The noisy underwater world : the effect of sound on behaviour of captive zebrafish

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Citation

Shafiei Sabet, S. (2016, April 5). *The noisy underwater world : the effect of sound on behaviour of captive zebrafish*. Retrieved from https://hdl.handle.net/1887/38704

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Title: The noisy underwater world : the effect of sound on behaviour of captive zebrafish
Issue Date: 2016-04-05

Chapter 1

General introduction and aim of the thesis

General introduction

Man-made noise as a pollutant in natural habitats

Human activities are increasing rapidly in terrestrial and aquatic habitats. Marine and freshwater habitats are being affected by a variety of anthropogenic pollutants. Urbanization, transportation and industrialization have continuously increased ambient noise levels with different temporal and spectral patterns (Slabbekoorn et al. 2010; Radford et al. 2014). Anthropogenic noise, as an environmental pollutant, is ubiquitous in, on and near aquatic habitats and potentially may have detrimental effects on aquatic animals. Over the past few decades, public attention, activities in the field of conservation and animal welfare by non-governmental organizations (NGOs), and scientific exploration are raising awareness on the potential effects of sounds on marine mammals and fish species.

Origin of sound in aquatic habitats

Aquatic habitats, including marine and freshwater systems, are similar to terrestrial habitats in that they are filled with a variety of biotic and abiotic sound sources (Wenz 1962; Wysocki et al. 2007). Firstly, natural abiotic sound sources such as water waves and tides, surf, submarine volcanic eruptions and seismic activity are prevalent in marine habitats and riffles, waterfalls and rapids are ubiquitous in freshwater habitats. Secondly, there are also many biotic sources such as animal vocalizations, sound produced during feeding and other activities in both marine and freshwater habitats. Furthermore, sound generating human activities are responsible for so-called “anthropogenic noise”, which has spread in time and space in the last few decades and is now recognized as potential driver of environmental changes in many aquatic habitats (Slabbekoorn et al. 2010).

Next to the abundance of sounds from various sources, there are several reasons why sounds play an important role in the life of aquatic animals and why artificial elevation of ambient noise may have detrimental consequences. Firstly, sound travels almost five times faster in water than in air and therefore potentially spreads over a large area. Secondly, sound has the capacity to carry information and species may extract signals and exploit cues from ambient sounds to find prey and avoid predators, especially in dark and murky waters. Thirdly, many fish species are also able to produce sounds and use it as a tool for conspecific communication during territory defense, mate choice and reproduction. The presence of anthropogenic noise may interfere with these functions through masking, disturbance and deterrence.

Noise may affect fish species in marine and freshwater habitats

Man-made sounds are generated by a variety of human activities that vary among different waterbodies. Sound sources in marine and offshores habitats include seismic surveys, pile driving, navy sonars, shipping activities and detonation of explosives. In addition, anthropogenic noise in coastal and freshwater habitats include pumping systems, cruise vessels, motorized recreational activities, weirs and building activities (Wysocki et al. 2007). All these activities elevate ambient noise levels and potentially decrease relevant signal-to-noise ratios (typically important for both senders and receivers of signals) and relevant cue-to-noise ratios (cue reception is useful for receiver and potentially harmful for the cue-emitting animals). Consequently, anthropogenic noise elevating natural ambient noise levels may have behavioural and ecological consequences in aquatic habitats.

