composed of fishes having a swim bladder anteriorly connected to the inner ear by a series of bony structures or linked to the inner ears otolith organs by a series of gas-filled vesicles (Popper et al. 2003). The second group is composed of the fish having a swim bladder but with no specific connection with the inner ear, and within the third group the fish without a swim bladder. These two last groups are generally described as “hearing generalists/non-specialists”.

Hearing specialists, have a series of bony structure named the Weberian ossicles/apparatus (Figure 2.4) that is connected to the inner ear and is helping in sound transmission. It has been confirmed by Poggendorf (1952) and Finneran & Hastings (2000), that surgical disruption of the Weberian ossicles could result in a reduction of hearing sensitivity up to 30-40 dBs.

In addition of the Weberian ossicles found in Ostariophysi fish, some species (mainly from the Clupeidae family) have developed other structures that are paired gas-filled vesicles (or bullae) positioned in contact with the inner ear. The bullae can either be connected to the swim bladder via small tubes, or not be connected at all (Fletcher & Crawford 2001).

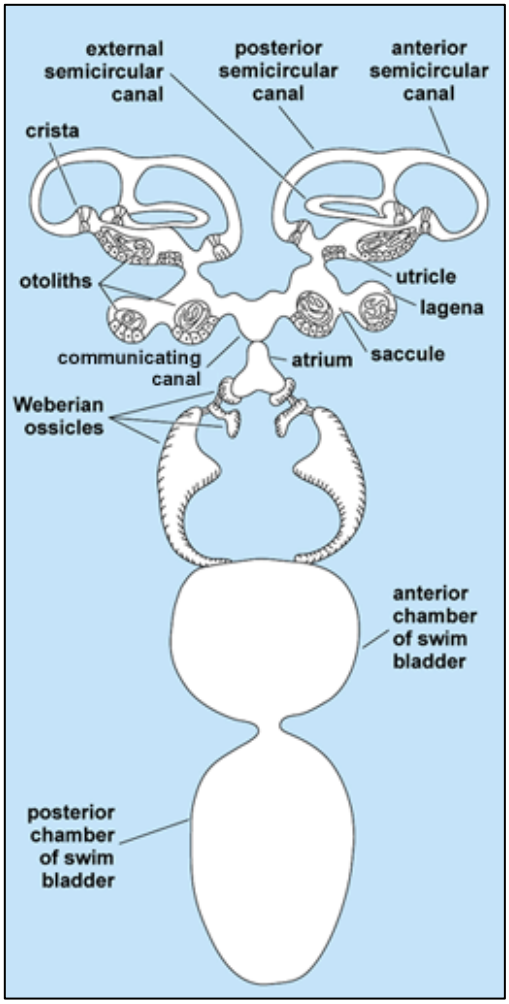


Figure 2.4 - Drawing of the three connected structures, the inner ear, the Weberian ossicles and the Swim bladder. (http://www.accessscience.com/loadBinary.aspx?filename=508100FG0020.gif)

2.2.4 **Sound generation by fish**

In terms of communication fishes are able to produce certain type of sounds by different methods. Fishes contrary to other vertebrates like birds, frogs or mammals do not possess a dedicated organ (larynx or syrinx) for producing sound (Ladich & Bass 2003). Two mechanisms of sound production have been so far identified, one based on the the stridulation and the other on the vibration of the swim bladder. It is recognized (Fine & Parmentier 2015) that is possible that other mechanisms exist and play a role in social communication. Gobiidae, for instance are well-known sound producers but the method by which they produce sound has still not been clearly identified (Ladich & Bass 2003).

Stridulatory sounds can be produced in different ways, by rubbing pharyngeal teeth against each other for instance, or by using pectoral spines and rubbing their ridges. These mechanisms are generally described in Cichlidae and Centrarchidae (teeth) and in Siluriformes (catfish) (pectoral spines).

The swim bladder mechanism required the presence of extrinsic or intrinsic "drumming muscles" that can contract very fast and then extend or compress the swim bladder generating some low-frequency sounds.

Again, these two mechanisms are reported in this report as they have been widely studied but other mechanisms have been documented and did not fit in any of these two categories (for review see Ladich & Bass 2003).

2.2.5 **Hearing capabilities**

The most common method used to represent fish hearing capabilities consists in building its audiograms. Audiograms are a graphical representation of hearing sensitivity, plotting "the lowest detectable level for tones as a function of frequencies" (Popper et al. 2003; Ladich & Fay 2013). In 2005, Popper (2005) reported that audiograms of around 50 fishes were available in the literature. Seven years later, Ladich & Fay (2012), in their review on Auditory Evoked Potential (AEP), a physiological method to build audiograms, reported that by that time around a "100 papers on more than 100 fishes have been published".

Strict non-specialists fish (lacking air cavities) are able to detect sounds ranging between 1 and 500 Hz. On the other hand hearing specialists can detect sound ranging between 1 and 2000 Hz (Coombs & Montgomery 1999; Popper & Fay 1999).

The most recent review of hearing capabilities of fish (Ladich & Fay 2012) show that most of the experiment currently conducted on fish and their hearing capabilities are focused on adult fishes, rarely considering neither ultrasound (>20kHz) or infrasound (<20Hz) and mainly targeting hearing specialists.

It is very important to consider hearing capabilities at all stages of a fish life. The auditory system develops in the early and has been shown to play a role in several aspects of fish behaviour, such as orientation and settlement of larval stages (Tolimieri et al. 2000, 2002).

The effects of sound on fish can be observed at different stages of the life cycle of fish. Therefore, this statement raises new questions in terms of management of anthropogenic sound with a need to consider not only the adult fish but also its juveniles forms.

Infrasound systems have been deployed as a fish barrier on Atlantic salmon (*Salmo salar*) smolts (Knudsen et al. 1994) and Silver eel (Sand et al. 2000) to prevent them from entering hazardous areas. Nevertheless, the effects of such systems on other surrounding species still have to be investigated in order to preserve the ecosystem integrity and to be sure that beneficial effects on

one species are not detrimental to others. The same thing applies for ultrasonic signals that have been, for example, used as deterrent for shad, herring and other species (Popper & Carlson 1998).

2.3 **Underwater acoustics & Physics of sound**

In this project the main focus is on the capacity of animals to receive and utilize sound underwater. In order to design appropriate experiments and better understand how fish deal with sounds it is necessary to get a good background on general physics and underwater acoustics.

Sound is a form of mechanical energy transmitted by the periodic compression and expansion permitted by the elasticity of the medium (Simmonds & MacLennan 2008; Johansson 2011). In water, sound energy propagates in term of particles motion generating a travelling pressure wave oscillating and often represented as a sinusoid with changes in amplitude around a baseline (Figure 2.5).

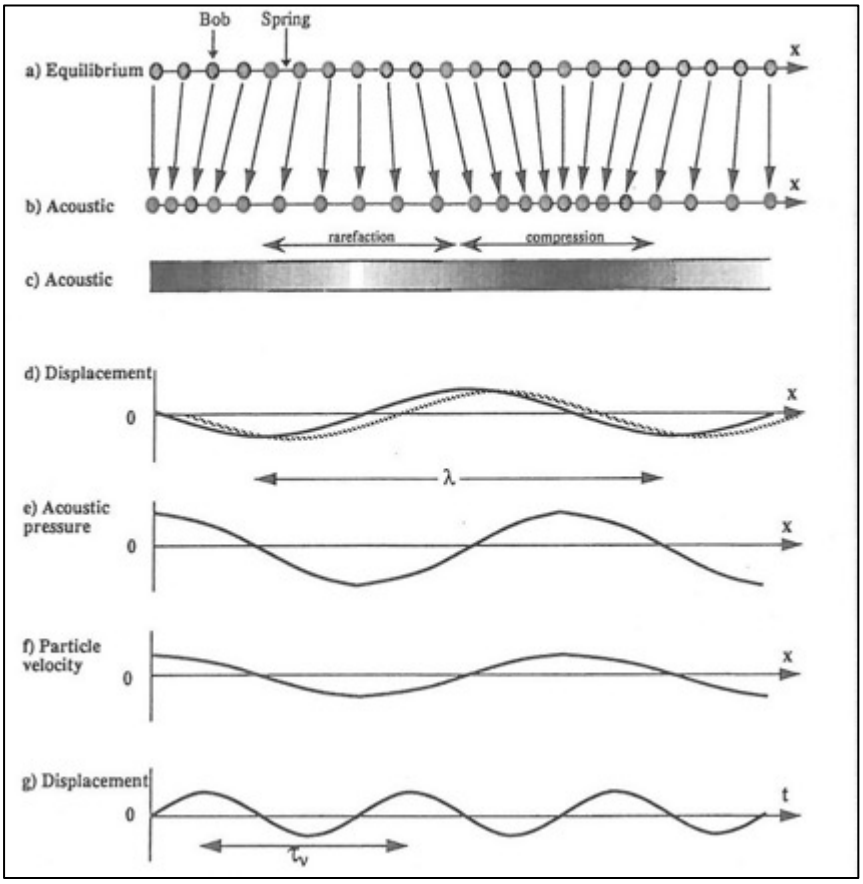


Figure 2.5 - Illustration of a propagating sound wave with the example of springs and bobs: (Diagrams and legends from Leighton 2012)

The frequency (*f*) of the sound wave corresponds to the number of cycles per second observed at a fixed point and is expressed in Hertz (Hz) and the duration of one cycle is called the period (*T*), measured in seconds and linked to the frequency by the equation: $T=1/f$

The wavelength of sound (λ), is the spatial period of the wave, that means the distance (expressed in meters) covered by a full cycle. This can be described by the expressions $\lambda=c/f$ or $\lambda=cT$ where *c* is the speed of sound propagation. In water the relative speed of sound usually ranges between 1450 and 1550 ms^{-1} (compared to 343 ms^{-1} in air) and varies depending of temperature, depth and salinity.

The acoustic pressure (the sound pressure level = SPL) is expressed in dB (decibels) based on a logarithmic scale to accommodate the high range of amplitudes. By definition, the decibel corresponds to ten times the base-10 logarithm of the ratio of two powers (P_1 and P_2)

SPL = 20log(P_1/P_2)

To obtain the absolute pressure in dB, a reference level (P_{ref}) is necessary. This value corresponds to 1 µPa. The SPL is therefore expressed in "dB re 1 µPa".

Therefore, the SPL equation becomes:

SPL = 20log(P/P_{ref})

It is important to notice that the reference pressure for air is 20 µPa and 1 µPa for water meaning that these two cannot be directly compared (Finfer et al. 2008; Simmonds & MacLennan 2008).

As mentioned earlier, fish are sensitive to the particle motion component of sound that can be described as the local oscillation of the molecules with the rate of one particle also called particle velocity (*v*) is measured in m/s (particle acceleration is in m/s^2). The expression p=v*Z* describes the relation between the particle velocity (*v*) and the pressure (*p*) in a free field (i.e. away from reflecting boundaries). In that same equation *Z* is the value of the specific acoustic impedance.

2.4 **Behaviour and Physiology of stress**

Stress can be defined as a state of threatened homeostasis that is re-established by a complex suite of adaptive responses (Chrousos 1998; Barton 2002). The threatening factors or more commonly called stressors can have different intensities more severe or long-lasting, resulting in different physiological responses that can be totally adapted to the stressor intensity or on the

other hand completely maladaptive. In the last case, fish wellbeing and health can be severely impacted and this state can be so called “distress” (Selye 1974; Barton & Iwama 1991).

This can be associated with the model built by Selye (1974) of the General Adaptation Syndrome (GAS) where an organism goes through three stages of responses because of stress exposure. The first stage is an alarm stage where the organism detects and recognizes the stimulus as an homeostasic threat. Then, the second stage also called “resistance” when the organism is collecting its resources to maintain its homeostasis. Finally, a third stage qualified of “exhaustion” where even with the stress response and mobilization of resources the organism cannot cope with the disturbance and re-adjust its homeostasic level.

Nowadays, and from a physiological point on view, responses of fish to environmental stressors have been split into primary and secondary physiological responses (Barton & Iwama 1991; Galhardo & Oliveira 2009).

The first physiological response, involves the initial neuroendocrine responses with the release of catecholamines from chromaffin tissues but also the release of corticosteroid hormones into the general circulation due to the stimulation of the hypothalamic-pituitary-interrenal axis (HPI) (Reid & al. 1998; Barton 2002).

The second physiological response, leads to physiological adjustments such as respiration, metabolism, hydromineral balance, immune function and cellular responses, due to changes in plasma and tissue ions, levels of metabolite, release of heat-shock proteins (known as stress proteins) (Pickering 1981: Mommsen et al. 1999)

Barton (2002) also suggests that a tertiary response can be considered even if less physiological but still correlated to stress levels and physiological changes and affecting the general performances of the animal like growth, resistance to disease, behaviour and more dramatically survival (Wedemeyer et al. 1990).

Interestingly, even if this mechanism of stress responses has been studied and integrated into the literature for decades, investigations on stress in fish have been mainly focused on teleost fishes (Barton 2002; Pankhurst 2011), specifics factors like temperature, water quality, pH, but rarely on the effects of sound.

Finally, to make the link with behaviour and the type of responses expected in this study, Cannon (1929) introduced the concept of “fight or flight” (also called acute-stress response). This concept describes the behavioural response of an individual facing a stressor/threat and is currently associated to the first stage of the GAS, described earlier. This is the type of response that would

be expected through this study, with fish being exposed to sound stimulus and either deciding to "fly" or "fight" the potential acoustic stressor. In the last decades, the concept of "fight or flight" response has been gradually replaced/renamed into two specific strategies of coping behaviour opposing on one side the proactive (fighters) individuals and on the other side the reactive ones ("flyers") (Koolhaas et al. 1999).

In this study, we offer to investigate the effects of sounds as a potential stressor, on behavioural responses of the non-teleost species, the river lamprey (*Lampetra fluviatilis*) but also in the European eel (*Anguilla anguilla*).

2.5 Sound pollution and mitigation techniques

In the Introduction of this thesis (Chapter 1), the general concern around anthropogenic acoustic pollution on fish environments and paradoxically the possibilities that sound offered as a behavioural system to protect fish at hazardous passage, were both presented. This section will jointly review, the present legislation and mitigation methods to reduce acoustics pollution but also the current knowledge on acoustics as a fish passage mitigation.

2.5.1 Acoustic pollution reduction

Several international agreements have been established primarily to monitor underwater sound pollution and also to limit its impacts on the aquatic life. In Europe, this includes for instance the Marine Strategy Framework Directive (MSFD) (Directive 2008), the Helsinki Commission (1988) (HELCOM) and the OSPAR Convention (1992). All these international agreements aim to protect marine ecosystems and work from the common agreement that underwater noise is a serious environmental stressor. Nevertheless, they also share a common limitation, which is the lack of specific guidelines and regulations that relate to individual countries. Some explicit guidelines have only been produced for pile driving and seismic surveys but are only addressing the potential impacts of such activities on marine mammals (Erbe 2013). Common practices, for instance, include a selection of an adapted source (minimal practical power for seismic surveys) or the use of alternative foundation techniques instead of pile driving (offshore renewables) (e.g. Byrne et al. 2002). Operators of seismic or pile driving activities are also required to work out of breeding seasons (marine mammals) and also to perform a "ramp-up" start when running their activities (Compton et al. 2008). Another requirement is to send a "warning signal" to allow animals to retreat outside of the exposure/mitigation area. In the United Kingdom, the Joint Nature Conservation Committee (JNCC), has set the minimal mitigation area to a 500 m (radius) circle

around the source during pile driving activities (JNCC 2010). Finally, with the increasing body of research around developing new methods or techniques to reduce the impacts of such practices, some have actually emerged and become part of the best practice guidelines in Europe. For instance, as introduced previously (Chapter 1), the use of a bubble curtain as a mean to scatter sound coming from a pile has been well-studied (Nehls et al. 2016; Tsouvalas & Metrkine 2016, Dähne et al. 2017) and has become a requirement during impact piling activities in almost all European countries (Erbe 2013). However, even if some forms of anthropogenic acoustic pollution have been mostly mitigated, there is still a need for further research and development in this area especially regarding noise emission from powerboat engines.

2.5.2 **Sound as mitigation**

As described in the previous section, sounds propagate well in water and fish are capable of using hearing to assay their environments (Fay & Popper 2000). Therefore, many authors have suggested and investigated the use of acoustics stimuli as a means to alter the behaviour of fish (Chapter 1). The ultimate goal is to develop a guiding system based on fish behavioural response to sound and capable of direct fish away from hazardous areas (e.g. water intakes, turbines, screens). Compared to most mammals, fish have their best hearing sensitivity located in the low-frequency ranges generally below a few kHz (Schilt 2007).

Consequently, a few studies have focused their investigations on the response of fish to (very) low-frequency sounds (generally <20 Hz = infrasound) and have reported some positive results. Amaral et al. (2001), observed strong avoidance of three different infrasound frequencies by caged northern pikeminnow (Ptychoceilus oregonensis). The authors suggested that these frequencies could be used as a repellent to this specific species. Mueller et al. (2001) successfully tested avoidance response to infrasound, this time on wild and hatchery reared chinook salmon (*Oncorhynchus tshawytscha*). Finally, Sand et al. (2000), deployed an infrasound (11.8 Hz) source at European eel (*Anguilla anguilla*) trapping site and observed significant changes in trajectories of fish due to the sound stimulus. Nevertheless, possibly due to the cost of deploying a reliable infrasound source and the difficulty to predict sound propagation in a more natural environment such findings have not been further followed up.

Contrary to what was initially thought, some fish species are capable of hearing high frequency sound and even ultrasound (> 20kHz). One early study was conducted by Kynard & O'Leary (1990) who successfully created avoidance of 161.9 kHz acoustic field by American shad (*Alosa sapidissima*). Later, several studies have shown that the capability to hear ultrasound is shared by most members of the Alosinae (subfamily of the Clupeidae). Furthermore, most of the research

on alosine herrings reported their common avoidance response to ultrasonic stimulus (Dunning et al. 1992, Platcha & Popper 2003; Higgs et al. 2004).

Finally, a few studies have actually investigated sounds which are audible to humans (Schilt 2007). Of most relevance to this thesis, Patrick et al. (2001) have described an attractive effect of sound on American eel (*Anguilla rostrata*). As illustrated through the previous sections and Chapter 1, underwater bioacoustic is a novel science. Moreover, most of the work that has been carried out has often focused on the biological aspect; understanding how and what fish were capable of hearing. A few studies have also investigated the potential effect of sound as a deterrant device. Most of these researches has only been based in very confined environment (tanks) or directly in the field with sometimes a weak evaluation and control of the acoustic scenery. Furthermore, a lot of the bioacoustics work on the anthropogenic impacts on marine life has been mainly targeting mammals or very recurrent species.

This thesis offers a new approach, focusing on two sensitive species, the river lamprey and the European eel. A combination of both restricted (tank) and semi-restricted (flume) test environments will allow a good assessment of sound effects on fish with a good control of the acoustic environment. This research combines several aspects of classic bioacoustics studies by investigating: 1) the hearing capabilities of both species, 2) the influence of anthropogenic sounds on their behavior and 3) the potential use of sound as a behavioral guidance system.

3 Research methodology

3.1 Study species

3.1.1 European eel – Ecology & History

The European eel (*Anguilla anguilla*) (Figure 3.1), is one of the 19 species of the family Anguillidae that are catadromous fishes.

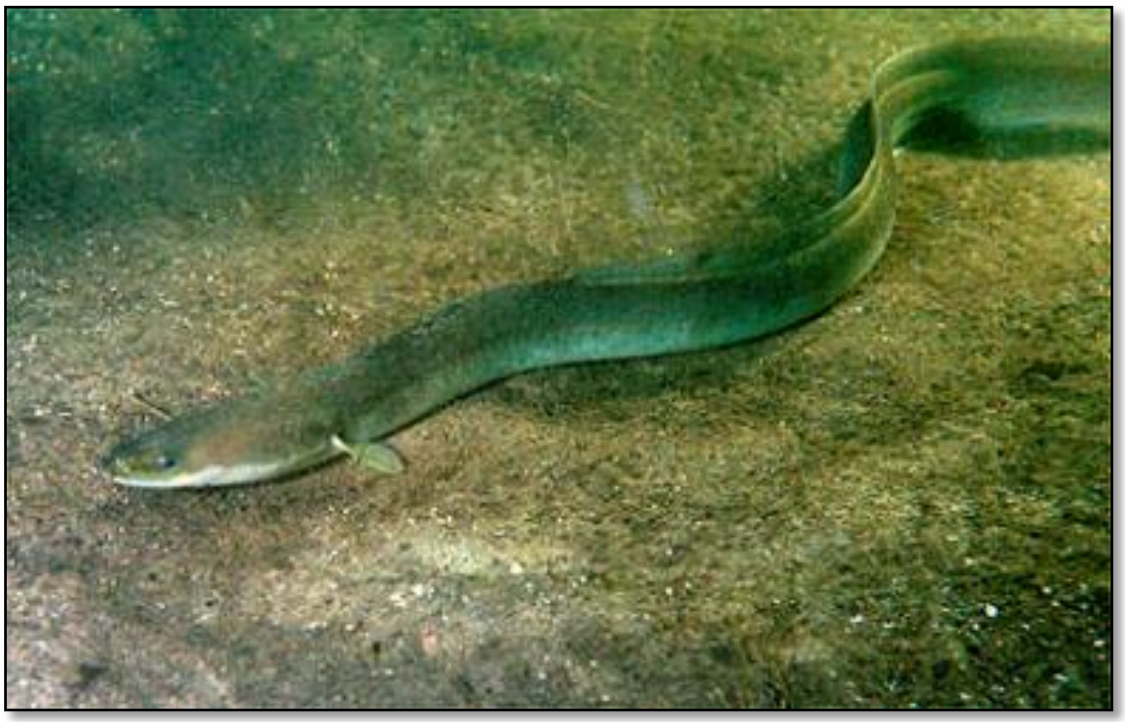


Figure 3.1- Picture of an adult silver stage European eel (*Anguilla Anguilla*).

Catadromous fishes spend a part of their life cycle (Figure 3.2) in freshwater and go to the ocean to spawn (Sargasso Sea). The young eel larvae also called Leptocephali are carried back to the European coast by the Golf Stream currents following a 7 to 11 months period and settle in estuaries while reaching the next stage of their life cycle, also called glass eel (Rochard & Elie 1994). Then the glass eel either stay in estuaries or migrate upstream to freshwater areas to grow up (yellow eel) and reach maturity (silver eel) entering in a long feeding period (6-12 years for males and 9-20 years for females) (Hureau et al. 1984).

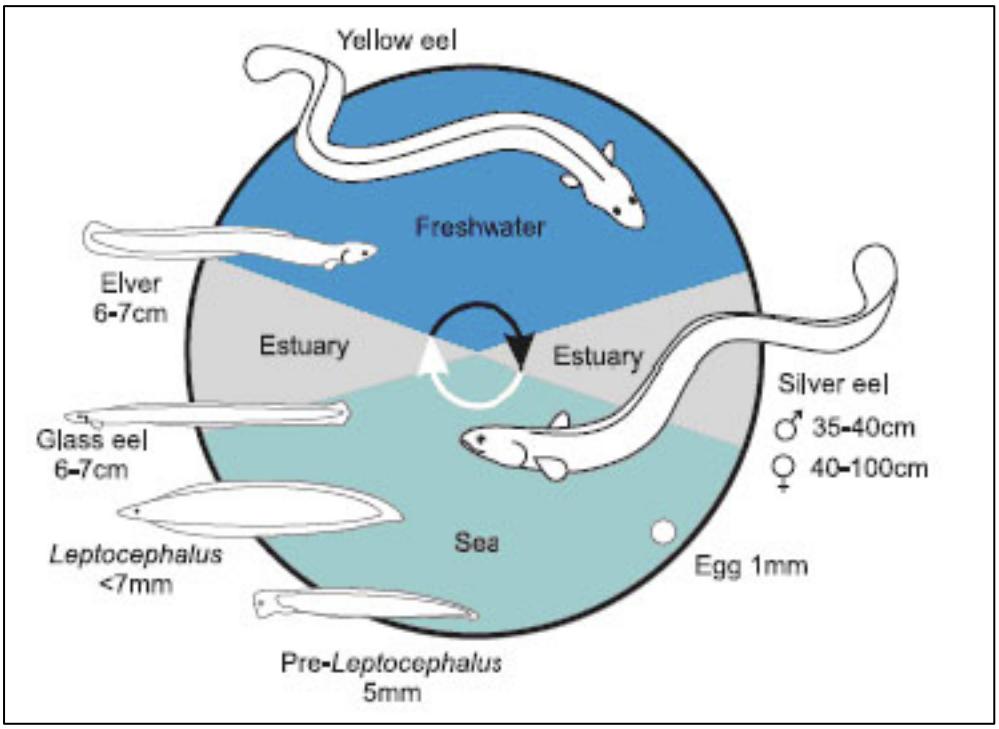


Figure 3.2 - Diagram of the life cycle of the European eel (from : http://www.cefas.defra.gov.uk/our-science/fisheries-information/eel/eel-lifecycle.aspx)

Nowadays European eel has been listed as a Critically endangered species by the IUCN (International Union for Conservation of Nature). Since the seventies, it appears that the number of eel reaching Europe has encountered an estimated 90% drop off. This decrease of the population could be caused by several factors like overfishing and illegal fishing (Belpaire et al. 2011), but also climate change (Knights 2003), the presence of power stations intakes, or also diseases and parasites outbreaks (Jakob et al., 2009; Geeraerts & Belpaire, 2010), and finally habitat destruction and pollution.

Eel are important commercial fish in Europe. For decades, they have been fished and exported all around the world, especially to Asia. It is also a cultural dish in some countries with for instance the jellied eel in the United Kingdom (UK), the Kabayaki in Japan (Prosek 2010) and also in other European countries where eel can be served smoked (e.g. Iceland, Norway, Sweden).

In the UK, due to this dramatic decrease in eel stocks, a management plan has been adopted (EC 1100/2007). The management plan aims to get at least a 40% of the potential biomass of silver eel emigrating each year if they were no anthropogenic influence in their life cycle (fishing, changes in water quality, barriers to migration, etc.).

3.1.2 **European eel – Sound**

From a bioacoustics point of view, eel can be classified as hearing non-specialists (or generalists). They possess a classic octavolateralis system (inner ear and lateral line) and a swim bladder but this last one is not connected to the inner ear by any additional features like bullae or Weberian ossicles. According to this short description, the frequency hearing range for the eel should be comprised between 100 Hz and 500Hz. Nevertheless, it has been suggested that eel would be capable to hear infrasound (Jerkø et al. 1989; Sand et al. 2000, 2002). However the literature treating about eel auditory abilities is not extensive and therefore, there is a need to further investigate on this topic.

3.1.3 **River lamprey – Ecology & History**

The river lamprey (*Lampetra fluviatilis*) (Figure 3.3) looks very much like the European eel. They share a similar body shape and locomotion. Nevertheless, these two species are evolutionary very different. The river lamprey is part of the family of the Petromyzontidae, which main caracteristic is a jawless mouth, and in the case of the lamprey, the mouth is replaced by a powerful sucker with some rasping teeth organized in several sets of disks.

Figure 3.3 – Picture of two adult river lamprey (*Lampetra fluviatilis*).

Petromyzontidae do not have any bones or vertebra and are considered has the closest living ancestors of vertebrates (Docker 2014). In fact, one of the closest class of fish to the lamprey is the Elasmobranchii (shark, chimera and ray) sharing also a couple of anatomical features with them. For instance, lamprey have a set of seven gills freely opening on the back of their eyes

(sharks and rays having five) without any operculum covering them (unlike eel which possess one set of gills with operculum). Similarly to the eel, lamprey have a migratory life cycle (Figure 3.4) with an upstream and a downstream migration. However, lamprey spawn in freshwater around March and their larvae (Ammocoete larvae) migrate downstream (15 to 30 days after spawning) until reaching an adult size and getting to the sea where they stay until reaching complete maturity. Lamprey are anadromous fishes (Figure 3.2), as opposed to eel which are catadromous. The young river lamprey feed in the estuaries, mainly on Atlantic herring (*Clupea harengus*), European sprat (*Sprattus sprattus*) or European flounders (*Platichthys flesus*) using they powerful sucker and their teeth to rasp important amount of flesh (Kelly & King, 2001). They are parasitic fish.

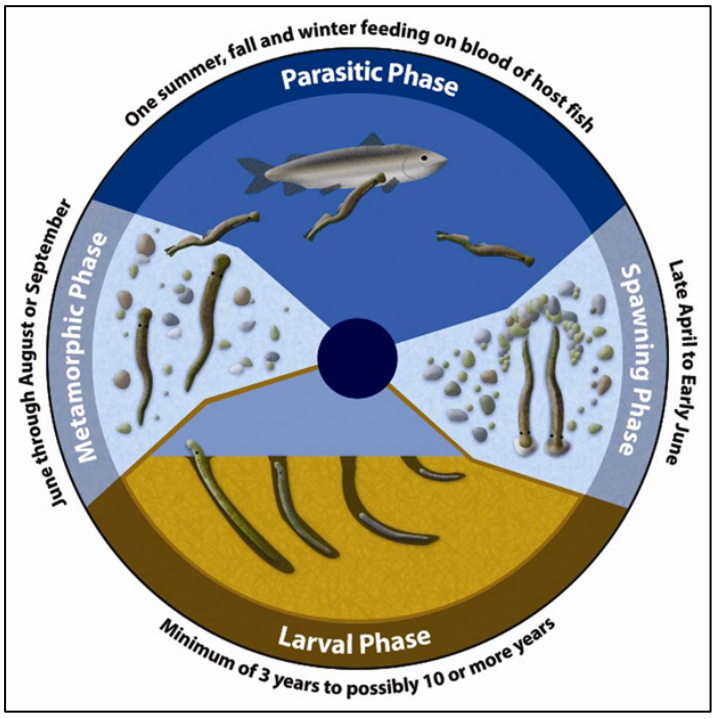


Figure 3.4 - Diagram of the life cycle of the River lamprey (Lampetra fluviatilis). From http://www.glfc.org/

River lamprey, as opposed to European eel are not currently an endangered species even if they were listed as “Near Threatened“ until 2008, by the IUCN due to a global reduction in populations.

Lamprey are commercial fish in a couple of countries in Europe, but in the United Kingdom there are no commercial fisheries so far. Nevertheless, their population can be impacted by several factors like pollution (Kelly & King 2001), engineering works and physical barriers like dams, weirs, etc. being obstacles to their migrations (Igoe et al. 2004). There is currently no specific management plan for river lamprey but the population is regularly monitored by delegated

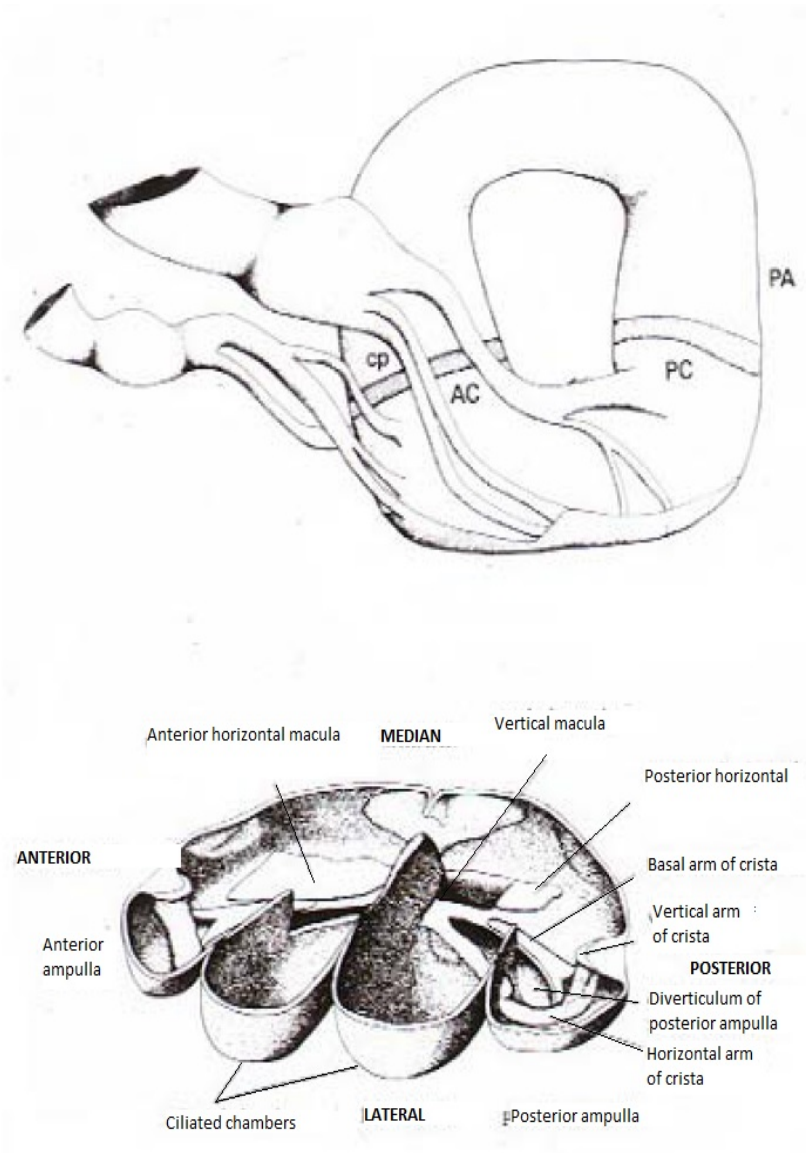
authorities. In the UK, these authorities include the Environment Agency, the Scottish and Northern Ireland Fisheries. They run regular surveys every 5 years in rivers designated as Special Area of Conservation (SAC) (Cowx et al. 2010).

3.1.4 River lamprey – Sound

The literature concerning lamprey and sound is not extensive. Lamprey, like eel can be considered as "hearing non-specialists" as they do not possess a swimming bladder. Therefore, lamprey should not be affected by sound pressure and would be no be capable of quantifying it. From an anatomical point of view, the lamprey inner ear is slightly different from the one of bony fishes. The inner ear of the lamprey (Figure 3.3) is formed of two semi-circular canals and a single epithelium or macula communis divided in three regions, the anterior, the middle and the posterior maculae (Osborne & Thornhill 1968: Fay & Popper 2000). The solid otolith of more taxonomically "evolved" fishes is replaced by a thick otoconial layer overlaying the same ciliary bundles and hair cells of the macula than in other fish species.

In a paper from 2005 untitled "A review of hearing by Sturgeon and Lamprey", Popper clearly stated at that time that there were "no studies to determine the responses of the ear to sound or whether lamprey respond to sound behaviourally" (Popper 2005)

With regards to sound, there is currently no work that has been done on the lateral line of the lamprey. A paper from Ayali et al. (2009), describes the potential implication of lateral line of the lamprey in the correction of their swimming behaviour. Basically, the lamprey lateral line appears to be able to detect the changes in surrounding particles motion generated by the animal and therefore get an idea of its own movements. This mechanism might be useful in improving or optimizing swimming movements (Ayali et al. 2009).



From Popper (2005):

"The ear consists of two semicircular canals and a single epithelium that makes up the otolithic end organ.

Top shows the complete ear with the anterior canal (ac) and posterior canal (pc) and their associate sensory epithelia, or cristae (ac, pc). The ear is innervated by the eighth cranial nerve, as it is in all other vertebrates.

Bottom shows a cross section of the ear with the different regions labelled. Note that the sensory epithelium is referred to as having three parts, but the significance of the three parts in terms of function is not known. One unique feature of the ear of lamprey is the presence of large "ciliated chambers" which contain numerous cells, each having many kinocilia, that appear to move with fluid-flows in the ear (see Popper & Hoxter 1987). The function of the ciliated chambers is not known. "

Figure 3.5 - Diagram and captions from Popper (2005) representing the inner ear of the lamprey.

3.2 Fish collection and husbandry

European silver eel (*Anguilla Anguilla*) were collected in during the month of November from the River Stour, with the agreement of the Environment Agency, by a third part located in Longham (50°46'32.2"N 1°54'38.3"W) (Figure 3.4).

Figure 3.6 - Collection site for the European eel – location of the eel rack circled in red.

The collected fishes were brought back by car, stored in highly oxygenated containers, to Chilworth Research Park (50°57'44.6"N 1°25'27.6"W). Fishes were relocated in a 4000 litres holding tank and four external tanks of 1000 litre (Figure 3.7). The 4000 litres tank was equipped with three recirculating biological filters and their pumps. Each of the 1000 litre tank was equipped with individual gravity fed biological and mechanical filtration system and inline ultraviolet filter. Some air stones and their pumps were also added to the system to improve oxygen levels.

Water quality and temperature were checked on a daily basis and water changed regularly to maintain optimal conditions. Also, fish have been treated with a 10 days course of Eradick© (NT Labs) prior to experiment in order to prevent any breakout of infectious diseases.

Figure 3.7 – External holding tanks (4 x 1000 litre) – ICER facility, University of Southampton.

3.3 **Fish PIT Tagging**

Before being used in any experimental setup, fish morphometrics data (e.g. weight, body length) were collected (Figure 3.8a). To ease fish handling and measurements, fish were sedated using either 2-phenoxy-1-ethanol or a preparation of clove oil diluted in ethanol.

For the purpose of some experiments, fish were also tagged with PIT (Passive integrative transponder) tags. European eel were generally tagged with 24 mm tags, while river lamprey, due to their smaller size, were tagged with 12 mm tags.

Figure 3.8a & 3.8b – Measuring setup and Sterilisation process for surgical instruments and 12 mm PIT tags.

Surgical tools and Tags (Figure 3.6b) were preserved in a bath of 99% ethanol to be sterilised and subsequently rinsed with sterile water in order to not harm the fish when cutting through its skin.

Figure 3.9 - Surgery table with sterile drapes and containers filled with anaesthetic.

Fish were anaesthetised using a 1/5 L concentration of 2-phenoxy-1-ethanol dissolved in tap water. Fish were dump individually in a container and left for a couple of minutes until reaching deep anaesthesia (verified by turning the fish upside down). Containers were closed with a lead and a heavy weight to prevent any escape from the fish (Figure 3.9).

When anaesthetised the fish was collected and placed on a piece of foam covered with surgical drapes. A small incision was performed mid-ventrally in the posterior quarter of the peritoneal cavity of the fish (Figure 3.10a, 3.10b).

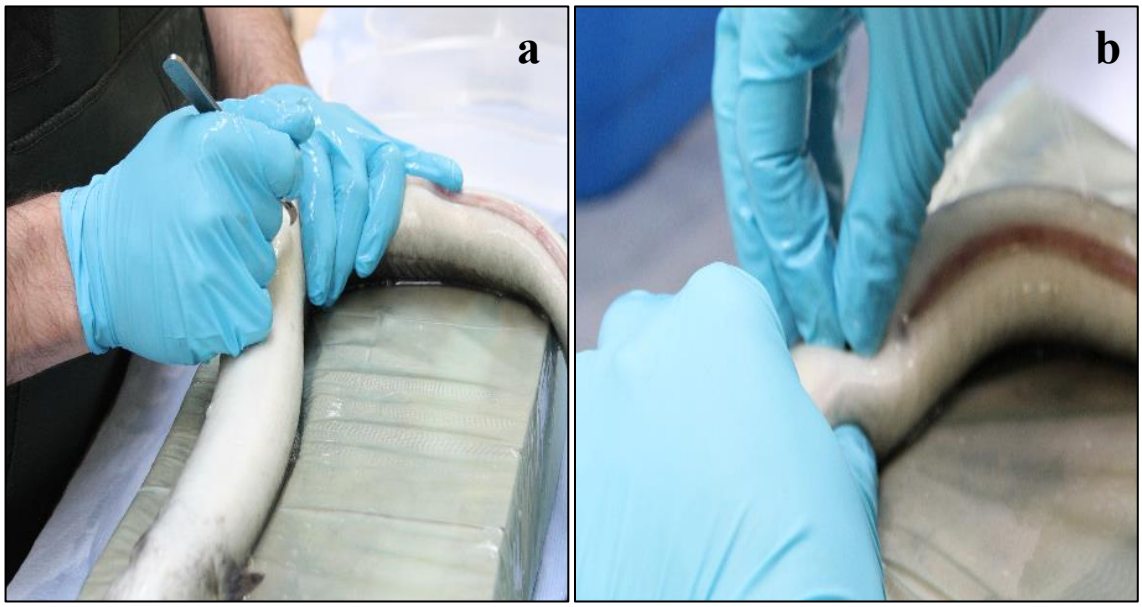


Figure 3.10a & 3.10b - Incision & insertion of a 24 mm PIT tag in the body cavity of a European eel.

The tag was then pushed inside the incision hole and gently rubbed through the skin to make sure it will not come out. Medical cyanoacrylate glue was used to seal the incision, preventing any inflammatory response and granting higher survival rates (Baras 1998).

After the surgery, fish were released in a large highly oxygenated container, to recover. Some "Banish" solution (Bermuda©) was also added in the recovery container in order to prevent any infection and enhance the healing.

At the end of the tagging session and after all the fish have recovered from anaesthesia they were returned to their holding tanks and monitored for a couple of days. Several studies have shown that fish PIT-tagging did not affect their swimming performance or impact on their behaviour (Newby et al. 2007; Ficke et al. 2012, Hirt-Chabbert & Young 2012).

3.4 **Measuring and representing hearing in fish**

Underwater Bioacoustics is a novel science, which mainly triggered a real interest about 50 years ago. As previously explained it is a combination of two sciences, Biology and Acoustics, and the general focus is about understanding the mechanisms and capabilities of hearing in species that live (partially or totally) underwater.

One of the most common tools to represent hearing capabilities in Bioacoustics is the audiogram. An audiogram is a graphical representation of the hearing threshold of a single subject, for example a human person being diagnosed, or more generally to an entire species when measurements have been replicated on multiple subjects.

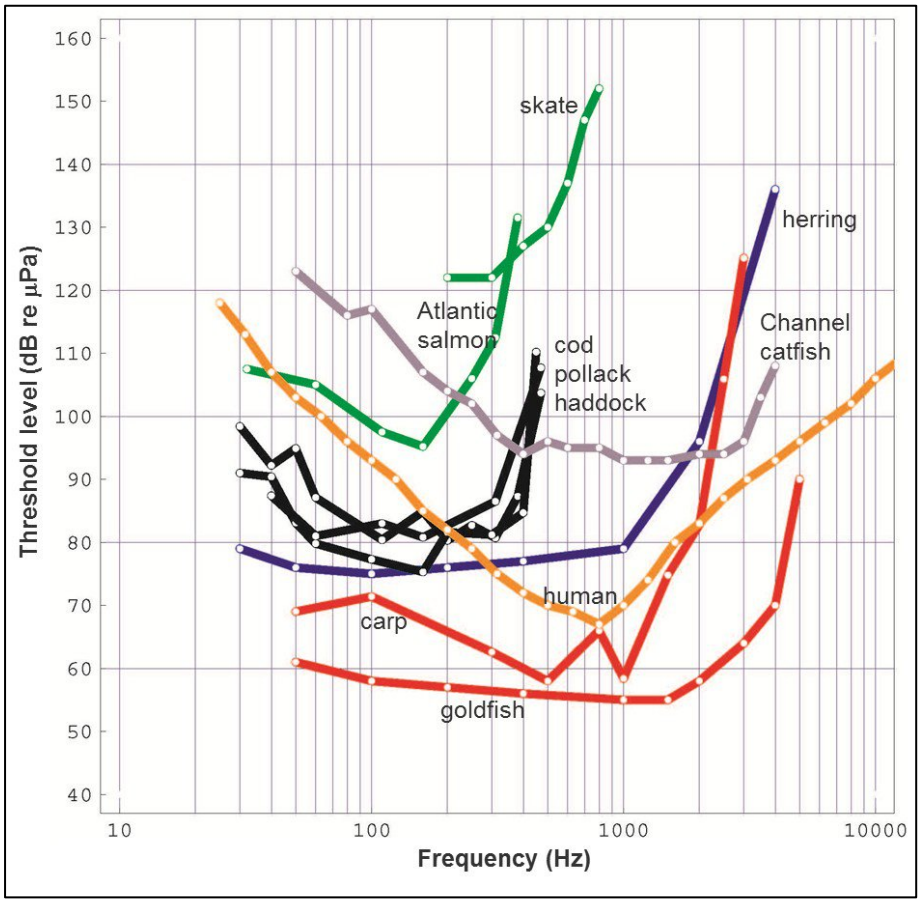


Figure 3.11 - Audiograms of several species of fish and humans (from: http://www.geoexpro.com/articles/2011/03/marine-seismic-sources-part-viii-fish-hear-a-great-deal).

Figure 3.11 is an example of a combination of audiograms from several fish species that have been compared to humans. On the X axis the sound frequencies are represented in Hertz (Hz) and on the Y axis the sound level (Sound Pressure Level) in Decibels (dBs). Following the example of the goldfish (*Carassius auratus*), the hearing range of the species is between 50Hz at 60 dBs and 5 kHz at 90dBs. These values correspond to the extremities of the goldfish curve. The curve represents the hearing threshold; it means the smallest level for which goldfish can hear a specific frequency, nothing under this curve can be "heard" by the species.

Currently, there are more than a 100 audiograms that have been published for fish over about 33000 known species. Audiograms can be obtained following two different methods, the first one being based on the behaviour of the fish and consisting in recording any potential behaviour

triggered by a sound stimulus. The second method is based on electrophysiology and is very similar to the method generally used on humans also called AEP (Auditory Evoked Potential) or ABR (Auditory Brainstem Response). This last method consists in exposing a subject to sound stimuli and to record the changes in brain/nerves activity associated to the stimuli. Chapter 4 of this thesis uses the first method based on behaviour to investigate silver eel and river lamprey hearing capabilities. Audiograms of both species have been produced as a result of this experiment.

It was initially planned to use the ABR method and compare its outcome with the behavioural method in a dedicated Chapter. Unfortunately, due to non-significant results during the ABR trials this Chapter has been move to the Appendix Section.

3.5 **Video & Data analysis**

Both Chapters 5 and 6 experiments have been undertaken in the outdoor flume at the International Centre for Ecohydraulics Research flume facility part of the Southampton University and located at Chilworth, UK. Trials for these two setups were carried out during night times when the fish were the most active (Kelly & King 2001; Calles et al. 2010).

Trials for the Behaviour Audiogram and ABR experiment detailed in Chapter 4 and in the Appendix were carried out during daytime at Highfield Campus.

For all these experiments, fish activity was video recorded using different numbers of CCTV infrared cameras capable to record under low/no light conditions. Also, when the in-built infrared lights from the cameras were not sufficient, additional infra-red lights were added to the setup. Both river lamprey and European eel share the same spectral sensitivity to humans and therefore are not capable of detecting infrared lights (respectively, Andjus et al. 1998 and Hope et al. 1998).

Sony 500TVL underwater cameras in combination to Sony Effio 580TVL in-air cameras were used in Chapter 4 experiment to monitor fish reaction to sound. The Sony Effio 580TVL were used in Chapters 5 and 6 experiments. All the cameras were connected to a PC computer capable of running up to 8 cameras at the same time and running under Microsoft Windows 7©.

All the video recording, live monitoring, data backup and video analysis were realised using the NUUO Mainconsole and Playback © softwares.

3.6 Sound production

Sound stimuli for the different experiments and the mapping of the experimental areas were produced using a basic setup (Figure 3.10). A program written under MatLab 2014 © was used to generate the stimuli and setting up all its parameters (periodicity, frequencies, sound level, etc.). The stimuli were not only used for the experiment but also to perform the initial sound calibration and measurements during the mapping of the sound field. A laptop (Dell © Latitude E6430) was connected to a Data Acquisition Box – "DAQ" (National Instruments © USB-6251) that was used as interface between the laptop and the recording or sound producing elements of the setup. One of the DAQ outputs was then connected to an amplifier (SkyTronic © Mini AV Digital Surround Amplifier 103.100) that was itself connected to at least one underwater speaker (Electro Voice © UW30).

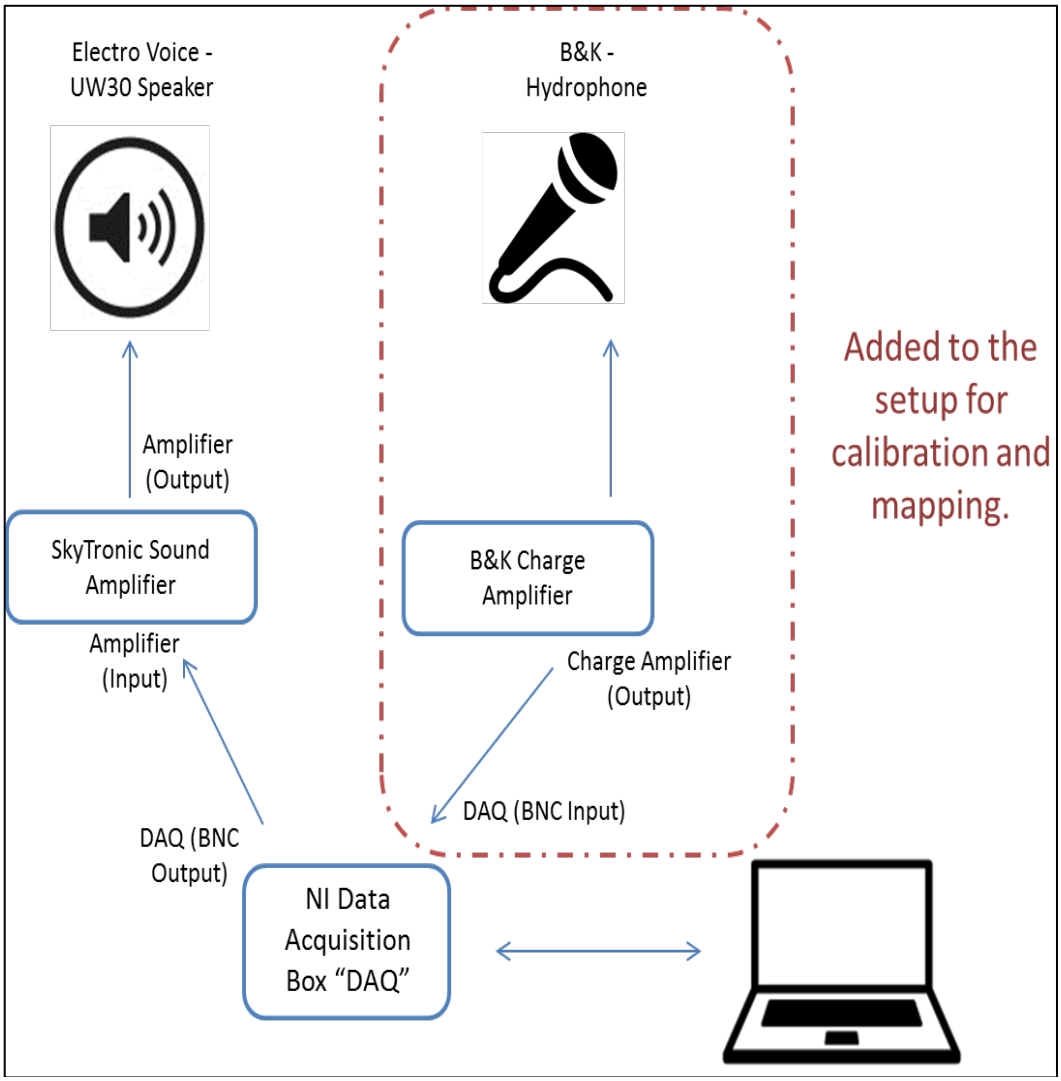


Figure 3.12 - Diagram of the sound setup for generating and recording sound during experiments.

3.7 Acoustics and Hydraulics Mapping

To measure water velocity in the outdoor flume under the experimental conditions, two methods were used.

An electromagnetic flow meter (Valeport, 801 –flat) calibrated to record over 10 seconds and averaging the values to the mean velocity was used in the "Maze Experiment" (Chapter 5) to get a general idea of the flow velocity within the maze.

In the "Screen Experiment", a more detailed mapping of the velocity was undertaken using a Nortek Vectrino+ Acoustic Doppler Velocimeter (ADV). Sampling rate was set to an optimal frequency of 50Hz with a sampling period of 30 seconds, giving about 1500 measurements for each of the four coordinates.

Sound measurements for both Maze and Screen Experiment have been obtained by re-using the same setup (Figure 3.10) previously described in the "Sound generation" section and adding a hydrophone (Brüel & Kjær © 8105 or 8103 hydrophones) combined with a modified code running under MatLab. The program code was adjusted to play the sound through the underwater speaker while simultaneously recording it with the hydrophone. Except for the "Behaviour Audiogram" experiment the code was set to play consecutively 1 second single tones for each of the frequency of interest and also a 1 sec broadband noise that will be the stimulus used for these two experiments. Values as recorded by the hydrophone on MatLab were then stored on a Microsoft Excel © spreadsheet to be later converted into a map using R Studio©.

3.8 Statistical Analysis – Mixed Effect Model

The data analysis was also performed using the statistical software R Studio©. In the following Chapters 4 and 5, a Mixed-Effect model was used to analyse the data. The choice of this specific type of analysis came from the fact that in both chapters' experiments, one or more variables (behavioural responses) were potentially influenced by the separate or cumulative action of Random or Fixed Factors.

Fixed Factors, are generally constant, controlled parameters while Random Factors are more uncontrolled. The distinction between both of them can be difficult and is sometimes more obvious when illustrated by an example (Crawley 2012) (see. Table 3.1).

Fixed effects	Random effects
Drug administered or not	Genotype
Insecticide sprayed or not	Brood
Nutrient added or not	Block within a field
One country versus another	Split plot within a plot
Male or female	History of development
Upland or lowland	Household
Wet versus dry	Individuals with repeated measures
Light versus shade	Family
One age versus another	Parent

Table 3.1 - Table extracted from the R Book (Crawley 2012) showing a list of examples for Fixed and Random Effects/Factors.

In this study, through different experimental conditions (treatments), behaviour of eel and lamprey were collected. The behavioural response of fish corresponded to the measured variable and, the type of treatment, for instance “With” or “Without an acoustic stimulus” was the Fixed Factor. The experiments undertaken in both Chapters involved multiple tagged test subjects/fish with potentially different behavioural responses (due to genetics, life history, etc.), This constituted a Random Factors, the factor “Individual” for instance.

The use of this statistical method allowed to assess how much the variable (behavioural response) differed between the individuals (in this specific example). In addition, due to the experimental design, trials were undertaken over several days. This was also another Random Factor, the factor “Day”. Because from one day to another, some parameters could not be controlled (e.g. weather, moon phase) the impacts of these parameters could be assessed by including this factor in the model.

How the model works is very similar to running a classic ANOVA (ANalysis Of Variance) or General Linear Model (GLM). In fact a Mixed- Effect model is a particular case of GLM. A set of data for a specific variable is analysed according to different factors. The more factors you add into your model, the more complex it is to run. According to the software used, the display of the data can be different but generally some key values are conserved across the different outputs.

Among them the p-value is probably the most used as it is considered to be the reference number to validate statistical results (show the significance of the results). In this specific case, the p-value allowed to validate or reject the null hypothesis that the selected Fixed Factors had no effect on the variable/ behavioural response. The threshold of the p-value is set to 0.05. When the p-value

is higher than 0.05 the null hypothesis is validated meaning in that specific case that the Fixed Factor did not have any effect on the variable variation (behavioural response). On the other hand a p-value lower than 0.05 indicates the rejection of the null hypothesis and the fact that the variation of the variable (behavioural response) is affected by the Fixed Factor.

When running the model, the p-values are given for every Fixed Factors that were input into the model; if 7 factors were input, the outcome will display 7 p-values.

To obtain the final model, it is common practice to first run a complex model with all the tested factors, then to gradually remove the terms/factors that did not return a significant p-value (<0.05). In the present study, final models were built in the same way, preserving the key factors of interest (significant or not) (e.g. Sound On Vs Sound Off) and discarding the less relevant ones (e.g. interactions) when not significant at all (see. Table 4.3 – Lampreys).

CHAPTER 4

4 River lamprey and European eel behaviour-based audiograms

4.1 Summary

Hearing capabilities of anguilliform fish species including the threaten European eel (*Anguilla anguilla*) and the declining river lamprey (*Lampetra fluviatilis*) are poorly known. With a global increase in anthropogenic underwater sounds and a concern by the scientific community, there is a need to investigate the impacts of such sounds on fish species. A series of experimental trials was conducted on European eel and river lamprey in a confine environment (two separate tanks). Fish were tested in pair (9 pairs per species), with one fish exposed to a specific sound frequency which intensity was gradually increased, and the other fish placed in a control tank. The frequency of the trial was randomly selected from a series of 9 defined frequencies including 60, 80, 100, 200, 400, 800 ,1000, 2000 Hz and produced by an underwater speaker located at the surface of the tank and facing down to the bottom of the tank. A total of 18 fish from each species were tested once with every frequency. Fish response to sound were monitored and recorded using a combination of video cameras. An ethogram common to both species was built from the observed behaviour. Based on their behavioural responses to sound, audiograms were produced for both species. A Mixed-Effect model was used to analyse the variation in behaviours due to frequencies and additional factors (Weight, Length, trials duration) but also to assess the variability of the response between individuals. European eel did not show much difference in their behavioural response to sound compare to control treatments. On the other hand, river lamprey showed a significant reduction in their response during the ensonified treatments. The audiograms indicate that both species best hearing threshold is located around low frequencies (≤ 100 Hz). Nevertheless, due to high variability in the behavioural response among the individuals (confirmed by the Mixed-Effect Model), these results have to be considered with care. Further researches, potentially based on electrophysiological methods (ABR/AEP) could represent a potential tool to support these findings.

4.2 Introduction

For the past decades, there has been a global growing concern about the impacts of anthropogenic sounds in the marine and freshwater environment, with more and more studies published on that topic (Stocker 2002; Wahlberg & Westerberg 2003; Popper & Hastings 2009; Slabbekoorn et al. 2010; Dahl et al. 2015). However, the majority of researches tends to focus on marine mammals and only a small portion consider fish and even fewer freshwater fish species.

The implementation of European regulations on freshwater environments (e.g. the Water Framework Directive, see: Directive 2000), and the need to meet International standards in terms of sustainable energy sources like hydropower stations or offshore windfarms, means there is pressing concern to understand what the potential impacts of such structures on the environment could be. Very little is known about the impacts of the acoustic field associated with the implementation and functioning of hydropower and other structures in the freshwater environment.

Over recent decades, two commercially important migratory species, the European eel (*Anguilla anguilla*) and the river lamprey (*Lampetra fluviatilis*) suffered from a severe drop in their populations (Kelly & King 2001; EIFAC 2006; Masters et al. 2006; Aalto et al. 2015; Bevacqua et al. 2015). Several contributory factors were proposed including pollution, climate change and over fishing. River industrialisation with the deployment of infrastructures is also recognise as a critical factor of this decline. The implementation of structures like dams and weirs result in habitat fragmentation and creates barriers to these species migration (Calles et al. 2010).

On a different approach, the use of sound as a mean to control fish behaviour, mainly as a deterrent, has been a focus of recent activities. Ultimately, sound could be used as a guiding system during fish migration to prevent them from approaching hazardous manmade structures (e.g. dams, water intakes, screens). Of great interest has been the use of ultrasounds, in particular their deterrent properties towards clupeid (Popper et al. 2005) and salmonid fish (Knudsen et al. 1997; Bui et al. 2013). Furthermore, infrasounds have also been tested on the same species (Knudsen et al. 1994, 1997) with some studies also investigating on the European eel (Sand et al. 2000, 2002).

Nevertheless, the development of such guiding or deterring systems along with appropriate regulations on infrastructures requires some fundamental knowledge on fish hearing capabilities. There have been a few studies on hearing capabilities in eel with especially the research undertaken by Jerkø et al. (1989) that produced the only available audiogram of European eel.

However, despite the previously mentioned studies from Sand et al. (2000, 2002), there has been no further investigation or development on this topic regarding eel. On the other hand, there is currently no work published on the hearing capabilities of river lamprey with the exception of a review by Popper (2005). In this paper detailing the anatomy of their inner ear the author emphasizes the need of researches looking at the behavioural response of lamprey to acoustics.

The aim of this study was to consider some of the fundamentals of bioacoustics and behavioural studies by investigating the possible link between sounds and behavioural response. This was achieved by exposing fish to basic acoustic stimuli, similar to the ones used in human hearing testing (use of a range of single frequency tones). The main goal of this research was to assess the hearing capabilities of European eel and river lamprey to better understand what impacts anthropogenic sound can have on them. To achieve this one goal, the experimental approach consisted in recording fish behavioural response to sound in a controlled environment (holding tanks), in order to build an audiogram. Audiograms of fish are more commonly constructed using an electrophysiological technique (ABR/AEP – Auditory Brainstem Response/Auditory Evoked Potential) but this requires one to immobilise the fish and does not provide any details on the behavioural response of fish, despite what it might tell us about the hearing capabilities of the subject (Ladich & Fay 2013). This research used a different approach to conventional sound and behaviour studies. To assess the hearing capabilities of a species, these traditional studies either use a pre-experimental conditioned behavioural response (usually feeding and cardiac response) discarding any potential natural behaviour (Fish et al. 1972; Casper et al. 2003; Kojima et al. 2010; Ladich & Fay 2013), or use sounds that fish were assumed to hear and expect an anticipated behavioural response (Maes et al. 2004). Here the intention is to (1) assess the behavioural repertoire of these fish and (2) to build the audiogram based on the occurrence of these behaviours in presence or absence of sound in a confined environment. A statistical model (Mixed Effect model) is used to analyse the changes in behavioural response in presence or absence of sound and to associate these changes to specific test frequencies. The model also allowed to quantify the degree of variation of the behavioural response to sound per individual.

4.3 Materials and Methods

4.3.1 Fish collection and maintenance

European eel (N=18, Total Length: 534–666 mm, mean = 597,11 mm, S.D. = 40.14; mass: 267 - 573 g, mean = 388.4 g, S.D. = 92.89) were caught during their seaward migration using a fixed eel trap on the River Stour near Longham, United Kingdom (50°46'31.6"N 1°54'38.1"W), in December

2013. Migrating adult river lamprey (N=18, Total Length: 317– 379 mm, median = 367 mm, IQR = 18.25; mass: 48- 89 g, median = 76 g, IQR = 14.25) were trapped in the River Ouse (53° 53’ 26.2”N, 1° 5’ 36.8”W) by a commercial fisherman in December 2013. Fish were transported to the International Centre for Ecohydraulics Research Facility, University of Southampton (50° 57’42.6”N,, 1°25’26.9”W), in two large transportations tank filled with aerated river water. The fish were maintained in two separate 3000 L holding tanks equipped with individual filtration systems and separate air pumps. Water was monitored daily and maintained through regular water changes (50% weekly) using dechlorinated tap water (pH = 7.8, Nitrate: <40ppm). Mean water temperatures was 9.3 °C, S.D. = 1.7 °C for the lamprey and silver eel, and 12.1 °C, S.D. = 2.6 °C for the yellow eel.

Eel and lamprey were tagged with half-duplex Passive Integrated Transponder (PIT) tags. Tags of 24 mm were used on eel and 12 mm were used on river lamprey. Tags were inserted through a small mid-ventral incision in the posterior quarter of the peritoneal cavity of anaesthetized fish (2-Phenoxy-1-ethanol, 1 ml L^{-1}). Fish were also weighed and measured during the tagging procedure and at least a 48 hours recovery period was allowed before being used in an experiment.

18 eel were first brought to Highfield Campus, AB Woods Laboratory, using the same transportation tank as described above and stored in a 900 litres tank equipped with a recirculating filtration system. After running the trials with eel, the same procedure was repeated with the lamprey.

4.3.2 **Experimental setup**

For the purpose of the experiment, fish have been grouped in pairs of similar weight and length just in case these two parameters have an effect on sound perception. During a trial, each fish in the pair are allocated to one tank, the fish in one tank is exposed to sound and in the other tank no sound is replayed; this will be a control.

In this experiment nine frequencies (60, 80,100, 200, 300, 400, 800, 1000, 2000Hz) have been selected, based on the range of frequencies commonly used in audiograms experiments (Yan 2004; Ladich & Fay 2012).

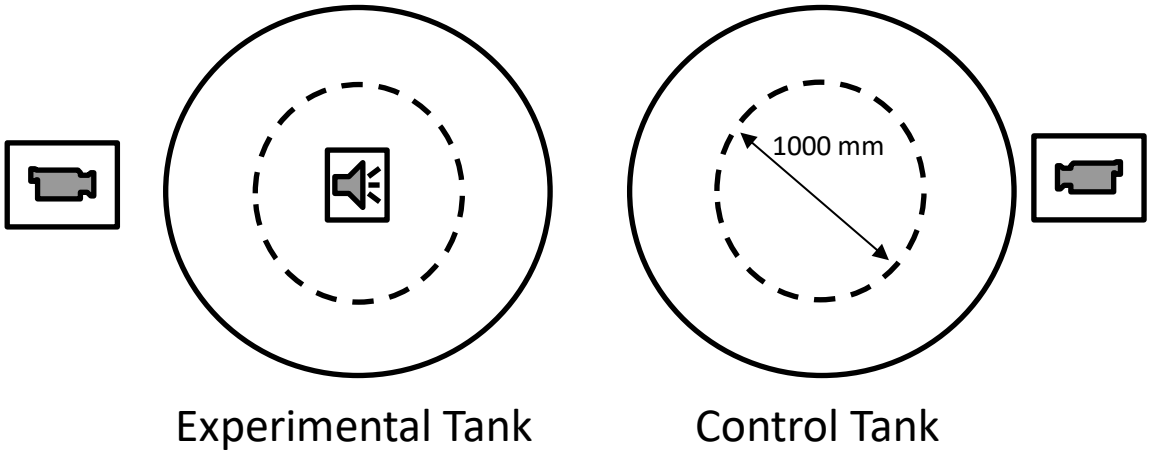


Figure 4.1 - Plan view representation of the experimental setup for the Behavioural audiogram experiment with cages represented by dashed lines. The underwater speaker was located in the centre of the cage at a distance of 42 cms from the bottom of the tank.

Fish were video-recorded using a combination of underwater and in-air cameras equipped with infrared lights. In order to eliminate any visual clues, each tank was covered with thick black material to block exterior lighting.

The available tanks being not identical, two circular cages (diameter equal to 1 meter) made of metal mesh were built and setup in each tank, providing an identical confinement for both fish and securing a recapture zone within the tanks in case any fish displayed an evasive behaviour.

Fish were introduced in the tank and left there for 30 minutes before the sound stimulus start. This provided an acclamation period.

A randomised schedule for each pair of fish was allocated. Every day for nine days, one specific fish from the pair was tested for one frequency, then a different frequency on the following day and so on. At the end of this schedule, the roles of the two fish were reversed and a further 9 days of testing was undertaken. Fish that were used in the Experimental tank on the first schedule became Control on the new round of experimentation and respectively, fish that were used in the Control tank became Experimental fish on the second round.

4.3.3 **Ethogram**

From the extraction of data from the recorded trials, a series of recurrent behaviours have been listed and used for the final analysis. The following behaviours, and the way they have been recorded, are described in the Table 4.1.

Table 4.1 - Ethogram used in the collection of behavioural data from European eel and river lamprey exposed or not to sound. The Ethogram was drawn up after watching the recordings as no Ethogram were available in the literature for these two species.

Name of the behaviour as recorded	**Description**	**Additional recorded data**
"Moving"	Described as the general swimming of the fish, involving a displacement of the individual in any direction. The head of the fish is used as a reference point and the behaviour is validated when the head of the fish moves in a straight line movement from its initial point to another point within a head length distance.	*Duration of the behaviour* (in seconds): **Start time** is defined as the moment when the head of the fish moves in a straight line from its initial point to another point within a head length distance. **End time** is defined as the moment when the head of the fish is still for 3 seconds.
"Escape Attempt"	Described as the moment when the fish raise more than 75% of its body upward trying to swim out of the cage. The body of the fish is perpendicular to the bottom of the tank.	*Duration of the behaviour* and *Success of the attempt* (in seconds): **Start time** is defined as the moment when the fish raise at least 3/4th of its body upward, with an angle of 90 degrees with the bottom of the tank. **End time** is defined as the moment when the head and at least half of the body of the fish reach the bottom of the tank.
"Forcing"	Described as the moment when the fish puts the front region of its	*Duration of the behaviour*: Described as **small** when less than

	head ("nose") against or in between the grid of the cage and apply pressure on it as an attempt to break through.	3 seconds and **obvious** when going above 3 sec. *Effort of the attempt:* Described only as **strong** when the fish is intensively swimming and shaking its body seemingly to push through the mesh.
"Head Raise (toward speaker)"	Described as the moment when the fish orientates its head in the direction of the speaker.	*Duration of the behaviour* (in seconds): **Start time** is defined as the moment when the fish is "looking" in the direction of the speaker, the "neck to nose" axis orientated straight up to the speaker. **End time** when the "neck to nose" axis does not anymore point at the speaker.
"Speaker Check"	Described as the moment when the fish is raising its body under the speaker and often touching it with its nose. **Note: This behaviour has only been observed with the European eel during this study.**	*Duration of the behaviour* (in seconds): **Start time** is defined as the moment when 3/4th of the body of the fish is raised under the speaker. **End time** is defined as the moment when the head of the fish is not anymore directed toward the speaker or that the fish is not anymore in physical contact with the speaker for at least 2 seconds.

"Other Behaviours"	Other recurrent behaviour have been originally identified but regrouped under this category to facilitate the analysis. For instance, behaviours categorised as "Back Swim", "Digging behaviour", "Listening", and others have been regrouped under that label.	

4.3.4 **Data analysis**

All video recordings were played back and analysed without sound in order to stay objective and not attribute any potential reaction due to sound. Video recordings have been watched one by one and every occurring behaviour was recorded with its start time, end time, and additional comments were added when needed. The transcribing process of watching every recording and noting the observed behaviour was done by the author itself. All the data were pooled on a Microsoft Excel workbook. The statistical analysis was conducted using R Studio®.

Because the experimental design implicitly required that tagged fish were used repeatedly as Control and Experimental fish in the same environments/tanks, a Mixed-Effect Model (MEM) was more appropriate for the analysis. This type of approach allows one to include the variability between the individuals in the analytical process, a thing that would not been included in a more classic analysis like an ANOVA. In addition, the MEM can also integrate fixed factors, either numerical or categorical, in a similar manner as an ANOVA.

In this study, the different behaviours noted in the video playback formed the main variables of the models. Of central interest was the estimation of the effect of Sound on these variables and the analysis was performed using the following factors:

- Fixed factors:

"Frequency" (10 levels): the type of frequency used during a trial (ranging from 0 to 2000 Hz).

"Order" (2 levels): either "First" or "Second" expressing if the data were taken during the First 9 days of experiments or the Second series of 9 days when fish were swapped between Control and Experimental.

"Length" and "Weight" (18 levels): respectively the weight (in grams) and the length (in millimeters) of each tested fish.

"Duration" (4 levels): The total duration of a trial in seconds.

- Random factors:

"Identity" (18 levels): Identity of each fish (tag number)

"Date" (18 levels): Date of each trial

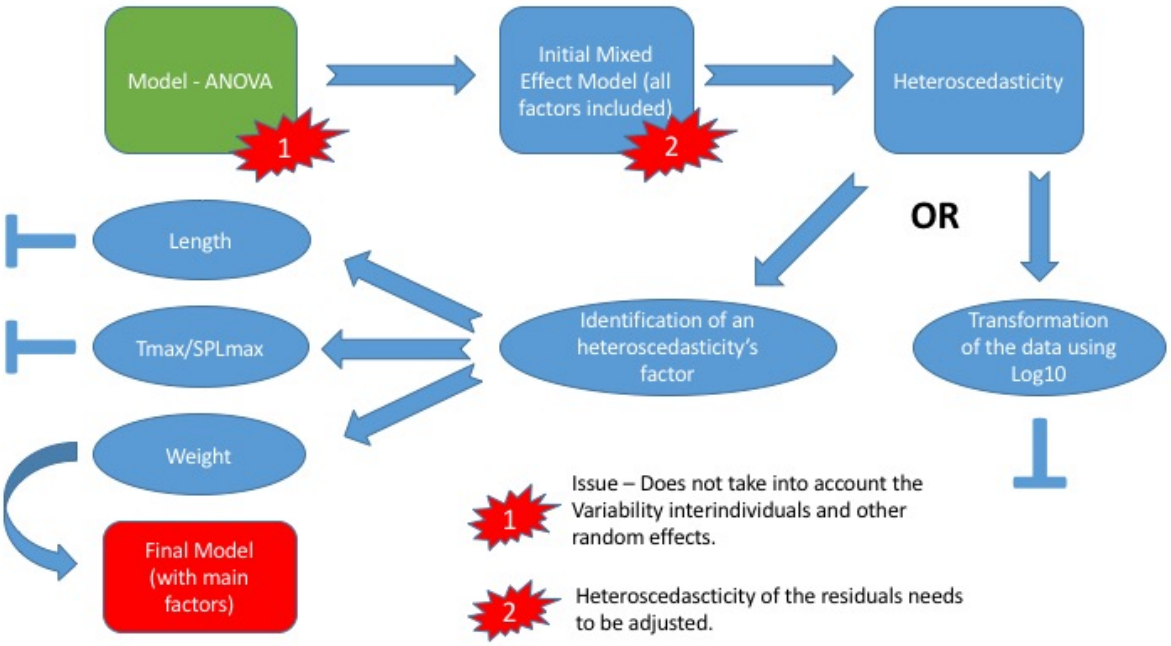


Figure 4.2 – Organigram of the process undertaken to create the final version of the MEM. This process applies for all the behaviours (variables) for both species, European eel and River Lamprey and will therefore be detailed only for one behaviour.

The final model is presented here with only the factors that were found statistically significant after running several models. Initially the first model included others factors but these have been removed from the final model as they were not statistically significant at any point with any of the behaviours tested. For instance, the interaction between Length and the Frequency has been removed in this model for all the behaviour as it was never found significant.

This mixed-effect linear model was fitted to each of the behavioural data collected using Restricted Maximum Likelihood (REML) to assess the Random effects and the Maximum Likelihood (ML) method for the fixed factors. The Least-squares means or predicted marginal means ("lsmeans" function in R) was used as a post-hoc test to discriminate the significant level of significant factors, in this case to identify which frequency could affect the most the variation of a behaviour.

4.4 Results

4.4.1 Impacts of sound and others factors on behaviour

One of the intermediate model, did not include the Frequency, factor that allows the discrimination of the data per frequency used for each trial. Initially, before considering frequencies, another model was built using a factor specifying which tank was being employed (Control or Experiment) which allowed verification of whether the presence or absence of sound in the tank made a difference to the behaviours. The results are presented in the following section.

4.4.1.1 Forcing and Escape Attempt

No significant results were observed for the Forcing in any of the two species (only a tendency for the European eel, df = 1, F = 3.52, p-value = 0.062). This behaviour was rarely observed for the lamprey (Figure 4.3).

For the river lamprey the model showed a significant difference for the Escape attempt behaviour (df = 1, F = 11.58, p-value = 0.001) with fish displaying this behaviour more often in the Control tank (Figure 4.3). A trend is noticeable with the Escape Attempt for European eel with statistical value close to the significance threshold (df = 1, F = 3.12, p-value = 0.078).

Regardless to the significance of the statistics, European eel seemed to display more often the “Escape Attempt” behaviour in the Experimental tank with sound compared to the Control tank.

The average time spent per Escape attempt per trial was also analysed and a significant difference observed according to the type of treatment. European eel put more effort (spent more time) in their Escape Attempts when they were in the Experimental tank (df =1, F = 6.36, p-value = 0.012). On the other hand, the opposite scenario was observed with the lamprey that displayed longer Escape Attempt behaviour when in the Control tank (df = 1, F = 8.3, p-value = 0.004).

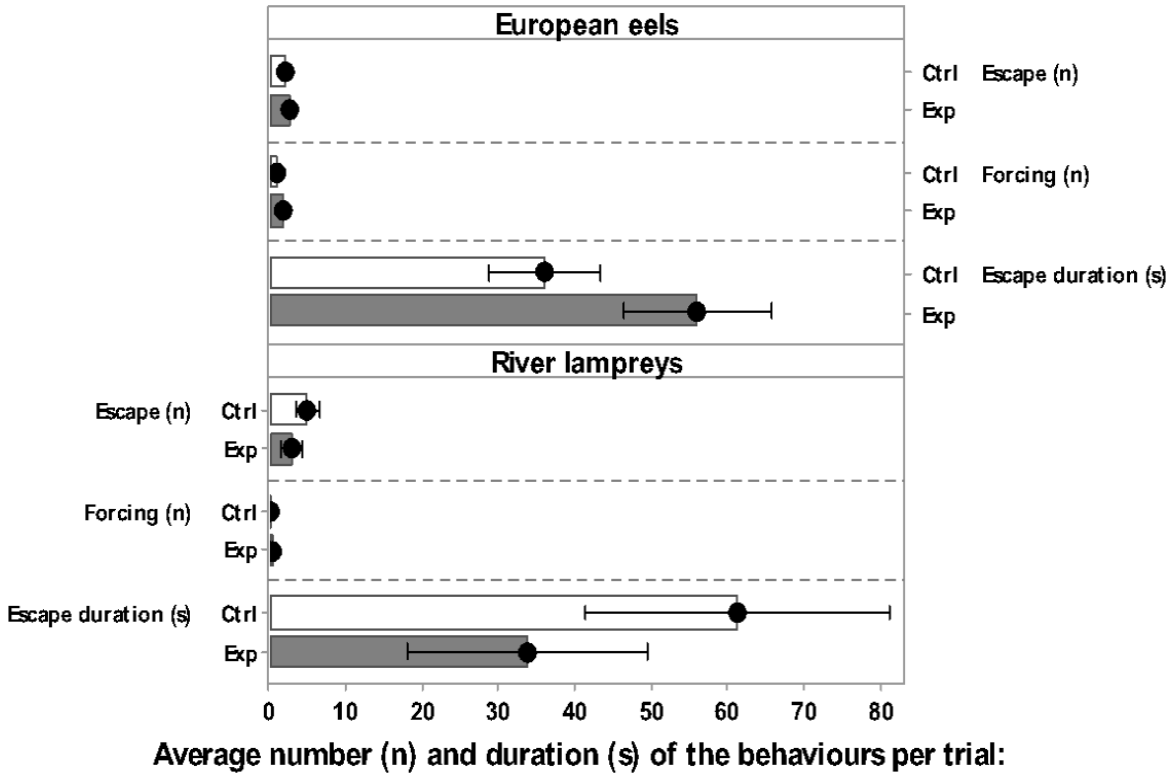


Figure 4.3 – Horizontal bar plot showing the difference between Control "Ctrl" (white bars) and the Experimental "Exp" tank (dark grey bars) for the averaged total number of "Escape attempt" and "Forcing" behaviour and the average duration of an "Escape attempt" for both European eel and river lamprey. The same scale is used on the X axis but "Escape" and "Forcing" are expressed in number of behaviours (n) and "Escape duration" in seconds (s). The values along the bar plot correspond to the means.

4.4.1.2 **Moving**

European eel did not show any difference between the amount of time spent swimming in the Control and the Experimental Tank (df = 1, F = 0.54, p-value = 0.461). On the other hand, river lamprey appeared to be significantly more active in the Control tank compare to the Experimental one (df = 1, F = 5.67, p-value = 0.018). This is illustrated by Figure 4.4 representing the average time spent per trial by both species "Moving" (as in swimming) during experimental and control conditions.

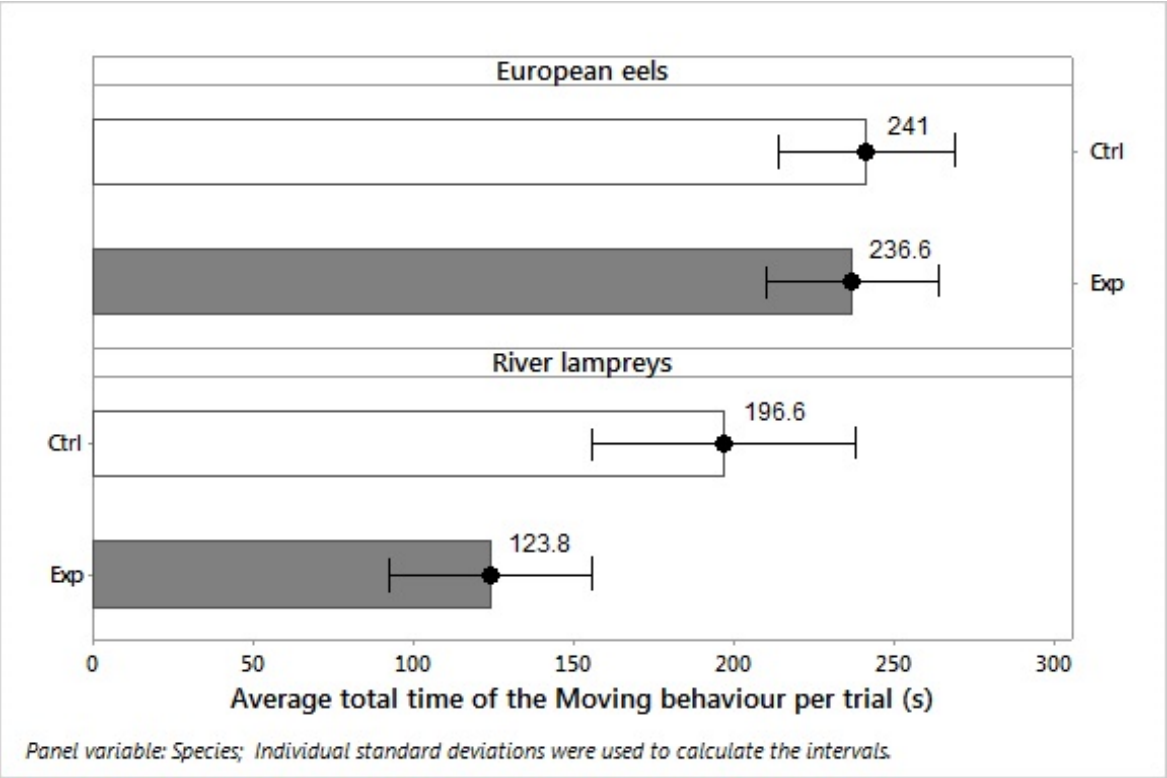


Figure 4.4 – Horizontal bar plot showing the averaged time spent by a fish "Moving" in the Control "Ctrl" and the Experimental "Exp" tank for both European eel and river lamprey. Values are expressed in seconds (s) and correspond to the means. White bars represent the Control tank "Ctrl" with no sound and the dark grey bars represent the Experimental tank "Exp" where 9 different frequencies were randomly tested.

4.4.2 **Frequency and behaviours**

As revealed by the previous results, there were some difference in the behaviour obtained in the Control and the Experimental Tank. Therefore, the analysis was carried a step deeper and the influence of Frequency evaluated. Frequency was noted in the Control trials using a value of 0 (no sound) and other trials were assigned a value corresponding to one of the 9 frequencies tested. Instead of having data/behaviours with "Sound On" or "Sound Off", behaviours were analysed based on these Frequency values. When running the model with this new factor, p-values were obtained and according to their significance it was possible to say if the Frequency was responsible for some of the variation among the behaviour tested.

The Mixed-Effect Model was evaluated for the frequencies to determine which was the more involved in the behaviour variation.

These results and the one from the previous section are summarised in the Table 4.3.

4.4.2.1 **Forcing, Escape attempt, Moving**

For the Forcing behaviour, the frequency was only significant in European eel (df = 9, F = 2.45, p-value = 0.01), while it showed some tendency with the river lamprey (df = 9, F = 1.72, p-value = 0.084). For the European eel, the posthoc test (Least-squares means) revealed that 400 Hz was the frequency that most triggered this behaviour (having the lowest p-value). Nevertheless, this result was not significant (400 Hz, p-value = 0.106).

With river lamprey only, the model returned a significant effect of the frequency on the number of escape attempts displayed by the fish (river lamprey, df = 9, F = 3.91, p-value = 0.0001). In addition, the posthoc test revealed that 1000 Hz was (almost significantly) the frequency triggering the most this behaviour (p-value = 0.061).

On the duration of the Escape Attempt, the outcome was significant for both species (European eel: df = 9, F = 5.06, $p < 0.0001$; lamprey: df = 9, F = 3.11, p-value = 0.001). For the European eel the 80 Hz and 100 Hz frequencies were significant (respectively $p < 0.0001$ and 0.008) for this specific behaviour. For the River lamprey, 1000 Hz was the frequency closest to the significance level (p-value = 0.161).

Finally, for the Moving behaviour the frequency did not have any effect in river lamprey but was significant in the European eel (Eel: df = 9, F = 2.07, p-value = 0.032). Nevertheless, with the European eel the closest frequency from the significance threshold was 800 Hz (p-value = 0.055).

4.4.2.2 **Speaker Check**

The Speaker Check is a particular behaviour only observed in European eel and only during the trials when eel were exposed to sound.

According to the model this behaviour was significantly affected by the frequency (df = 8, F = 3.64, p-value = 0.0007). The p-value being significant, the post-hoc test was run to verify which frequency would be involved in such significance. In this specific case, the lsmeans function only compared in pair the 9 tested frequencies (from 60 to 2000 Hz). This test showed that among the nine frequencies compared, 200 Hz was almost significantly generating more variation than 400 Hz (p-value = 0.062) and 2000 Hz (p-value = 0.079) (Figure 4.5). This suggest that with more replicates a significant difference might have been observed for the Speaker Check between the frequencies and especially with 400Hz.

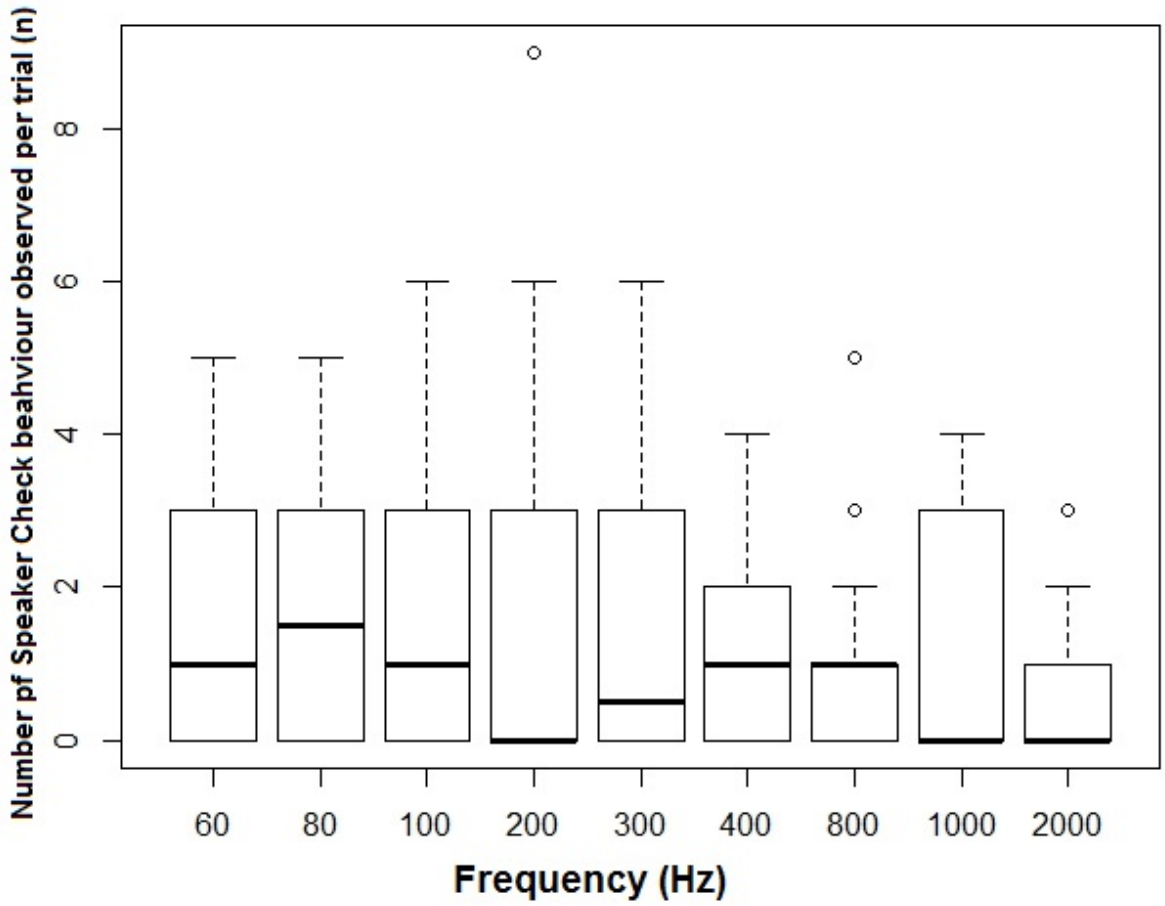


Figure 4.5 - Boxplot showing the distribution of the number of Speaker Checks per trial over the 9 tested frequencies.

The effect of the frequency on the duration of the Speaker Check behaviour was also investigated and returned a significant result (df = 8, F = 4.44, p-value = 0.0001). With this behaviour, the post-hoc test showed a significant difference between 80 Hz and two others frequencies 200 Hz (p-value = 0.012) and 800 Hz (p-value = 0.01). Also some nearly significant results were observed between 80 Hz and 2000 Hz (p-value = 0.074) and also between 200 and 400 Hz (p-value = 0.0833).

4.4.3 **Order effect: First Vs Second**

For the European eel the only significant difference was revealed by the model for the "Order" parameter with the Forcing behaviour (Figure 4.6). European eel expressed significantly less Forcing behaviour in the Second round of experiments (df = 1, F = 28.35, p <0.0001) than in the First.