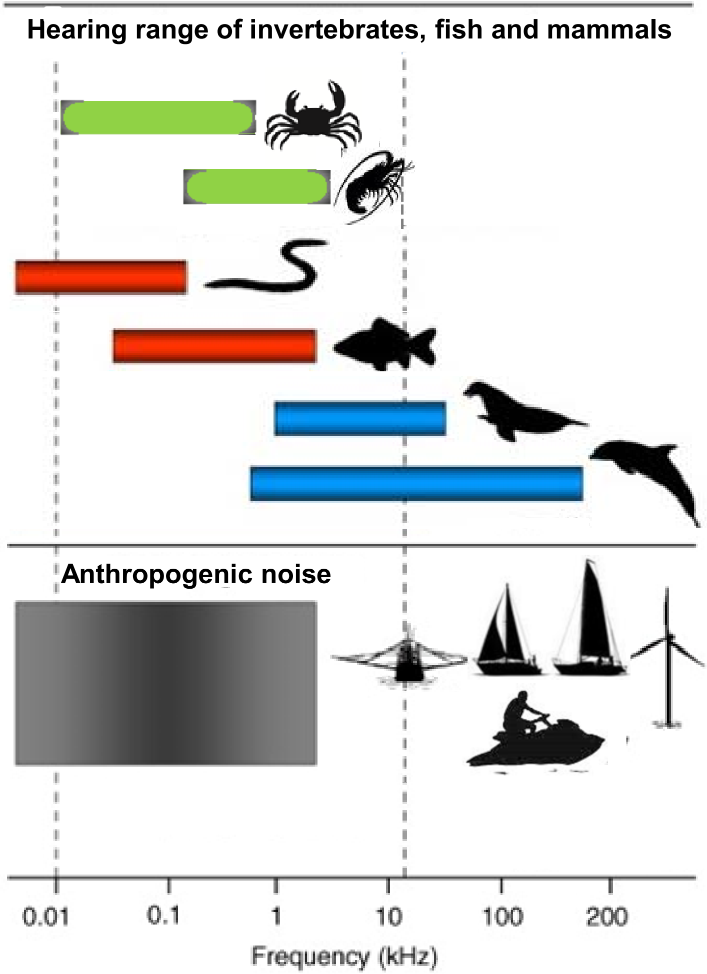

Fig. 1. Hearing range of invertebrates, fishes and mammals in aquatic habitats. The crab and prawn are representative of aquatic invertebrate species Lovell et al. (2005) and Morley et al. (2014). The eel is a representative of fish species with a bias to low-frequency sensitivity. The goldfish is a representative of the cyprinid fish, which also include the zebrafish (*Danio rerio*), that are a large relatively sensitive group of fish. Anthropogenic noise is largely overlapping the hearing range of aquatic animals and especially those of invertebrates and fishes. Modified from Slabbekoorn et al. (2010).

In natural habitats, artificially elevated ambient noise may have a variety of detrimental effects that can be described as a continuum of relative severity in sound-related effects on marine mammals and fish species. Depending on the amplitude of the sound source and the proximity of the animal, extreme levels of sound exposure may lead to elevated mortality and immediate death. Further away from loud sound sources, elevated ambient noise may still cause physical damage and physiological stress, hearing threshold shifts (permanent or temporary) , mask relevant sounds and interfere or change behavioural patterns (sound-related disturbance and deterrence). All the effects are correlated with the species-specific hearing ability of fish species, both in terms of absolute thresholds and the audible frequency range.

Very little is known about which specific sound field features are triggering changes in behaviour, especially in fish tank conditions, where sound fields can be complex. Behavioural parameters that can be used to investigate effects of sound exposure on fish are sudden rises in swimming speed, startle responses and erratic swimming movements, reduced swimming speed and freezing, going down in the water column and staying in the bottom layer, and effects on group coherence and feeding efficiency (e.g. Purser & Radford 2011; Voellmy et al. 2014; Neo et al. 2014; 2015). These measurements are all well-known indicators of physiological stress, disturbance and deterrence (see reviews: Blaser et al. 2010; Egan et al. 2009; Maximino et al. 2010).

Fish species vary in sound detection abilities

All fishes can detect sound using various sound sensitive organs (Slabbekoorn et al. 2010; Popper & Fay 2011; Ladich 2014). Unlike terrestrial animals, fish species are sensitive to the particle motion component of sound. Depending on the species-specific hearing system, they perceive sounds via different organs, including the inner ear, which consists of three semi-circular canals (utricle, saccule and lagena) and three otoliths (lapillus, sagitta and asteriscus), and peripheral structures such as the lateral line system. Moreover fishes belonging to the Ostariophysi, including zebrafish from the family Cyprinidae, are more specialized and well-known as hearing specialists, as they have a connection between swim bladder and inner ear via a set of small bones (Weberian Ossicles). Pressure fluctuations in the water cause size fluctuations of the gas-filled swim bladder. This pressure-to-motion conversion and the improved conduction via the Weberian ossicles provides fish with lower absolute sensitivity thresholds and a broader frequency hearing range. However, in contrast to the Ostariophysi families many fish species, including cichlids from the family Cichlidae, do have a swim bladder, but not that connection or other special conductors and are less specialized hearing generalists.