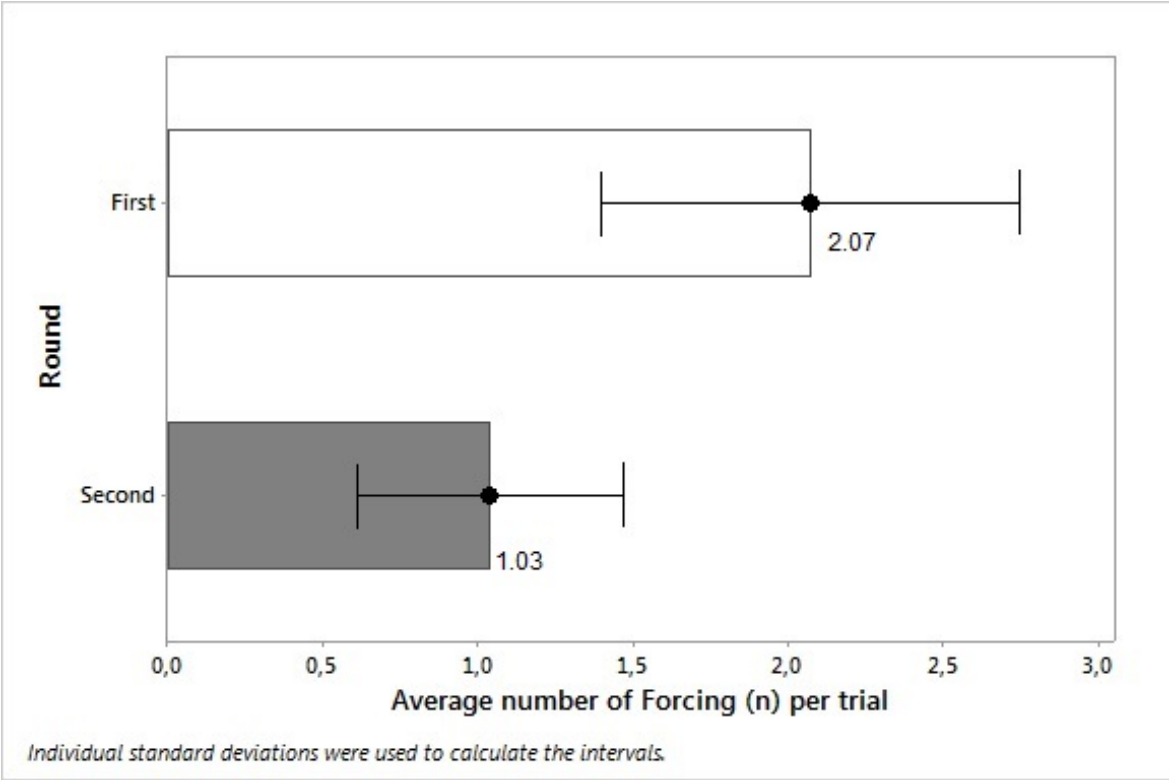
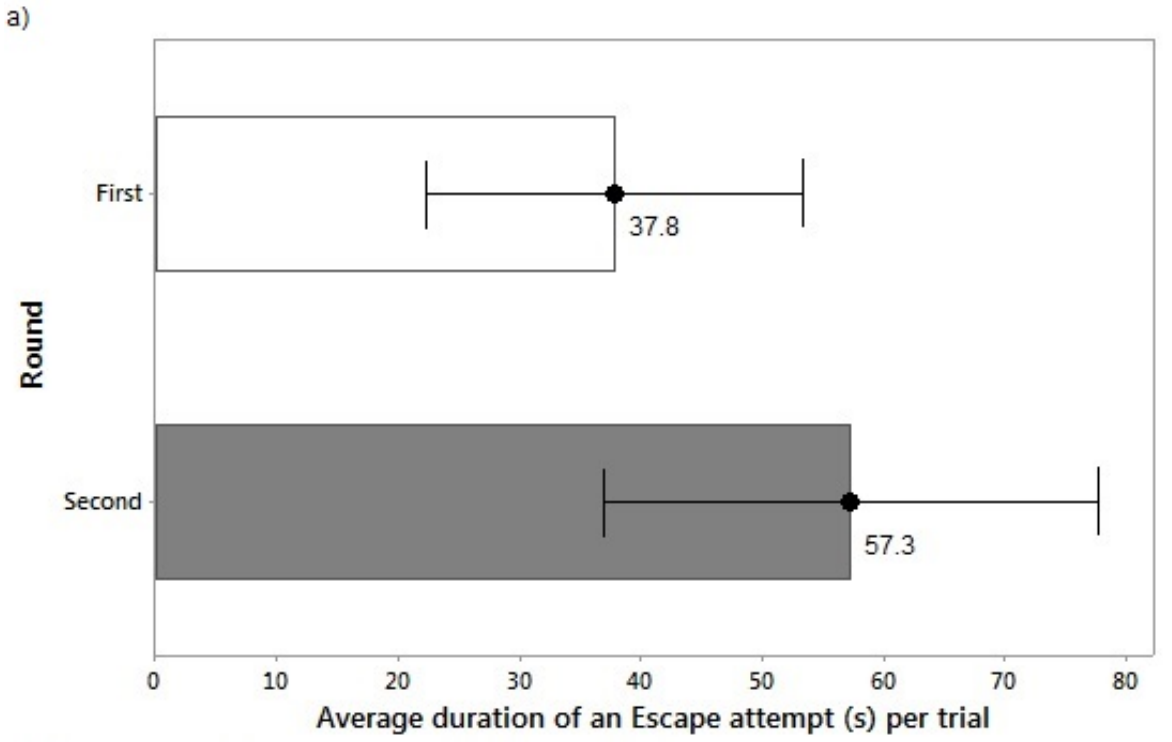


Figure 4.6 – Horizontal bar plot representing the average number of "Forcing" behaviour displayed by European eel for each Round of experiment. The white bar corresponds to the "First" round of experiment (9 first days) and the dark grey are for the "Second" round.

For the lamprey a significant difference was observed on that same parameter with the Escape Attempt numbers (df = 1, F = 5.56, p-value = 0.019) (Figure 4.7a) and duration (df = 1, F = 5.52, p-value = 0.019) (Figure 4.7b) with the behaviour being more numerous and on average longer in the First round of experiments.



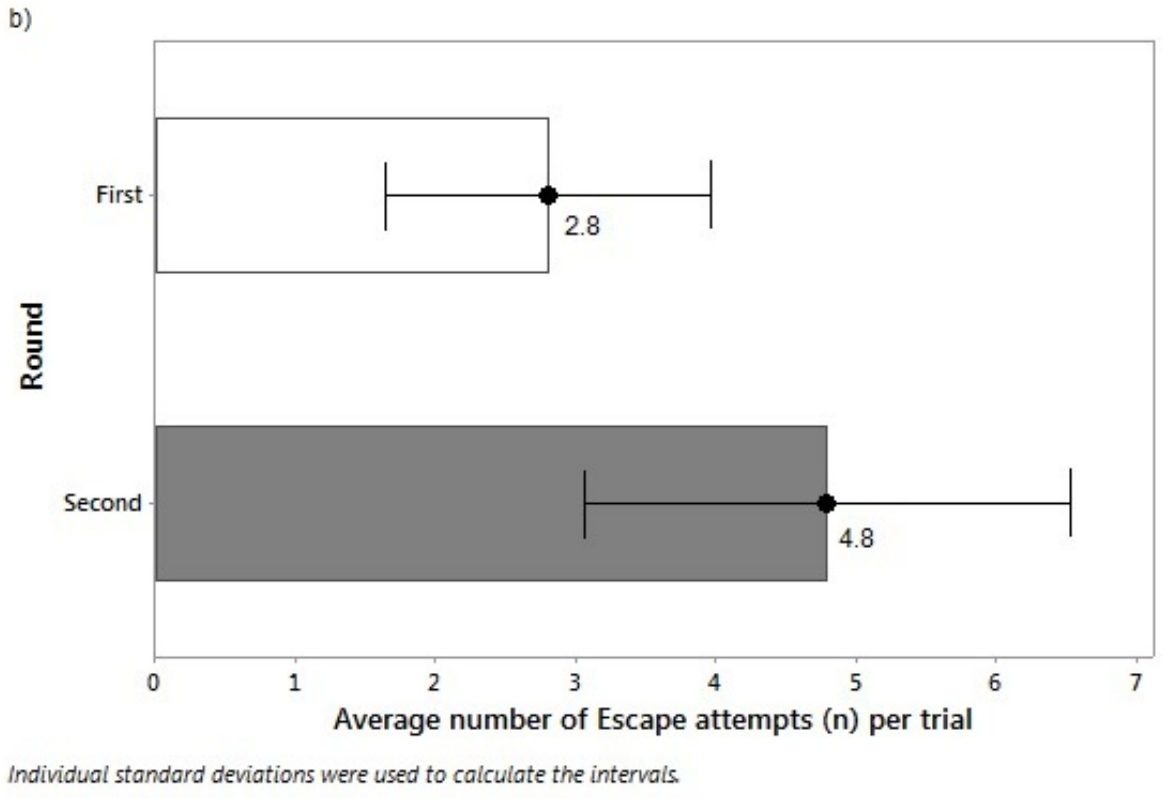


Figure 4.7a & 4.7b – Horizontal bars plots representing the average number and duration of Escape attempts displayed by river lamprey for each Round of experiment. The white bar corresponds to the "First" round of experiment (9 first days) and the dark grey are for the "Second" round.

4.4.4 **Length, Weight, Trial duration and Interaction "Length x Frequency"**

The analysis also considered the effects of different factors on the behavioural variables. This included morphological factors (e.g. weight and length of the fish), the duration of the trial and also the interaction between the length of the fish and the tested frequency.

The weight of the fish was significant for the European eel for both Escape Attempt numbers and duration (respectively df = 1, F= 7.26, p-value = 0.007 and df = 1, F = 51.1, $p < 0.0001$). Similarly, the "Trial duration" (Tmax) was also significant for these two behaviours (df = 3, F = 4.92, p-value = 0.002 and df = 3, F = 12.29, $p < 0.0001$). The length of the fish was significant for Moving (df = 1, F = 4.25, p-value = 0.04).

For the lamprey the weight of the fish did not have any significant effect on any of the behaviours. The length was significant with the Escape Attempt duration (df = 1, F = 16.31, p-value = 0.0001) and the Tmax was significant for the Forcing (df = 3, F = 3.29 , p-value = 0.0209), Escape attempt numbers (df = 3, F = 4.22, p-value = 0.006) and duration (df = 3, F = 3.38, p-value = 0.019).

Finally, for the European eel, the Interaction between Length and Frequency was found almost significant for all the behaviours except the Forcing (p-value = 0.057), see Table 3. No significant results were found for that same interaction in the river lamprey.

4.4.5 **Interindividual variability**

The model allowed percentages calculation of the influence of the interindividual variability (Identity) in the distribution of the data. The higher the percentage, the stronger the effect of interindividual variability. It also allows determination of the role played by the variation between the days (Date) of experiments. Table 4.2 summarizes the percentage found for these two random factors. Date was treated as a random factor because even if there was a high level of control on all the environmental parameters of the experiment, this control is not absolute and one day of experiment will always slightly differ from the other.

Identity is more obvious as a random effect, even if the fish are all from the same species and that the main physical parameters of all the individuals are known and input in the model, individual reaction due to intrinsic factors excluded from the model can be different. The percentage calculated from this corresponds to the variability interindividual, which is the percentage of variation of the data explained by the fact that the tested individuals are different.

Table 4.2 - Percentage of the data variation due to the Interindividual variability (Identity) and the Variability between days of experiments (Date) for each variable (behaviour) and for each species.

	European eel		**River lamprey**	
Random Effect Behaviour	%ID	%Day	%ID	%Day
Speaker Check	23,8%	4,8%	nc	nc
Speaker Check Duration	18,0%	13,7%	nc	nc
Moving	16,8%	0,4%	40,1%	0,9%
Escape Attempt	18,4%	4,1%	6,7%	1,0%
Escape Attempt Length	11,0%	2,2%	6,2%	1,0%
Forcing	6,7%	1,0%	1,0%	1,0%

Except for the Speaker Check and Speaker Check duration behaviours, the effect of the individual (Identity) is greater than the influence of the day (Date). It shows that the interindividual variability is quite strong for both species (see Figures 4.8 and 4.9).

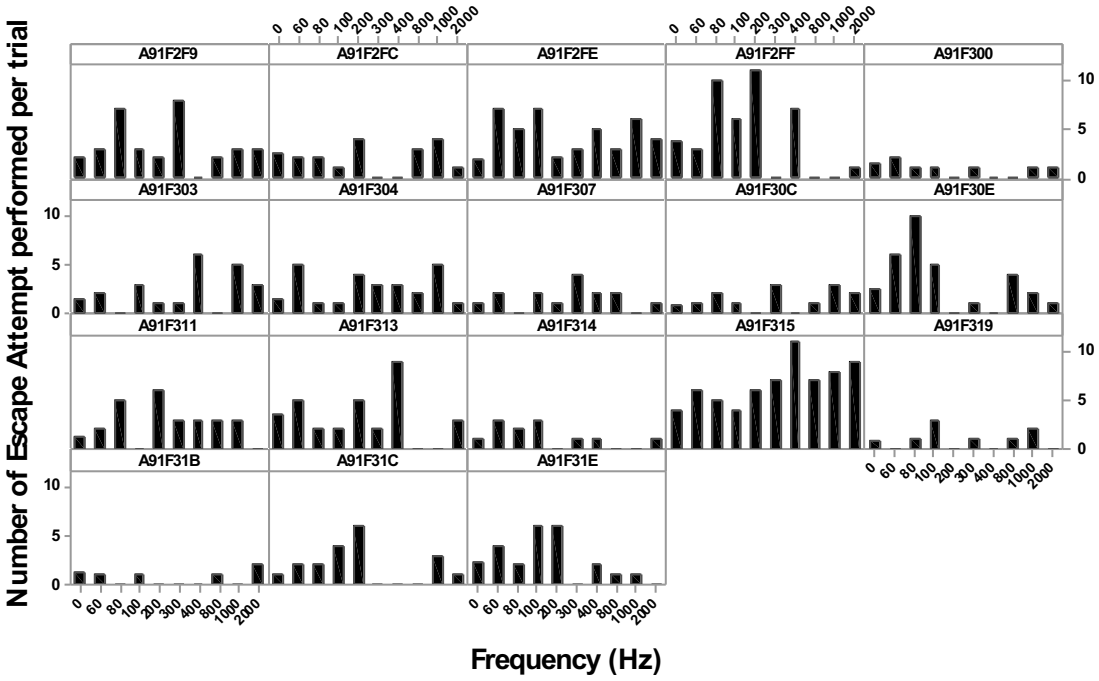


Figure 4.8 - Barplot showing the variation in the Escape Attempt behaviour per frequency for each individual for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

This figure gives the total number of Escape Attempts performed by each fish for each treatment. The figure emphasizes that the distribution of the data is different for each individual with for example individuals highly receptive (A91F315) and some providing weaker reactions (A91F31B). These graphs have been produced for all the behaviours, for both species and both random factors (Identity and Date) and can be found in the Appendix section.

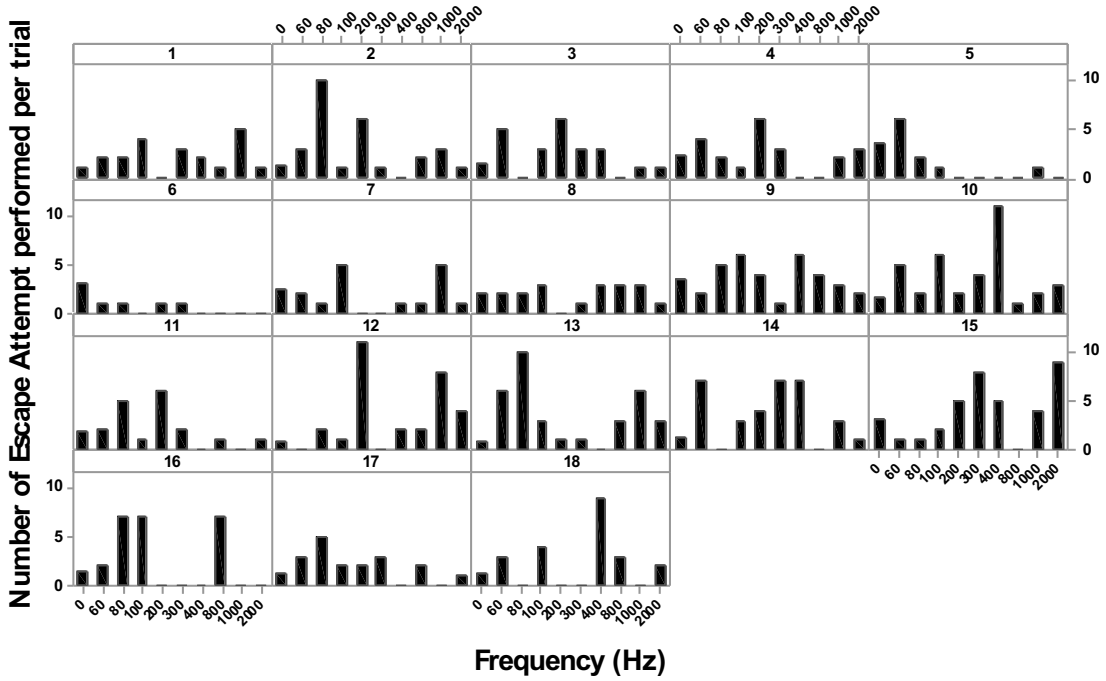


Figure 4.9 - Barplot showing the variation in the Escape Attempt behaviour per frequency for each day of experiment for the European eel.

Figure 4.9 shows for the same behaviour (Escape Attempt), the variation of the collected data by day of experiment. The distribution of the data is more balanced than for the Identity and illustrates the 4.1% of variability found in the model for that factor on this behaviour.

Table 4.3 - Table summarizing all the main findings from the result section with their main conclusions. These results will be discussed in the next section of this thesis.

Frequency[1]: the first value is the p-value from the model, the second ones are from the post-hoc test to identify the significant frequency.

Factor / Species / Behaviour	Sound ON Vs OFF		Frequency[1]		Round		Weight / Length, Trial Duration (Tmax), Intéraction Length x Frequency**		Interindividual Variability (ID) Variability between Days (Day)	
	E. Eel	*R. Lamprey*	*E. Eel*	*R. Lamprey*	*E. Eel*	*R. Lamprey*	*E. Eel*	*R. Lamprey*	*E. Eel*	*R. Lamprey*
Forcing	p = 0,0616 ON ≈ OFF	p = 0,2658 ON ≈ OFF	p = 0,0105 400Hz (0,1062)	p = 0,0842	p = 0,0001 First **>** Second	p = 0,8192	Weight (0,0896) **Inter. (0,0573)**	**Tmax (0,0209)**	ID = 6,7% Day = <1%	ID = 1% Day = 1%
Escape Attempt	p = 0,0783 ON ≈ OFF	p = 0,0008 OFF **>** ON	p = 0,2374	p = 0,0001 **1000Hz (0,0606)**	p = 0,9138	p = 0,0190 First **<** Second	**Weight (0,0075)** **Tmax (0,0024)** **Inter. (0,04)**	**Length (0,0011)** **Tmax (0,0061)**	ID = 18,4% Day = 4,1%	ID = 6,7% Day = 1%
Escape Attempt Duration	p = 0,0122 OFF < ON	p = 0,0042 OFF **>** ON	p <0,0001 **80Hz (<0,0001)** **100Hz (0,0086)**	p = 0,0013 **1000Hz (0,1613)**	p = 0,4588	p =0,0194 First **<** Second	**Weight (<0,0001)** **Tmax (<0,0001)** **Inter. (<0,0001)**	**Weight (0,0618)** **Length (0,0001)** **Tmax (0,0187)**	ID = 11% Day = 2,2%	ID =6,2% Day = 1%
Moving	p = 0,4612 ON ≈ OFF	p = 0,0179 OFF **>** ON	p = 0,0311 **800Hz (0,0552)**	p = 0,7162	p = 0,4186	p = 0,1466	**Length (0,0401)** **Inter. (0,0392)**	p > 0,1	ID = 16,8% Day = 0,4%	ID = 40,1% Day = 0,9%
Speaker Check	nc	nc	p = 0,0007	nc	p = 0,3453	nc	**Inter. (0,0042)**	nc	ID = 23,8% Day = 4,8%	nc
Speaker Check Duration	nc	nc	p = 0,0001	nc	p = 0,3711	nc	**Inter. (0,009)**	nc	ID =18% Day = 13,7%	nc
Comments	Tend to see most behaviour increase during the ensonified trials.	Most of the behaviours decreased when the sound was active.	80 & 100Hz seems to be the frequencies generating higher behavioural response in European eel.	1000Hz seems to be the frequency the most involved in behavioural responses in river lamprey.	Only the "Forcing" decreased between the two rounds of experiments.	Only change between the two rounds of experiments is the duration of the Escape Attempt.	Weight, Length and Trial duration (Tmax) seem to have an impact on European eel behaviour.	Duration of the trial had a significant impact on almost all the behaviours.	For all the variables, the variation between the individuals "ID" is very strong.	For 3 behaviours, the percentage of variation in the data explained by "ID" is much higher than for the "Day".

4.4.6 **Audiograms**

From the collected data, two audiograms have been produced for each species based on the observed behaviour during the trials. For this purpose, the Escape Attempt behaviour has been selected as it was common to both species and offered good amount of data (as opposed to the Forcing for instance). The first audiogram is based on the whole dataset and for each frequency includes all the behaviours observed in all the acoustic trials. The second audiogram was made of a dataset where only the behaviours were recorded during an interval when sound was played (15 seconds interval).

4.4.6.1 **European eel**

Figure 4.10 represents the two audiograms produced for European eel from the two datasets previously described. The overall shape of the hearing threshold is conserved with low variations (not exceeding 4 dB re 1 µPa for 400Hz) between the two audiograms. This suggests that between the two datasets there were not many differences and that generally the behaviour occurred during sound intervals (and was potentially triggered by the stimulus).

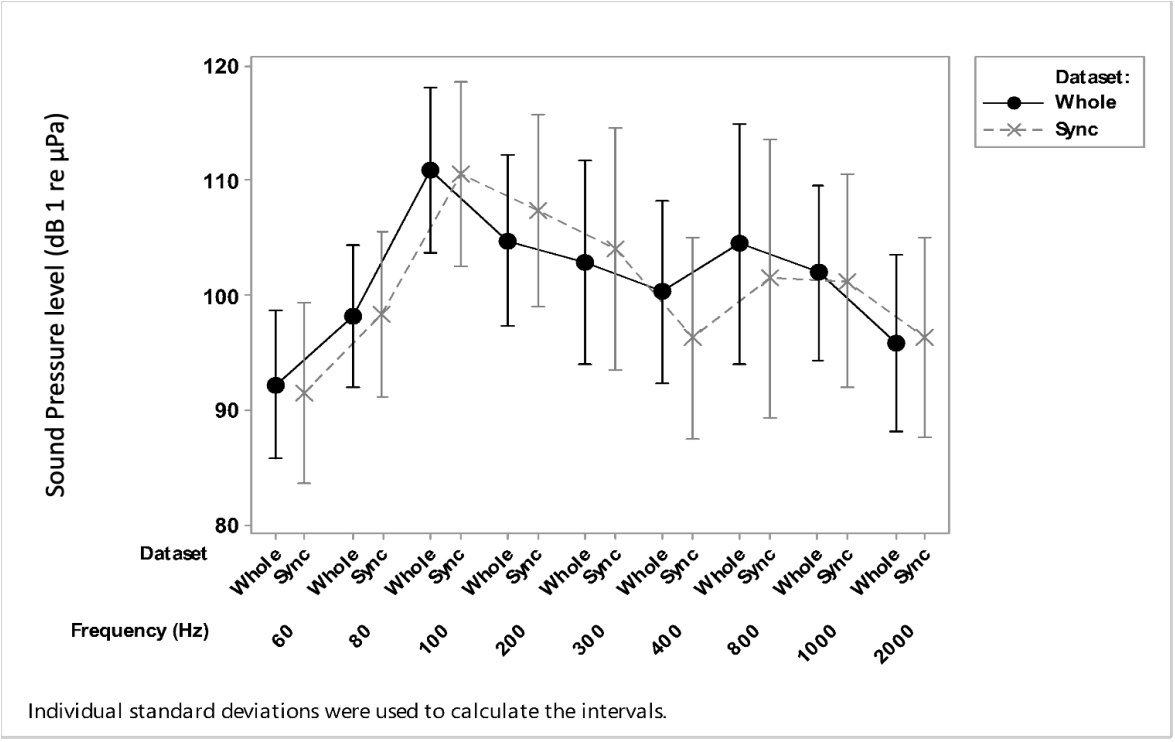


Figure 4.10 - Two different audiograms created from the data collection of European eel Escape Attempt behaviours during acoustic trials. The plain black line corresponds to the complete dataset "Whole" including all the behaviour recorded over a trial. The grey dashed lined corresponds to the dataset including only behaviours synchronised with the sound interval "Sync". The connecting-line, links the mean values between frequencies, of all the Sound Pressure Level (SPL) at which the Escape behaviour has been observed for the 18. The full line represents the hearing threshold of the fish. Error bars represents the 95% interval of confidence for each mean.

Regardless to the error bars, the audiograms indicates that 60, 80 and 2000 Hz are the most sensitive frequencies in terms of hearing capabilities with respective threshold being, 91, 98, 96 dB re 1 µPa (based on both audiograms). In addition, if only considering the audiogram with the "synchronised" behaviours to sound, 400 Hz is potentially a sensitive frequency with a threshold level at 96 dB re 1 µPa.

4.4.6.2 **River lamprey**

Figure 4.11 shows the two audiograms built from the different dataset of Escape behaviour collected for the river lamprey. If the general shape of the audiogram does not change dramatically between the two dataset, there are so more obvious difference compare to the European eel ones. For instance, with the 80 Hz and 300 Hz means, there are respectively a difference of 7 and 9 dB re 1 µPa between the two datasets.

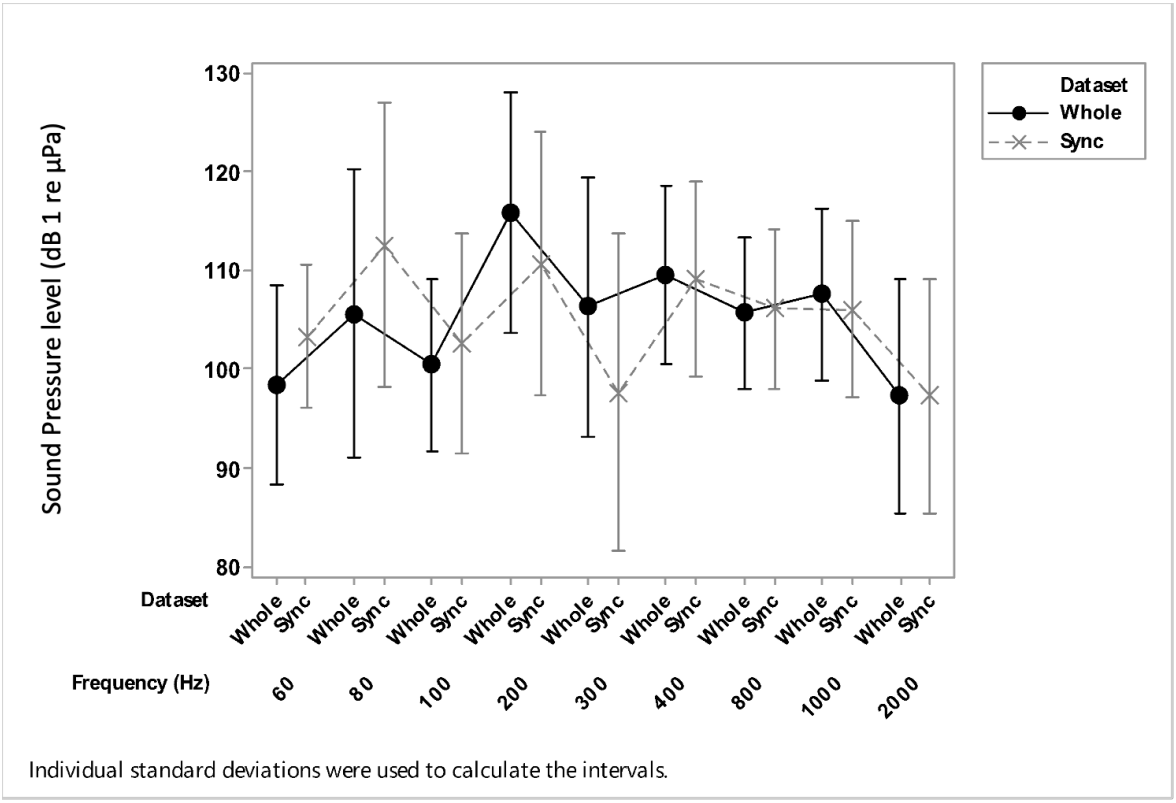


Figure 4.11 - Two different audiograms created from the data collection of river lamprey Escape Attempt behaviours during acoustic trials. The plain black line corresponds to the complete dataset "Whole" including all the behaviour recorded over a trial. The grey dashed lined corresponds to the dataset including only behaviours synchronised with the sound interval "Sync". The connecting-line, links the mean values between frequencies, of all the Sound Pressure Level (SPL) at which the Escape behaviour has been observed for the 18. The full line represents the hearing threshold of the fish. Error bars represents the 95% interval of confidence for each mean.

Regardless to the error bars, 60, 100 and 2000 Hz appear to be the three frequencies common to both audiograms resulting with the lowest hearing thresholds with mean values respectively equal to 98, 100 and 97 dB re 1 µPa (values from the "whole" audiogram). If considering the "synchronised" audiogram, 300 Hz is an additional frequency with a hearing threshold equal to 97 dBs µPa.

4.5 Discussion

4.5.1 Behaviours

This study is the first to have extracted a series of behaviour from an in-situ experiment on European eel and river lamprey and presenting a generic ethogram for both species. Also it is the first study introducing a potential audiogram for river lamprey and also introducing an alternative audiogram for European eel to the one from Jerkø et al. (1989). Finally, the potential impacts of sound on these generic behaviours is also studied and does not limit to fish activity or passage rate, that are the most common fish behaviours studied in the literature.

The results tend to show that European eel displayed more behaviours in the experimental tank compared to the control tank. It seems that being exposed to sound triggers more evasive and aggressive responses from the eel. Sand et al. (2000), on their experiment on European eel have recorded some behavioural reactions to infrasound. Unlike our experiment, Sand et al. (2000) have used a continuous sound and have only observed a startle response of the fish followed by "motionless period of 1-2 mins". In this study, no significant difference in the activity of the fish associated to the "Moving" behaviour has been observed between sound exposed and control fish. Another difference between these two studies is that trials were run with single fish in each tank compare to Sand et al. (2000) that ran trials with 60 eel in their tank. However, Peters et al. (1980) found out that European eel gets less stressed and aggressive when kept in small density.

On the other hand, river lamprey significantly displayed the opposite reaction, with decreased behavioural response in the experimental tank compare to the control tank. This is true for all the behaviours except the Forcing behaviour that did not show any changes in response between the two treatments. Nevertheless, compared to European eel this appears to not be a key behaviour of their repertoire. Lamprey seem to be a much more "shy" species compare to eel and lets us think that as a response to a stress stimulus they would probably adopt a motionless/freeze response. One simple explanation to this would be their anatomical difference especially in matter of hearing structures. As stated by Popper (2005), the inner ear of lamprey is simpler to the one of other bony-fish species and in addition Lamprey do not possess a swim-bladder and might therefore be less sensitive to variations in sound pressure. These differences might affect lamprey's perception of sound and also their behavioural reactions to it.

An ecological explanation can also be considered with the fact that European eel, especially when reaching their full maturity do not have many predators in the freshwater environment. On the other hand, river lamprey probably due to their smaller size experienced a wild range of predators

including fish (Brown trout, *Salmo trutta* and Burbot, *Lotta lotta*) and also birds (Common Merganser, *Mergus merganser* and members of the Ardeidea family like herons and egrets) that have been reported eating river lamprey (Sjöberg 1980; Maitland et al. 2015).

In fact, this kind of motionless response from the Lamprey has been for a long time known as an adaptive way to cope with a threat and increase the survival of the individual as generally described Lima & Dill (1990) and later shown in another fish species by Krause & Godin (1995). Toms et al. (2010) also stated that the expression of certain behaviours in fish is linked to their past experience and ecology (predation, environmental conditions, confinement) and therefore influence their "bold-shy axis" even at an individual level ("personality" of the fish) (Magnhagen & Staffan 2005). This raise an interesting question, would the fish have reacted differently if they have been raised in a lab and not experienced any predation or more related to our study any "major" sound events ?

The previous statement on fish personality and individual with specific reaction can potentially be applied to these data. When looking at behaviour expression at an individual level, it appears that some fish displayed much more behaviours compared to others. With these data, this statement seems valid for both species but has been much more observed with the Escape Attempt behaviour in European eel with the interindividual variability accounting for 18.4% of the data variation for this specific behaviour. Nevertheless, except for the Forcing behaviours, the variability among the individuals in both species is a very important parameter to consider for data interpretation and for further experimental work. However, the low number of replicates has to be considered in this experiment. Even if the total number of trials is greater than 100, only 18 fish in total from each species have been used for this experiment and this could have slightly biased the results. In order to consolidate these findings, it would be necessary to perform similar experiments with a larger number of fish and to compare the evolution of the interindividual variability but also of the general behavioural response. More trials could also be a good way to isolate more significantly/precisely any key frequencies linked to specific behaviours and also to reinforce the results from the post-hoc test and reduce the error bars in the audiograms.

For the "Date" parameter and especially its importance with the expression of the Speaker Check duration, it is difficult to find an explanation. When looking at the graphical representation for these data, a difference between the different days is observable but there is no clear pattern appearing in the distribution of them. It looks like the first 9 days of experiment had less Speaker check behaviour displayed than the second round but this has not been significantly confirmed by the model. If this experiment is reproduced it would be interesting to add other potential

parameters (moon phase, pH, etc.) that were not included in the original experiment into the model and to ultimately compare if the Speaker Check behaviour has a significant daily variation.

Finally, no obvious sign of habituation has been observed during the trials. Tort et al. (2001), suggested that when a stressor was applied daily for short period of time, a decrease in the physiological indicators of stress (and the behavioural response) could be observed and categorised as habituation. The random factor Day that did not account for much of the variation of the data can be used as an indicator. As the percentage of the data variation explained by the factor Day is for all the behaviour very low (except "Speaker Check duration"), it seems that the daily exposure of the fish did not affect their behaviour. Nevertheless, during further similar experiments it would be interesting to add measurements of the physiological parameters of the fish (cortisol, glucose and lactate for instance).

4.5.2 **Audiograms**

The audiogram produced for the European eel is relatively in accordance with the one from Jerkø et al. (1989), with its first three frequencies closely following a similar trend. Jerkø et al. (1989), suggested that 80 Hz is the frequency with the lowest hearing threshold for this species, with a threshold value closed to 100 dB re 1 µPa. In our audiogram we found for that same frequency a mean value of 98 dB 1re µPa.

As there are no other audiogram of river lamprey available in the literature, the one produced in this study is hardly comparable in terms of findings. If compared to the European eel one, the audiogram of the lamprey seems to follow the same trend with the lower hearing threshold found with the lowest frequencies 60 and 80 Hz. From an evolutionary point of view, Elasmobranchii (sharks and ray) are closely related to lamprey. According to Hart & Collin (2015), shark hearing capabilities range between 20 and 1000 Hz, with their best hearing threshold for frequencies around 100Hz. Based on this paper, it seems that our result point in the same direction.

However, the two audiograms produced in the present study need to be considered with great care due to the importance of the error bars. Indeed, the means values have been used as hearing threshold in our analysis but cannot in any case be assumed as an absolute reference for future usage due to the range of the error bars (reaching for instance almost 20dB re 1 µPa at 300 Hz for the lamprey). Furthermore, these audiograms are based on behavioural responses and once again cannot be used as a reference defining the real hearing capabilities of the species. These audiograms have to be considered as basic indicators of the triggering threshold of a specific behaviour by an acoustic stimulus and give a basic idea of the hearing capabilities of the species. Nevertheless, they are not as accurate as an electrophysiological based audiogram (Yan 2004;

Ladich & Fay 2012). Further investigations using this method (ABR or AEP) would be needed to comfort our results.

5 Use of an acoustic maze to assess the behavioural response of anguilliform fish to underwater sound

5.1 Summary

Traditional physical screens designed to prevent fish entering dangerous areas (e.g. cooling water intakes at power stations) can have negative impacts due to impingement or mechanical abrasion at high velocities (de Bie et al. 2018). Behavioural deterrents (e.g. light, bubble curtains, acoustics) may provide an alternative or complementary approach to screening. This study investigated the potential for a continuous broadband sound (CBS: 60 to 1000 Hz) to modify the behaviour of two endangered species of anguilliform fish, European eel (*Anguilla anguilla)* (both migratory silver and non-migratory yellow life stages), and river lamprey (*Lampetra fluviatilis*). Experiments were conducted in an outdoor experimental channel. Eel and river lamprey were, respectively, released upstream and downstream of an "acoustic maze" that consisted of two chambers positioned in series. By testing the fish twice, the number of replicates were doubled thus reducing the number of experimental fish used in line with the principles of ethical research. A single individual released during a trial encountered a choice of route on entering each chamber through either a treatment corridor ensonified with the CBS, or a control corridor in which a speaker was turned off. Trials were conducted under one of two possible configurations to control for any left/right bias. Under 'configuration 1' only the right-hand choice of the two channels in the first chamber, and the left-hand route in the second, was ensonified. This was reversed under 'configuration 2'. The influence of chamber, configuration, and treatment on route selection, rejection of the speakers, and time to pass were tested. For eel, there was no influence of any of the three factors on route selection. River lamprey tended to pass through the ensonified corridor more often under configuration 2, but only in the first chamber encountered. Once fish had selected a route, rejection of the speaker was observed for all species and occurred more frequently in the ensonified corridors. Time to pass was greater for fish that rejected than those that did not. For eel, variation in time to pass was greater for the non-migratory (yellow phase) life-stage. While the acoustic signal used in this study influenced fish behaviour, the

response observed would likely be insufficient to induce a strong deterrent effect in the field if used in isolation.

5.2 Introduction

Many populations of diadromous anguilliform fish have experienced substantial declines over recent decades. For example, since the 1980s recruitment of the catadromous European eel (*Anguilla anguilla*) has reduced by nearly 90% throughout Europe (EIFAC 2006; Aalto et al. 2015; Bevacqua et al. 2015). Dekker (2003a) reported a reduction in the fisheries landings in the preceding two decades and suggested this to be consistent with a decline in the continental stock (Dekker 2003b). Similarly, anadromous river lamprey (*Lampetra fluviatilis*) populations across Europe have suffered severe declines over the past 30 years (Kelly & King 2001; Masters et al. 2006), with human activity identified as one of the main causes (Kelly & King 2001). Multiple factors have been suggested to explain the collapse of populations of these species, including climate change (Bonhommeau et al. 2008), over fishing (eel - Feunteun 2002; lamprey - Masters et al. 2006), pollution (eel - Maes et al. 2005; lamprey - Kelly & King 2001), and fragmentation of habitat due to the placement of river infrastructure (eel - Kettle et al. 2011; lamprey - Igoe et al. 2004).

In response to concerns about declining populations, multiple pieces of legislation have been enacted to protect these species. These include the EU Eel Regulation (EC 1100/2007) that establishes a framework for the protection and sustainable use of stocks in the member states. It requires them to achieve escapement equivalent to 40% of the silver eel biomass expected to be produced under pristine conditions that would occur in the absence of human activity. Further, European eel are listed under Appendix II of the Convention on International Trade in Endangered Species (CITES) which places strict restrictions on their trade. Lamprey are listed under Annex II & V of the EU Habitats Directive (EC 92/43/EEC) that requires member states to contribute towards ensuring biodiversity through conservation of natural habitats and of wild fauna and flora. Species listed benefit from the implementation of Special Areas of Conservation (SACs) (Annex II) and measures to manage their exploitation (Annex V). River lamprey are also protected under Annex III of the Bern Convention. Despite this legislation, the major anthropogenic threats to population persistence remain.

River infrastructure, such as dams and weirs, negatively impact populations of diadromous fish, many of which are of high commercial interest, by delaying their migration or blocking access to critical habitats (Kemp 2016). Furthermore, fish are likely to suffer physical injury and mortality if

they enter dangerous areas such as turbine intakes (Calles et al. 2010) or water abstraction points (Piper et al. 2013). Physical screens are installed in an effort to reduce entrainment and guide fish towards alternative routes (e.g. bypass systems).

Although developed to mitigate environmental impacts, screens carry their own risks. If poorly designed, or incorrectly installed, and when water velocities are high relative to swimming capability, fish can become impinged on the screen face where they suffocate if unable to escape. Moreover, small bodied fish can be entrained through the screens if mesh / gap size is too large. Because of their elongated morphology, low aspect ratio of their body cross-section, and relatively weak burst swimming capabilities, anguilliform fish are at greater risk of impingement and entrainment than many other species (Russon et al. 2010). In the face of this challenge, one option to better protect anguilliform fish is to install very fine spaced screens (e.g. 2 mm slot width for juvenile eel; Sheridan et al. 2015), which require larger surface areas to maintain equivalent through-flows of water at greater costs to the owners and operators (e.g. water supply and electricity generating companies) of the associated infrastructure. Devices that employ behavioural stimuli (e.g. acoustics, lights, bubbles, hydrodynamics or multiple stimuli in combination) to induce an avoidance response and deter fish have the potential to enhance the efficiency of traditional physical screens if used in combination.

The development of behavioural deterrents for anguilliform fish is not new, and devices that are based on a range of stimuli, such as infrasound (Sand et al. 2000; Sand et al. 2001) and strobe lights (Patrick et al. 1982), are commercially available but tend to be used in isolation to screens. Unfortunately, in many instances they have been advanced through a process of trial-and-error and their effectiveness seldom quantified by robust experimental studies; when evaluation has taken place, the results are often contradictory and inconclusive. As a result, behavioural deterrents are generally considered less efficient than physical and mechanical screens. Nevertheless, behavioural screening devices remain appealing, should high efficiencies be attainable, as they represent a much sought-after solution to the challenge of developing sustainable water and electricity generating infrastructure systems in a cost-effective manner.

For European eel, the use of strobe lights has recently returned promising results (Elvidge et al., 2018), while infrasound (< 20 Hz) is suggested to influence their downstream swimming trajectories (Sand et al. 2000). For lamprey, however, there is little published information on their response to behavioural deterrents.

Focusing on acoustics as a potential deterrent, this study aimed to investigate the possibility of developing a device that would deter multiple species of anguilliform fish (eel and lamprey). We quantified the effectiveness of a Continuous Broadband Sound (CBS) (60 to 1000 Hz) to influence: (1) route selection; (2) rejection; and (3) time to pass, exhibited by European eel and river lamprey in an acoustic maze that consisted of a series of two chambers, each with a choice of route through either an ensonified or control corridor. Two chambers were used to enable the number of replicates to be increased by twice presenting each fish with a choice of route. This is in-line with the principles of ethical research which dictates that efforts should be made to reduce the numbers of individuals used, an ethos that is especially important when studying endangered species. This design, however, requires the importance of any influence of repeated exposure to be evaluated, e.g. because processes such as learning or habituation may confound the results obtained. Furthermore, to control for any lateral bias (e.g. of flume characteristics or fish behaviour), tests were conducted under two configurations: 1) right and left hand channels ensonified in chambers 1 and 2, respectively; and 2) the reverse. Each individual fish was tested under both configurations. The results obtained will be of value to those interested in designing behavioural deterrents for anguilliform fish in an effort to enhance their conservation.

5.3 Materials and Method

5.3.1 Fish collection and maintenance

Silver phase European eel (N = 81, Total Length: 300–782 mm, median = 547 mm; mass: 29–850 g, median = 261 g) were caught during their seaward migration using a fixed eel trap on the River Stour (50°46'31.6"N 1°54'38.1"W), UK, in December 2014. Migrating adult river lamprey (N = 82, Total Length: 312–419 mm, mean = 368.2 mm, S.D. = 20.34; mass: 51–109 g, mean = 80.6 g, S.D. = 13.45) were trapped in the River Ouse (53° 53’ 26.2”N, 1° 5’ 36.8”W), UK, by a commercial fisherman in December 2014. Non-migratory (yellow phase) eel (N = 67, Total Length: 332–681 mm, mean = 447.5 mm, S.D. = 70.61; mass: 56–617 g, median = 133 g) were collected by electric fishing a small stream near Funtington (50°50'47.9"N 0°50'45.5"W), UK, at the beginning of March 2015. Fish were transported to the International Centre for Ecohydraulics Research Facility, University of Southampton (50° 57’42.6”N, 1°25’26.9”W), in two large transportation tanks filled with aerated river water. The fish were maintained in five separate holding tanks (silver eel and river lamprey: 4 x 1000 L outdoor tanks; yellow eel: 1 x 2000 L indoor tank) equipped with individual filtration systems and separate air pumps. Water was monitored daily and maintained through regular water changes (50% weekly) using dechlorinated tap water (pH = 7.8, Nitrate: <

40 ppm). Mean water temperature was 8.9 °C (S.D. ± 0.8 °C) for the lamprey and silver eel, and 12.1 °C (S.D. ± 0.6 °C) for the yellow eel. As yellow eel were expected to continue feeding during this phase of their life-cycle, they were fed live *Dendrobaena* worms.

5.3.2 **Experimental setup**

A concrete block experimental channel (7.16 m length, 1.39 m width, 0.56 m depth) was constructed within an existing outdoor flume at the International Centre for Ecohydraulics Research (Figure 5.1 & 5.2). The channel was divided into two identical chambers (3.15 m length, 1.39 m width), each comprised two corridors (2.25 m length, 0.37 m width) separated by blocks. A 0.52 m wide entrance/exit was located centrally at the start/end of each chamber, and wire-mesh release/recapture enclosures (13 x 13 mm mesh, 1 mm gauge) installed at the ends of the experimental area to facilitate safe introduction and retrieval of fish at the start and end of each trial.

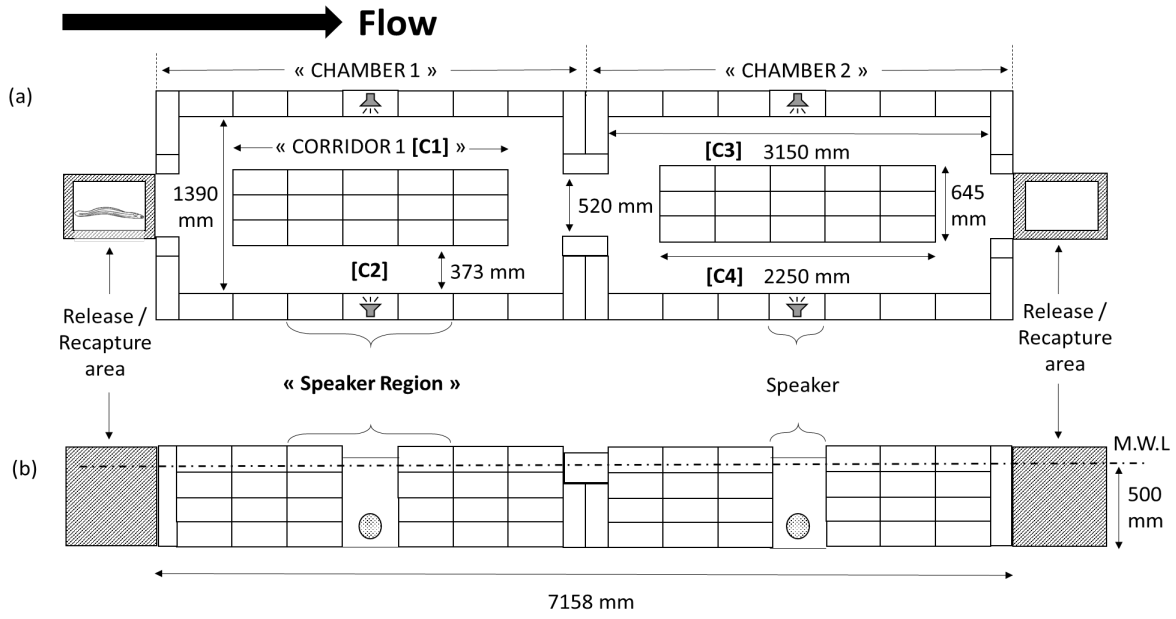


Figure 5.1- An "acoustic maze" installed in an outdoor recirculating flume at the International Centre for Ecohydraulics Research, University of Southampton. During experimental trials the acoustic maze twice presented European eel and river lamprey with a choice of route through either an ensonified or control corridor. Plan (a) and elevation (b) views are depicted with the Maximum Water Level (M.W.L.) represented by a dashed line. The grey boxes at both ends represent the release/recapture enclosures that restrained the fish within the experimental area of the maze.

Figure 5.2 - Picture of the maze setup in the outdoor flume. Orientation from downstream facing upstream.

5.3.3 **Acoustic stimuli**

The stimulus selection was based on an audiogram constructed for European eel (Jerko et al. 1989) that indicated sensitivity between 60 and 400 Hz, with a peak at approximately 80 Hz. There is currently no available audiogram in the literature for river lamprey, but Teague and Clough (2013) speculate that neither low frequency nor ultrasound deterrents would be effective for lamprey as they are hearing non-specialists. Nevertheless, Popper (2005) encourages further investigation into the hearing capabilities of lamprey and their behavioural response to sound.

During a pilot study, a series of experiments were conducted to define an appropriate acoustic stimulus relevant for the test species. Fish response to a series of specific test tones was assessed and behaviours compared with those observed under a control in which there was no externally generated sound.

Use of an acoustic maze to assess the behavioural response of anguilliform fish to underwater sound

Preliminary results indicated that sounds with frequencies that ranged from 60 – 2000 Hz may have the potential to deter fish. In the current study, a continuous broadband (60 – 1000 Hz) sound was generated by four underwater speakers (ElectroVoice UW30), one installed in each corridor. During trials the set-up was divided into one of two test configurations in which one of two speakers in each chamber was turned on. The position of the ensonified corridor alternated between chambers under each configuration (Figure 5.3). The sound production system consisted of a laptop (Dell © Latitude E6430) linked to a National Instruments Data Acquisition Box (National Instruments © USB-6251) driving a Power Amplifier (SkyTronic © Mini AV Digital Surround Amplifier 103.100) to which the speakers were connected. The sound field was measured using a calibrated hydrophone (Brüel & Kjær © 8105) and recorded at two depths (1 and 25 cm) between the tip of the hydrophone and the channel floor.

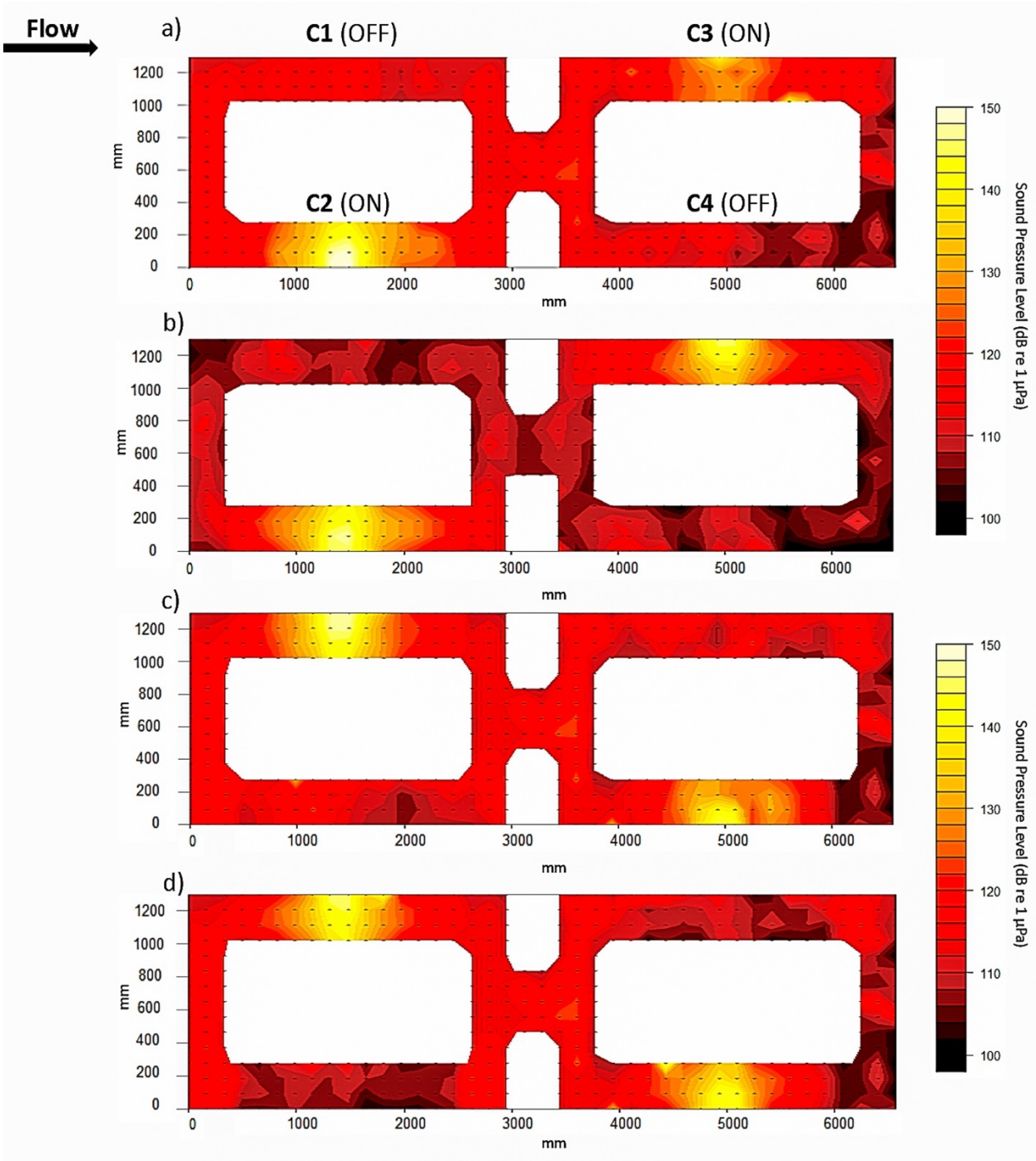


Figure 5.3 - Acoustic field generated within the maze at two different depths (a and c = 1 cm; b and d = 25 cm from the channel floor) under configuration 1 (a and b), in which corridors (C) 2 and 3 were ensonified, and the reverse configuration 2 (c and d) in which speakers C1 and C4 were turned on. The sound field was measured at regular spatial intervals along a grid using a hydrophone (dots indicate measurement points). White areas correspond to the concrete blocks structures delimiting the chambers and corridors.

Fish movements were recorded using a series of eight CCTV cameras (2 cameras per corridor) with integrated infrared light units (AV-TECH 245 Sony Effio 580TVL CCD) mounted 1.5 m above

the Maximum Water Level (0.5 m). Six additional 15.0 W infrared lights provided additional illumination to enhance the contrast of the video recordings.

A constant flow (depth: 48-50 cm; mean velocity: 0.1 m s^{-1}, S.D. ± 0.01 m s^{-1}) was maintained using three centrifugal pumps. Note: the noise generated by the pumps was not an issue as the maze was located far enough downstream of the flume channel to not be affected by it (no radiation of noise was recorded on the acouistic mapping). Depth and velocity were measured using a rule and an electromagnetic flow meter (Valeport, 801 –flat) recording over 10 seconds. Measurements were obtained for two different depth (1cm above the flume floor and 25 cm above) (Figure 5.4).

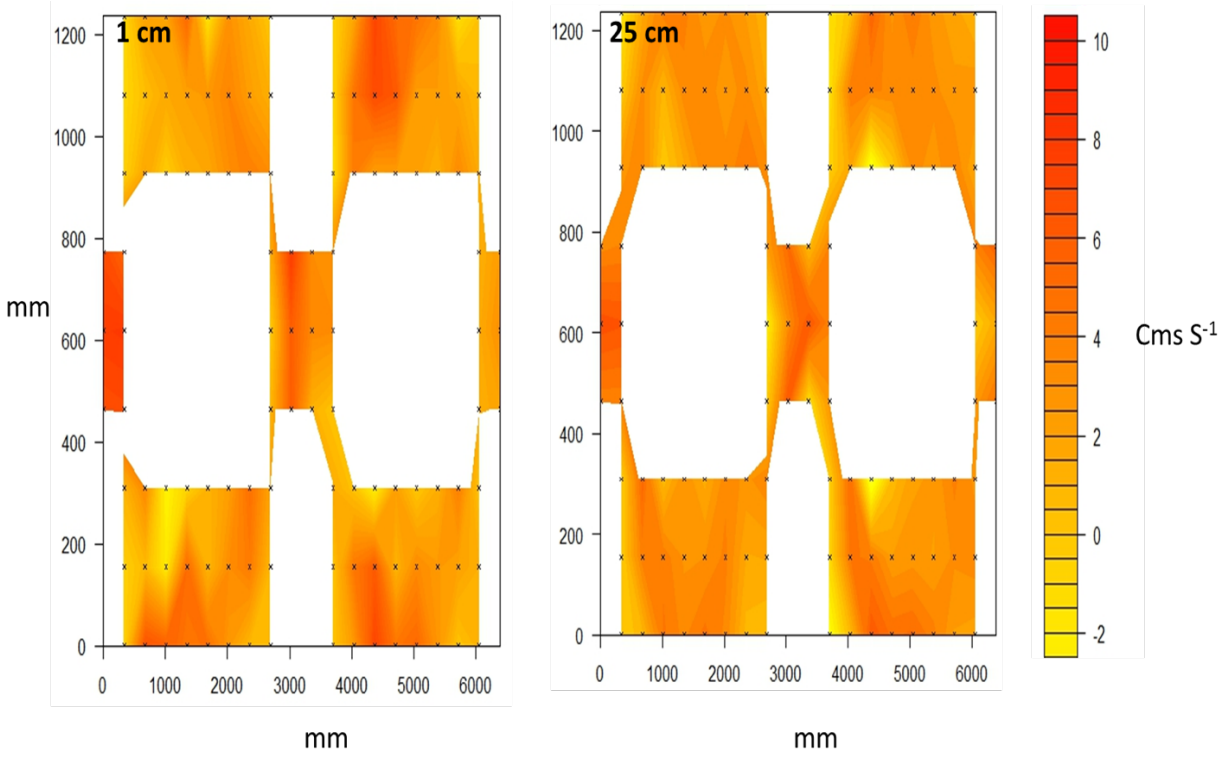


Figure 5.4 - Hydrodynamic profile within the maze at two different depths, 1 and 25 cm above the floor of the maze. Each dot on the map is a measurement point taken with the Valeport.

5.3.4 **Experimental trials**

Trials took place between the 26 February and 18 March 2015 for river lamprey and silver eel (mean flume temperature: 9.8°C S.D. ± 0.8°C), and between the 19 and 21 March 2015 for yellow eel (mean flume temperature: 10.6° S.D. ± 0.5°).

A total of 460 trials (162 silver and 134 yellow eel; 164 river lamprey), each using a single fish, were conducted during hours of darkness (between 18.00 and 06.00); half under configuration 1 (stimulus activated in C2 and C3) (see Figure 5.3) and half under configuration 2 (stimulus

activated in C1 and C4). Thus, fish were presented with the option of moving through each chamber via either an ensonified or control corridor.

Following the guiding principles of ethical research using animals, we reduced the number of fish used while increasing statistical power by exposing all fish to both configurations. Fish from each of the three groups (silver and yellow eel and lamprey) were randomly divided into two separate batches (B1 and B2). B1 were first tested under configuration 1, followed by B2 under configuration 2. After a period of recovery that ranged from 2 to 10 days, B1 was tested under configuration 2, followed by B2 under configuration 1.

European eel were released from the upstream end of the maze while river lamprey were released downstream, reflecting the migratory life history of the species. Trials were completed once the fish had passed through the maze or 60 minutes had elapsed.

Prior to the start of each trial, water temperature in the flume was compared to that of the holding tanks to ensure that temperatures never differed by more than 2°C to limit stress to the fish. During both experimental periods, the temperature difference between tanks (silver eel and lamprey: mean = 9°C, S.D. ± 0.8°C; yellow eel: mean = 12.3°C, S.D.± 0.6°C) and the flume (silver eel and river lamprey: mean =9.8°C S.D. ± 0.8°, and yellow eel: mean = 10.6°C S.D. ± 0.5°C) never exceeded the 2°C limit. In addition, fish were acclimated to flume conditions in a porous container in the channel for at least 1 hour before transfer to the "release area" (Figure 5.1).

5.3.5 **Fish behaviour and Data analysis**

Route selection: The movement of fish through the maze was recorded and video data analysed. Only trials in which fish passed the entire maze (i.e. both chambers) were included in analysis (98.8% and 100% of the silver and yellow eel, respectively, and 85.9% of river lamprey). Passage was deemed to have occurred when the test fish passed through the entire length of a corridor. In cases when a fish passed through a single chamber multiple times (after returning in the opposite direction), only the first pass was recorded and included in further analysis. The number of passes that occurred through the ensonified corridors was calculated as a proportion of the total number of passes through both control and treatment routes. Pearson's Chi-squared tests were used to evaluate whether the number of passes differed with chamber, configuration and treatment.

Rejection: The number of fish that expressed a rejection, when the direction of travel was reversed, within the speaker region (extending 450 mm upstream and downstream from the speaker edges – total length 1350 mm, Figure 5.1) was quantified. Eel only were observed

swimming backwards, while both eel and lamprey were observed to turn around and swim away from the speaker. The number of trials in which a rejection was displayed was expressed as a proportion of the total conducted. Chi-squared tests were used to assess whether the observed number of fish displaying a rejection differed between chambers, configurations and acoustic treatments.

Time to pass: The time taken to pass the region of the speaker was calculated. For fish that rejected the region of the speaker, "time to pass" was calculated as the duration from first entering to leaving the same speaker region, or the alternative speaker within the same chamber for those fish that, having rejected, selected the opposing route. A mixed-effect model was used to interpret these data by including the following factors: 1) "Wall" (fixed factor with 2 levels) = "Speaker" or "Opposite" indicating whether or not the fish passed along the wall in which the speaker was installed; 2) "Sound" (fixed factor with 2 levels) = "ON or "OFF"; 3) "Configuration" (fixed factor with 2 levels) = 'Config. 1" or "Config. 2" indicating the acoustic configuration fish were tested under; 4) "Chamber" (fixed factor with 2 levels) = "First" or "Second"; 5) "Batch" (fixed factor with 2 levels) = "B1" or "B2" indicating the batch each individual had been assigned to; 6) body "Length" and 7) body "mass" (continuous factors); 8) "Rejection" (fixed factor with 2 levels) = "Yes" or "No" indicating if the fish performed a rejection during the trial; 9) "ID" (discreet random factor) identifying each individual; and 10) "Day" discrete random factor defining the day on which the trial was conducted. "ID" and "Day" were included in the model as random uncontrolled factors to test whether response differed among fish and day.

In order to tackle any problem of heteroscedasticity of the data, it is common practice to perform a transformation of the dataset. For this specific experiment a Box-Cox transformation was applied to the data.

5.4 Results

5.4.1 Route selection

For both yellow and silver eel, route selection did not differ between chamber, configuration or treatment (Figure 5.5). For river lampreys, the route selection did not differ between chambers. Nevertheless, a difference was observed for the route selection between configurations (X^2 = 6.82, df = 1, p-value = 0.009), with more lampreys passing through the ensonified corridor of the first chamber in configuration 2 compare to configuration 1 (Figure 5.5). River lamprey were more

likely to pass through the ensonified treatment corridor than the control in the first chamber (X^2 = 19.18, df = 1, p-value = 0.001), but not in second (X^2 = 3.18, df = 1, p-value = 0.0743) (Figure 5.6).

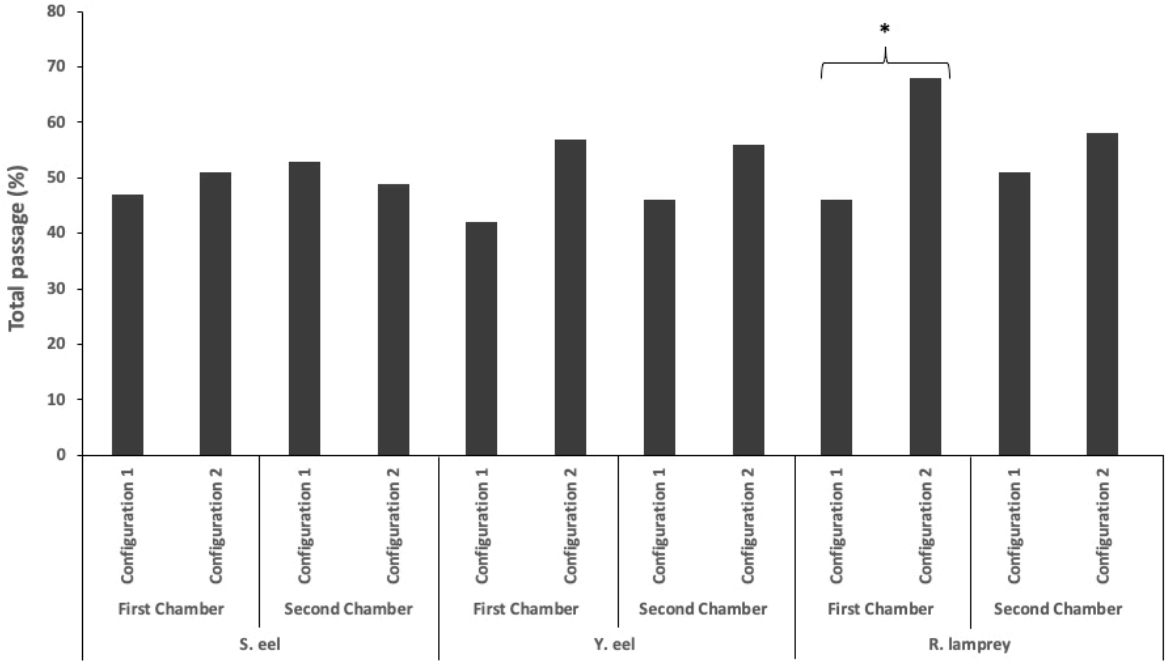


Figure 5.5 - The percentage of total passes by silver and yellow eel and river lamprey through the ensonifed treatment corridor presented in two chambers of an acoustic maze under two configurations tested in which treatment route was alternated. Asterisk indicates a significant difference.

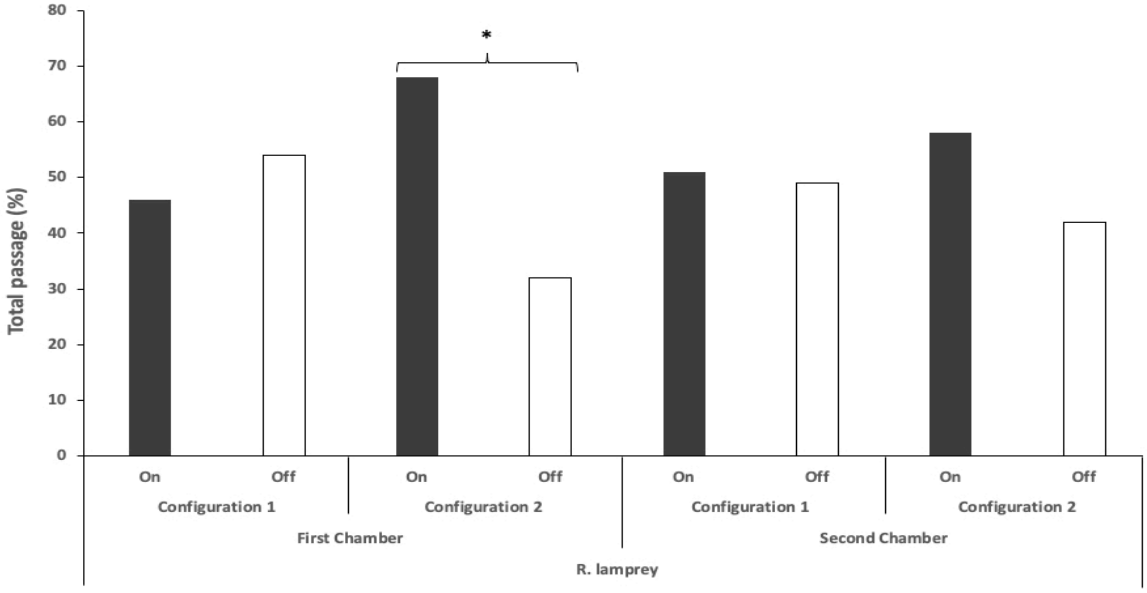


Figure 5.6 - The percentage of total passes of river lamprey through the ensonified treatment and control corridor for each chamber of an acoustic maze under two different configurations. Asterisk indicates a significant difference.

5.4.2 Rejection

For all species / life-stages, neither chamber nor configuration influenced the number of fish exhibiting a rejection. For European silver eel and river lamprey there was an influence of treatment, with greater rejection in the ensonifed corridor (Figure 5.7). For yellow eel there was no effect of treatment under configuration 2. Only one rejection of the control corridor was observed, that being by a silver eel.

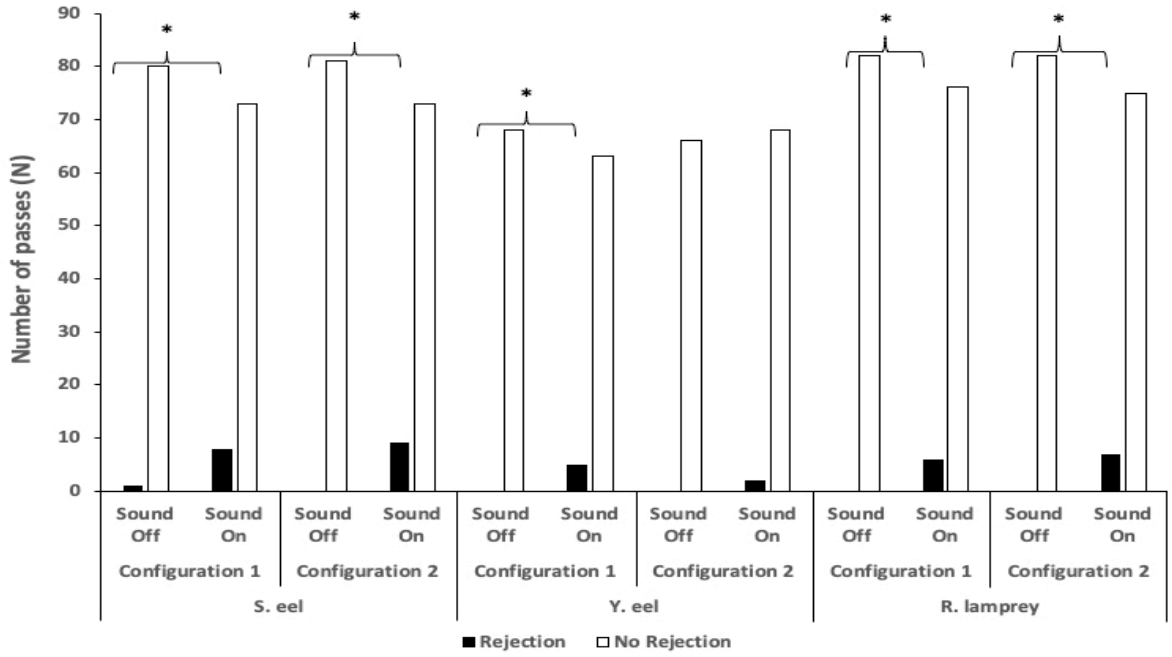


Figure 5.7 - Number of passes (clear bars) (all species) and rejections (solid bars) of the speaker in the treatment and control corridors under both configurations. Asterisks indicate a significant difference.

5.4.3 Time to pass

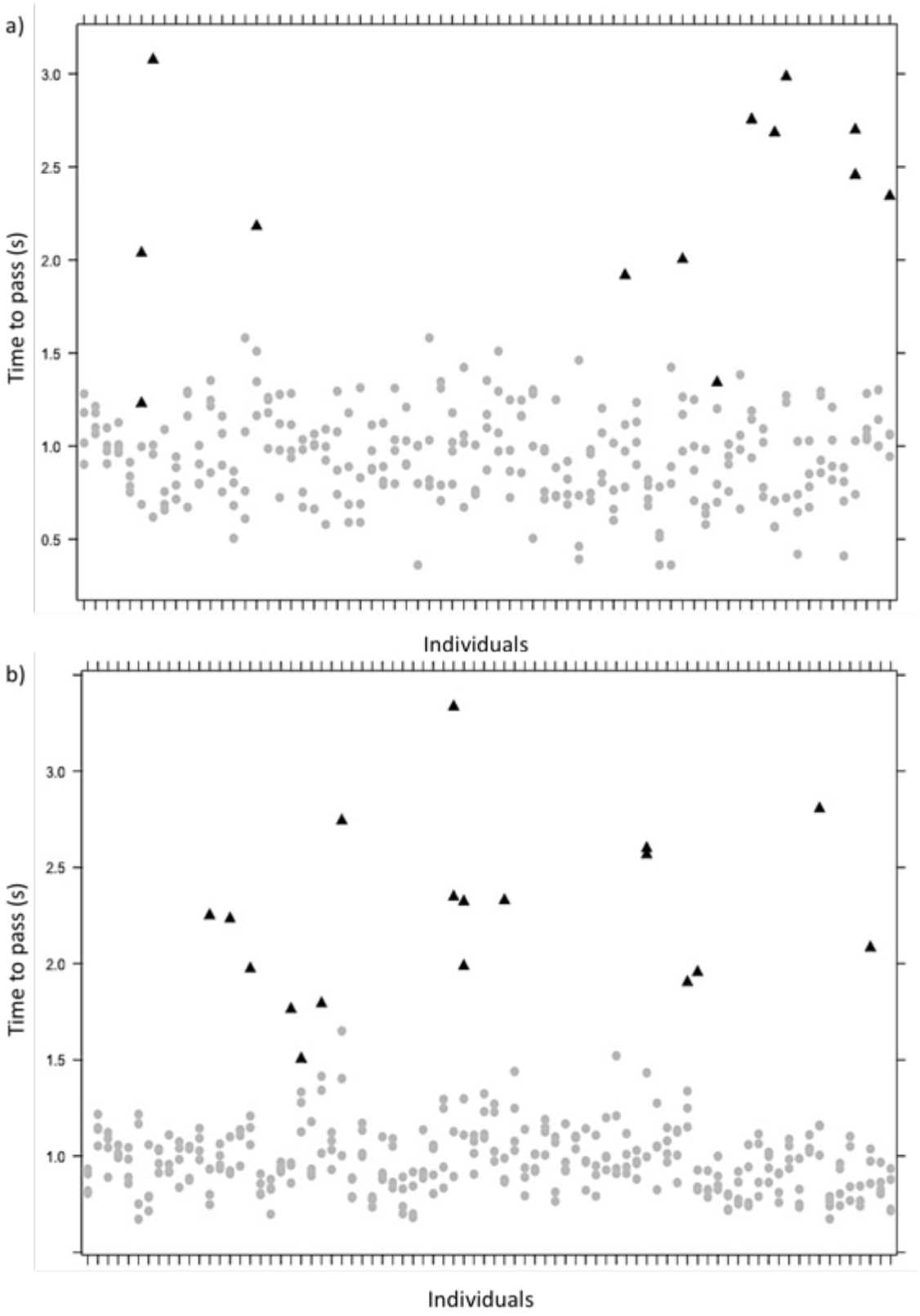
With the exception of river lamprey that passed more rapidly through the first chamber (df = 276, F-value = 3.91, p-value = 0.05), no difference was observed between chambers for European eel. Time to pass was not influenced by configuration for either eel or lamprey. For both life stages of European eel, and river lamprey, time to pass was not influenced by treatment (Table 5.1). Body length had an effect on time to pass in silver eel (df = 312, F-value = 4.05, p-value = 0.04) and river lamprey (df = 276, F-value = 4.56, p-value = 0.03), but not for yellow eel, with longer individuals being generally slower.

Both life stages of eel tended to pass more rapidly along the wall with the speaker (Silver eel, df = 312, F-value = 9.72, p-value = 0.002; yellow stage eel, df = 259, F-value = 4.58 , p-value = 0.03), and time to pass was greater when both eel and lamprey exhibited a rejection (Silver eel, df = 312, F-value = 135.55, p-value < 0.0001; yellow stage eel, df = 259, F-value = 80.70, p-value < 0.0001; river lamprey, df = 276, F-value = 109.3, p-value < 0.0001) (Figure 5.8).

The inter-subject variability was high for eel, especially the yellow phase, and greater than that for day (ID = 50% and Day < 1%) in yellow eel. This was not the case, however, for silver eel for which a greater day effect was evident (51.9% versus 40.4%). For river lamprey, the percentage of variation in the data explained by the difference among individuals (ID) and day was not as pronounced as that for eel (ID = 13.4% and Day = 19.9%). There was no influence of batch.

Table 5.1 - Results from the fixed factors model for fish passage time and percentages of variability due to the random factors (ID and Day). - *Asterisks mark the statistically significant results (at the 5% level)

Variable	**Passage Time**		
Species	*S. Eel*	*Y.Eel*	*R. Lamprey*
Factors :			
Sound	p = 0.7254	p = 0.1829	p = 0.5503
Chambers	p = 0.0628	p = 0.4406	p = 0.0492*
Configuration	p = 0.2778	p = 0.1003	p = 0.2490
Weight / Length	Weight (0.0564) Length (0.0452*)	Weight (0.7229) Length (0.9511)	Weight (0.5920) Length (0.0339*)
Wall	p = 0.0020*	p = 0.0334*	p = 0.8728
Rejection	p ≤ 0.001	p ≤ 0.001	p ≤ 0.001
Interindividual Variability (ID) &	ID = 40.4%	ID = 50%	ID = 13.4%
Variability between Days (Day)	Day = 51.9%	Day < 1%	Day = 19.9%


a)
Time to pass (s)
3.0
2.5
2.0
1.5
1.0
0.5
Individuals
b)
Time to pass (s)
3.0
2.5
2.0
1.5
1.0
Individuals

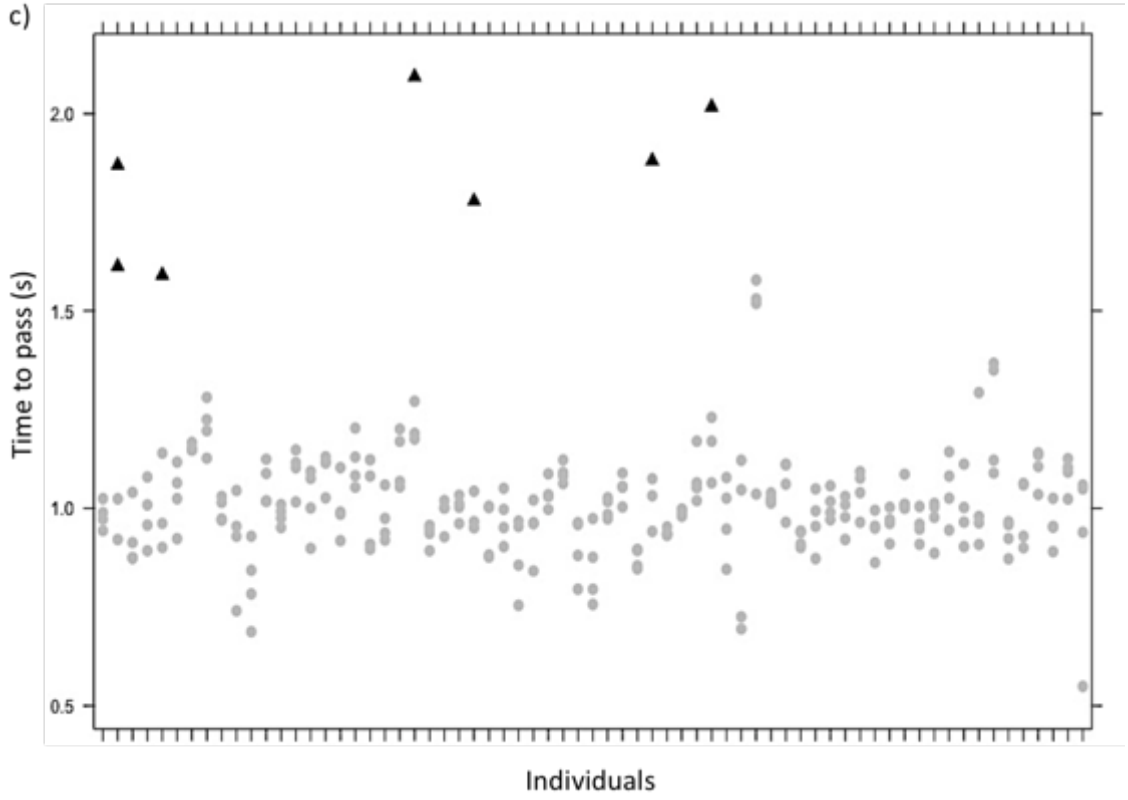


Figure 5.8 - Time to pass for (a) river lamprey, (b) silver eel, and (c) yellow eel. A Log10 has been applied to the data to optimise graphs resolution (Y-axis). Black triangles and grey circles indicate time to pass during attempts with and without a rejection, respectively. Data shown have been transformed via a Box-Cox transformation used in the Mixed Effect Model.

5.5 Discussion

In this experimental study we investigated the behavioural response of European eel (silver and yellow stages) and adult river lamprey to a broadband stimulus (60 to 1000 Hz) using a test that twice offered a choice between an ensonified corridor and a control route. No preference for treatment or control was evident, with the exception of lamprey that tended to pass through the ensonified route in the first chamber encountered only under configuration 2. Furthermore, there was no influence of treatment on the time taken to pass the maze for any of the species / life-stage tested, but passage took longer for those fish that exhibited a rejection, which was observed in the ensonified corridor on all but one occasion.

Our results indicate that despite inducing an avoidance response (rejection) in both species, the acoustic stimulus encountered did not alter the overall probability of passing a particular route, or time taken to do so. This is the first study to our knowledge that tested river lamprey response to

sound. The apparent preference for the ensonified channel in the first chamber encountered under one configuration was unexpected. This could have reflected the weaker acoustic signal close to the channel floor where lamprey may have travelled (see Russon et al. 2010 for description of river lamprey utilising boundary zones at the channel walls), a suggestion supported by more rapid passage observed under that treatment. For eel, previous studies investigating response to sound, however, have provided inconclusive and sometimes contradictory results. While Sand et al. (2000) describe the potential of low frequency (11.8 Hz) sound to divert downstream migrating eel in the field, MacNamara (2012) and Piper et al. (in press) were unable to replicate their findings at other sites. As a result, the justification for employing infrasound deterrents remain uncertain, especially considering their cost and difficulty in installing what are typically large units that generate limited sound fields due to the weak propagation under shallow water conditions associated with fluvial environments (Noatch & Suski 2012).

Identification of appropriate acoustic stimuli is the first challenge facing those that intend to develop acoustic deterrents for fish. High frequency sound (e.g. > 20 kHz) can elicit responses within some families, such as the salmonids and clupeids (Popper & Carlson, 1998), although this has not been reported for anguilliform species. The sound source used in this study was designed to provide a small and relatively easy to deploy unit capable of generating broadband acoustic signals in the range of 60-1000 Hz. This ensured that the frequencies employed corresponded with those identified to be within the hearing sensitivity of eel based on the audiogram produced by Jerko (1989), in which greatest sensitivity was determined to be between 80 and 100 Hz. We are unaware of any audiogram published for river lamprey, and recommend that clarification of hearing sensitivity in this species is an area worthy of future investigation.

Earlier work suggests that frequencies above the infrasound range can influence eel response, as observed for juveniles and adult American eel (*Anguilla rostrata*) (< 1000 Hz; Patrick et al. 2001). However, better understanding is needed on how intensities and temporal patterns of acoustic signal likely influence response, with suggestions that intermittent sounds may inhibit rates of recovery in some species (e.g. European seabass, *Dicentrarchus labrax*, Neo et al. 2014). Indeed, in a follow up experiment that explored the use of sound to improve the effectiveness of physical screens, Deleau et al. (under review) compared the response of eel to the continuous broadband signal (60 – 1000 Hz) used in this study and an intermittent pulsed stimulus (100 Hz). Overall, they observed similar responses, although passage times were shorter for eel exposed to the pulsed stimulus. Further research is required to test a wider variety of frequencies, intensities, and

temporal patterns of sound to help select those most appropriate in the development of effective fish guidance systems.

When developing conservation technology, such as behavioural deterrents designed to protect populations of migratory fish, intraspecific variability in response is an important determinant of overall operating efficiency. Unlike for lamprey, eel in our study, and particularly the yellow life-stage, exhibited high levels of inter-subject variability in passage time. Furthermore, in silver eel and lamprey body length influenced passage time, with large silver eels taking longer. Previous research has also demonstrated intraspecific variability in European eel response to sound. In one study, eel that were in poor condition were more likely to exhibit negative physiological (increased ventilation rates) and behavioural (reduced startle in the presence of a looming predatory stimulus) responses to playbacks of shipping noise (Purser et al. 2016). Further research is needed to quantify intraspecific variability in response to stimuli tested and to incorporate this into design and operation of future deterrent devices developed to protect fish populations.

This study demonstrated that acoustic stimuli were able to induce behavioural avoidance, all be it limited when viewed from a holistic perspective, in some European eel and river lamprey under the experimental conditions described. This is important because it indicates that sound may have potential in the development of behavioural deterrents that might be used either in isolation or in combination with other stimuli, or traditional screening devices (Noatch & Suski, 2012). However, more work is needed to identify which frequencies, intensities, and temporal patterns are most effective in this endeavour.

CHAPTER 6

6 Use of acoustics to enhance the efficiency of physical screens designed to protect downstream moving European eel (*Anguilla anguilla*)

6.1 Summary

During their seaward migration, European eel (*Anguilla anguilla*) are vulnerable to entrainment at a variety of man-made intakes, including those that lead to hydropower turbines or other abstraction points, e.g. for irrigation. Physical screens traditionally employed to protect fish by preventing entrainment and guiding them to safer bypass systems suffer from variable and often low efficiencies. Two experiments were conducted during hours of darkness to investigate the potential for acoustic stimuli to improve the efficiency of a vertical bar-screen placed at 45° to the direction of flow to guide downstream moving eel to a bypass in an external flume. A series of three underwater speakers were installed along the channel wall immediately upstream of the screen. In the first experiment (1), screen guidance efficiency recorded in the presence (treatment, n = 79) and absence (control, n = 78) of a continuous broadband stimulus (CBS: 60 to 1000 Hz) was compared. Adopting a "before-after" design, the second experiment (2) assessed the guidance of the control eel used in experiment 1 when exposed to a 100 Hz pulsed stimulus. During both experiment 1 and 2 the majority of eel were guided to the bypass, with three passing through the screens during the control compared to one during each acoustic treatment. Rejection of the area immediately adjacent to the speakers by moving back upstream was more common during the acoustic treatment (CBS: 16.4%; pulsed: 12.8%; control: 2.5%). Eel moved past the speakers more rapidly in the presence of sound (median time to pass: CBS = 6.8 s; pulsed = 5.4 s) than during the control (8.1 s). Although improvements in guidance were subtle, the results suggest there may be potential for employing acoustic stimuli to enhance the guidance efficiency of physical screens for eel. However, more experimental work is needed to define the most effective acoustic characteristics for eel guidance and to validate improvements in screen efficiency under field settings.

Use of acoustics to enhance the efficiency of physical screens designed to protect downstream moving European eel (Anguilla anguilla)

6.2 Introduction

Historically, the European eel (*Anguilla anguilla*) sustained a large number of small-scale fisheries throughout its range (Dekker 2003), and thus was highly valued for its socio-economic importance. Over recent decades, stocks have declined to levels considered to be outside safe biological limits (Astrom & Dekker 2007). Some estimate that glass eel recruitment declined to 5% of the pre-1980 level (EIFAC 2006), while others suggest that the abundance of seaward migrating adult silver eel decreased by as much as 90% between 1975 and 2010 (Bevacqua et al. 2015). Concerns over the potential extinction of the European eel led to its protection under international legislation. This included the European Council Regulation (EC 1100/2007) which established a framework for the protection and sustainable use of stocks in the member states through administration of local eel management plans. European eel is also listed under appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which places controls on international exports.

Several contributory factors for the decline of eel have been proposed (Feunteun 2002), including changes in oceanic currents (Baltazar-Soares et al. 2014), habitat loss (Bevacqua et al. 2015), pollution (Maes et al. 2005), non-native parasites (Wielgoss et al. 2008; Newbold et al. 2015), overfishing (Aalto et al. 2015), and river engineering (Piper et al. 2013). Water and energy infrastructure, in particular, has received much attention, with concerns over the loss of migratory juveniles and adults at intakes to water supply facilities, pumping stations, fish farms, power station cooling systems, and hydroelectric turbines (Calles et al. 2012; Piper et al. 2013). In response, regulatory agencies have imposed stringent guidelines and criteria for eel protection at these points of abstraction (Sheridan et al. 2014). Increased interest in constructing new, or replacing existing, energy infrastructure that depend on water use, largely associated with a drive towards renewables (e.g. small-scale hydropower), highlights the need for a parallel advance of environmental impact mitigation strategies so that adverse effects of resource development on other ecosystem services are minimised.

Traditional fish protection approaches at water intakes have tended to focus on physical and mechanical screens (Kemp 2016). There is a wide variety of designs, some reflecting a long-term process of incremental development at specific sites for a limited number of target species of interest (Gessel et al. 1991). Unfortunately, these screens can negatively impact the fish they are designed to protect. Fish may become impinged and suffocate on the screen surface if the local current velocities are greater than their burst swimming capabilities (Calles et al. 2012), or they

may suffer physical abrasion after making contact (Swanson et al. 2005), potentially leading to secondary infection and delayed mortality. Inefficient screens installed to guide fish to alternative bypass routes, but that fail to do so, can have negative consequences in terms of elevated energetic costs and predation risk associated with a delayed migration (Schilt 2007). For eel, screens can be particularly problematic. They have an elongated body morphology and relatively low burst swimming capability, both of which may increase the probability of impingement. Further, owing to their small size as juveniles, and low aspect ratio, eel are more easily entrained through some screen types than many other species, resulting in calls to retrofit existing facilities with extremely narrow spaced designs (1-2 mm mesh size: Sheridan et al. 2014). This is of concern to industry because of the economic implications of doing so, both in terms of cost of installation and maintenance, and the limited evidence on which such guidance is based. Therefore, the provision of alternative less costly options that have been validated is a subject of great interest.