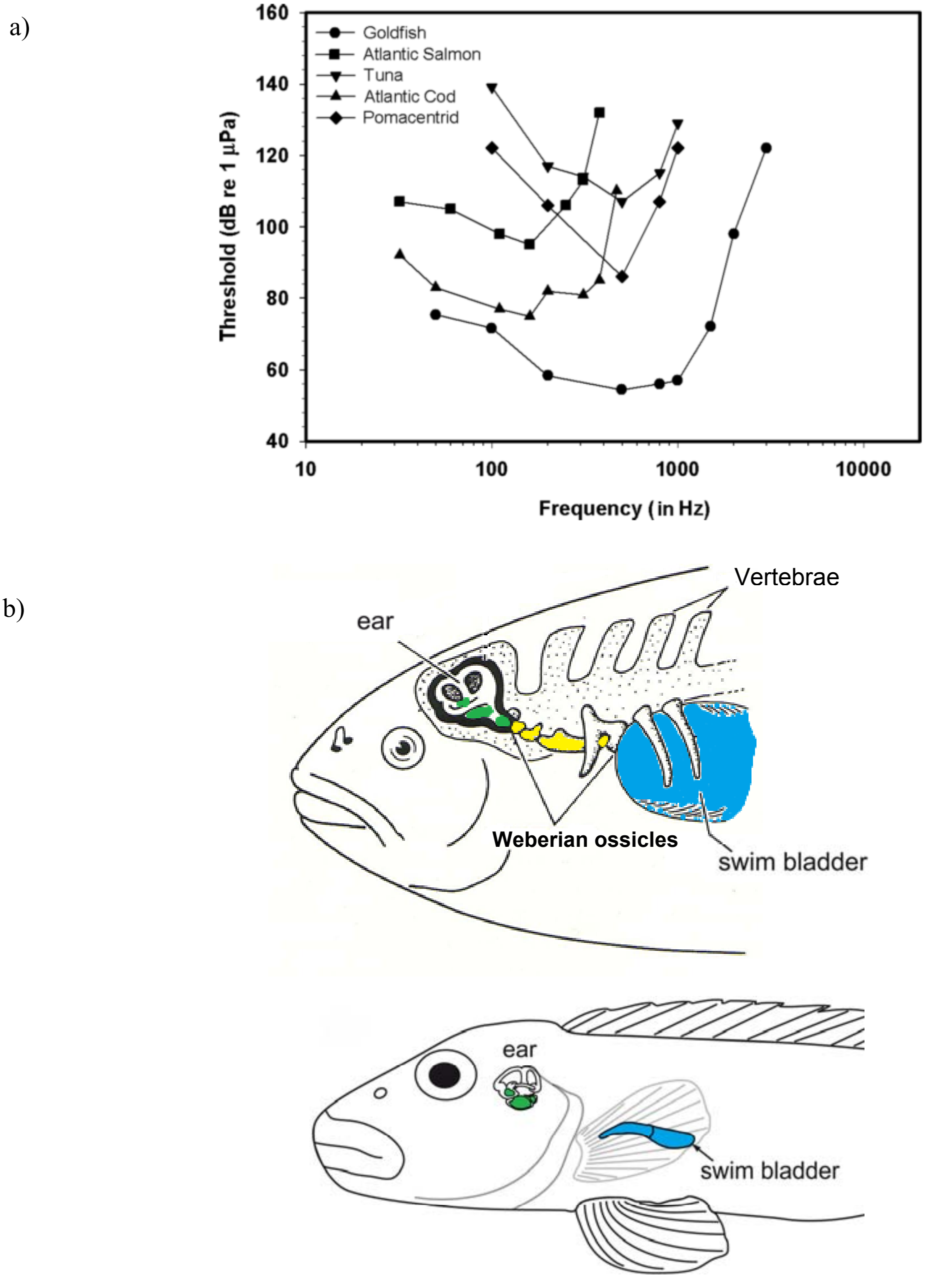

Fig. 2. a) Audiograms for a group of teleost fishes depicting the hearing thresholds across the spectral range of audible sound. Goldfish are similar in hearing abilities to zebrafish, the

model species of this thesis, while Pomacentrids are more like cichlids, also addressed in one comparative study, and have lower hearing abilities. (Audiogram originates from Popper & Schilt 2008; all data from Fay 1988). b) The fish drawings show lateral views of a species with a large swim bladder and Weberian ossicles and another species with a small swim bladder and no special adaptation to connect it to the inner ear (drawings used with permission originate from Wake 1979; Schulz-Mirbach et al. 2012). The swim bladder is shown in blue; the otoliths of the inner are shown in green and the Weberian ossicles are shown in yellow.