Devices that employ behavioural stimuli (e.g. acoustics, lights, bubbles, hydrodynamics or combinations of these) to induce an avoidance response and so deter fish from entering dangerous areas, such as intakes, may provide an alternative to traditional screens, or be used in combination to improve their efficiency. The development of behavioural deterrents for eel is not new, and devices that are based on a range of stimuli, such as infrasound (Sand et al. 2000; Sand et al. 2001) and strobe lights (Patrick et al. 1982), are commercially available. Unfortunately, in many instances they have been developed through a process of trial-and-error and their effectiveness seldom quantified by robust experimental studies; when evaluation has taken place, the results are often contradictory and inconclusive. As a result, behavioural deterrents are generally considered less efficient than physical and mechanical screens. Nevertheless, behavioural screening devices remain appealing, should high efficiencies be attainable, as they represent a much sought-after solution to the challenge of developing sustainable water and electricity generating infrastructure systems in a cost-effective manner.

There are several possible explanations for the ambiguous results obtained when the efficiency of behavioural deterrents is evaluated. Considering acoustic stimuli, previous studies conducted *in situ* invariably fail to control confounding variables. Furthermore, the sound field can be highly heterogeneous (e.g. as a result of reflection from walls, the sediment and the air/water interface) in ways difficult to predict. In the body of water in which an acoustic fish deterrent is located, there will be multiple reflecting surfaces, the most ubiquitous being the air-water interface which, unlike almost all others, reflects as an approximate ‘pressure-release interface’, generating low acoustic pressures (Leighton, 2012). The acoustic properties of an environment are commonly

poorly characterised a priori, particularly in regards of the sediment, and are typically time-varying (Wysocki et al. 2007), depending on factors like water temperature and depth. Recognition of the variation in the acoustic fields generated by a source varies between studies. Some authors completely fail to define the sound field (Maes et al. 2004), while others conduct mapping, but with insufficient resolution to capture key variations in intensity that are likely to occur (Nestler et al. 1993; Ross et al. 1993). This is problematic because the assumption that an active sound source will create a predictable acoustic field is likely to be incorrect, especially in relatively shallow river or estuary environments, or near infrastructure such as intake channels. Furthermore, different fish species exhibit different sensitivities to the components of an acoustic field, e.g. some fish, in some frequency ranges, are more sensitive to particle motion than to acoustic pressure (Radford et al. 2012). It is important to identify to what component of an acoustic field the fish of interest is most sensitive to and to ensure that the mapping conducted captures the variation in that component. This complexity may explain, at least in part, why different studies report conflicting results. There is a need to conduct robust, replicable and controlled experiments in which fish response to a well-defined acoustic gradient is quantified.

This study adopted an experimental approach to test the potential of acoustic stimuli to improve the efficiency of traditional physical screens to guide downstream migrating silver eel to a bypass channel, a commonly installed structure at many in-river barriers. The behaviour of eel in response to encountering the sound field was quantified, in terms of avoidance (rejection) and time taken to pass the area of acoustic influence. During the design of the study we addressed two key challenges to real world application of an acoustic deterrent. First, many previous studies focus on marine species of fish and their response to acoustics propagation under relatively deep-water conditions (Fewtrell & McCauley 2012). In comparison, the downstream fluvial migration of eel occurs in shallow water in which acoustic fields are strongly influenced by the riverbed and banks. We, therefore, conducted the study in a large open-channel external flume. Second, although infrasound (<20 Hz) has long been promoted as a potential deterrent for eel (Sand et al. 2000), there are several limitations. Expensive commercial devices capable of generating infrasound at amplitudes sufficient to deter fish have needed to be large, and thus challenging to deploy in shallow water environments. Further, the zone where the infrasound is loud enough to act as a deterrent is often limited to within a couple of metres from the source (Popper and Carlson 1998). Based on an audiogram constructed for European eel (Jerkø et al. 1989), hearing ranges between 60 and 400 Hz, with a peak in sensitivity at around 80 Hz. In the interest of developing a small and relatively cost-effective deterrent, two experiments were conducted in which we presented: 1) a continuous broadband stimulus with frequencies ranging from 60 to

1000 Hz, and 2) a 100 Hz pulsed stimulus. The amplitude of the two test signals reached a maximum of 160 dB re 1 µPa at the measurement point closest to the sound source, decreasing to 135 dB re 1 µPa at 50 cm from the source with an attenuation of almost 5 dB every 10 cm. In experiment 1, the behaviour of downstream moving eel under the continuous broadband treatment was compared with that obtained under a control (ambient background sound only). In line with the principles of ethical science that challenges the researcher to reduce the number of individuals used in experimental studies, experiment 2 adopted a "before-after" design in which the control fish used in experiment 1 were exposed to the pulsed treatment. The results of this study provide important insight into the use of acoustics to supplement traditional technologies designed to protect downstream moving eel at river infrastructure.

6.3 Materials and Methods

6.3.1 Fish collection and maintenance

European eel (length: 305–815 mm; mass: 57-1352 g) were caught during their seaward migration using a fixed eel trap on the River Stour near Longham, United Kingdom (50°46'31.6"N 1°54'38.1"W), in November 2015. Fish were transported to the International Centre for Ecohydraulics Research Facility, University of Southampton (50° 57'42.6"N, 1°25'26.9"W), in two large transportations tanks filled with aerated river water. The fish were maintained in four separated 3000 litre holding tanks, equipped with individual filtration systems and separate air pumps. Water was monitored daily and maintained through regular water changes (50% weekly) using dechlorinated tap water (pH = 7.8, Nitrate: <40ppm). Mean water temperature was 10.5 °C (S.D. ± 0.9 °C).

6.3.2 Experimental setup

A concrete block channel (5.28 m length, 1.66 m width, 0.56 m depth) was constructed within an outdoor recirculatory flume (Figure 1). Wire mesh screens (13 x 13 mm mesh, 1 mm gauge) were installed at either end of the channel to prevent escape of the subject fish. A concrete block wall (1.32 m length) longitudinally divided the downstream section of the channel to create a 0.52 m wide bypass. A vertical bar screen (bar-spacing 12 mm) was installed between the channel wall and bypass entrance at an angle of 45° to the direction of flow (Russon et al. 2010; Figure 6.1).

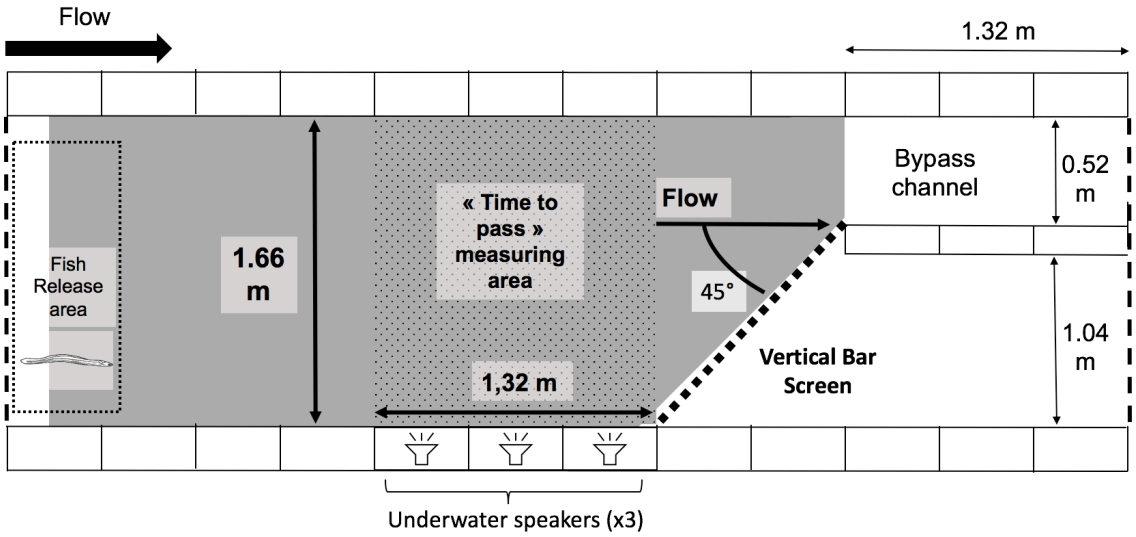


Figure 6.1 - Plan of a concrete block channel installed within an outdoor recirculating flume. The dashed lines indicate the position of wire mesh screens that contained adult silver European eel (*Anguilla anguilla*) within the experimental area. A bar screen installed at 45° to the direction of flow (bold dotted line) was designed to guide fish to the entrance of a bypass channel. The shaded zone represents the area where the acoustic (Figure 6.2) and hydrodynamic (Figure 6.3) fields were mapped using a hydrophone and acoustic Doppler velocimeter (ADV), respectively. The speakers were installed in a horizontal series at the channel floor. The dotted zone represents the area for which "time to pass" was measured.

An array of three underwater speakers (ElectroVoice UW30) were installed within the channel wall immediately upstream of the screen (Figure 6.1). Because previous experiments indicate downstream moving silver eel maintain position at, or close to, the substrate (Russon et al. 2010), the speakers were positioned close to the channel floor at a depth of 0.5m. During treatments, the speakers generated a continuous broadband sound (CBS: 60 - 1000 Hz) (experiment 1) and a 100 Hz pulsed (experiment 2) acoustic field (Figure 6.2), and were turned off during the control trials. The sound production system consisted of a laptop (Dell © Latitude E6430) linked to a National Instruments Data Acquisition Box (National Instruments © USB-6251) connected to a Power Amplifier (SkyTronic © Mini AV Digital Surround Amplifier 103.100) to which all three speakers were connected. The acoustic field was measured along 20 cm transects at 17 equidistant points 10 cm apart (Figure 6.2) using a calibrated hydrophone (Brüel & Kjær © 8105) at a depth of 3 cm (S.D. ± 2 cm due to channel floor irregularities) above the floor.

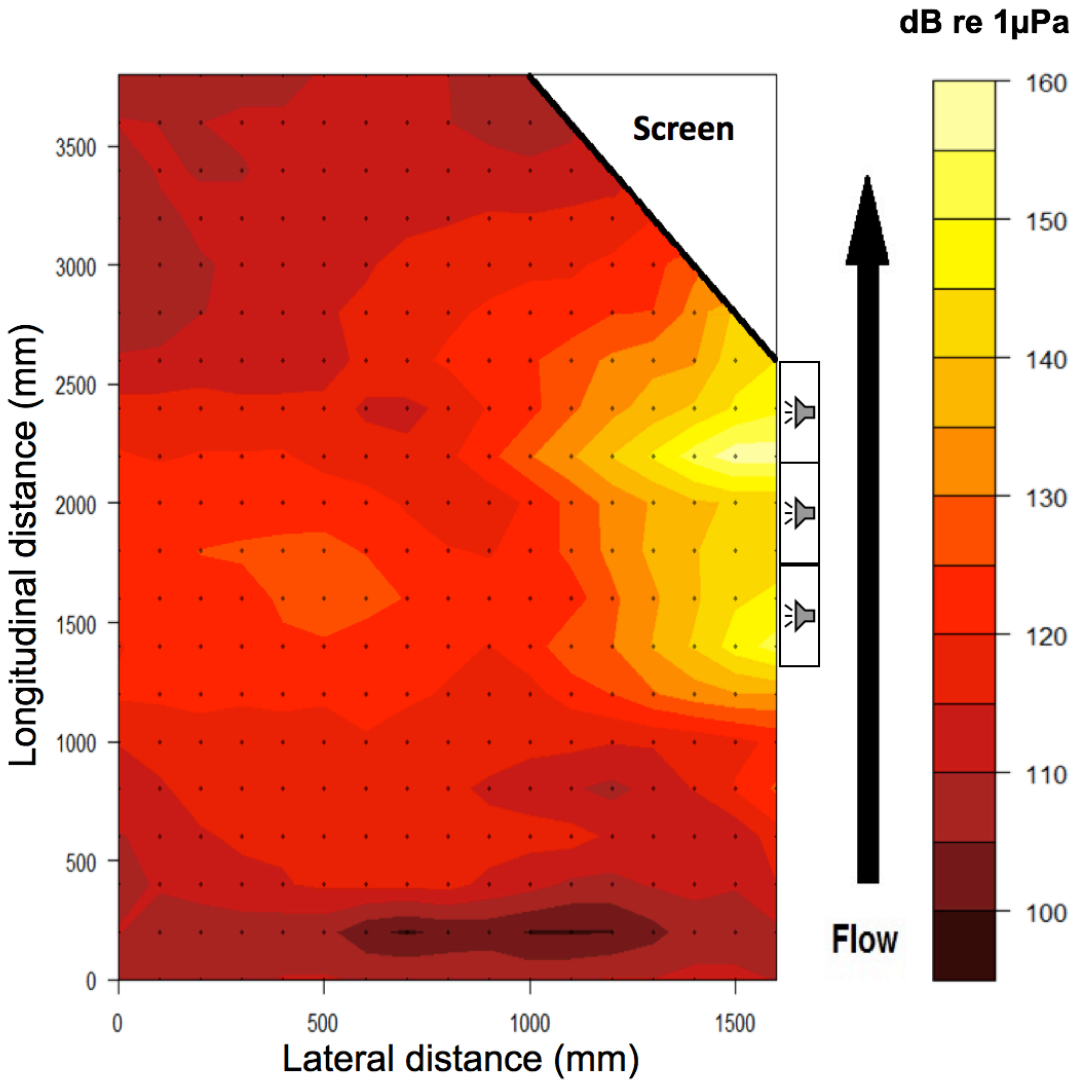


Figure 6.2 - Acoustic field generated in the experimental area upstream of the bar screen using continuous broadband noise (CBS). Sound was recorded at regular intervals (dots) along 20 cm transects at 17 equidistant points 10 cm apart using a hydrophone. The position of the speakers is indicated by the three boxes on the right side of the map. Note: High intensities in the sound field are localised to the vicinity of the speakers. This is because the water depth is much less than one quarter of a wavelength and therefore the propagation modes excited are evanescent (White 2004).

Fish movements were recorded using a series of five CCTV cameras with integrated infrared light units (AV-TECH 245 Sony Effio 580TVL CCD) mounted 2.9 m above the channel floor. Four additional 15.0 W infrared lights provided additional illumination to enhance the contrast of the video recordings.

During trials a constant flow (48-50 cm depth; 0.1 m s^{-1} mean velocity S.D. ± 0.01 m s^{-1}) was maintained using three centrifugal pumps. Depth and velocity was measured using a rule and a

Nortek Vectrino+ Acoustic Doppler Velocimeter (Figure 6.3). Mean flume water temperature during the experimental period was 10.2 °C (S.D. ± 0.8 °C).

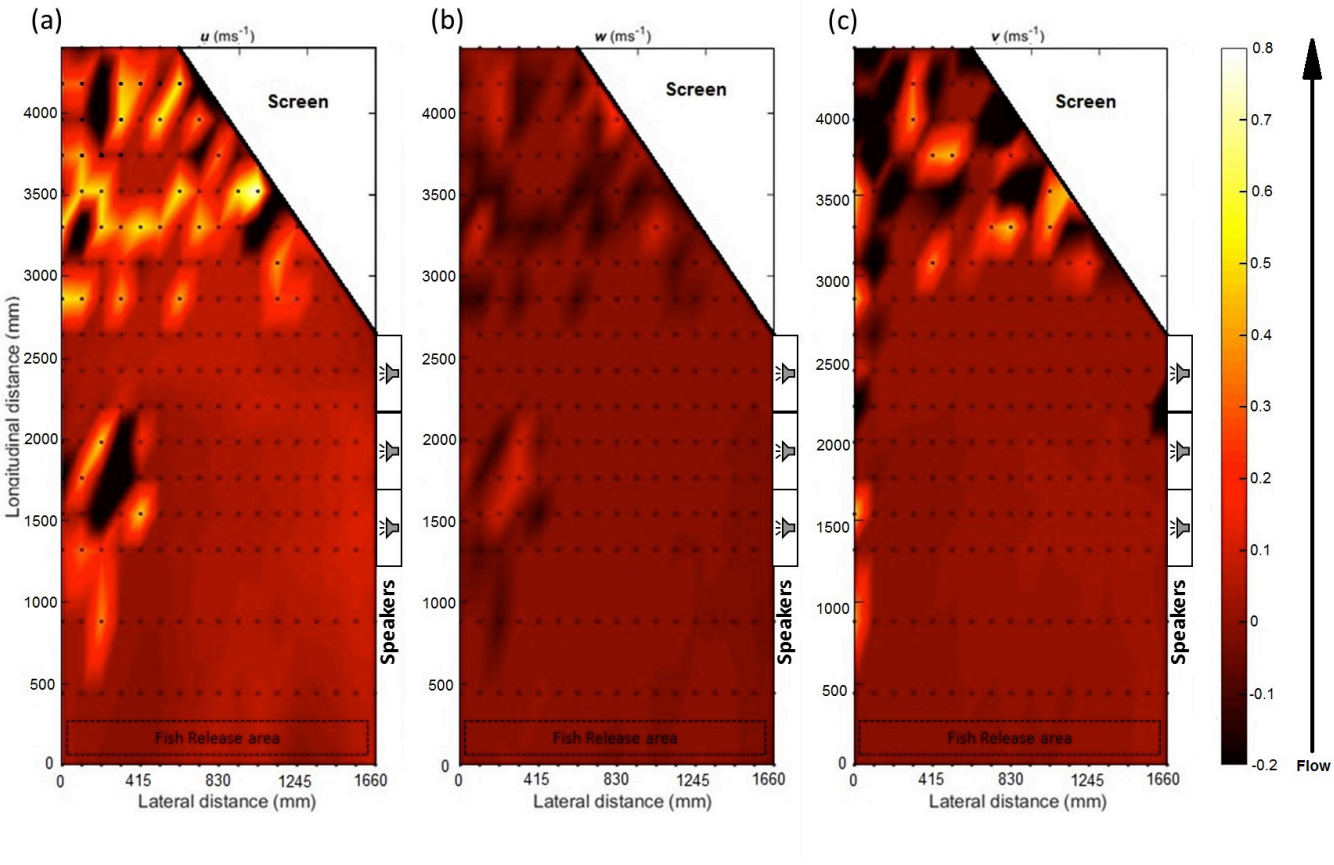


Figure 6.3 - Colour maps of water velocity recorded in an experimental channel used to investigate the response of European eel to acoustic fields encountered upstream of a bar screen. a, b and c illustrate the longitudinal (u), vertical (w) and lateral (v) components of the flow, respectively. Dots indicate the positions of velocity measurements. The scale is expressed in m s-1.

6.3.3 **Experimental trials**

During experiment 1, a total of 78 control and 79 treatment (CBS) trials were conducted during hours of darkness (between 17.00 and 02.00). Adopting a "before-after" experimental design and in-line with the principles of reducing the numbers of individuals of a threatened species used in research, the 78 control fish used in experiment 1 were exposed to the 100 Hz treatment in experiment 2. Eel were introduced into a submerged container situated upstream of the experimental area and allowed to acclimatise for at least one hour prior to the start of the trials. A trial commenced when an individual fish was released from the container close to the upstream end of the right wall and allowed to move volitionally downstream towards the speakers. The trial ended once the fish had passed downstream either via the bypass channel or through the screen,

or after one hour had elapsed. At the end of each trial, the eel was recaptured, measured and weighed.

6.3.4 Fish behaviour

Video recordings of the downstream movements of eel was analysed. The selected passage route (through the screen or via the bypass) was recorded. On approaching the screen, a *rejection* was deemed to occur if on reaching the area immediately adjacent (< 20 cm) to the speakers the eel exhibited a clear change in direction and moved towards the opposite channel wall, swam backwards in a reverse direction to the flow, or turned around and swam back upstream. A Pearson chi-square (X^2) test was used to determine whether there was a difference between the expected and observed frequency or *rejections* for the control and treatments. The *time to pass* the speakers area (1.32 m longitudinal distance, Figure 1) was recorded for each fish. Each dataset was tested for normality by using the Shapiro–Wilk-test. When data were not normally distributed, the Mann–Whitney-U-test was used to test for differences between the treatments.

6.4 Results

6.4.1 Experiment 1

Rejections were higher than expected under the acoustic treatment (N = 13, 16.4%) and lower than expected under the control (N = 2, 2.5%) (Pearson X^2 = 7.231, df = 1, p-value = 0.007) (Table 6.1). Multiple rejections within the same trial were observed for two individuals in the acoustic treatment, with fish respectively exhibiting 2 and 4 rejections before passing through the bypass channel. Eel that did not reject continued downstream to encounter the screen, where the majority were diverted to the bypass channel. Three (3.9%) and one (1.3%) individuals passed through the screen under the control and treatment trials, respectively.

Table 6.1 - Contingency table summarising the number of observed versus expected rejections exhibited by downstream moving adult European eel on encountering either a continuous broadband sound field (treatment) or ambient background noise (control).

	Treatment	
Behaviour	Continuous Broadband Sound (CBS)	Control (Sound Off)
Rejections (observed counts)	13	2
Rejections (expected counts)	7.55	7.45
No Rejections (observed counts)	66	76
No Rejections (expected counts)	71.45	70.55
Total observed	79	78

The median time to pass the speakers was higher under the control (8.1, Interquartile Range (IQR) = 6.58 sec) than treatment (6.8, IQR = 5.54 sec) (Mann–Whitney-U-test: W = 2325.5, p-value = 0.008) (Figure 6.4).

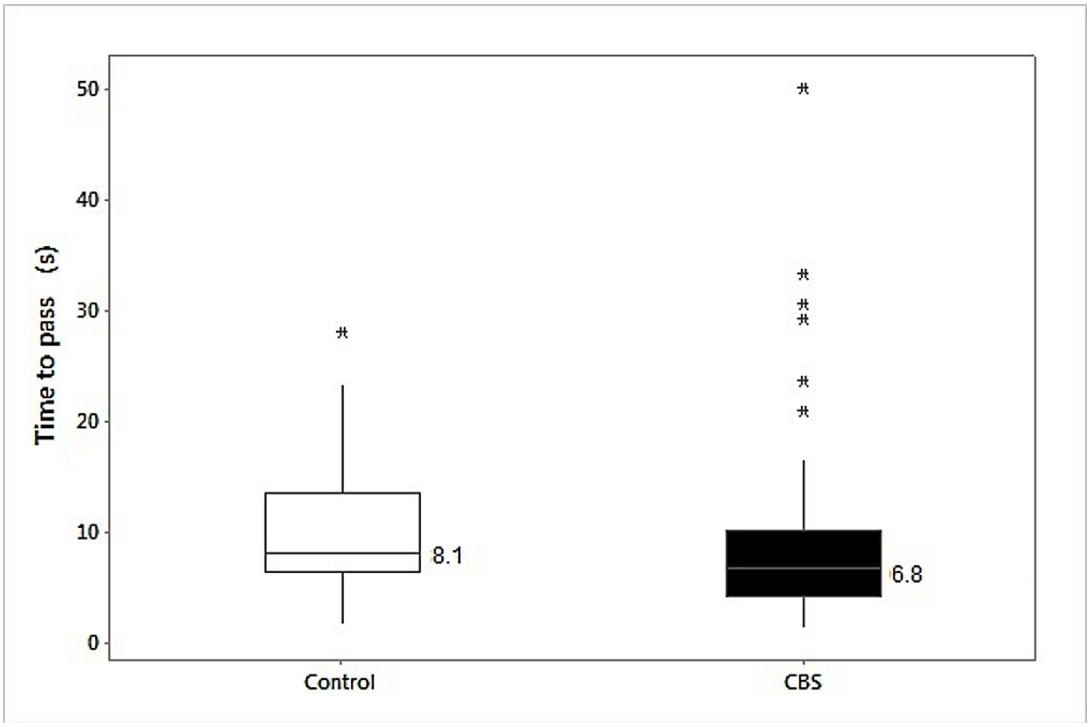


Figure 6.4 - Time taken by downstream moving European eel to pass three speakers positioned immediately upstream of a bar screen in an experimental flume when continuous broadband sound was on (treatment: solid box) or off (control: clear box). The boxes represent the interquartile range, with the bottom and top indicating 25% and 75% quartile. The horizontal line in the middle of the box indicates the median with its value labelled. The whiskers span the highest and lowest observations with the exceptions of outliers indicated by asterisks.

6.4.2 **Experiment 2**

During the 100Hz pulsed treatment, one eel passed through the screen (98.7% of the fish reached the bypass). All the other fish that did not reject the acoustic stimulus and encountered the screen swam to the bypass channel. Rejections were more frequent than expected under the acoustic treatment (N = 10, 12.8%) and less frequent than expected under the control (N = 2, 2.5%) (Pearson X^2 = 8.55, df =1, p-value = 0.014) (Table 6.2). One fish exhibited two rejections during a single trial under the acoustic treatment.

Table 6.2 - Contingency table summarising the number of observed versus expected rejections exhibited by downstream moving adult European eel on encountering either a pulsed 100 Hz sound field (treatment) or ambient background noise (control).

	Treatment	
Behaviour	Treatment 100Hz pulsed	Control
Rejections (observed counts)	10	2
Rejections (expected counts)	6.00	6.00
No Rejections (observed counts)	68	76
No Rejections (expected counts)	72.00	72.00
Total observed	78	78

Time to pass was higher under the control (8.1, IQR = 6.58 sec) than the treatment (5.6, IQR = 3.86 sec) (Mann–Whitney-U-test: W = 2878, p <0.001) (Figure 6.5).

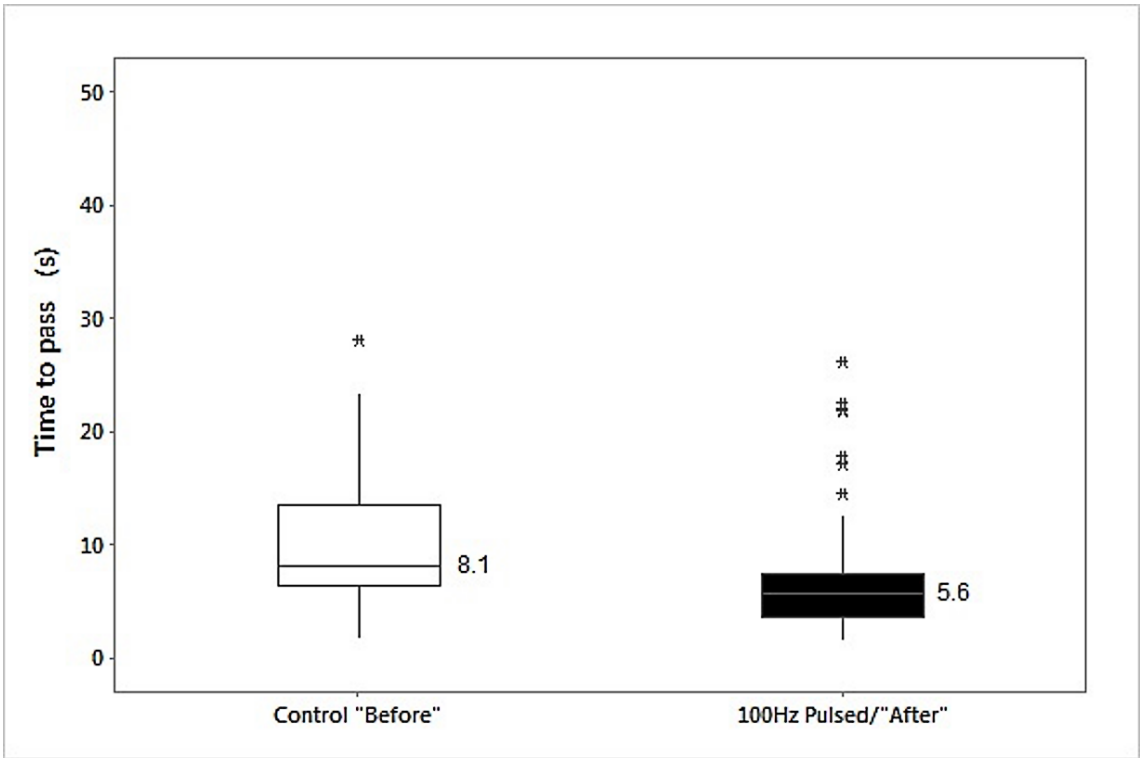


Figure 6.5 - Time taken for downstream moving eel to pass three speakers positioned immediately upstream of a bar screen in an experimental flume in the absence (control: clear box) and presence (treatment: solid box) of a 100 Hz pulsed sound field during Experiment 2. The same fish were used twice in this experiment using a before (control) and after (treatment) design. The boxes represent the interquartile range, with the bottom and top indicating the 25% and 75% quartile, respectively. The horizontal line in the middle of the box indicates the median with its value labelled. The whiskers span the highest and lowest observations with the exceptions of outliers indicated by asterisks.

6.5 Discussion

In this study we investigated the potential of using acoustic stimuli, continuous broadband (CBS: 60 -1000 Hz) and 100 Hz pulsed sound, to enhance the efficiency of a physical screen to guide European eel away from water intakes towards an alternative bypass route. The effects of sound on overall guidance efficiency were subtle under the experimental conditions described, with the majority of eel entering the bypass independent of treatment. Nevertheless, differences in behaviour provide an explanatory mechanism for improved guidance efficiency observed under the acoustic treatments. Eel tended to avoid the acoustic field encountered, either by exhibiting a rejection response during which they altered their swim path to a direction away from the sound source, or by moving downstream rapidly to the bypass entrance. As a consequence, and despite

higher rejection behaviour, the time to pass the zone of influence was shorter and the probability of interacting with the screen lower during the acoustic treatments, resulting in reduced passage through the screen and enhanced guidance to the bypass channel.

This study built on efforts to enhance protection of European eel at river infrastructure. Using a screen configuration and angle observed to effectively guide downstream migrating silver eel to a bypass channel under similar experimental settings (Russon et al. 2010), we added acoustic stimuli in an attempt to enhance screen efficiency still further. In essence, this approach combined multimodal stimuli (hydrodynamic and acoustic) in an effort to improve guidance. Compared to other families, such as the salmonids and clupeids (Popper & Carlson 1998), there has been limited published research related to the development of acoustic deterrents for eel. A notable exception is the work conducted by Sand et al. (2000) that focused on the application of infrasound for this purpose. In their study, a single 11.8 Hz infrasound source was used to manipulate the trajectories of downstream migrating European eel in the River Imsa, Norway. Avoidance of infrasound appeared clear, with the number of eel trapped close to the sound source during exposure reduced to 43% of that obtained during a control period, with a corresponding increase of 144% for eel collected in a trap farthest away. However, contradictory results have also been obtained indicating no (e.g. MacNamara 2012) or limited (Piper et al. in prep) avoidance response exhibited by silver eel migrating in an Irish and English river, respectively. This highlights the need for rigorous controlled experiments in which eel response to well defined acoustic fields are quantified at appropriate scales of resolution.

Infrasound deterrents have several limitations. Commercial devices are often large and as a consequence challenging to deploy under shallow water conditions, while the deterrent effects of the acoustic fields generated tend to be spatially limited due to weak sound propagation across hard substrates and through shallow depths (Noatch & Suski 2012). The potential to use higher frequency sound to guide eel has received little attention in the scientific literature, although some preliminary experimental results for American eel (Anguilla rostrata) indicate that, under some situations, low frequency sound (<1000 Hz) might act as an attractant for both juveniles and adults (Patrick et al. 2001), although to the best of our knowledge no further follow up work has been published to date. Considering the wider body of research that includes investigation of potential environmental impact of sound on fish behaviour, interesting insight is gained from controlled experimental studies that expose European eel to sound of anthropogenic origin. Juvenile eel are less likely to startle in response to a looming predatory stimulus during exposure to playback of recordings of ships passing through harbour (frequency range 100 to 10 000 Hz)

when compared to control treatments that used recordings of the same harbours without ships (Simpson et al. 2015). The depressed startle response is relatively short lived, however, as indicated in a follow up study in which recovery occurred within 2 min after the noise stopped (Bruintjes et al. 2016). Such findings demonstrate the potential to manipulate eel behaviour through exposing them to higher frequency sound than that used by Sand et al. (2000).

Returning to a focus on protecting eel, our experiments used higher frequency acoustic stimuli that encompass the range of sensitivity defined by the Jerkø et al. (1989) audiogram for European eel, and that might be more easily applied to field settings than infrasound devices. In an effort to identify frequencies and temporal structure of sound that elicit a behavioural response in eel, we used two different sound types; continuous broadband (60 -1000 Hz) sound and an intermittent pulsed stimulus (100 Hz). Previous studies have demonstrated that response and recovery can differ depending on frequency and intermittency of exposure. For example, groups of four European seabass (*Dicentrarchus labrax*) were exposed to either continuous or intermittent sound of consistent or fluctuating amplitude in an outdoor basin (Neo et al. 2014). Fish exhibited slower recovery to pre-exposure levels of behaviour under the intermittent sound treatment. In our study, both sound treatments resulted in similar responses, with greater rejection and lower passage through the screen when compared to the control, although eel exposed to the continuous broadband sound exhibited shorter passage times. More research is needed to test a wider variety of frequencies, intensities, and temporal patterns of sound to help select those most appropriate to advance eel protection technology.

The European Commission's Eel Regulations (Council Regulation No. 1100/2007) requires EU Member States to establish measures for the recovery of the stock of European eel. In England and Wales this requirement is brought into law through The Eel (England & Wales) Regulations 2009, and as part of these there is a requirement to install effective eel screens at any water intake capable of abstracting >20 m3 day-1 from a water body where eel may be present. In England and Wales, the guidance provided by the regulatory authority, the Environment Agency, is that where glass eel or elvers may be present a mesh size of 1-2 mm is required (Sheridan et al. 2014). This increases to 15-20 mm for silver eel. From the perspective of water supply and electricity generating industries, retrofitting existing infrastructure and maintaining such fine meshed screens will be costly, and potentially unviable under some circumstances due to the risk of blockage. Improving the efficiency of existing physical screens by combining them with appropriate behavioural deterrents may provide an alternative approach if they can be demonstrated to work as well as, or better than, the fine meshed alternatives proposed.

Use of acoustics to enhance the efficiency of physical screens designed to protect downstream moving European eel (Anguilla anguilla)

Combined physical and behavioural guidance systems that employ multimodal stimuli (e.g. in this case hydrodynamics and acoustics) are likely to be more efficient than those that employ a single factor operating in isolation because they enhance detection and increase the probability of a response by operating on more than one sensory modality. For example, downstream moving juvenile Chinook salmon (*Oncorhynchus tshawytscha*) were more likely to avoid a section of experimental flume when hydrodynamic (velocity gradient) and visual cues were employed in combination, than when hydrodynamics were manipulated in isolation (when dark) (Vowles et al. 2014). In the current study, acoustic stimuli enhanced the efficiency of a physical screen to guide eel to the bypass and reduced the number that passed through the screen itself. The improvement in efficiency were relatively small, indicating subtle modifications in behaviour, but significant. We propose that further enhancement will be achieved by investigating the influence of a wider range of frequency, intensity, and temporal structure of sounds used. Rather than simply replacing physical screens with behavioural deterrents as is commonly proposed, it is likely that fish protection technology will progress by following the principles of aggregation of marginal gains (e.g. Hall and al., 2012), a common approach adopted in elite sports engineering in which small incremental improvements of multiple aspects of the whole system lead to substantial advance. In addition to acoustics, the combined used of other deterrents should also be considered, including those that have previously been developed for the purpose of deterring eel, such as strobe lights (Patrick et al. 1982, 2001 for American eel) and electric fields (Alex Haro, USGS, pers. comm.; International Centre for Ecohydraulics Research unpublished data), while recognising the advantage of sound fields that extend over larger spatial scales and can remain effective under turbid conditions that are common in many river systems during the eel migration.

CHAPTER 7

7 Thesis discussion

The global rise of underwater sound levels and their impacts on marine and freshwater life has been a major concern for several decades (Slabbekoorn et al. 2010; Williams et al. 2015). There has also been a growth of interest in the utilization of guidance systems to protect migratory fish from hazardous areas, notably (in reference to the topic of this thesis) in the development of guidance systems that are based on the behavioural responses of fish to acoustic stimuli (Schilt 2007). This thesis has considered acoustic guidance systems for the protection of both migratory eel and river lamprey, because of their specific ecology and conservation status. The experimental research conducted as part of this thesis was undertaken to enhance the scientific knowledge on the hearing capabilities, and the behavioural responses to sound, of these two species. This research also aimed to lay the foundations for filling specific critical knowledge gaps in the development of guidance techniques to improve migratory fish passage at risk locations. Those knowledge gaps are:

- A lack of details on the hearing capabilities of European eel and river lamprey and their behavioural reactions to sound.
- The identification of key frequencies and adapted acoustic stimulus for both species in the potential development of a guiding system.
- Testing the efficiency of a combination of a conventional mitigation technique (vertical screen) with an acoustic system.

This chapter discusses the key findings of this research including the fundamental outcomes, the limitations, the extent to which new knowledge was discovered to begin the process of reducing the above knowledge gaps, and suggestions for future research.

The literature review revealed that European eel (*Anguilla anguilla*) hearing capabilities ranged from 40 to 300 Hz with the most sensitive hearing threshold at 80 Hz (Jerkø et al. 1989), although there is clear evidence of hearing sensitivity away from this most sensitive frequency band: Sand et al. (2000) observed behavioural responses (change of trajectories) with an infrasound source centred at 11.8 Hz.

It is germane at this point to recognize that these data come from a small number of individuals (how many?), and we know amongst human individuals can vary enormously (e.g in excess of 60 dB), even between individuals of the same age and experience. This is not simply a philosophical point, but one that is now causing the guidelines for Maximum Permitted Exposures to high frequency to be questioned, because they were based on a subset of mainly adult males (Leighton 2016, 2017, 2018; Fletcher et al. 2018a, 2018b). If, with decades of audiological experience and tens of millions of hearing tests, we are lulled into a false sense of security when referring to the 'hearing threshold' at a given frequency for humans, caution is clearly required when applying such terms to a given fish based on measurements on a much smaller set of individuals.

Recognizing therefore that the number of individuals tested is vanishingly small compared to those on which human audiological norms are based, the results obtained in the Chapter 4 seem to corroborate the findings of Jerkø et al. (1989): the best hearing thresholds determined by our analysis of eel behaviour appeared to be for 60 and 80 Hz (at respectively 91 and 98 dB re 1 re 1 µPa). In addition, we further investigated the hearing range of eel by using test frequencies higher than 320 Hz, which was the maximum frequency used by Jerkø et al. (1989). Our results seem to indicate that the hearing range upper limit of eel also goes beyond 300 Hz and that 400 Hz and 2000 Hz are also some very sensitive frequencies (hearing thresholds estimated at 96 dB re 1 µPa amongst the individuals tested here).

On the other hand, the literature review failed to provide any information on the hearing capabilities of river lamprey (*Lampetra fluviatilis*). As mentioned previously, the only available work on lamprey hearing is the early anatomical work from Lowenstein (1968). To the author's best knowledge, this study, and the experimental work described in Chapter 4, represent the only published attempts to produce an audiogram of a river lamprey. Teague & Clough (2013), in their work on the survival of lamprey at travelling screens, suggest that because of their lack of swim bladder and otolith organ, lamprey fell into the non-hearing specialists group and consequently are supposedly unable to perceive high frequency sounds (greater than 1,5 kHz) and low-frequency sounds (below 100Hz). Contrarywise, an earlier paper from Popper (2005) stated that lamprey do possess an otolith organ but that no studies at that time have been undertaken to reveal the possibility of behavioural response to sound. Based on these two arguments, the experimental work carried on lamprey in Chapter 4 attempted to fill that research gap by investigating the hearing capabilities and behavioural response to sound of river lamprey. Following the results from this work, three main frequencies (60, 100 and 2000 Hz), were selected because of their low hearing threshold (respectively, 98, 100 and 97 dB re 1 µPa). From an evolutionary point of view, sharks (Elasmobranchii) are the closest related group to lamprey.

According to Hart & Collin (2015), the hearing range of a shark is between 20 and 1000 Hz with their greatest hearing sensitive being to frequencies below 100 Hz. Chapter 4 results are consistent with the suggestion that lampreys exhibit a similar frequency-dependent sensitivity.

The utilization of a confined lab environment in Chapter 4, allowed us to extract some generic behaviours and to create a joined ethogram for eel and lamprey. The presence of a control treatment confirmed that most of these behaviours were part of a spontaneous/natural panel (independent from sound) with the exception of the "Speaker Check" (observed only in eel). Based on these behaviours and their analysis, the audiograms mentioned above have been built. Nevertheless, this study has some limitations (stated below), and therefore its results needs to be treated with care, and not extrapolated to all members of the species without additional data.

The main limitations are: (1) the lack of realism (would the response be the same in the natural environment?), (2) the lack of precision (use of small number of replicates, and approximation of the hearing threshold in the presence of large error bars) and (3) the difficulty of interpretation of the observed responses (was the sound really causing the observed behavioural response?).

As reviewed by Ladich & Fay (2012), in recent years there has been a tendency to favour the use of Auditory Evoked Potentials (AEPs) to measure hearing thresholds in fish, as opposed to traditional behavioural methods used by Jerkø et al. (1989) and Sand et al. (2000) (the only two teams to publish on the hearing thresholds of eel prior to this thesis). This AEP method is based on electrophysiological parameters and requires specific equipment (sometime invasive for the fish) but, if it works, it allows the researcher to obtain accurate audiograms with a small number of replicate (generally 10 fish) (note that the 'accuracy' of an audiogram, as used here, refers to the fact that the variation of hearing threshold with frequency is representative of the samples tested and not a random fluctuation: it does not mean that it is representative of the wider population of a species that have not been tested by AEP). Tests undertaken in this study (Appendix 8.2) were unable to obtain reliable AEP data from eel for the type of testing ethically allowed in thus study (i.e. avoiding penetration of the fish by electrodes). Although it was disappointing not to obtain AEPs from non-penetrating electrodes, this was not a critical hinderance, because in many ways the behavioural hearing thresholds are more germane to this thesis. This is because the goal of this thesis was to investigate the impacts of sound on fish, and to gain sufficient knowledge to help in the development of behavioural guidance systems. One limitation of the AEP method is that its results do not directly, or in any detail, serve as a predictor of the behavioural response. Use of the experimental design in Chapter 4 allowed us to obtain direct data regarding the behaviour of the two species (data limited to those individuals tested, of

course) and to quantify the changes in these behaviours in presence of sound, whilst identifying frequencies of importance.

In the experiments undertaken in Chapter 5 and 6, a large open-channel flume was used to obtain conditions more representative to the field (e.g. hydrodynamics), allowing fish to display more natural behaviours. The experimental design in Chapter 5, brought a new method of investigation of behavioural responses to stimulus by using an underwater maze. Nevertheless, the tested hypothesis (that a Continuous Broadband Sound provided by a single source would be able to modify significantly the swimming trajectory of fish) was unsuccessful. In the Chapter 4, the swimming behaviour (Moving) in eel was not affected by the acoustic stimulus. However, a significant number of rejections was observed in the ensonified corridors of the maze experiment in Chapter 5, suggesting that the stimulus had the potential to acts as a deterrent on eel. In both Chapters 4 and 5, a Mixed-Effect model was used to compare the inter-subject variability for specific behaviours. In both studies, the inter-subject variability was very high (especially for the "Passage time" in Chapter 5). This suggests that the response to sound is to some extent hardly generalizable to the whole population in eel, as some individuals might react differently than others. The concept of "personality" in fish, as evoked by Magnhagen & Staffan (2005) in their work on perch (*Perca fluviatilis*), seems to be a possible explanation to these results.

In addition, in the Chapter 5, yellow stage eel were also tested through the maze to compare if any difference in behaviour was observable between them and the silver eel. The main difference resulted in the inter-subject variability in "Passage time". Yellow stage eel variability was more pronounced (50%) compared to the silver (40.4%). Through their maturation and change of stage, eel experience profound metabolic changes (Van Ginneken et al. 2007) and presumably behavioural changes. This variation in the inter-subject variability between both stages for the "Passage time" might be a consequence of these metabolic changes. Mature migrating silver eel, aiming to reach their reproduction ground, share a common goal while yellow stage eel are more sedentary fish and only display local migrations generally for foraging purpose. Furthermore, the mixed- model also emphasized the implication of the "Day" factor in the "Passage time" variability. Because all of the trials could not be performed on a single day due to experiment duration and number of trials, a factor "Day" was also included in the model to compare if it could play a role on the behavioural response of the fish. The factor "Day" accounts for all the uncontrolled parameters that could have fluctuated from one day of experiment to another (e.g. moon phase, temperature, rainfall). As reviewed by Stein et al. (2015), some of these factors have been proven to be critical in the migration process (Elie & Rochard 1994) of silver eel and lamprey. The results from Chapter 5 seem to confirm the importance of daily variation on the

"Passage time". Furthermore, the result emphasizes the importance of these environmental parameters on the migrating fish with, moderate level of influence on river lamprey (19.9%), and important level in silver eel (51.9%). Non-migrating yellow stage eel barely reached 1% suggesting that these factors were irrelevant for them in term of "Passage time".

The global decline of eel and lamprey populations (lamprey: Kelly & King 2001; eel: Dekker et al. 2007) has been mainly linked to anthropogenic pressures (e.g. infrastructures, pollution, habitat fragmentation). Water and energy infrastructure, in particular, has received much attention, with concerns over the loss of migratory juveniles and adults at intakes to water supply facilities, hydroelectric turbines, fish farms. In addition to strict regulations, protective measures have been deployed to reduce fish mortality. Traditional protective measures at water intakes generally involve physical and mechanical screens (Kemp 2015) and aim to prevent fish from entering dangerous areas. The main problematics with traditional screens are their inadaptability to species, and their potential risks (e.g. impingement) for anguilliform fish (Bruijs & Durif 2009) (Figure 7.1).

Figure 7.1– Juvenile Pacific lamprey in the bypass grates at a Columbia River dam. Copyright - http://www.critfc.org/

Behavioural guidance systems (based on acoustics, lights, bubbles, or a combination of them) have been suggested as an alternative mitigation to protect fish (Schilt 2007).

More relevant to this thesis, in the Chapter 6 efficiency of two different acoustics stimuli (Continuous Broadband Sound - CBS and 100 Hz Pulse) played by a series of three underwater speakers in combination to a conventional bar-screen was tested. The tested hypothesis was that the acoustic stimulus will guide fish toward a bypass channel before encountering the screen. The obtain results did not show any obvious difference in terms of guiding. Even if no fish swam through the screen in the experimental conditions compared to the control conditions, this

difference could not be related to the presence of the stimulus. Nevertheless, the number of rejection behaviours (fish swimming back or away from the acoustic source) was significantly different between control and ensonified treatments. Each time eel displayed this type of behaviour, individuals never encountered the screen and swam directly to the bypass. With respectively 16.4% and 12.8% of fish displaying this rejection behaviour in the CBS and the 100 Hz Pulse treatments, these results are not as clear as the one obtained by Sand et al. (2000). In their study, the avoidance of an infrasound source by European eel reached 57%.

In Chapters 4 and 5, the models showed that some behaviours (e.g. "Swimming/Moving", "Escape attempt", "Passage time") were highly dependent from the inter-subject variability. One possible explanation to the results obtained in Chapter 6 could be that some individuals were more inclined to respond to sound than others (sensitivity to sound, past experience, anatomy and physiology). A "flight or fight" type of response (Cannon 1929; Romero et al. 2007) would possibly illustrate these observations. The small proportion of individuals that decided to avoid the stimulus would express a "flight" response, the rest of them would be "fighters". In absence of sound fish do not have to make this type of choice and adopt a standard swimming speed (time to pass) relatively slow. When a fish opted for a fighting response, facing the sound stressor, it will potentially increase its speed to overcome that stress event rapidly. From an energetic point of view, it might also be more energy consuming to swim against the flow (as opposed to swimming along with the flow) to move away from the stressor (Tort 1991). Nowadays, this concept has been revisited and "fighters" and "flyers" are more often respectively designated as "proactive" and "reactive" individuals (Koolhaas et al. 1999).

7.1 Future research

The results obtained through Chapter 4 need to be applied carefully. This experiment was carried out only on 18 individuals per species. With a consequent interindividual variability, the behavioural response was very variable among the tested frequencies, leading to unbalanced numbers of observations for some frequencies. To increase the power of the audiograms, a similar methodology using more tanks and more replicates (unachievable in this study due to tank and individuals limitation) would potentially offer a reduction of the error bars observed on the audiograms and ascertain the hearing threshold for the frequencies tested. Nevertheless, this type of experimental design can be re-used as preliminary study to establish the repertoire of behavioural response to sound and to draw a first picture of the hearing capabilities of the tested species. For future work, it is suggested that physiological sampling be added to the protocol (e.g. glucose, lactate, cortisol) to further investigate the short and long terms stress response of the

species. Finally, performing an electrophysiological audiogram via AEP or ABR methods to further confirm the hearing threshold obtained via the behavioural method would be an asset (see Kenyon et al. 1998). As reported by Fay & Ladich (2012), behavioural and electrophysiological results can sometimes be very different and the choice of method needs to reflect the main purpose of the study. In the present research, the results from the European eel audiogram (regardless of the error bars) were in accordance with the audiogram produced by Jerkø et al. (1989). However, there is currently no available audiogram (neither behaviourally or electrophysiologically built) for lamprey species. There is a need for this kind of research in order to corroborate our findings.

To the author's best knowledge, the use of a behavioural maze in Chapter 5, is a novel approach inspired by conventional studies on terrestrial animal behaviour. The flexibility of this design allows testing of more complex setups (e.g. multiple speakers in the corridor, different speaker position and orientation), including additional behavioural stimulus (e.g. bubble curtain, strobe light), and to assess their impacts on the swimming and exploratory behaviour of fish. For future work involving acoustic stimuli in a similar setup, it would be better to bring the speaker closer to the entrance of the chamber to increase the chances for the fish to perceive the stimulus before entering a corridor (Figure 7.2).

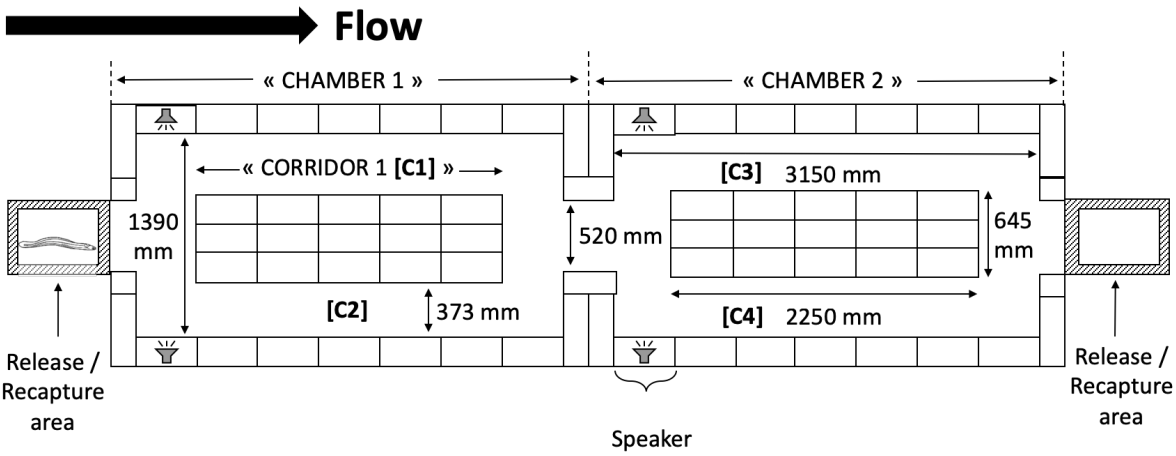


Figure 7.2 - Plan view of an "acoustic maze" with speaker positions moved to the entrance of the corridors (as opposed as Figure 5.1 in Chapter 5). This configuration is adapted for European eel in their downstream migration.

In both Chapters 4 and 5, the use of PIT tagged fish has been a key element for the statistical analysis allowing the use of a Mixed Effect Model and the investigation on inter-subject variability. From the obtained results, it was suggested that inter-subject variability played an important part in the expression of certain behaviours. Further behavioural research on fish behaviour should undertake the same kind of analysis. This method could be of great use in determining the percentage of expected behavioural response, among a target species, to a specific stimulus for instance produced by a deterring or guiding device.

This approach is also of great use as it allows to include several additional factors. In Chapter 5, the random factor "Day" was used as generic factor accounting for the daily variation of uncontrolled parameters (e.g. temperature, moon phase, rainfall). Further researches, especially on species known for their correlation between their behavioural response and those parameters, need to add up these parameters to their analysis/model.

To add up on this research, as the Home Office regulation requires the euthanasia (Schedule 1 procedure) of animals that have been used in experimental research to limit the risk of contamination to the wild population, some dissections have been performed on the eels used in Chapter 5. Our main interest was to investigate their swim bladders, looking for potential parasites, especially *Anguillicola crassus*, a non-native species of nematodes (results were forwarded to the Environment Agency). Our observations indicated that more than 61% of the dissected European eel (N=76) had their swim bladder infected by *Anguillicola crassus*. This raise new questions in terms of swim bladder sensitivity to acoustic stimuli in presence of the parasite. Is the presence of the parasite altering the swim bladder functioning and its sensitivity to

acoustics? Is the parasite sensitive to acoustics? An inescapable conclusion is that future work should consider these questions and to investigate if parasitized eels are more sensitive to acoustics than the non-infected.

In Chapter 6, the guidance efficiency of the acoustic stimuli was subtle but significant. It nevertheless triggered some rejections and change of trajectory that led a small proportion of the fish to reach directly the bypass channel without encountering the screen. The use of a single pulsed frequency as opposed to a continuous broadband noise emphasized the need for further work to better tune the acoustic stimulus parameters (e.g. gap duration between the pulse, length of the pulse). In order to develop the appropriate signal, future work should prioritize obtaining a clear picture of the hearing range and sensitivity limits of the test species. This need to be done by using an appropriate methodology like AEP. However, the cautions in extrapolating one set of audiogram tests to a different population of the same species (mentioned at the start of this chapter) remain. Unfortunately, the effect of the signal periodicity (speed of the pulse) on the fish behavioural response needs to be tested via a trial-and-error process.

Pulsed and CBS signal had the same accelerating effect on the *"*Passage time*"* of European eel. However, the pulsed signal made fish pass even faster than the CBS while generating fewer rejections. This suggest that both types of signal could be used separately to achieve distinct aims, one for deterring fish, the other to speed them up. New research on the signal parameters are therefore strongly recommended, and could offer new perspectives in terms of guiding or deterring systems (Schilt 2007; Slabbekoorn 2016).

7.2 Conclusion

The aims of this thesis were to 'Investigate the potential impacts of anthropogenic sounds on European eel and river lamprey behaviour' and 'Develop or improve mitigation techniques based on acoustics in order to enhance the migrations of European eel and river lamprey'. To meet this aim, experimental research was undertaken to address three knowledge gaps: 1) A poor understanding of the hearing capabilities of anguilliform fish, and of their behavioural response to sound in a confined environment. 2) A lack of knowledge on the effects of sound on the migratory behaviour of eel and lamprey in controlled environment (flume). 3) A lack of investigation on the effectiveness of the combined use of a conventional mitigation (bar-screen) with an acoustic stimulus during downstream passage.

Conventional studies investigating the hearing capabilities of fish, based on behavioural response, generally go through the training or the conditioning of the test subjects (Fay & Ladich 2012).

Such methodology had considerable success in terms of hearing capabilities determination (reliable audiograms) but does not bring any insight on the natural behavioural response of the fish in relation to sound. This issue was overcome in Chapter 4 by using a simpler approach and eliminating any form of conditioning with the test species. As expected, both species displayed a sufficient amount of behaviour to allow the creation of the first ethogram, listing some of the typical reactions of anguilliform fish in a confined environment. The statistical analysis allowed to further emphasize the influence of sound of the previously identified behaviours. Interestingly, the impact was more pronounced with river lamprey than European eel. Nevertheless, in both species, the behavioural response seems to be very variable among the test subjects. Therefore, these results need to be taken with care and cannot be easily extended to a wider population of individuals of the same species without further testing.

The audiograms suggest that the best hearing sensitivity to these species is located in the range of frequencies around 100 Hz, as previously suggested by Jerkø et al. (1989) for the European eel.

Despite the unsuccessful observation of a clear effect of sound on the migratory behaviour of eel and lamprey, the experimental work in Chapter 5 introduced a new research method by using a behavioural maze. Sound did have an effect on the exploratory behaviour of the species, as rejections behaviours were observed in presence of the acoustic signal. Moreover, sound also had an effect on the *"*Passage time*"* of fish. Finally, this experiment seemed to indicate that other factors were also significantly involved in the behavioural response (e.g. daily environmental variations, life stage, "personality"). More research of this type need to be carried out with a modified and potentially more advanced/complicated setup taking into account the previous factors.

Finally, the joint effect of a conventional bar-screen combined to an acoustic source was not as successful as expected in terms of guiding efficiency. Nevertheless, as a potential deterrent, both acoustics stimuli performed well with a significant percentage of fish passing to the bypass channel without encountering the screen. Moreover, the analysis of the *"*Passage time*"* confirmed that both stimuli had a significant impact on the *"*Passage time*"* of the fish. Therefore, the results indicate that conventional screening techniques can perform efficiently in combination to acoustic signals, and potentially increase the chances of fish to reach the bypass without encountering the screen. However, the small proportions (≈ 13 and 16.5%) of avoidance emphasize the need for further researches on developing a more efficient stimulus that magnify this type of response.

7.3 Research impact

As a result of this thesis, a number of original contributions to existing knowledge and thinking have been made to the fields of bioacoustics and behavioural study, as well as on the development of mitigation system for fish passage:

- The literature review (Chapter 2) highlighted the complexity of behavioural and acoustics studies on fish. Furthermore, this complexity is mainly due to the need to combine several bodies of science (e.g. ethology, acoustics, biology, statistics) and to incorporate different techniques in order to obtain the most realistic approach.
  A piece of this work was presented as an introductory presentation on "*Impacts of sound on fish*" at a regional conference: *Chalkstream Research Conference, Southampton, UK, June 2013*.

- Chapter 4 brought in a novel approach to jointly study the hearing capabilities and the behavioural response of fish in a confined environment. From this experiment, new audiograms were respectively produced for European eel and River lamprey along with an ethogram of both species.

- Chapter 5 introduced a new methodology to assess fish behavioural response to sound in a controlled environment using an experimental design inspired from conventional behavioural studies. The maze design, offers new possibilities by providing a very adaptive design, easy to set up and capable to reach a high level of complexity (e.g. multiple stimulus, multiple corridors, multiple camera).

- Chapter 6 also used a novel design by using a series of three underwater speakers to extend the ensonified area. Two types of stimuli especially a pulsed one were tested in combination to a traditional bar-screen.

  The results of these chapters have been presented at an international conference:
  "Impacts of anthropogenic sounds on fish" - Fourth International Conference on "The effects of Aquatic Noise", Dublin, Ireland, July 2016.
  By invitation, at a regional conference:

"Mixing stats and biology, not such a fishy idea…" – Cafés de l'IMT, Institut de Mathématiques de Toulouse, Toulouse, France, Janvier 2017

In addition, a paper summarising Chapter 6 findings and titled, "*Use of acoustics to enhance the efficiency of physical screens designed to protect downstream moving European eel (Anguilla anguilla)" has been submitted and is under review by the journal "Ecological Engineering".*

A second paper based on Chapter 5 findings has been submitted to the journal "River Research and Applications". This paper is titled "*Use of an acoustic maze to assess the behavioural response of anguilliform fish to underwater sound*"

8 Appendix

8.1 Chapter 4 (continuation) - River lamprey and European eel behaviour-based audiograms

This section regroups the diagrams (barplots) representing the different behavior variations among the test subjects "ID" and the day "Day" of experiment for the different frequencies. These diagrams have been produced for both European eel and river lamprey. Escape attempt diagram is missing for the European eel as it is used as reference in the Chapter 4 – Result section of this thesis.

8.1.1 European eel – inter-subject variability:

8.1.1.1 Moving Vs ID

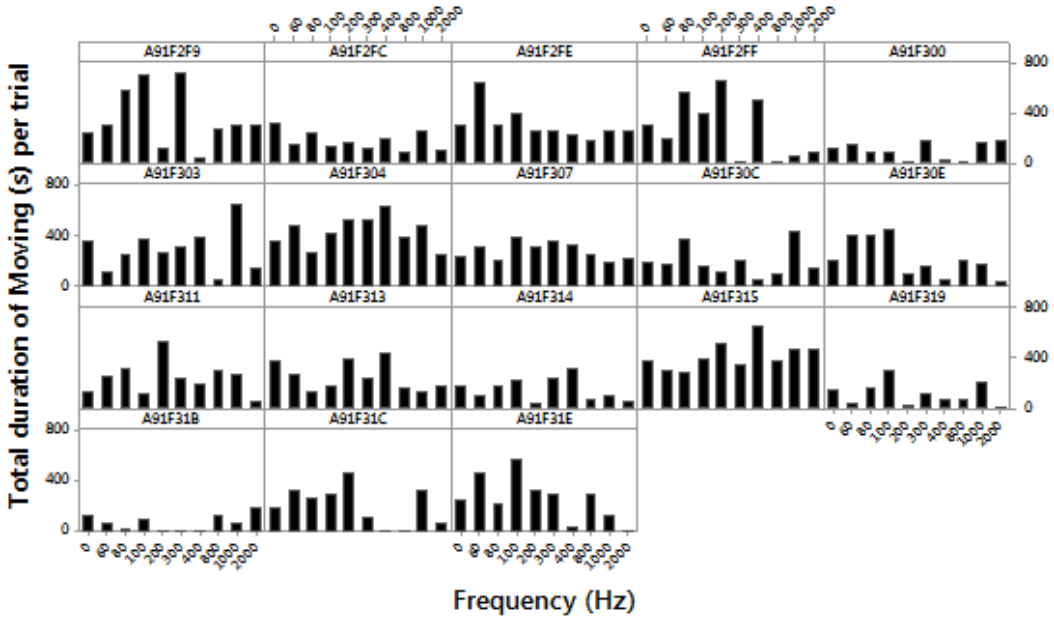


Figure 8.1 - Barplot showing the variation in the Moving behaviour per frequency for each individual for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.1.2 **Forcing Vs ID**

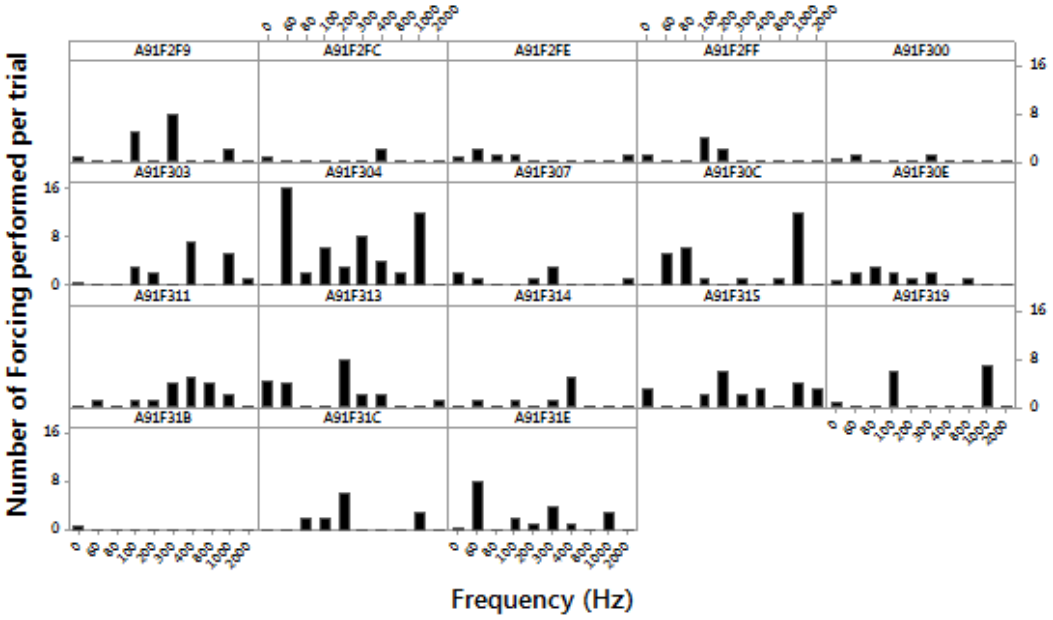


Figure 8.2 - Barplot showing the variation in the Forcing behaviour per frequency for each individual for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.1.3 **Escape Length Vs ID**

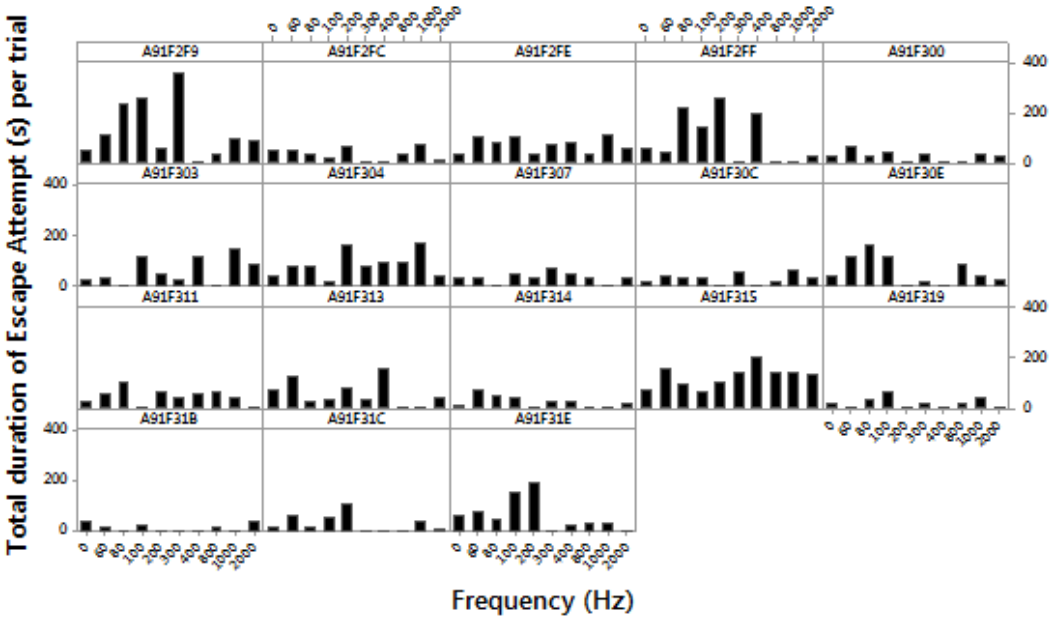


Figure 8.3 - Barplot showing the variation in the Escape attempt behaviour duration per frequency for each individual for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.1.4 **Speaker Check Vs ID**

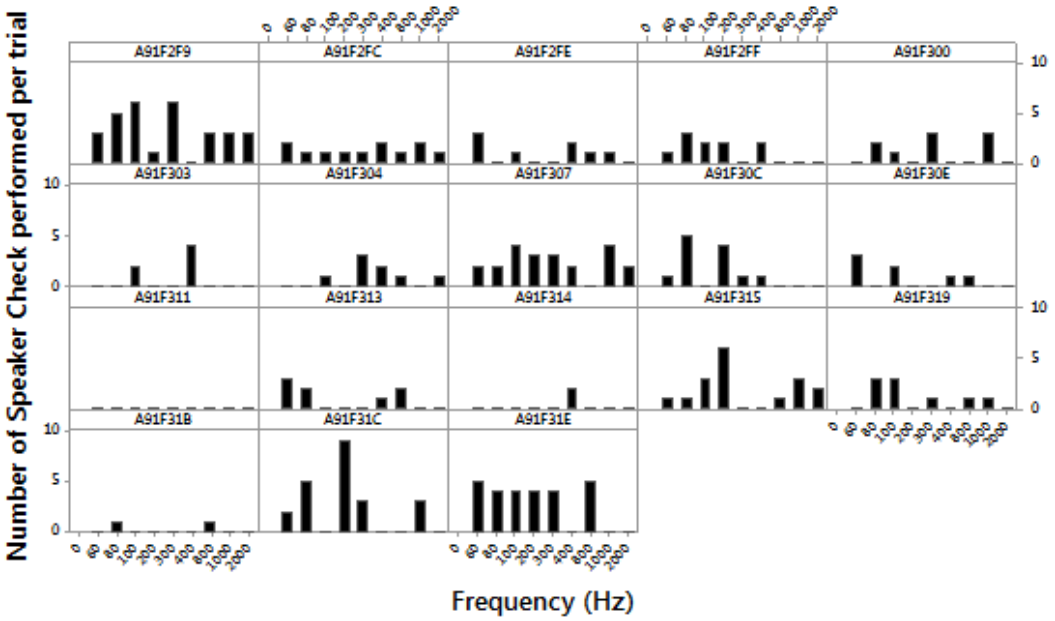


Figure 8.4 - Barplot showing the variation in the Speaker Check behaviour per frequency for each individual for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.1.5 **Speaker Check Length Vs ID**

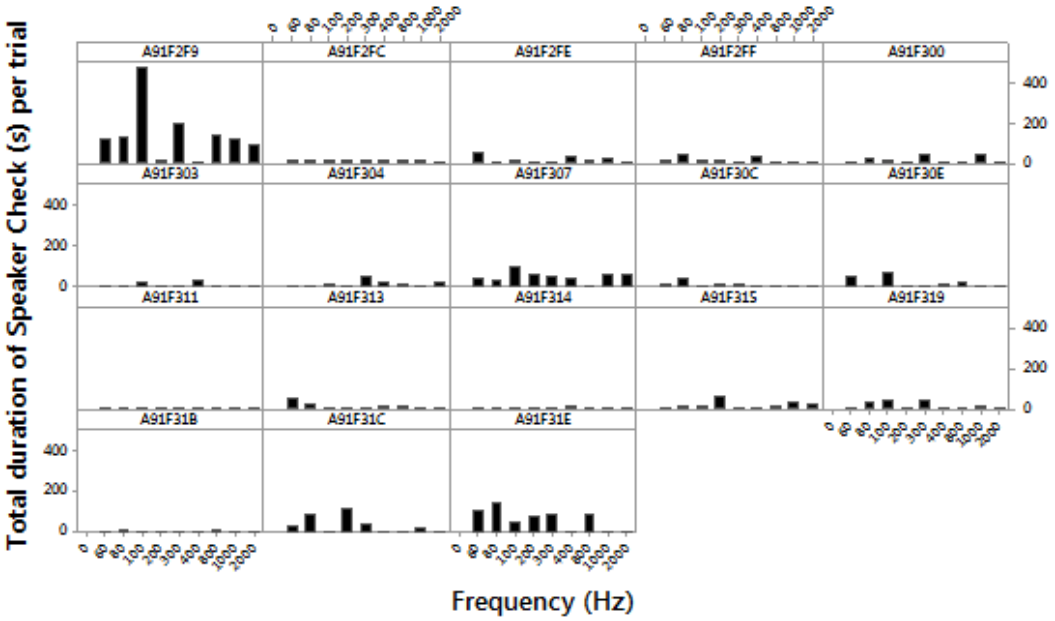


Figure 8.5 - Barplot showing the variation in the Speaker Check behaviour duration per frequency for each individual for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.2 European eel – Daily Variation

8.1.2.1 Moving Vs Day

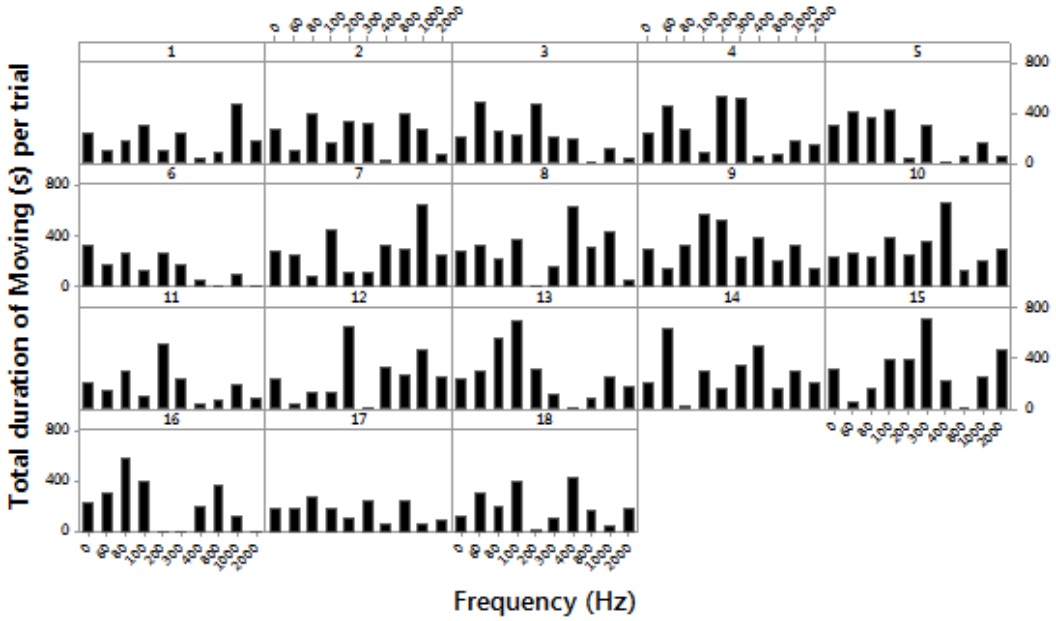


Figure 8.6 - Barplot showing the variation in the Moving behaviour per frequency for each day of experiment for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.2.2 Forcing Vs Day

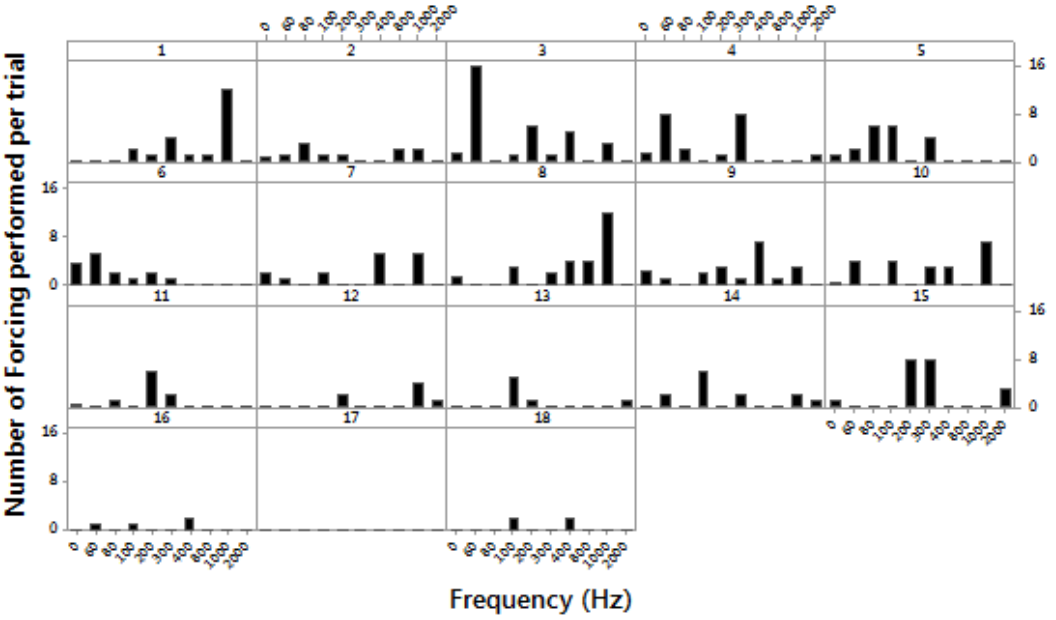


Figure 8.7 - Barplot showing the daily (Day) variation of the Forcing behaviour per frequency for each day of experiment for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.2.3 **Escape Length Vs Day**

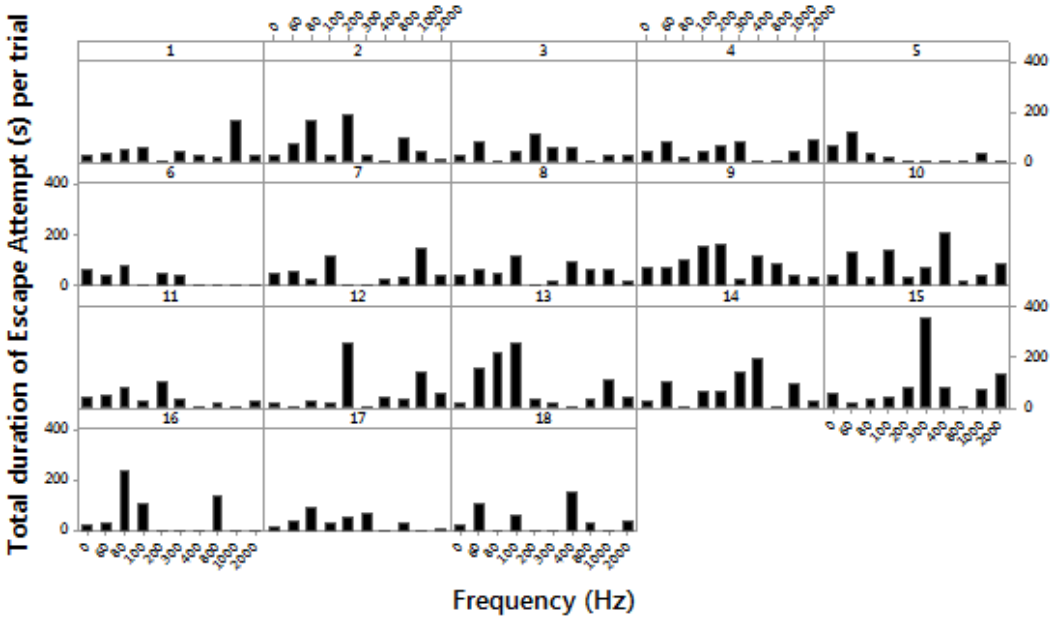


Figure 8.8 - Barplot showing the daily (Day) variation of the Escape attempt behavior duration per frequency for each day of experiment for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.2.4 **Speaker Check Vs Day**

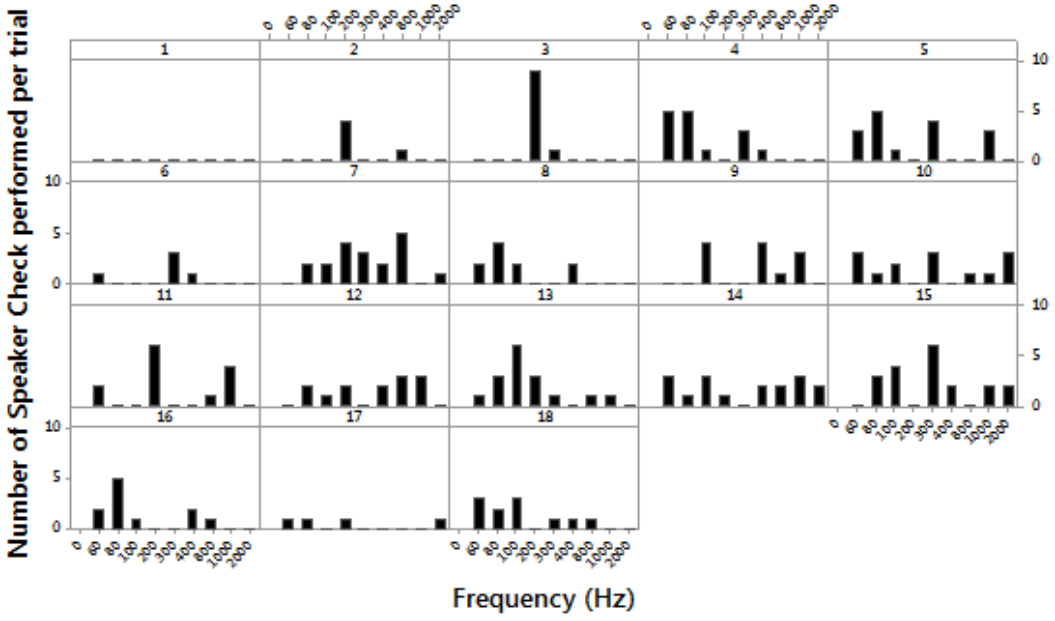


Figure 8.9 - Barplot showing the daily (Day) variation of the Speaker Check behavior per frequency for each day of experiment for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.2.5 **Speaker Check Length Vs Day**

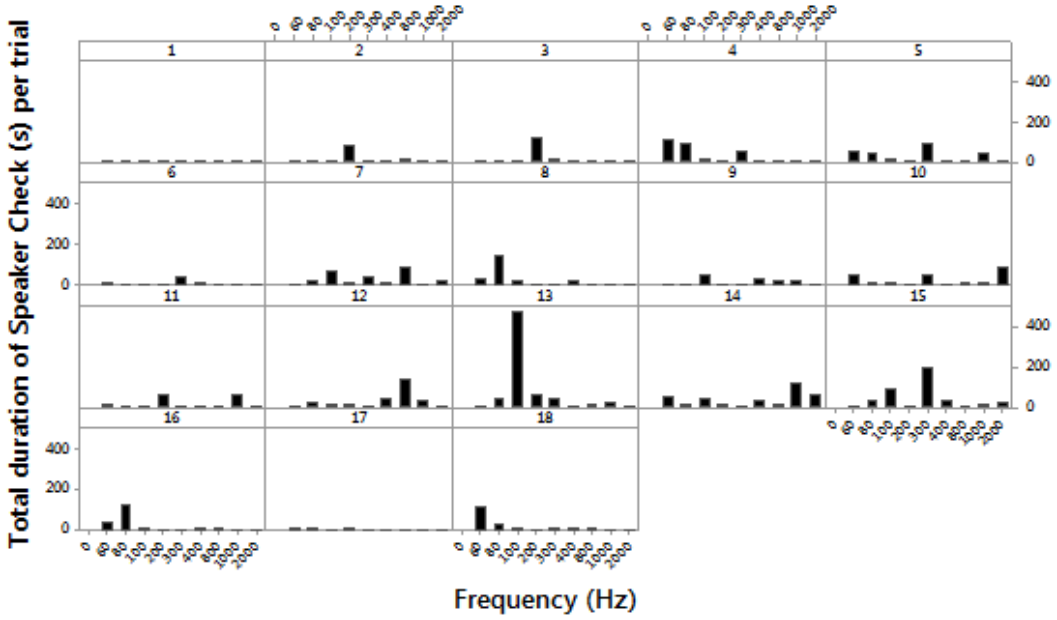


Figure 8.10 - Barplot showing the daily (Day) variation of the Speaker Check behavior duration per frequency for each day of experiment for the European eel. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.3 **River lamprey – inter-subject variability**

8.1.3.1 **Moving Vs ID**

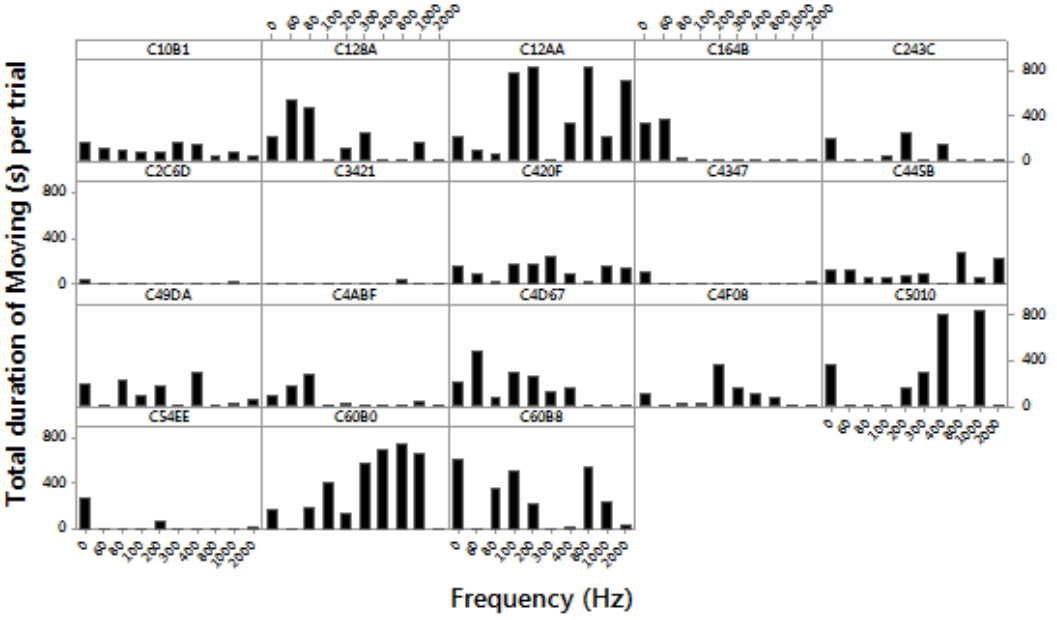


Figure 8.11 - Barplot showing the variation in the Moving behaviour per frequency for each individual for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.3.2 **Forcing Vs ID**

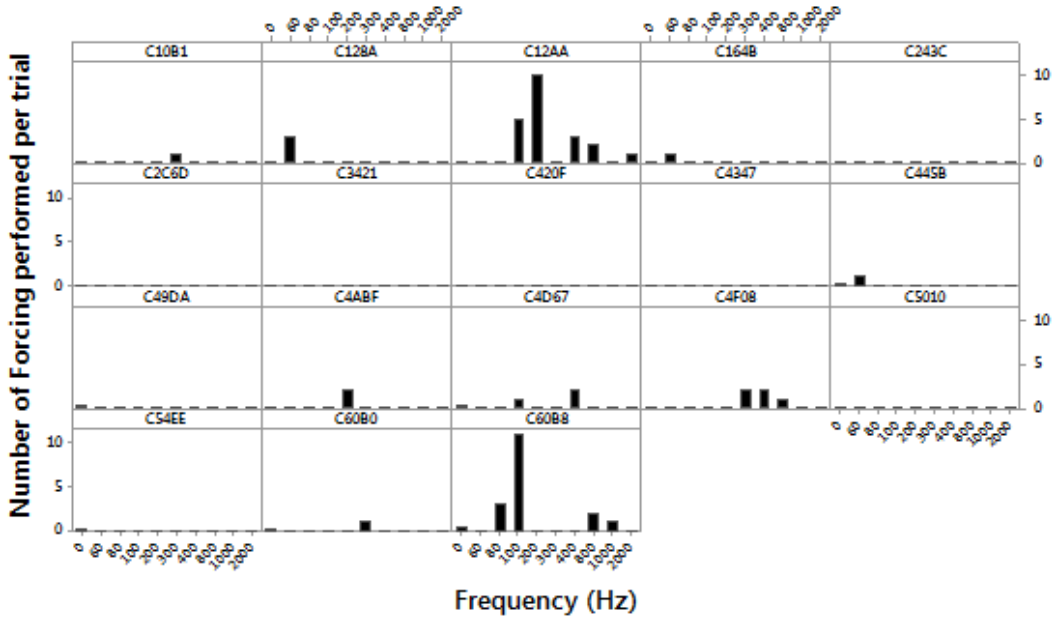


Figure 8.12 - Barplot showing the variation in the Forcing behaviour per frequency for each individual for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.3.3 **Escape Attempt Vs ID**

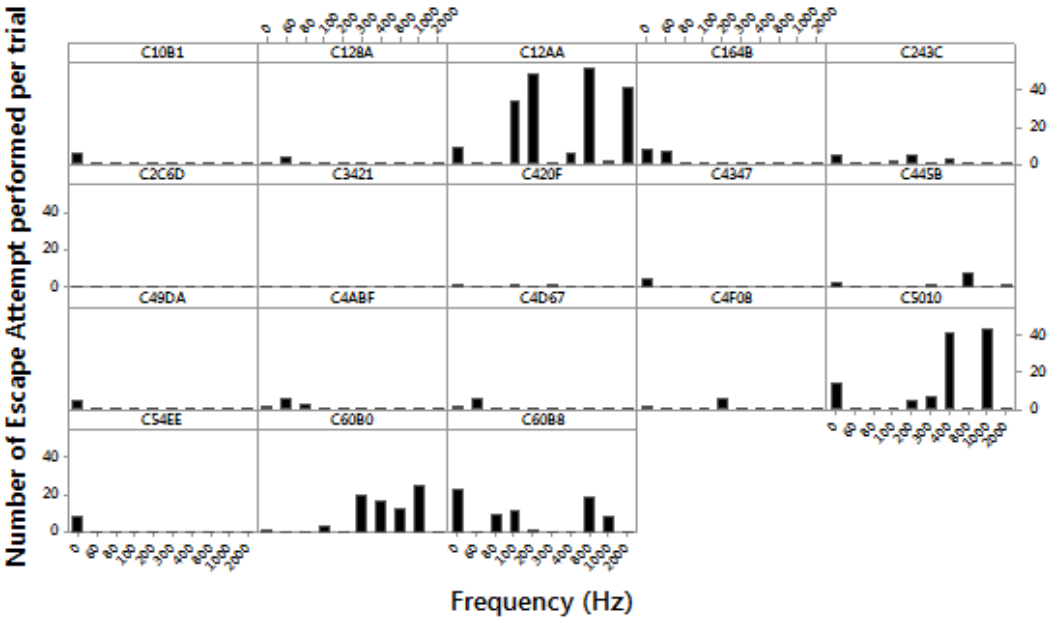


Figure 8.13 - Barplot showing the variation in the Escape attempt behaviour per frequency for each individual for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.3.4 **Escape Length Vs ID**

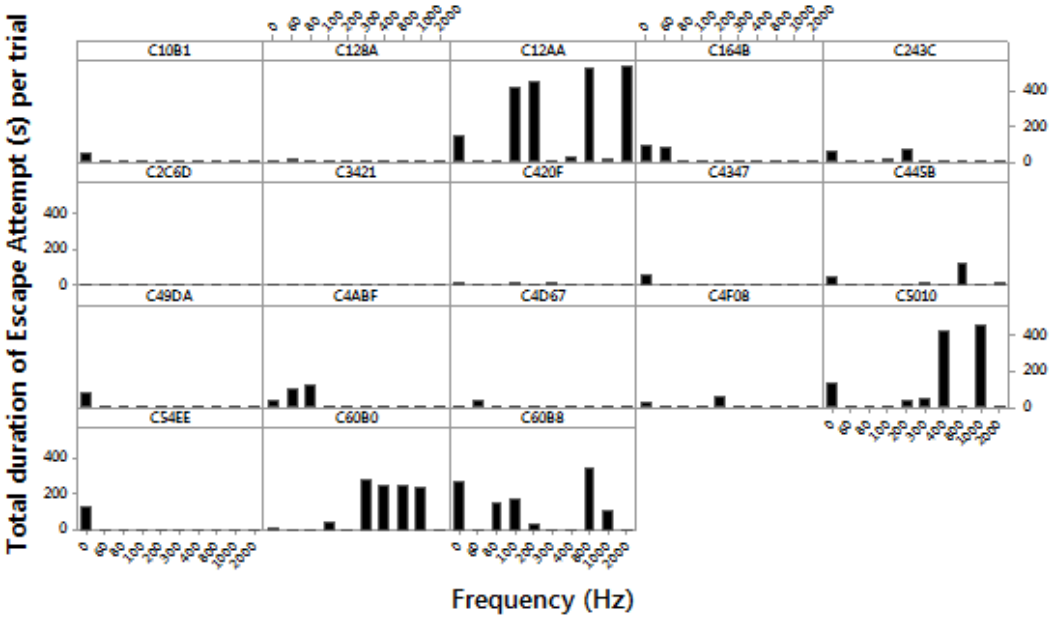


Figure 8.14 - Barplot showing the variation in the Escape attempt behaviour duration per frequency for each individual for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.4 **River lamprey – Daily variation:**