Multimodal complexity and ecosystem level effects

Natural habitats of fish not only vary in sound conditions but also in light conditions (Longcore & Rich 2004; Brüning et al. 2011). Fish species use their auditory and visual systems along with other environmental modality receptors for optimal perception of their surroundings (Halfwerk & Slabbekoorn 2015). They extract relevant signals and cues in this multimodal sensory context to mediate essential behaviours, including territory defense, mate choice, reproduction, finding prey and avoiding predators (Swaddle et al. 2015). Changes in artificial light levels at night are also becoming more wide-spread on a global scale and, like the impact of artificial sound, may have potentially negative consequences for fish activities and their spatial distribution (Becker et al. 2013; Swaddle et al. 2015). Elevated light levels at night have the potential to affect fish behaviour directly or indirectly when the effect of sound is altered by light level. Light pollution, like noise pollution, may go beyond single species effects. For instance, artificial lighting at night may affect biological rhythms of hormone cycles potentially leading to: higher physiological stress levels (Brüning et al. 2015), delay in dispersal timing and disrupted diel patterns in captive species (Riley et al. 2015). However, artificial lighting may also affect predator-prey interactions in coastal habitats; both large predator and small prey fish species were reported to aggregate at nocturnal light sources, which resulted in predator benefits from locally elevated prey abundance and possibly overall shifts in abundance in multiple trophic levels (Becker et al. 2013) (see Fig. 3).

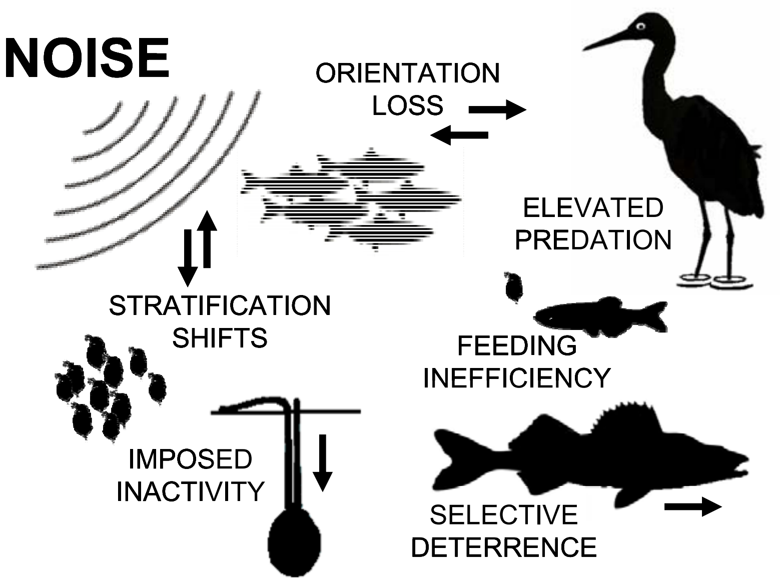

Fig. 3. Anthropogenic noise may have consequences that go beyond single species effects. Species interactions among predators and prey or among competitors may be affected in various ways and have cascading effects through different trophic levels in the underwater food chain. The figure illustrates several examples through which anthropogenic noise may cause shifts in relative species densities in the horizontal and vertical pane (Shafiei Sabet et al. 2016).

Captive fish and sound

Many fish species have been artificially introduced to confined areas for different purposes. Fishes are being used in laboratory conditions for scientific research, in aquaria and zoos for fun and entertainment, in aquaculture facilities (cages, races, pens etc.) for breeding, restockment and harvesting. For instance, in China alone there are already at least 532 species belonging to 24 families of marine fish that have been used for artificial breeding and reproduction purposes in captivity (Hong & Zhang 2003). Several fish species, including zebra fish, are used for scientific research in large numbers for a wide range of investigations in laboratories around the world. Therefore, also many fish in captivity may be continuously exposed to a variety of sound sources.