8.1.4.1 **Moving Vs Day**

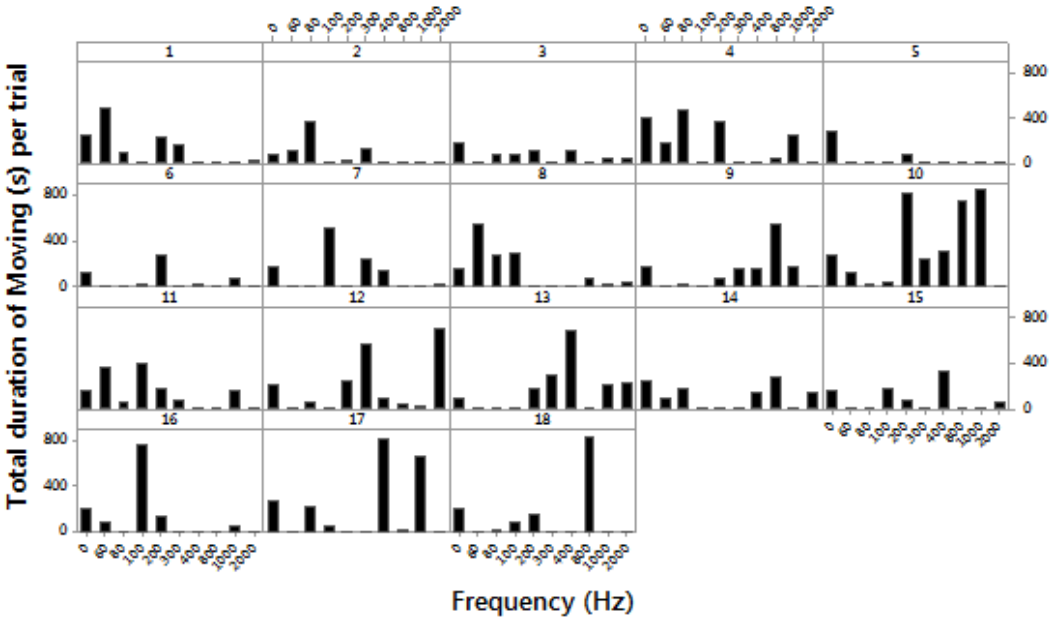


Figure 8.15 - Barplot showing the daily (Day) variation in the Moving behaviour per frequency for each day of experiment for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.4.2 **Forcing Vs Day**

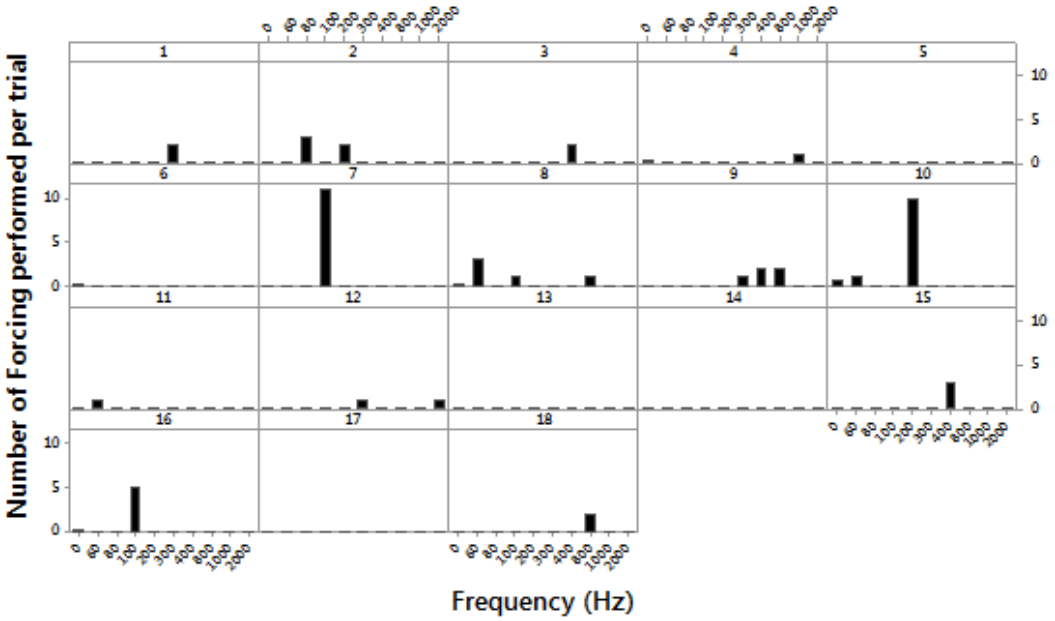


Figure 8.16 - Barplot showing the daily (Day) variation in the Forcing behaviour per frequency for each day of experiment for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.4.3 **Escape Attempt Vs Day**

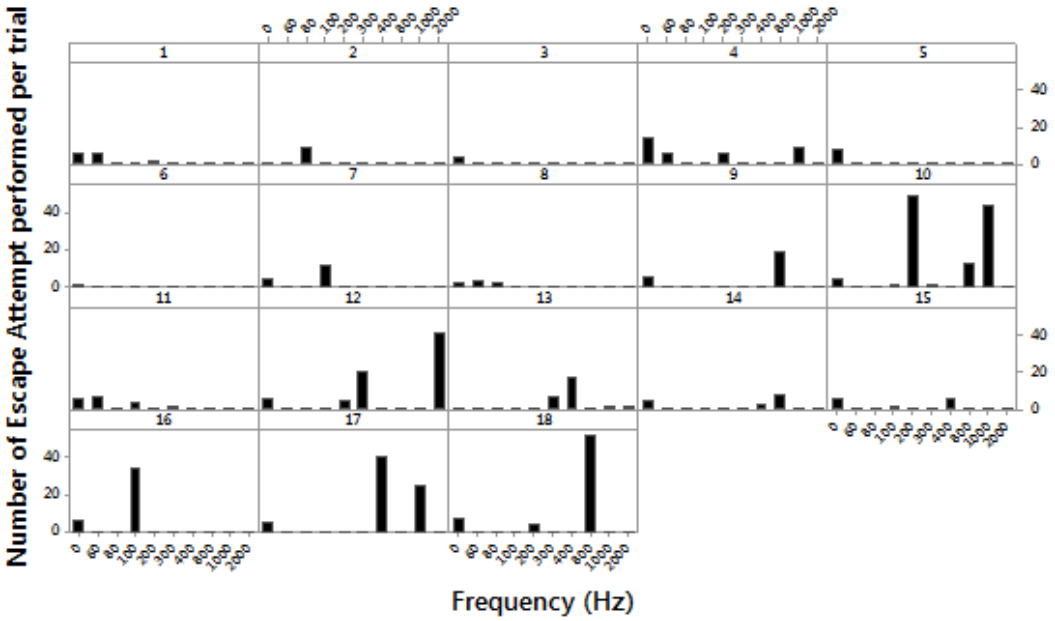


Figure 8.17 -Barplot showing the daily (Day) variation in the Escape attempt behaviour per frequency for each day of experiment for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.1.4.4 **Escape Length Vs Day**

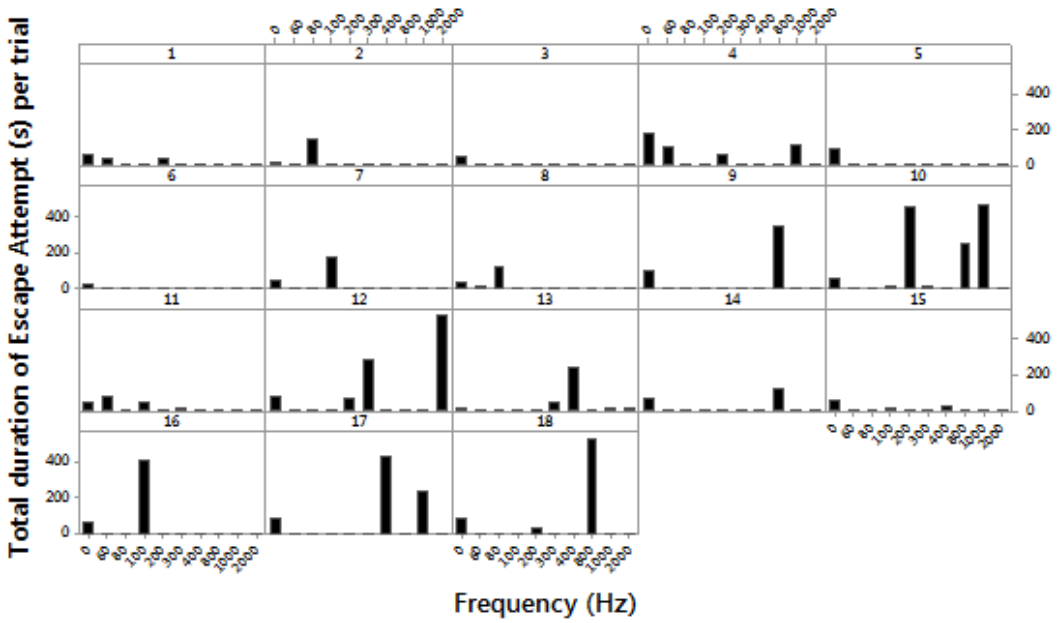


Figure 8.18 - Barplot showing the daily (Day) variation in the Escape attempt behavior duration per frequency for each day of experiment for the river lamprey. On the X-axis the frequencies from 0 to 2000 Hz.

8.2 - Auditory Evoked Potential (AEP) method

The AEP method, originally named ABR (Auditory Brainstem Response), has been for long used as an alternative to behavioural experiment to determine the hearing capabilities of fish. Behavioural studies generally imply a long conditioning of the fish and therefore measurements can take up to weeks (Yan 2004). On the other hand, the AEP/ABR method is a rapid, non-invasive way to investigate fish hearing with the possibility of repeated measurements on the same individual.

This technique consists in a "far-field recording of synchronous neural activity in the eighth nerve and brainstem auditory nuclei elicited by acoustic stimuli" (Kenyon et al. 1998). This method is inspired from clinical assessment of hearing in humans (Kenyon et al. 1998).

In this research, it was initially planned to assess the hearing capabilities of both European eel and river lamprey using this technique and to compare the outcome with the results behaviourally obtained in Chapter 4. Nevertheless, due to technical, financial and time limitations, this part of the research was not successfully achieved. However, the author desired to include this "unfinished" study into the present thesis to help potential research that will be taken over from this work.

This section reviews the methodology that was used during that research, the limitation and problem encountered but also the suggestions for further work.

8.2.1 **Experimental setup**

The experimental design selected for this experiment was inspired by works from Kenyon et al. (1998) (Figure 8.19) and Suga et al. (2005). Work was undertaken in a semi-anechoic chamber located in the Institute of Sound and Vibration, Highfield Campus, Southampton.

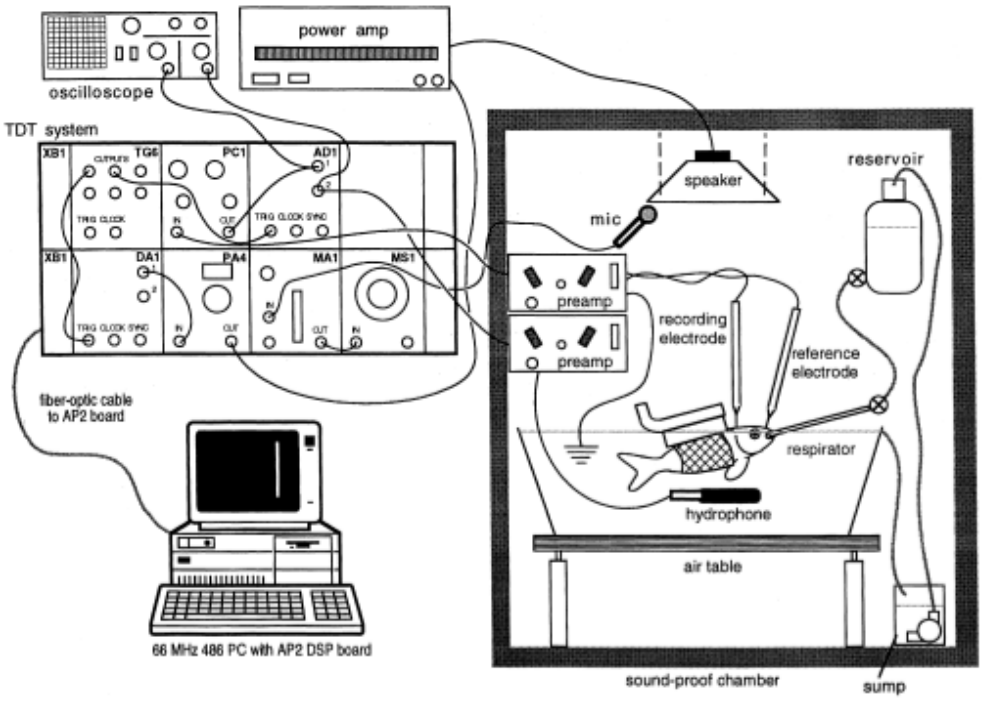


Figure 8.19 - Diagram of Auditory Brainstem Response (ABR) experimental setup from Kenyon et al. 1998.

One of the initial steps consisted in building some recording electrodes to monitor brain activity and the auditory response of the fish. The design was inspired from Suga et al. (2005) (Figure 8.20).

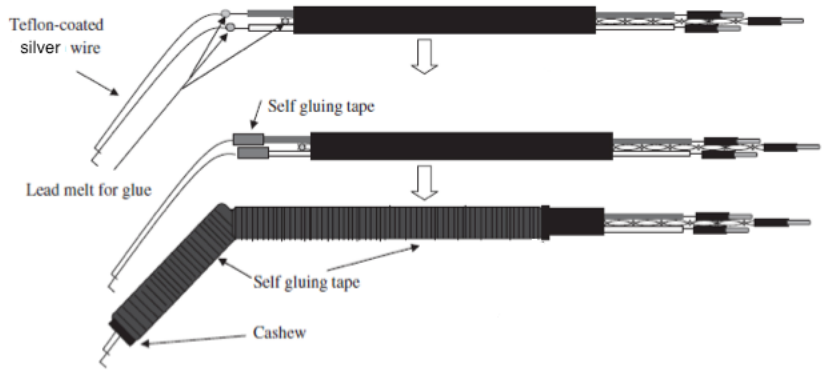


Figure 8.20 - Diagram of the recording electrodes adapted from Suga et al. 2005.

The only difference was that silver wire was used instead of tungsten wire and that micro capillaries were used to keep the wire straight and prevent it from bending when pressed against the test subject cranial region.

A large plasterer bath (1200 x 600 x 300 mm) was used as experimental tank and was filled with dechlorinated tap water. To cancel any potential vibrations resulting in some unwanted stimulation of the electrodes, four large air chambers were installed under the tank and acted as air cushions.

A large U-shaped frame made of wood and steel mesh was used as a "Faraday cage" and to support the speaker placed above the tank at 90 cm from the water surface. Following Yan H. recommendations (exchange via email), the speaker was wrapped in some fine copper mesh to cancel any electromagnetic noise that could disturb the recorded signal.

A smaller wooden frame, fixed to the top of the tank was used to support the micromanipulators and the electrodes (Figure 8.21 & 22).

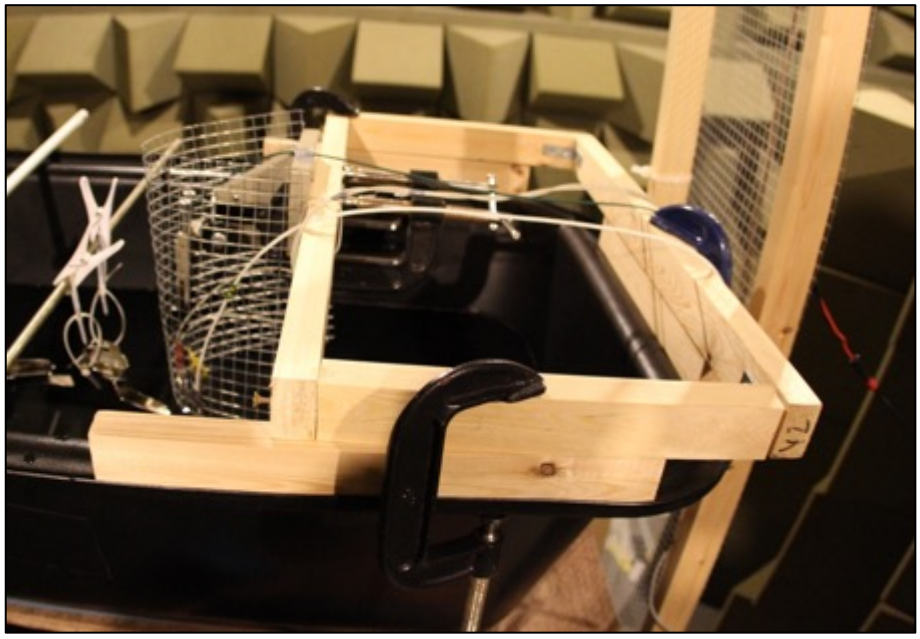


Figure 8.21 – Picture of the experimental setup of the ABR/AEP experiment – Wooden frame holding the micromanipulators.

Similarly to the setup by Kenyon et al. (1998), the fish was secured in some fine-mesh nylon screen, wrapped around it and maintained by two metal clamps (Figure 8.23). The clamps were attached to two fiber glass bars placed on the top of the tank and going across its width. Spacing between the clamps could be adapted according to the length of the fish by moving the bars along the length of the tank.

Figure 8.22 – Picture of the experimental setup for the ABR/AEP experiment held in the semi-anechoic chamber at the Institute of Sound and Vibration.

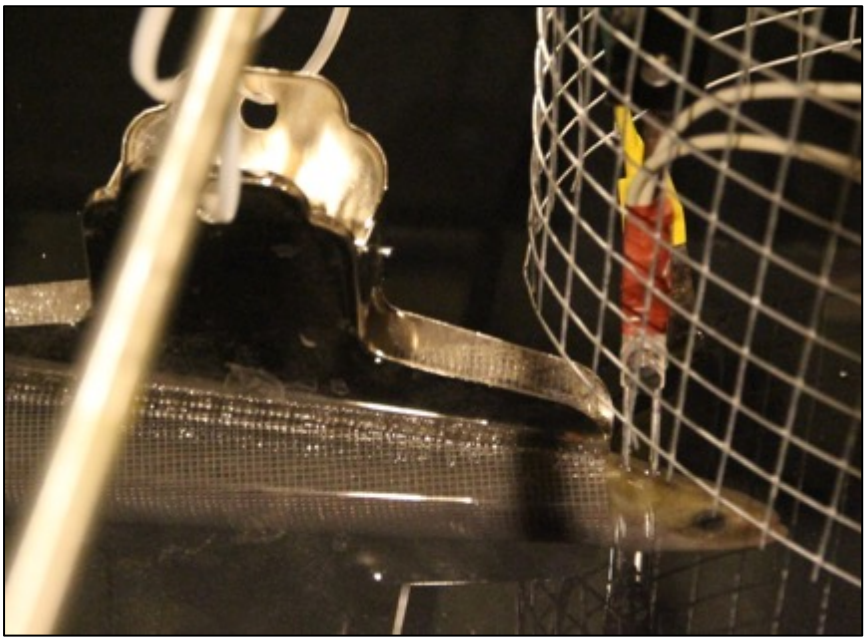


Figure 8.23 – Picture of the ABR/AEP experimental setup with a silver European eel. Silver wires Electrodes are in place and in contact with the midbrain region of the head.

In the majority of the ABR experiments, the authors injected fish with some immobilant, generally (gallamine triethiodide - Flaxedil) to prevent them to move during the recording procedure. Unfortunately, the Faculty of Engineering and Environment did not allow this procedure. Therefore, fish were sedated before being wrapped in the nylon mesh and being placed in the setup. A preparation of clove oil (diluted in ethanol) was used with a concentration of 1 mL / 5 Litre of water. Some of this preparation was also added, with a lower concentration, to the experimental bath in order to keep the fish sedated. The degree of sedation, allowed fish to perform weak opercular movements. After the experiment, the fish was transferred to a bucket of water highly oxygenated by an air pump and some air stones.

The Institute of Sound and Vibration provided the technical equipment to produce the stimulus and record the brainstem response. Sound was produced using a loud speaker, connected to a CED (Cambridge Electronic Design) 1902 pre-amplifier, itself connected to a CED Micro 1401-3 data acquisition unit, finally connected to a laptop. Electrodes were connected to the pre-amplifier via 4 channel electrode adaptor box (CED). Acoustic signal and brain response were respectively generated and monitored using CED Signals software installed on the laptop.

To test the efficiency of the setup, a couple of experiments were first undertaken on goldfish (*Carassius auratus*) that is generally considered as a reference model for this type of experiments. Goldfish is the most commonly studied species in the scientific literature and several audiograms have been produced for them using the AEP/ABR methods (Ladich & Fay 2013). Figure 8.24, illustrates the outcome of an ABR experiment carried out on goldfish at 600 Hz (Kenyon et al. 1998).

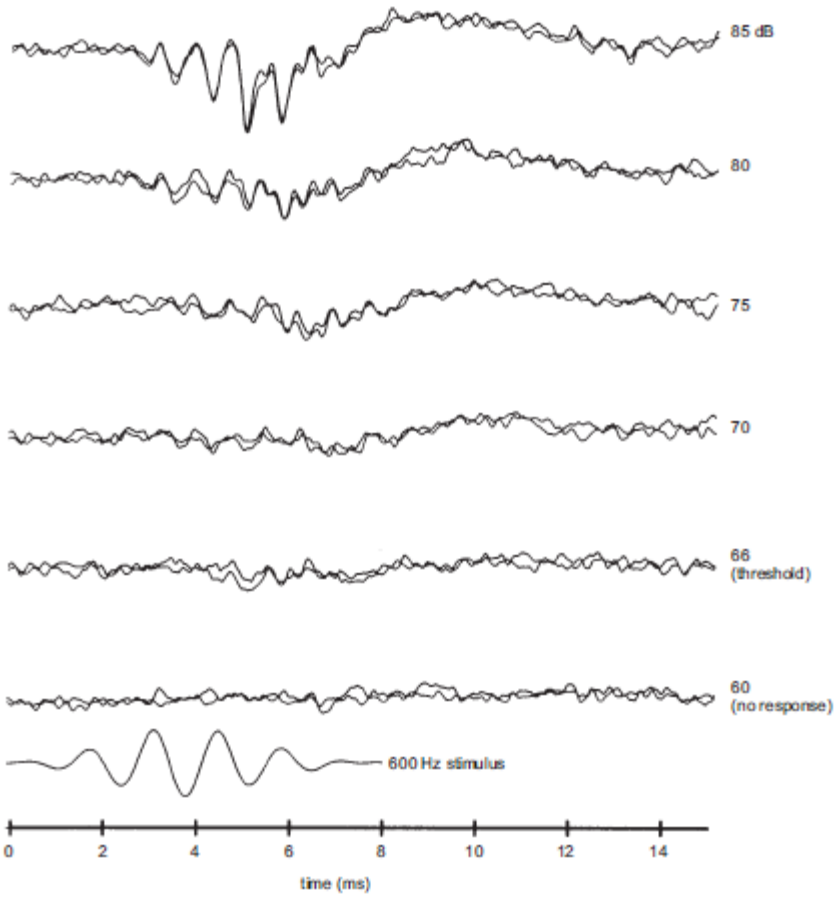


Figure 8.24 – Acoustically evoked brainwaves of a goldfish in response to a 600 Hz tone burst attenuated in 5 dB steps. Averaged traces of two different runs for each sound pressure level are overlaid. dB here is in relative scale (figures and caption from Kenyon et al. 1998)

8.2.2 **Results**

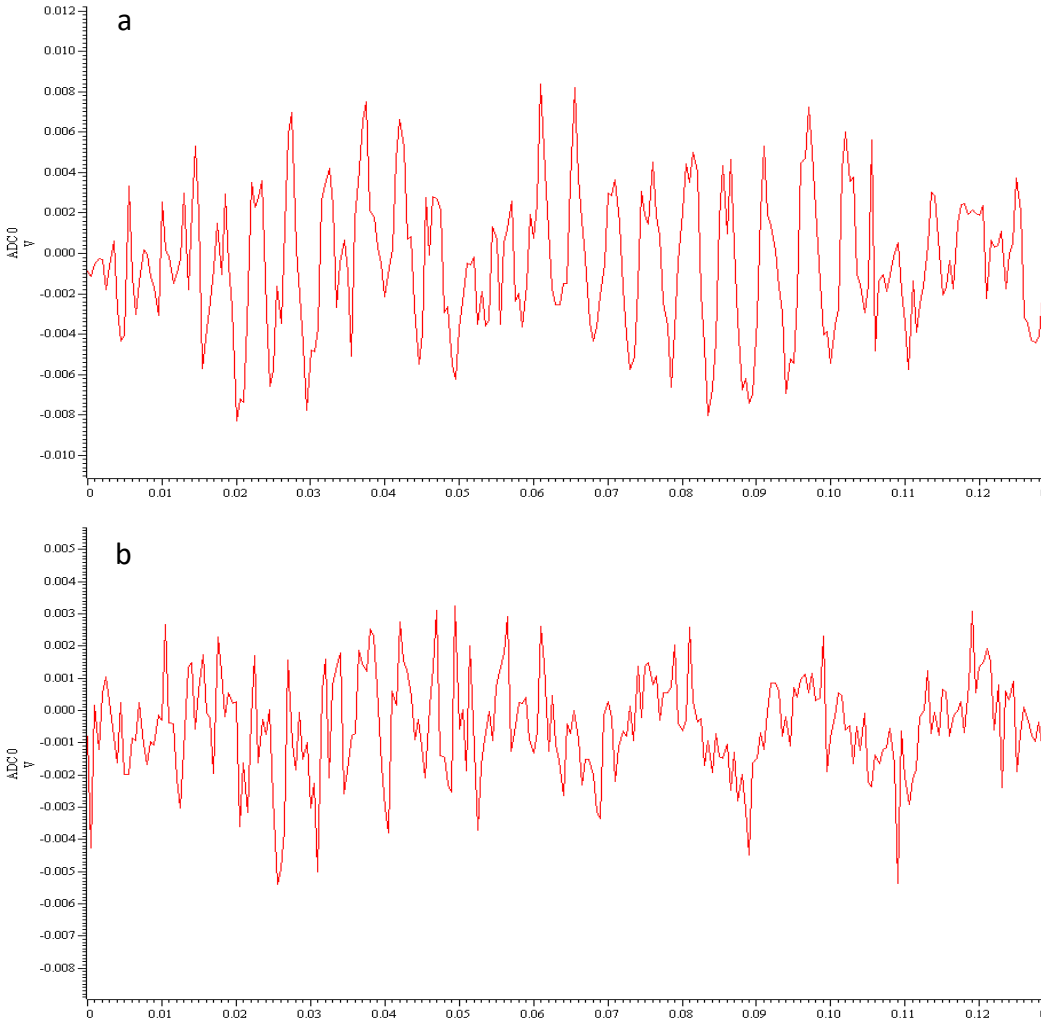


Figure 8.25a & 8.25b – Waveforms recordings from the Auditory Brainstem Response experiment undertaken on a goldfish (*Carassius auratus*) tested with a 100 Hz stimulus. Figures **a** & **b** respectively show the response at 130 and 120 dB. The X axis represents the time in seconds (s) and the Y axis is the level of the response in Volts (electrical potential).

Unfortunately, the outcome of this experiment was not consistent with the audiograms produced by other authors. Between Figures 8.25a and 8.25b the signals of the waveforms are very different. Even if an attenuation of the response is expected with the decrease of the acoustic stimulus intensity (-10 dB), a portion of the waveform should be (almost) identical between the two measurements (as in Kenyon et al. 1998).

Figures 8.25a & b, are just examples of the kind of readings that were obtained on multiple trials with goldfish. Nevertheless, the results were never conclusive even after several modifications of the setup and assistance from other researchers.

8.2.3 **Discussion and suggestions**

This experiment initially intended to produce two distinct audiograms for European eel and river lamprey and to further compare them with the one built with the behavioural method from Chapter 5. Nevertheless, the experimental setup was unsuccessful after many attempts. Therefore, the experiment was aborted due to a lack of time and possible exhaustion of the fish, kept for too long in captivity. Several factors were responsible of this failure and as part of this discussion, they are listed below as well as potential solution to overcome them and not repeat the same mistakes in the future.

This research was mainly inspired by the work from Kenyon et al. (1998), and the experimental design adapted from it. The first problem encountered during this experiment was that even if instructions from Kenyon et al. (1998) were carefully followed some details are missing in the description of their work and have been obtained by contacting directly one of the authors. For instance, the silver wire electrodes have to be "activated". This can be done by removing a small portion of the coating from the silver wire end tip (side in contact with the skin of the fish) and placing it in some bleach for several hours. The tip will be blackened meaning they have been chlorinated and therefore will be able to conduct the evoked potentials.

The second identified problem, and what is apparently appearing on the recording presented in the previous section is the electromagnetic noise. To overcome this problem multiple solutions can be applied to the setup. All the grounds from electric equipment need to be connected to a single grounding point. In addition, if using a loud speaker (as opposed of an underwater speaker), it is advised to wrap it up with some copper mesh to reduce/cancel that phenomena.

The third problem was that most of the studies of ABR in fish using exactly the same equipment from the same provider Tucker Davies Technology (TDT) especially configured for fish recordings in this case. The equipment used in our experimental setup was initially developed for recording ABR in humans and could hardly be compared to the one used in other studies. Furthermore, it is possible that the equipment that was used in this experiment was not sensitive enough to pick up the change in evoked potentials due to sound as the electric signal should be weaker in fish than in humans. For future experiments, if the budgetary conditions allow it, it would be suggested to use exactly the same type of equipment commonly used in other published paper (see Kenyon et al., 1998, Ladich & Fay, 1993).

Finally, the use of anaesthetic (clove oil or 2-phenoxy-ethanol) as a replacement to gallamine triethiodide - Flaxedil is not recommended. Flaxedil is injected directly to the fish providing a fast and long-lasting immobilization of the test subject, while both clove oil and 2-phenoxy-ethanol

are inhaled by the fish and therefore more difficult to control. The use of a respiratory system with re-circulating water is also very much recommended.

8.3 Chapter 5 (continuation) - Use of an acoustic maze to assess the behavioural response of anguilliform fish to underwater sound – 2nd Model

In Chapter 5, a Mixed Effect model was used to analyse the "passage time" of European eel (silver and yellow stages) and river lamprey. Initially, it was decided to split the "speaker region" into three separate sections (Block) representing the area preceeding the speaker, the speaker region (shorter version) and the area following the speaker (Figure 8.21). Each section or Block were equal in distance (45 cms) and corresponded to the length of the speaker box and of the concrete blocks constituting the maze. The idea was to "split" the passage behavior and to verify if fish potentially slowed down their approach to an active speaker or accelerate when passing at the closest point to the speaker where the sound level was the highest.

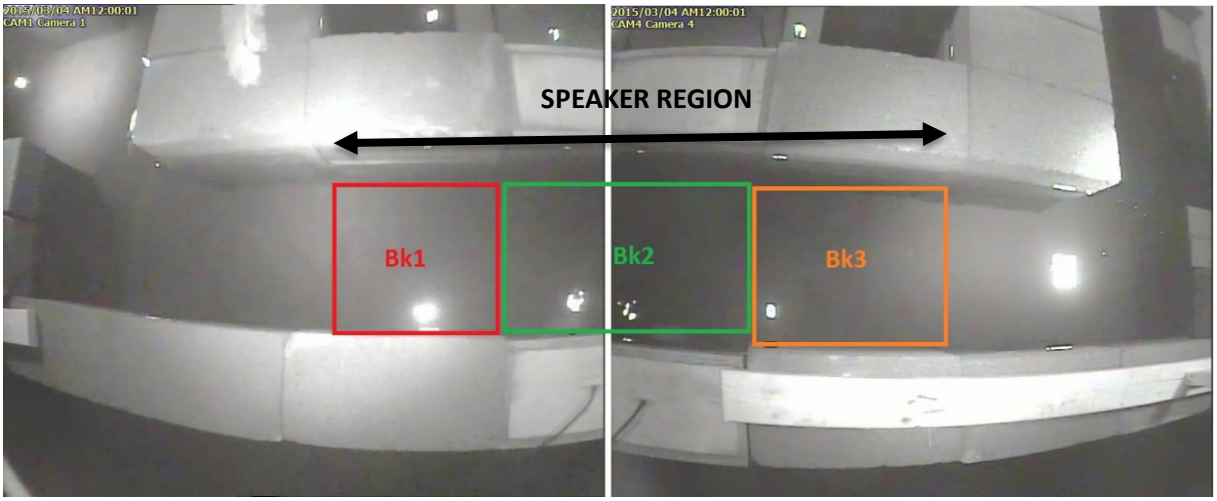


Figure 8.26 - Print Screen from NUUO Surveillance software used to monitor fish movements through the maze. The system of "Blocks" used to investigate changes of "passage time" for the fish are represented by the 3 different rectangles. Bk1 in red is the block preceding the Speaker, Bk2 in green is the block of the speaker and Bk3 in orange is the block after the speaker.

A mixed-effect model was used to interpret the set of data, " "Passage Time with Blocks" (Time) using the following factors: "Wall" fixed factor (2 levels, "Speaker" or "Opposite", indicates if the fish passed the speaker region swimming along the wall side of the speaker or opposite to it), "Sound" fixed factor (2 levels, "On" or "Off", indicates if the stimulus was playing or not), "Configuration/Config" (2 levels, "Cf1" and "Cf2" standing for the configuration speakers were in), "Length" and "Weight" (as many levels as fish), "Blocks" (3 levels, "Bk1", "Bk2", "Bk3"), "ID" random factor (as many levels as fish, individual tag number) and "Day" random factor (as many levels as days of experiments). "ID" and "Day" were used as random factor as it was anticipated

that the response would be different from a fish to another and also from one Day of experiment to another due to non-controlled parameters.

Interactions between factors have been also treated in this model and the lsmeans function (Least-squares means or predicted marginal means) in R, was used to discriminate the significant level of significant interactions.

8.3.1 **General results:**

When using the variable Passage Time with Blocks, the results differ slightly from the first model. Results are presented in Table 4. Contrary to the previous analysis the Sound factor only had a significant effect on the River Lamprey with a passage time of the blocks being longer when the sound was ON.

Similarly to the previous model, Yellow eel show a significant difference in the passage time of the blocks between the two Configurations, passage time being shorter in Configuration 1.

For both types of eel, the time to pass each block was significantly different. Silver eel spent the same amount of time in bk1 (before the speaker) and bk2 (speaker level) but much faster to pass the third block (bk3). On the other hand, yellow eel showed similar passage time for both bk1 and bk3 but were much slower in bk2, in front of the speaker.

Weight and Length did not have much effect on the passage time of the blocks except for the Silver eel for which Length seemed to have a significant effect (p-value = 0.0136)

Finally, the passage time per block was also significantly different for the factor Wall with the two types of eel. Both yellow and silver eel were significantly faster when swimming along the “Speaker” wall compare to the “opposite” one.

Table 8.1 -Results from the Fixed factors tests and percentages of variability due to random factors (ID & Day) from the mixed effect model for fish passage time through the "Blocks" of the "Speaker region".

Variable	**Passage Time**		
Species / **Factors**	*S. Eel*	*Y.Eel*	*R. Lamprey*
Sound	p-value = 0.3628	p-value = 0.5537	**p-value <0.0001**
Configuration	p-value = 0.5629	**p-value = 0.0075**	p-value = 0.6251
Block	**p-value = 0.0350** Bk1 = Bk2 Bk1 > Bk3 Bk2 > Bk3	**p-value <0.0001** Bk1 < Bk2 Bk2 > Bk3 Bk1 = Bk3	p-value = 0.4461
Weight / Length	Weight (0.1393) Length (0.0136*)	Weight (0.7632) Length (0.8740)	Weight (0.7840) Length (0.7379)
Wall	**p-value <0.0001** **Speaker < Opposite**	**p-value = 0.0004** **Speaker < Opposite**	p-value =0.5588
Interactions			
*Block*Sound*	**p-value = 0.0075**	**p-value <0.0001**	p-value = 0.7397
*Wall*Sound*	**p-value = 0.0002**	**p-value = 0.0248**	p-value = 0.1327
*Block*Wall*	**p-value <0.0001**	**p-value = 0.0365**	p-value = 0.1753
*Config*Wall*	p-value = 0.0677	p-value = 0.1590	p-value = 0.4322
*Block*Config*	**p-value = 0.0002**	p-value = 0.8882	p-value = 0.5141
*Config*Sound*	p-value = 0.3259	**p-value <0.0001**	**p-value = 0.0133**
*Block*Wall*Sound*	p-value = 0.0989	p-value = 0.0538	p-value = 0.4886
*Block*Config*Wall*	**p-value <0.0001**	p-value = 0.0980	p-value = 0.4578
*Config*Wall*Sound*	**p-value = 0.0063**	**p-value = 0.0003**	**p-value = 0.0012**
*Block*Config*Sound*	**p-value = 0.0046**	**p-value <0.0001**	**p-value = 0.0163**
*Block*Config*Wall*Sound*	**p-value <0.0001**	p-value = 0.8904	p-value = 0.1402

8.3.2 **Interactions**

Table 8.1 also includes results from the interactions of factors, also treated by the model. Results from the ls-means function are displayed in Table 8.2.

- *Block*Sound:*

For both type of eel no effect of sound on passage time per blocks was observed when considering only the "Sound" factor (absence or presence of sound) (see Table 4). Nevertheless, when considering the interaction Blocks*Sound it appears that passage time for specific Blocks differs between Sound On and Sound Off.

Silver eel are "faster" to pass the block preceding the speaker (Bk1) when the stimulus is not active (Sound Off) (p-value = 0.0232).

This is also the case for the Speaker block (Bk2) for both type of eel, fish were faster at passing "Bk2" when the sound was "Off" (p-value < 0.0001 for the Silver and p-value = 0.0024 for the Yellow eel).

Additionally, Yellow eel were spent more time to cross the Bk2 compare to the Bk3 (block following the Speaker) (p-value = 0.0001). They also showed a tendency (p-value= 0.0671) at passing Bk1 faster than Bk2 when the Sound was "On".

- *Wall*Sound*:

Generally, both Yellow and Silver eel showed a smaller passage time along the Speaker side ("spkr") when Sound was ON compare to the "opposite" side (respectively, p-value <0.0001 and 0.0017).

In addition, Yellow eel only showed a difference in passage time on the "opposite" side between Sound "On" and Sound "Off" trials, being faster when sound was "Off" (p-value = 0.0298).

- *Block*Wall*:

Both types of eel have shown a faster passage time of the Speaker block (Bk2) along the "opposite" wall compared to the Speaker (spkr) one (Silver eel, p-value < 0.0001 and Yellow eel, p-value = 0.0033).

Silver eel also tended to be longer at passing the first block (Bk1) along the "opposite" wall (p-value= 0.0549).

Finally, Yellow eel were longer at passing the Speaker block (Bk2) compared to the following one (Bk3) when swimming along the Speaker wall (spkr) (p-value = 0.0001).

- *Block*Config:*

This interaction was only significant in Silver eel with passage time of the speaker block (Bk2) being slower in the first Configuration (Cf1) compared to the second one (Cf2) (p-value = 0.0005). On the other hand, the opposite effect was observed at the level of the third block (Bk3) with Silver eel being faster in the "Cf1" compared to "Cf2" (p-value=0.0392).

- *Config*Sound:*

Yellow eel only showed a significant difference in passage time between "Cf1" and "Cf2" when the Sound was "Off" (p-value = 0.0023). They also tended to be slower at passing blocks when Sound was "Off" compared to Sound "On" in the first Configuration "Cf1" (p-value = 0.05).

River lamprey were faster at passing blocks when the Sound was "Off" in the Configuration 2 "Cf2", compared to when the stimulus was "On" (p-value <0.0001).

- *Block*Config*Wall:*

Only Silver eel have shown a significant response with this interaction. Passage time of the Speaker block (Bk2) along the "opposite" wall to speaker was slower in Configuration 1 (Cf1) compare to Configuration 2 (Cf2) (p-value < 0.0001). Also passage time in "Bk1" during Configuration 2 was smaller along the speaker wall ("spkr") compared to the "opposite" wall (p-value = 0.0043).

- *Config*Wall*Sound:*

Silver eel only showed a significant difference for block passage time, between the Sound "On" and "Off" during Configuration 1 along the opposite wall, passage time being shorter when sound was "Off" (p-value < 0.0001).

Yellow eel on the other hand showed multiple differences. Between Configuration 1 and 2, along the opposite wall with the stimulus "Off", yellow eel tended to pass faster during Configuration 1 (p-value = 0.0074). The same way they also seemed to pass faster in Configuration 1 compared to Configuration 2, this time along the speaker wall (spkr) when sound was "On" (p-value < 0.0001).

Results also showed a significant difference in passage time while Yellow eel were in Configuration 1 and between Sound “On” and “Off”. When Yellow eel swam along the “opposite” wall they were passing the blocks slower when sound was “On” (p-value = 0.0129) while when swimming along the speaker wall they were slower when sound was “Off” (p-value = 0.0026).

Yellow eel also seemed to be faster at passing blocks along the opposite wall compared to the speaker wall, when sound was “Off” in Configuration 2 (p-value = 0.0028). The opposite result applies to Configuration 1 with Yellow eel passing blocks faster along the speaker wall (spkr) when Sound was “On” (p-value = 0.0047).

River lamprey also showed some interesting results for that specific interaction.

A difference in the passage time of the blocks along the opposite wall in Configuration, between Sound “On” and Sound “Off”, with a faster passage on the Sound “Off” corridors (p-value = 0.005). The same statement applies for the passage time of blocks along the speaker wall (spkr), again faster when sound was “Off” (p-value = 0.05). Also, in Configuration 1 when the Sound was “On”, River lamprey passed the blocks faster along the opposite wall compared to the speaker wall (spkr) (p-value = 0.0041).

- *Block*Config*Sound:*

Silver European eel only showed significant differences in passage time of the blocks during Configuration 1 trials.

Differences in passage time occurred between Sound “On” and Sound “Off” corridors at the level of the second/speaker block (Bk2), with passage time being faster when sound was “Off” (p-value < 0.0001). In addition, a significant difference was found when Sound was “On” and when passing “Bk1” compare to “Bk2”. Silver eel seemed to be faster at passing the first block (Bk1) compare to the second one (Bk2) (p-value < 0.0001).

Yellow stage European eel returned the most significant differences for this interaction. Even if most of the results occurred in Configuration 2 only, yellow eel showed a difference in passage time of the block preceding the speaker (Bk1) when sound was “Off” between both Configurations. Yellow eel were faster at passing Bk1 in the Configuration 1 compare to Configuration 2 (p-value = 0.0001).

As just mentioned, the rest of the results are only treating of Configuration 2 (Cf2). When the Sound was “Off” Yellow eel passed the first block (Bk1) faster than the third one (Bk3) (p-value = 0.0421). On the other hand, when the Sound was “On”, they seemed to pass the speaker block

(Bk2) slower than the third one (Bk3) (p-value = 0.0003). Finally, the passage time to pass the second block (bk2) was faster when the sound was “Off” compare to when it was “On” (p-value = 0.0011).

River lamprey only returned one result for this interaction, with a significant difference in passage time of the first block (Bk1), between Sound “On” and Sound “Off” corridors in the Configuration 2. Fish were faster when the Sound was “Off” (p-value = 0.0005).

*Block*Config*Wall*Sound:*

This interaction only returned a significant result in Silver European eel. In Configuration 1, the passage time of the first block (Bk1), along the wall opposite to the speaker was faster when the sound was ‘Off’ compared to when it was “On” (p-value < 0.0001).

Table 8.2 - Results from the interactions tests (ls-means) for the three types of fish. Red "<" and ">" designate the interaction for which the passage time was the shortest (tip) and the longest. Note: The interactions "Config*Wall" and "Block*Wall*Sound" are not included as they were not significant for any species (Table 8.1).