The sounds present in the breeding and rearing or experimental environment may affect production, reproductive success and potentially even non-behavioural results of any type of experiment. Sounds may not be detrimental, for instance when they learn that a particular sound, for instance from an automatic feeding system, indicates that they are likely to get food. Also, threats or uncertainties like in outdoor conditions are typically not present. However, novel sounds may induce behavioral changes due to anxiety or curiosity (Neo et al. 2015). Moreover, in aquaculture activities, sound-generating equipment may also affect both target and non-target species in surrounding marine and freshwater habitats (Lepper et al. 2004). For instance, pumping devices in aquaculture may produce high levels of background noise continuously. Also, in open water localities used for aquaculture, floating pen systems may be used in combination with commercial aquaculture acoustic devices (CAADs) that generate loud sounds to deter predator species (Lepper et al. 2004).

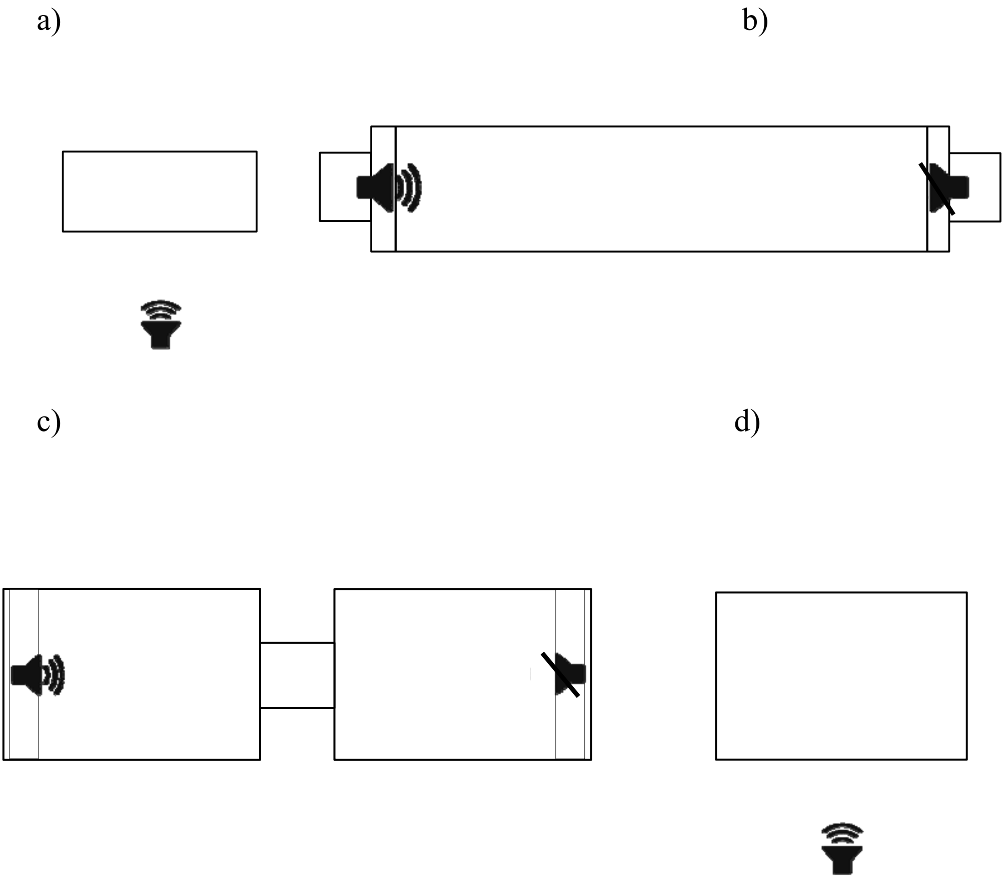

Fig. 4. Schematic overview of the four different set-ups used in this thesis in terms of relative size and shape of the experimental fish tank and the location of in air or in water speakers. (a) a small tank (Chapter 2); (b) a long tank (Chapter 3) (c) a dual- tank (Chapter 4) and (d) a standard 1 meter tank with an acoustically transparent enclosure to restrict the swimming arena for the target fish (Chapter 5).