Species / Interactions	*S. Eels*	*Y.Eels*	*R. Lampreys*
*Block*Sound*	Bk1*OFF < Bk1*ON (p-value = 0.0232) Bk2*OFF < Bk2*ON (p-value < 0.0001)	Bk2*OFF < Bk2*ON (p-value = 0.0024) Bk2*ON > Bk3*ON (p-value = 0.0001)	**not significant**
*Wall*Sound*	opposite*ON > spkr*ON (p-value < 0.0001)	opposite*OFF < opposite*ON (p-value = 0.0298) opposite*ON > spkr*ON (p-value = 0.0017)	**not significant**
*Block*Wall*	Bk2*opposite > Bk2*spkr (p-value < 0.0001)	Bk2*opposite > Bk2*spkr (p-value = 0.0033) Bk2*spkr > Bk3*spkr (p-value = 0.0001)	**not significant**
*Block*Config*	Bk2* Cf1 > Bk2*Cf2 (p-value = 0.0005) Bk3* Cf1 < Bk3* Cf2 (p-value = 0.0392)	**not significant**	**not significant**
*Config*Sound*	**not significant**	Cf1*OFF < Cf2*OFF (p-value = 0.0023) Cf1*OFF > Cf1*ON (p-value = 0.05)	Cf2*OFF < Cf2*ON (p-value <0,0001)
*Block*Config*Wall*	Bk2*Cf1*opposite > Bk2*Cf2*opposite (p-value < 0.0001) Bk1*Cf2*opposite > Bk1*Cf2*spkr (p-value = 0.0043)	**not significant**	**not significant**
*Config*Wall*Sound*	Cf1*opposite*OFF < Cf1*opposite*ON (p-value < 0.0001)	Cf1*opposite*OFF < Cf2*opposite*OFF (p-value = 0.0074) Cf1*spkr*ON < Cf2*spkr*ON (p-value <0,0001) Cf1*opposite*OFF < Cf1*opposite*ON (p-value = 0.0129) Cf1*spkr*OFF > Cf1*spkr*ON (p-value = 0.0026) Cf2*opposite*OFF < Cf2*spkr*OFF (p-value = 0.0028) Cf1*opposite*ON > Cf1*spkr*ON (p-value = 0.0047)	Cf2*opposite*OFF < Cf2*opposite*ON (p-value = 0,0050) Cf1*opposite*ON < Cf1*spkr*ON (p-value = 0,0041) Cf2*spkr*OFF < Cf2*spkr*ON (p-value = 0,05)
*Block*Config*Sound*	Bk2*Cf1*OFF < Bk2*Cf1*ON (p-value < 0.0001) Bk1*Cf1*ON < Bk2*Cf1*ON (p-value < 0.0001)	Bk1*Cf1*OFF < Bk1*Cf2*OFF (p-value = 0.0001) Bk1*Cf2*OFF > Bk3*Cf2*OFF (p-value = 0.0421) Bk2*Cf2*ON > Bk3*Cf2*ON (p-value = 0.0003) Bk2*Cf2*OFF < Bk2*Cf2*ON (p-value = 0.0011)	Bk1*Cf2*OFF < Bk1*Cf2*ON (p-value = 0,0005)
*Block*Config*Wall*Sound*	Bk2*Cf1*opposite*OFF < Bk2*Cf1*opposite*ON (p-value < 0.0001)	**not significant**	**not significant**

List of References

Aalto, E., Capoccioni, F., Terradez Mas, J., Schiavina, M., Leone, C., De Leo, G., & Ciccotti, E. (2015). Quantifying 60 years of declining European eel (Anguilla anguilla L., 1758) fishery yields in Mediterranean coastal lagoons. ICES Journal of Marine Science, 73(1), 101-110.

Allibone, R., David, B., Hitchmough, R., Jellyman, D., Ling, N., Ravenscroft, P., & Waters, J. (2010). Conservation status of New Zealand freshwater fish, 2009. New Zealand Journal of Marine and Freshwater Research, 44(4), 271-287.

Amaral, S., Giza, D., Sullivan, L., Timko, M., Jacobson, P., & Perry, E. (2016). Eels III: Evaluation of Behavioral Cues for Guiding Silver Eels at Hydro Projects.

Amorim, M. C. P., Fonseca, P. J., & Almada, V. C. (2003). Sound production during courtship and spawning of Oreochromis mossambicus: male–female and male–male interactions. Journal of fish biology, 62(3), 658-672.

Andersson, M. H. (2011). Offshore wind farms-ecological effects of noise and habitat alteration on fish (Doctoral dissertation, Department of Zoology, Stockholm University).

Andjus, R. K., Damjanović, I., Gačić, Z., Konjević, D. J., & Andjus, P. R. (1998). Electroretinographic evaluation of spectral sensitivity in yellow and silver eels (Anguilla anguilla). Visual neuroscience, 15(5), 923-930.

Aparicio, E., Vargas, M. J., Olmo, J. M., & De Sostoa, A. (2000). Decline of native freshwater fishes in a Mediterranean watershed on the Iberian Peninsula: a quantitative assessment. Environmental Biology of Fishes, 59(1), 11-19.

Åström, M., & Dekker, W. (2007). When will the eel recover? A full life-cycle model. *ICES Journal of Marine Science*, *64*(7), 1491-1498.

Ayali, A., Gelman, S., Tytell, E. D., & Cohen, A. H. (2009). Lateral-line activity during undulatory body motions suggests a feedback link in closed-loop control of sea lamprey swimming. Canadian Journal of Zoology, 87(8), 671-683.

Bailey, H., Senior, B., Simmons, D., Rusin, J., Picken, G., & Thompson, P. M. (2010). Assessing underwater noise levels during pile-driving at an offshore windfarm and its potential effects on marine mammals. Marine Pollution Bulletin, 60(6), 888-897.

Baltazar-Soares, M., Biastoch, A., Harrod, C., Hanel, R., Marohn, L., Prigge, E., ... & Eizaguirre, C. (2014). Recruitment collapse and population structure of the European eel shaped by local ocean current dynamics. *Current Biology*, *24*(1), 104-108.

Baras, E. (1998). Selection of optimal positioning intervals in fish tracking: an experimental study on Barbus barbus. In Advances in Invertebrates and Fish Telemetry (pp. 19-28). Springer, Dordrecht.

Barton, B. A. (2002). Stress in Fishes: A diversity of responses with particular reference to changes in circulating corticosteroids1. Integrative and comparative biology, 42(3), 517-525.

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

List of References

Bass, A. H., & McKibben, J. R. (2003). Neural mechanisms and behaviors for acoustic communication in teleost fish. Progress in neurobiology, 69(1), 1-26.

Belpaire, C., Geeraerts, C., Evans, D., Ciccotti, E., & Poole, R. (2011). The European eel quality database: towards a pan-European monitoring of eel quality. Environmental monitoring and assessment, 183(1-4), 273-284.

Bevacqua, D., Melià, P., Gatto, M., & De Leo, G. A. (2015). A global viability assessment of the European eel. Global change biology, 21(9), 3323-3335.

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

Bregman, A. S. (1994). Auditory scene analysis: The perceptual organization of sound. MIT press.

Brönmark, C., Skov, C., Brodersen, J., Nilsson, P. A., & Hansson, L. A. (2008). Seasonal migration determined by a trade-off between predator avoidance and growth. PloS one, 3(4), e1957.

Bruijs, M. C., & Durif, C. M. (2009). Silver eel migration and behaviour. In Spawning migration of the European eel (pp. 65-95). Springer Netherlands.

Bruintjes, R., Purser, J., Everley, K. A., Mangan, S., Simpson, S. D., & Radford, A. N. (2016). Rapid recovery following short-term acoustic disturbance in two fish species. *Royal Society open science*, *3*(1), 150686.

Bui, S., Oppedal, F., Korsøen, Ø. J., Sonny, D., & Dempster, T. (2013). Group behavioural responses of Atlantic salmon (Salmo salar L.) to light, infrasound and sound stimuli. PloS one, 8(5), e63696.

Byrne, B., Houlsby, G., Martin, C., & Fish, P. (2002). Suction caisson foundations for offshore wind turbines. *Wind Engineering*, *26*(3), 145-155.

Bythell, J. C., Hillis-Starr, Z. M., & Rogers, C. S. (2000). Local variability but landscape stability in coral reef communities following repeated hurricane impacts. Marine Ecology Progress Series, 204, 93-100.

Calles, O., Olsson, I. C., Comoglio, C., Kemp, P. S., Blunden, L., Schmitz, M., & Greenberg, L. A. (2010). APPLIED ISSUES: Size-dependent mortality of migratory silver eels at a hydropower plant, and implications for escapement to the sea. Freshwater Biology, 55(10), 2167-2180.

Cannon, W. B. (1929). Bodily changes in pain, fear, hunger, and rage. *New York, Appleton*.

Casper, B. M., & Mann, D. A. (2006). Evoked potential audiograms of the nurse shark (Ginglymostoma cirratum) and the yellow stingray (Urobatis jamaicensis). Environmental Biology of Fishes, 76(1), 101-108.

Casper, B. M., Lobel, P. S., & Yan, H. Y. (2003). The hearing sensitivity of the little skate, Raja erinacea: a comparison of two methods. Environmental Biology of Fishes, 68(4), 371-379.

Casper, B. M., Popper, A. N., Matthews, F., Carlson, T. J., & Halvorsen, M. B. (2012). Recovery of barotrauma injuries in Chinook salmon, Oncorhynchus tshawytscha from exposure to pile driving sound. PloS one, 7(6), e39593.

Cederholm, C. J., Kunze, M. D., Murota, T., & Sibatani, A. (1999). Pacific salmon carcasses: essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. Fisheries, 24(10), 6-15.

Chrousos, G. P. (1998). Stressors, stress, and neuroendocrine integration of the adaptive response: The 1997 Hans Selye Memorial Lecture. Annals of the New York Academy of Sciences, 851(1), 311-335.

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

Compton, R., Goodwin, L., Handy, R., & Abbott, V. (2008). A critical examination of worldwide guidelines for minimising the disturbance to marine mammals during seismic surveys. *Marine Policy*, *32*(3), 255-262.

Coombs, S., & Montgomery, J. C. (1999). The enigmatic lateral line system. In Comparative hearing: Fish and amphibians (pp. 319-362). Springer, New York, NY.

Costanza, R., d'Arge, R., de Groot, R., Farberk, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R.G., Sutton, P. and van den Belt, M. 1997. The value of the world's ecosystem services and natural capital. Nature, 387, 253-260.

Coutant, C. C., & Whitney, R. R. (2000). Fish behavior in relation to passage through hydropower turbines: a review. Transactions of the American Fisheries Society, 129(2), 351-380.

Cowx, I. G., Harvey, J. P., Noble, R. A., & Nunn, A. D. (2010). Monitoring fish populations in river SACs. In Conservation Monitoring in Freshwater Habitats (pp. 53-62). Springer, Dordrecht.

Crawley, M. J. (2012). The R book. John Wiley & Sons.

Dahl, P. H., de Jong, C. A., & Popper, A. N. (2015). The underwater sound field from impact pile driving and its potential effects on marine life. Acoustics Today, 11(2), 18-25.

Dähne, M., Tougaard, J., Carstensen, J., Rose, A., & Nabe-Nielsen, J. (2017). Bubble curtains attenuate noise from offshore wind farm construction and reduce temporary habitat loss for harbour porpoises. Marine Ecology Progress Series, 580, 221-237.

de Bie, J., Peirson, G., & Kemp, P. S. (2018). Effectiveness of horizontally and vertically oriented wedge-wire screens to guide downstream moving juvenile chub (Squalius cephalus). *Ecological Engineering*, *123*, 127-134.

Deegan, L. A. (1993). Nutrient and energy transport between estuaries and coastal marine ecosystems by fish migration. Canadian Journal of Fisheries and Aquatic Sciences, 50(1), 74-79.

Dekker, W., Pawson, M., & Wickström, H. (2007). Is there more to eels than slime? An introduction to papers presented at the ICES Theme Session in September 2006. ICES Journal of Marine Science, 64(7), 1366-1367.

Dekker, W. (2003a). Did lack of spawners cause the collapse of the European eel, Anguilla anguilla?. *Fisheries Management and ecology*, *10*(6), 365-376.

Dekker, W. (2003b). Status of the European eel stock and fisheries. In *Eel biology* (pp. 237-254). Springer, Tokyo.

Directive, S. F. (2008). Directive 2008/56/EC of the European Parliament and of the Council. *Official Journal of the European Union.*

List of References

Directive, W. F. (2000). Directive 2000/60. EC of the European Parliament and of the Council of, 23(1).

Docker, M. F. (Ed.). (2014). Lampreys: Biology, Conservation and Control (Vol. 1). Springer.

Dodds, W. and Whiles, M. 2010. Freshwater Ecology: Concepts and Environmental Applications of Limnology. Elsivier Inc., London, UK. pp. 829.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., ... & Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews, 81(2), 163-182.

Dunning, D. J., Ross, Q. E., Geoghegan, P., Reichle, J. J., Menezes, J. K., & Watson, J. K. (1992). Alewives avoid high-frequency sound. *North American Journal of Fisheries Management*, *12*(3), 407-416.

Esguícero, A. L., & Arcifa, M. S. (2010). Fragmentation of a Neotropical migratory fish population by a century-old dam. Hydrobiologia, 638(1), 41-53.

EC 1100/2007 (2007). Council Regulation (EC) No 1100/2007 of 18 september 2007 establishing measures for the recovery of the stock of European eel. Official Journal of the European Union, 50, 17-23.

EIFAC, J. (2006). Report of the 2006 session of the Joint EIFAC/ICES Working Group on EELS. Rome, Italy, 23-27 January 2006.

Elvidge, C. K., Ford, M. I., Pratt, T. C., Smokorowski, K. E., Sills, M., Patrick, P. H., & Cooke, S. J. (2018). Behavioural guidance of yellow-stage American eel Anguilla rostrata with a light-emitting diode device. Endangered Species Research, 35, 159-168.

Erbe, C. (2013). International Regulation Of Underwater Noise. Acoustics Australia, 41(1).

FAO, F. Aquaculture Department (2012) The state of world fisheries and aquaculture 2012. Food and Agriculture Organization of the United Nations, Rome.

Fay, R. R., & Popper, A. N. (2000). Evolution of hearing in vertebrates: the inner ears and processing. Hearing research, 149(1-2), 1-10.

Feunteun, E. (2002). Management and restoration of European eel population (Anguilla anguilla): an impossible bargain. Ecological engineering, 18(5), 575-591.

Fewtrell, J. L., & McCauley, R. D. (2012). Impact of air gun noise on the behaviour of marine fish and squid. *Marine pollution bulletin*, *64*(5), 984-993.

Ficke, A. D., Myrick, C. A., & Kondratieff, M. C. (2012). The effects of PIT tagging on the swimming performance and survival of three nonsalmonid freshwater fishes. Ecological Engineering, 48, 86-91.

Fine, M. L., & Parmentier, E. (2015). Mechanisms of fish sound production. In *Sound communication in fishes* (pp. 77-126). Springer, Vienna.

Finfer, D. C., Leighton, T. G., & White, P. R. (2008). Issues relating to the use of a 61.5 dB conversion factor when comparing airborne and underwater anthroprogenic noise levels. Applied acoustics, 69(5), 464-471.

Finneran, J. J., & Hastings, M. C. (2000). A mathematical analysis of the peripheral auditory system mechanics in the goldfish (Carassius auratus). The Journal of the Acoustical Society of America, 108(3), 1308-1321.

Fish, J. F., & Offutt, G. C. (1972). Hearing thresholds from toadfish, Opsanus tau, measured in the laboratory and field. The Journal of the Acoustical Society of America, 51(4B), 1318-1321.

Fisheries, F. A. O. (2016). Aquaculture Department (2010) The state of world fisheries and aquaculture. Food and Agriculture Organization of the United Nations, Rome.

Fletcher, L. B., & Crawford, J. D. (2001). Acoustic detection by sound-producing fishes (Mormyridae): the role of gas-filled tympanic bladders. Journal of Experimental Biology, 204(2), 175-183.

Fletcher, M. D., Lloyd Jones, S., White, P. R., Dolder, C. N., Leighton, T. G., & Lineton, B. (2018). Effects of very high-frequency sound and ultrasound on humans. Part I: Adverse symptoms after exposure to audible very-high frequency sound. *The Journal of the Acoustical Society of America*, *144*(4), 2511-2520.

Fletcher, M. D., Lloyd Jones, S., White, P. R., Dolder, C. N., Leighton, T. G., & Lineton, B. (2018). Effects of very high-frequency sound and ultrasound on humans. Part II: A double-blind randomized provocation study of inaudible 20-kHz ultrasound. *The Journal of the Acoustical Society of America*, *144*(4), 2521-2531.

Freyhof, J. (2013). Lampetra fluviatilis. The IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Accessed on 11 July 2014.

Freyhof, J., & Brooks, E. (2011). European red list of freshwater fishes (p. 61). Luxembourg: Publications office of the European Union.

Galhardo, L., & Oliveira, R. F. (2009). Psychological stress and welfare in fish. Annual Review of Biomedical Sciences, 1-20.

Geeraerts, C., & Belpaire, C. (2010). The effects of contaminants in European eel: a review. Ecotoxicology, 19(2), 239-266.

Gessel, M. H., Williams, J. G., Brege, D. A., Krcma, R. F., & Chambers, D. R. (1991). Juvenile salmonid guidance at the Bonneville Dam second powerhouse, Columbia River, 1983–1989. *North American Journal of Fisheries Management*, *11*(3), 400-412.

Gerald, J. W. (1971). Sound production during courtship in six species of sunfish (Centrarchidae). Evolution, 25(1), 75-87.

Hall, D., James, D., & Marsden, N. (2012). Marginal gains: Olympic lessons in high performance for organisations. HR Bulletin: Research and Practice, 7(2), 9-13.

Hart, N. S., & Collin, S. P. (2015). Sharks senses and shark repellents. Integrative zoology, 10(1), 38-64.

List of References

Haymes, G. T., & Patrick, P. H. (1986). Exclusion of adult Alewife, Alosa pseudoharengus using low-frequency sound for application at water intakes. Canadian Journal of Fisheries and Aquatic Sciences, 43(4), 855-862.

Helsinki Commission. (1988). *Report of the ninth meeting of the Baltic Marine Environment Protection Commission-Helsinki Commission (HELCOM). Convention on the Protection of the Marine Environment of the Baltic Sea Area*(No. INIS-XA-N--325). Helsinki Commission (HELCOM).

Henshilwood, C. S., d'Errico, F., Yates, R., Jacobs, Z., Tribolo, C., Duller, G. A., ... & Wintle, A. G. (2002). Emergence of modern human behavior: Middle Stone Age engravings from South Africa. Science, 295(5558), 1278-1280.

Higgs, D. M., Plachta, D. T. T., Rollo, A. K., Singheiser, M., Hastings, M. C., & Popper, A. N. (2004). Development of ultrasound detection in American shad (Alosa sapidissima). Journal of Experimental Biology, 207(1), 155-163.

Hirt-Chabbert, J. A., & Young, O. A. (2012). Effects of surgically implanted PIT tags on growth, survival and tag retention of yellow shortfin eels Anguilla australis under laboratory conditions. Journal of Fish Biology, 81(1), 314-319.

Hope, A. J., Partridge, J. C., & Hayes, P. K. (1998). Switch in rod opsin gene expression in the European eel, Anguilla anguilla (L.). Proceedings of the Royal Society of London B: Biological Sciences, 265(1399), 869-874.

House, I., & Street, B. (2017). JNCC guidelines for minimising the risk of injury to marine mammals from geophysical surveys.

Hureau, J. C., Bauchot, M. L., Tortonese, E., & Nielsen, J. (1984). Fishes of the North-eastern Atlantic and the Mediterranean Vol. 1.

Hutchings, J. A. (2000). Collapse and recovery of marine fishes. Nature, 406(6798), 882.

Hutchings, J. A., & Reynolds, J. D. (2004). Marine fish population collapses: consequences for recovery and extinction risk. AIBS Bulletin, 54(4), 297-309.

Igoe, F., Quigley, D. T. G., Marnell, F., Meskell, E., O'Connor, W., & Byrne, C. (2004). The sea lamprey Petromyzon marinus (L.), river lamprey Lampetra fluviatilis (L.) and brook lamprey Lampetra planeri (Bloch) in Ireland: general biology, ecology, distribution and status with recommendations for conservation. In Biology and Environment: Proceedings of the Royal Irish Academy (pp. 43-56). Royal Irish Academy.

Jacoby, D. and Gollock, M. (2014). Anguilla anguilla. The IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Accessed on 14 July 2014.

Jakob, E., Hanel, R., Klimpel, S., & Zumholz, K. (2008). Salinity dependence of parasite infestation in the European eel Anguilla anguilla in northern Germany. ICES Journal of Marine Science, 66(2), 358-366.

Janetski, D. J., Chaloner, D. T., Tiegs, S. D., & Lamberti, G. A. (2009). Pacific salmon effects on stream ecosystems: a quantitative synthesis. Oecologia, 159(3), 583-595.

Jerkø, H., Turunen-Rise, I., Enger, P. S., & Sand, O. (1989). Hearing in the eel (Anguilla anguilla). Journal of Comparative Physiology A, 165(4), 455-459.

Johansson, K. (2011). Impact of anthropogenic noise on fish behaviour and ecology. *Introductory Research Essay No. 12*, Department of Wildlife, Fish, and Environmental Studies ,Swedish University of Agricultural Sciences?

Joint Nature Conservation Committee. (2010). Statutory nature conservation agency protocol for minimising the risk of injury to marine mammals from piling noise. *Inverness: JNCC*.

Kasumyan, A. O. (2004). The vestibular system and sense of equilibrium in fish. Journal of Ichthyology, 44(2), S224.

Kelly, F. L., & King, J. J. (2001, December). A review of the ecology and distribution of three lamprey species, Lampetra fluviatilis (L.), Lampetra planeri (Bloch) and Petromyzon marinus (L.): a context for conservation and biodiversity considerations in Ireland. In Biology and Environment: Proceedings of the Royal Irish Academy (pp. 165-185). Royal Irish Academy.

Kemp, P. S. (2015). Impoundments, barriers and abstractions. *Freshwater fisheries ecology*, 717-769.

Kemp, P. S. (2016). Meta-analyses, Metrics and Motivation: Mixed Messages in the Fish Passage Debate. River Research and Applications, 32(10), 2116-2124.

Kenyon, T. N., Ladich, F., & Yan, H. Y. (1998). A comparative study of hearing ability in fishes: the auditory brainstem response approach. Journal of Comparative Physiology A, 182(3), 307-318.

Kerr, J. R. (2015). Quantification of new methods, behaviour and hydrodynamics for improving fish passage at anthropogenic barriers (Doctoral dissertation, University of Southampton).

Kettle, A. J., Asbjørn Vøllestad, L., & Wibig, J. (2011). Where once the eel and the elephant were together: decline of the European eel because of changing hydrology in southwest Europe and northwest Africa?. Fish and Fisheries, 12(4), 380-411.

Knights, B. (2003). A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. Science of the total Environment, 310(1-3), 237-244.

Knudsen, F. R., Enger, P. S., & Sand, O. (1992). Awareness reactions and avoidance responses to sound in juvenile Atlantic salmon, Salmo salar L. Journal of Fish Biology, 40(4), 523-534.

Knudsen, F. R., Enger, P. S., & Sand, O. (1994). Avoidance responses to low frequency sound in downstream migrating Atlantic salmon smolt, Salmo salar. Journal of Fish Biology, 45(2), 227-233.

Knudsen, F. R., Schreck, C. B., Knapp, S. M., Enger, P. S., & Sand, O. (1997). Infrasound produces flight and avoidance responses in Pacific juvenile salmonids. Journal of Fish Biology, 51(4), 824-829.

Kojima, T., Suga, T., Kusano, A., Shimizu, S., Matsumoto, H., Aoki, S., ... & Taniuchi, T. (2010). Acoustic pressure sensitivities and effects of particle motion in red sea bream Pagrus major. Fisheries Science, 76(1), 13.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., ... & Blokhuis, H. J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, *23*(7), 925-935.

Krause, J., & Godin, J. G. J. (1995). Predator preferences for attacking particular prey group sizes: consequences for predator hunting success and prey predation risk. Animal Behaviour, 50(2), 465-473.

Kujawa, R., Fopp-Bayat, D., Cejko, B. I., Kucharczyk, D., Glińska-Lewczuk, K., Obolewski, K., & Biegaj, M. (2018). Rearing river lamprey Lampetra fluviatilis (L.) larvae under controlled conditions as a tool for restitution of endangered populations. Aquaculture International, 26(1), 27-36.

Kynard, B., & O'Leary, J. (1990). Behavioral guidance of adult American shad using underwater AC electrical and acoustic fields. In Proceedings of the International Symposium on Fishways' 90 (p. 131).

Ladich, F., & Bass, A. H. (2003). Underwater sound generation and acoustic reception in fishes with some notes on frogs. In Sensory Processing in Aquatic Environments (pp. 173-193). Springer, New York, NY.

Ladich, F., & Fay, R. R. (2013). Auditory evoked potential audiometry in fish. Reviews in fish biology and fisheries, 23(3), 317-364.

Larinier, M., & Marmulla, G. (2004). Fish passes: types, principles and geographical distribution–an overview. In Proceedings of the second international symposium on the management of large rivers for fisheries (Vol. 2, pp. 183-206). RAP Publication.

Leighton, T.G. (1996) The Acoustic Bubble, (paperback edition), London, San Diego, Academic Press, 640 pages (ISBN 0124419216).

Leighton, T. G. (2012). How can humans, in air, hear sound generated underwater (and can goldfish hear their owners talking)?. *The Journal of the Acoustical Society of America*, *131*(3), 2539-2542.

Leighton, T. G. (2016). Are some people suffering as a result of increasing mass exposure of the public to ultrasound in air?. *Proc. R. Soc. A*, *472*(2185), 20150624.

Leighton, T. G. (2017). Comment on 'Are some people suffering as a result of increasing mass exposure of the public to ultrasound in air?'. *Proc. R. Soc. A*, *473*(2199), 20160828.

Leighton, T. G. (2018). Ultrasound in air—Guidelines, applications, public exposures, and claims of attacks in Cuba and China. *The Journal of the Acoustical Society of America*, *144*(4), 2473-2489.

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. Canadian journal of zoology, 68(4), 619-640.

Limburg, K. E., & Waldman, J. R. (2009). Dramatic declines in North Atlantic diadromous fishes. BioScience, 59(11), 955-965.

Lowenstein, O., & Osborne, M. P. (1964). Ultrastructure of the sensory hair-cells in the labyrinth of the ammocoete larva of the lamprey, Lampetra fluviatilis. Nature, 204(4954), 197.

Lowenstein, O. (1970). The electrophysiological study of the responses of the isolated labyrinth of the lamprey (Lampetra fluviatilis) to angular acceleration, tilting and mechanical vibration. Proceedings of the Royal Society of London. Series B, Biological Sciences, 419-434.

Lucas, M. C., & Baras, E. (2001). Migration of Freshwater Fishes Blackwell Science Ltd.

Lucas, M. C., & Frear, P. A. (1997). Effects of a flow-gauging weir on the migratory behaviour of adult barbel, a riverine cyprinid. Journal of Fish Biology, 50(2), 382-396.

MacNamara, R. (2012). Conservation biology of the European eel *(Anguilla anguilla)* on a hydropower-regulated Irish river (Doctoral dissertation).

Maes, J., Turnpenny, A. W. H., Lambert, D. R., Nedwell, J. R., Parmentier, A., & Ollevier, F. (2004). Field evaluation of a sound system to reduce estuarine fish intake rates at a power plant cooling water inlet. Journal of Fish Biology, 64(4), 938-946.

Maitland, P. S., Renaud, C. B., Quintella, B. R., Close, D. A., & Docker, M. F. (2015). Conservation of native lampreys. In Lampreys: biology, conservation and control (pp. 375-428). Springer, Dordrecht.

Magnhagen, C., & Staffan, F. (2005). Is boldness affected by group composition in young-of-the-year perch (Perca fluviatilis)?. Behavioral Ecology and Sociobiology, 57(3), 295-303.

Maruska, K. P., Boyle, K. S., Dewan, L. R., & Tricas, T. C. (2007). Sound production and spectral hearing sensitivity in the Hawaiian sergeant damselfish, Abudefduf abdominalis. Journal of Experimental Biology, 210(22), 3990-4004.

Masters, J. E., Jang, M. H., Ha, K., Bird, P. D., Frear, P. A., & Lucas, M. C. (2006). The commercial exploitation of a protected anadromous species, the river lamprey (Lampetra fluviatilis (L.)), in the tidal River Ouse, north-east England. Aquatic Conservation: Marine and Freshwater Ecosystems, 16(1), 77-92.

Mateus, C. S., Rodríguez-Muñoz, R., Quintella, B. R., Alves, M., & Almeida, P. R. (2012). Lampreys of the Iberian Peninsula: distribution, population status and conservation. Endangered Species Research, 16, 183-198.

Mazeroll, A. I., & Montgomery, W. L. (1998). Daily migrations of a coral reef fish in the Red Sea (Gulf of Aqaba, Israel): initiation and orientation. Copeia, 893-905.

McCauley, R. D., & Cato, D. H. (2000). Patterns of fish calling in a nearshore environment in the Great Barrier Reef. Philosophical Transactions of the Royal Society B: Biological Sciences, 355(1401), 1289-1293.

McCullagh, P. (2002). What is a statistical model?. *Annals of statistics*, 1225-1267.

Mommsen, T. P., Vijayan, M. M., & Moon, T. W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries, 9(3), 211-268.

Moore, J.W. 2006. Animal Ecosystem Engineers in Streams. BioScience, 56(3), 237-246.

Mueller, R. P., Neitzel, D. A., & Amidan, B. G. (2001). Evaluation of infrasound and strobe lights for eliciting avoidance behavior in juvenile salmon and char (No. PNNL-SA-31653). Pacific Northwest National Lab., Richland, WA (US).

Nehls, G., Rose, A., Diederichs, A., Bellmann, M., & Pehlke, H. (2016). Noise mitigation during pile driving efficiently reduces disturbance of marine mammals. In The Effects of Noise on Aquatic Life II (pp. 755-762). Springer, New York, NY.

Neo, Y. Y., Seitz, J., Kastelein, R. A., Winter, H. V., Ten Cate, C., & Slabbekoorn, H. (2014). Temporal structure of sound affects behavioural recovery from noise impact in European seabass. *Biological Conservation*, *178*, 65-73.

Nestler, J. M., Ploskey, G. R., & Weeks, G. (1995). Development of an operational, full-scale fish protection system at a major pumped-storage hydropower dam (No. CONF-9507190--). American Society of Civil Engineers, New York, NY (United States).

Newbold, L. R., Hockley, F. A., Williams, C. F., Cable, J., Reading, A. J., Auchterlonie, N., & Kemp, P. S. (2015). Relationship between European eel *Anguilla anguilla* infection with non-native parasites and swimming behaviour on encountering accelerating flow. *Journal of fish biology*, *86*(5), 1519-1533.

Newby, N. C., Binder, T. R., & Stevens, E. D. (2007). Passive Integrated Transponder (PIT) Tagging Did Not Negatively Affect the Short-Term Feeding Behavior or Swimming Performance of Juvenile Rainbow Trout. Transactions of the American Fisheries Society, 136(2), 341-345.

Nieto, A., Ralph, G. M., Comeros-Raynal, M. T., Heessen, H. J. L., & Rijnsdorp, A. D. (2015). European Red List of marine fishes. Publications Office of the European Union.

Noatch, M. R., & Suski, C. D. (2012). Non-physical barriers to deter fish movements. Environmental Reviews, 20(1), 71-82.

Northcote, T. G. (1998). Migratory behaviour of fish and its significance to movement through riverine fish passage facilities. Fish migration and fish bypasses, 3-18.

Olton, D. S. (1979). Mazes, maps, and memory. American psychologist, 34(7), 583.

Osborne, M. P., & Thornhill, R. A. (1968). The anatomy and ultrastructure of the labyrinth of the lamprey (Lampetra fluviatilis L.). Proc. R. Soc. Lond. B, 170(1019), 113-134.

Ovidio, M., & Philippart, J. C. (2002). The impact of small physical obstacles on upstream movements of six species of fish. In Aquatic Telemetry (pp. 55-69). Springer, Dordrecht.

Pankhurst, N. W. (2011). The endocrinology of stress in fish: an environmental perspective. General and comparative endocrinology, 170(2), 265-275.

Patrick, P. H., Poulton, J. S., & Brown, R. (2001). Responses of American eels to strobe light and sound (preliminary data) and introduction to sound conditioning as a potential fish passage technology. In Behavioral Technologies for Fish Guidance: American Fisheries Society Symposium (p. 1).

Patrick, P. H., Sheehan, R. W., & Sim, B. (1982). Effectiveness of a strobe light eel exclusion scheme. *Hydrobiologia*, *94*(3), 269-277.

Pauly, D., & Zeller, D. (2017). Comments on FAOs state of world fisheries and aquaculture (SOFIA 2016). Marine Policy, 77, 176-181.

Peters, G., Delventhal, H., & Klinger, H. (1980). Physiological and Morphological Effects of Social Stress on the Eel, Anguilla anguilla L. In Fish Diseases (pp. 225-227). Springer, Berlin, Heidelberg.

Pickering, A. D. (1981). Stress and fish. No. 04; QL615, P5.. London: Academic Press, 1981.

Pidwirny, M. (2006). Fundamentals of physical geography. Date Viewed, 19, 2009.

Piper, A. T., Manes, C., Siniscalchi, F., Marion, A., Wright, R. M., & Kemp, P. S. (2015, July). Response of seaward-migrating European eel (Anguilla anguilla) to manipulated flow fields. In Proc. R. Soc. B (Vol. 282, No. 1811, p. 20151098). The Royal Society.

Piper, A. T., Wright, R. M., Walker, A. M., & Kemp, P. S. (2013). Escapement, route choice, barrier passage and entrainment of seaward migrating European eel, *Anguilla anguilla*, within a highly regulated lowland river. *Ecological Engineering*, *57*, 88-96.

Plachta, D. T., & Popper, A. N. (2003). Evasive responses of American shad (Alosa sapidissima) to ultrasonic stimuli. Acoustics Research Letters Online, 4(2), 25-30.

Platt, C., & Popper, A. N. (1981). Fine structure and function of the ear. In Hearing and sound communication in fishes (pp. 3-38). Springer, New York, NY.

Poggendorf, D. (1952). The absolute threshold of hearing of the bullhead (Amiurus nebulosus) and contributions to the physics of the Weberian apparatus of the Ostariophysi. Adler HE, Cappelli B, Transl.). Zeitschr Vergleigh Physiol, 34, 222-257.

Polis, G. A., & Hurd, S. D. (1995). Extraordinarily high spider densities on islands: flow of energy from the marine to terrestrial food webs and the absence of predation. Proceedings of the National Academy of Sciences, 92(10), 4382-4386.

Polis, G. A., & Hurd, S. D. (1996). Linking marine and terrestrial food webs: allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. The American Naturalist, 147(3), 396-423.

Popper, A. N., & Carlson, T. J. (1998). Application of sound and other stimuli to control fish behavior. Transactions of the american fisheries society, 127(5), 673-707.

Popper, A. N., & Fay, R. R. (1999). The auditory periphery in fishes. In Comparative hearing: Fish and amphibians (pp. 43-100). Springer, New York, NY.

Popper, A. N., & Fay, R. R. (2011). Rethinking sound detection by fishes. Hearing research, 273(1-2), 25-36.

Popper, A. N., & Hastings, M. C. (2009). The effects of anthropogenic sources of sound on fishes. Journal of fish biology, 75(3), 455-489.

Popper, A. N., & Hoxter, B. (1987). Sensory and nonsensory ciliated cells in the ear of the sea lamprey, Petromyzon marinus. Brain, behavior and evolution, 30(1-2), 43-61.

Popper, A. N., Fay, R. R., Platt, C., & Sand, O. (2003). Sound detection mechanisms and capabilities of teleost fishes. In Sensory processing in aquatic environments (pp. 3-38). Springer, New York, NY.

Popper, A. N., Plachta, D. T., Mann, D. A., & Higgs, D. (2004). Response of clupeid fish to ultrasound: a review. ICES Journal of Marine Science, 61(7), 1057-1061.

Popper, A. N. (2005). A review of hearing by sturgeon and lamprey. Submitted to the US Army Corps of Engineers, Portland District, 12.

Pracheil, B. M., DeRolph, C. R., Schramm, M. P., & Bevelhimer, M. S. (2016). A fish-eye view of riverine hydropower systems: the current understanding of the biological response to turbine passage. Reviews in fish biology and fisheries, 26(2), 153-167.

Prosek, J. (2010). Eels: An Exploration, from New Zealand to the Sargasso, of the World's Most Mysterious Fish. Harper.

Purser, J., Bruintjes, R., Simpson, S. D., & Radford, A. N. (2016). Condition-dependent physiological and behavioural responses to anthropogenic noise. Physiology & behavior, 155, 157-161.

Radford, C. A., Montgomery, J. C., Caiger, P., & Higgs, D. M. (2012). Pressure and particle motion detection thresholds in fish: a re-examination of salient auditory cues in teleosts. *Journal of Experimental Biology*, *215*(19), 3429-3435.

Rice, J. (1995). Food web theory, marine food webs, and what climate change may do to northern marine fish populations. Canadian Special Publication of Fisheries and Aquatic Sciences, 561-568.

Rochard, E., & Elie, P. (1994). La macrofaune aquatique de l'estuaire de la Gironde. Contribution au livre blanc de l'Agence de l'Eau Adour Garonne. État des connaissances sur l'estuaire de la Gironde. Bordeaux, France: Agence de l'Eau Adour-Garonne, p 115.

Romero, M. L., & Butler, L. K. (2007). Endocrinology of stress. *International Journal of Comparative Psychology*, *20*(2), 89-95.

Ross, Q. E., Dunning, D. J., Thorne, R., Menezes, J. K., Tiller, G. W., & Watson, J. K. (1993). Response of alewives to high-frequency sound at a power plant intake on Lake Ontario. *North American Journal of Fisheries Management*, *13*(2), 291-303.

Russon, I. J., Kemp, P. S., & Lucas, M. C. (2011). Gauging weirs impede the upstream migration of adult river lamprey Lampetra fluviatilis. Fisheries Management and Ecology, 18(3), 201-210.

Sargent, J. R. (1997). Fish oils and human diet. British Journal of Nutrition, 78(1), S5-S13.

Sand, O., Enger, P. S., Karlsen, H. E., Knudsen, F., & Kvernstuen, T. (2000). Avoidance responses to infrasound in downstream migrating European silver eels, Anguilla anguilla. Environmental Biology of Fishes, 57(3), 327-336.

Sand, O., ENGER, P., Karlsen, H. E., & Knudsen, F. R. (2001). Detection of infrasound in fish and behavioral responses to intense infrasound in juvenile salmonids and European silver eels: a minireview. In American Fisheries Society Symposium (Vol. 26, pp. 183-193).

Schilt, C. R. (2007). Developing fish passage and protection at hydropower dams. Applied Animal Behaviour Science, 104(3), 295-325.

Selye, H. (1974). Stress without distress. Toronto, McClelland Stewart.

Sheridan, S., Turnpenny, A., Horsfield, D., Bamford, D., Bayliss, B., Coates, S., ... & Trudgill, N. (2014). Screening at intakes and outfalls: measures to protect eel (Anguilla anguilla). International Fish Screening Techniques, 201117.

Siegelbaum, S. A., & Hudspeth, A. J. (2000). Principles of neural science (Vol. 4, pp. 1227-1246). E. R. Kandel, J. H. Schwartz, & T. M. Jessell (Eds.). New York: McGraw-hill.

Simmonds, J., & MacLennan, D. N. (2008). Fisheries acoustics: theory and practice. John Wiley & Sons.

Simpson, S. D., Meekan, M. G., Jeffs, A., Montgomery, J. C., & McCauley, R. D. (2008). Settlement-stage coral reef fish prefer the higher-frequency invertebrate-generated audible component of reef noise. Animal Behaviour, 75(6), 1861-1868.

Simpson, S. D., Purser, J., & Radford, A. N. (2015). Anthropogenic noise compromises antipredator behaviour in European eels. *Global change biology*, *21*(2), 586-593.

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

Sjöberg, K. (1980). Ecology of the European river lamprey (Lampetra fluviatilis) in northern Sweden. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(11), 1974-1980.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., & Popper, A. N. (2010). A noisy spring: the impact of globally rising underwater sound levels on fish. Trends in ecology & evolution, 25(7), 419-427.

Slabbekoorn, H. (2016). Aiming for progress in understanding underwater noise impact on fish: complementary need for indoor and outdoor studies. In The effects of noise on aquatic life II (pp. 1057-1065). Springer, New York, NY.

Stein, F., Doering-Arjes, P., Fladung, E., Brämick, U., Bendall, B., & Schröder, B. (2016). Downstream migration of the European eel (Anguilla anguilla) in the Elbe River, Germany: movement patterns and the potential impact of environmental factors. River Research and Applications, 32(4), 666-676.

Stocker, M. (2002). Fish, mollusks and other sea animals' use of sound, and the impact of anthropogenic noise in the marine acoustic environment. Journal of the Acoustical Society of America, 112(5), 2431.

Swanson, C., Young, P. S., & Cech, J. J. (2005). Close encounters with a fish screen: integrating physiological and behavioral results to protect endangered species in exploited ecosystems. *Transactions of the American Fisheries Society*, *134*(5), 1111-1123.

Teague, N., & Clough, S. C. (2014). The survival of lamprey on travelling screens at potable water intakes. WIT Transactions on State-of-the-art in Science and Engineering, vol.71, pp.10.

Thomsen, F., Lüdemann, K., Kafemann, R., & Piper, W. (2006). Effects of offshore wind farm noise on marine mammals and fish. Biola, Hamburg, Germany on behalf of COWRIE Ltd, vol.62.

Tolimieri, N., Haine, O., Montgomery, J. C., & Jeffs, A. (2002). Ambient sound as a navigational cue for larval reef fish. Bioacoustics, 12(2-3), 214-217.

Tolimieri, N., Jeffs, A., & Montgomery, J. C. (2000). Ambient sound as a cue for navigation by the pelagic larvae of reef fishes. Marine Ecology Progress Series, 207, 219-224.

Toms, C. N., Echevarria, D. J., & Jouandot, D. J. (2010). A methodological review of personality-related studies in fish: focus on the shy-bold axis of behavior. International Journal of Comparative Psychology, vol.23(1), 1-25.

Tort, L., Montero, D., Robaina, L., Fernández-Palacios, H., & Izquierdo, M. S. (2001). Consistency of stress response to repeated handling in the gilthead sea bream Sparus aurata Linnaeus, 1758. Aquaculture Research, 32(7), 593-598.

Tsouvalas, A., & Metrikine, A. V. (2016). Noise reduction by the application of an air-bubble curtain in offshore pile driving. Journal of Sound and Vibration, 371, 150-170.

List of References

Tummers, J. S., Winter, E., Silva, S., O'Brien, P., Jang, M. H., & Lucas, M. C. (2016). Evaluating the effectiveness of a Larinier super active baffle fish pass for European river lamprey Lampetra fluviatilis before and after modification with wall-mounted studded tiles. Ecological Engineering, 91, 183-194.

Van Ginneken, V., Durif, C., Balm, S. P., Boot, R., Verstegen, M. W., Antonissen, E., & van Den Thillart, G. (2007). Silvering of European eel (Anguilla anguilla L.): seasonal changes of morphological and metabolic parameters. Animal Biology, 57(1), 63-77.

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

Verhelst, P., Buysse, D., Reubens, J., Pauwels, I., Aelterman, B., Van Hoey, S., ... & Mouton, A. (2018). Downstream migration of European eel (Anguilla anguilla L.) in an anthropogenically regulated freshwater system: Implications for management. Fisheries Research, 199, 252-262.

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

Vowles, A. S., Anderson, J. J., Gessel, M. H., Williams, J. G., & Kemp, P. S. (2014). Effects of avoidance behaviour on downstream fish passage through areas of accelerating flow when light and dark. *Animal Behaviour*, *92*, 101-109.

Wahlberg, M., & Westerberg, H. (2003). Sounds produced by herring (Clupea harengus) bubble release. Aquatic Living Resources, 16(3), 271-275.

Wedemeyer, G. A., Barton, B. B., & McLeay, D. J. (1990). Stress and acclimation. 451-489

Wielgoss, S., Taraschewski, H., Meyer, A., & Wirth, T. (2008). Population structure of the parasitic nematode Anguillicola crassus, an invader of declining North Atlantic eel stocks. *Molecular ecology*, *17*(15), 3478-3495.

Williams, R., Wright, A. J., Ashe, E., Blight, L. K., Bruintjes, R., Canessa, R., ... & Hammond, P. S. (2015). Impacts of anthropogenic noise on marine life: publication patterns, new discoveries, and future directions in research and management. Ocean & Coastal Management, 115, 17-24.

Winter, H. V., Jansen, H. M., & Bruijs, M. C. M. (2006). Assessing the impact of hydropower and fisheries on downstream migrating silver eel, Anguilla anguilla, by telemetry in the River Meuse. Ecology of Freshwater Fish, 15(2), 221-228.

World Commission on Dams. (2000). Dams and Development: A New Framework for Decision-making: the Report of the World Commission on Dams. Earthscan.

Wu, J., Huang, J., Han, X., Gao, X., He, F., Jiang, M., ... & Shen, Z. (2004). The three gorges dam: an ecological perspective. Frontiers in Ecology and the Environment, 2(5), 241-248.

Wysocki, L. E., Amoser, S., & Ladich, F. (2007). Diversity in ambient noise in European freshwater habitats: Noise levels, spectral profiles, and impact on fishes. *The Journal of the Acoustical Society of America*, *121*(5), 2559-2566.

Xie, P. (2003). Three-Gorges Dam: risk to ancient fish. Science, 302(5648), 1149-1151.

Yan, H. Y. (2004). The role of gas-holding structures in fish hearing: an acoustically evoked potentials approach. In The Senses of Fish (pp. 189-209). Springer, Dordrecht.