Aim of the thesis

The aim of this thesis was to explore sound-induced behavioral changes in fish using captive zebrafish as a model species. I explored short-term behavioural parameters, which are indicators of sound-related stress, disturbance and deterrence. Several behavioural states are likely to reflect considerable changes in underlying physiology, which would be interesting and feasible to investigate for more long-term consequences, but this was beyond the scope of the current study. Here, I examined in four different studies various sound exposure treatments to provide insights that may be useful for future explorations for indoor and outdoor sound impact studies as well as for assessing animal welfare and productivity in captive situations. Furthermore, my findings may also raise awareness for sound levels in laboratories and the potential effect on reliability for fish as a model species for medical and pharmaceutical studies. I also explored the complexity of sound fields in indoor fish tanks by selecting a different set-up for each study (Fig. 4), which makes behavioural analyses and direct comparisons not only relevant within each study, but also provides insight into the role of fish tank acoustics on ‘natural’ and experimental exposure conditions.

In **Chapter 2,** I investigated how sound exposure with different temporal patterns affected swimming behaviour and foraging performance for zebrafish preying on waterfleas. In **Chapter 3,** I examined how sound exposure affected two different fish species with different hearing ability (cichlids and zebrafish) in terms of swimming behaviour and spatial distribution in a long tank set up. In **Chapter 4,** I investigated the effect of two modalities (sound and light exposure) and their potential interaction on zebrafish swimming behaviour and spatial distribution in a special dual-tank set up (c.f. Neo et al. 2014). In **Chapter 5**, I collaborated with James Campbell to explore the detailed sound field characteristics in terms of sound pressure and particle velocity that are responsible for zebrafish startle and anxiety-related response patterns. And finally, in **Chapter 6**, the general discussion and conclusion, I summarized the results of all four experiments and put them in a more general context.

Animal species

Throughout this thesis experimental sound exposure effects were assessed using the invertebrate species waterfleas (Daphnia spp) and vertebrate fish species (zebrafish and a Lake Victoria cichlid) below:

Fig. 5. Waterfleas (*Daphnia spp*) were used in the chapter 2. Waterfleas are crustaceans and a typical food item for many fish species in freshwater habitats. Crustacean are sensitive to sound in the low frequency range (Lovell et al. 2005; Montgomery et al. 2006; Mooney et al. 2010; Stanley et al. 2011; Morley et al. 2014), which they can hear through sensitivity to movement and vibration, either through the presence of a statocyst or small tentacles on their body (See Fig. 1.). The exact hearing range for waterfleas is unknown, but they are not expected to hear beyond a few hundred Hz (Picture by G. Lamers).

Fig. 6. Zebrafish (*Danio rerio*) were used in the chapter 2, 3, 4 and 5. Zebrafish are a freshwater fish species native to the flood-plains of the Indian subcontinent where they inhabit shallow and slow flowing waters (Spence et al. 2008). They are a widespread model species in a broad range of research areas such as neurophysiology, biomedicine and behavioural biology studies in laboratory conditions. As a Cyprinid, zebrafish belong to the ostariophysan teleosts, which all have a special hearing adaptation. A series of bones, the Weberian ossicles, connect the swim bladder to the inner ear and lower absolute detection thresholds and extend the spectral range. Zebrafish can therefore hear over a relatively broad frequency range between 100-4000 Hz, with sensitivity declining sharply above 2000 hz (Higgs et al. 2002). (see also Fig.2.). (Picture by S. Shafiei Sabet).

Fig. 7. Lake Victoria Cichlids (*Haplochromis piceatus*) were used in the chapter 2. Cichlids represent a non-Ostariophysi species and they are less advanced in terms of special structures for improved hearing compared to zebrafish. Cichlid hearing is therefore restricted to a lower range of frequencies and different species vary between 100-3000 Hz, with sensitivity declining sharply above 700 or 1000 Hz, depending on the species (Schulz-Mirbach et al. 2012). There is no hearing curve available for the species used for this thesis, but there are no special extensions of the swim bladder towards the inner ear, nor a particularly big or small swim bladder. Hearing sensitivity is therefore likely to be somewhere intermediate to those reported by Schulz-Mirbach et al. (2012) (Picture by A. Ekenberg).

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