Measuring Behavioural Changes to Assess Anthropogenic Noise Impact in Adult Zebrafish (*Danio rerio*)

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Sounds underwater

Underwater habitats are full of natural sounds from abiotic sources, such as wind and water flow, and biotic sources, such as chorusing fish and snapping shrimps. However, human activities elevate these ambient noise levels artificially. The so-called anthropogenic noise from traffic, industry, and recreational activities concerns an only recently recognized pollution factor underwater which is expanding in time and space [1]. The artificially high noise levels are typically of relatively low frequency and can cause many different problems such as physical damage in cases of extreme overexposure, physiological stress, and auditory masking, which are all factors that an animal is more or less passively undergoing (see Figure 1). Furthermore, anthropogenic noise may lead to spatial deterrence, behavioural interruption, and signal modifications, which are all factors that involve some sort of active response from the animal. The three passive factors are typically positively related: if one is getting worse the others are likely to follow the same pattern. An increase in any of the three passive factors also increases the probability that any of the three active factors occurs. Vice versa the opposite may be the case: the active factors have the potential to provide relief on the passive factors by reducing the level and duration of exposure.

Many fish species generate sounds to be heard by conspecifics for communication about for example competition for resources and attraction of social or mating partners. Probably even more common is the use of hearing abilities to find prey or to detect predators. Furthermore, the geographic distribution of habitat-specific abiotic and biotic sound sources provides cues to fish for orientation and localization of specific areas for migrating, feeding, hiding, or spawning [2]. The majority of these biologically relevant sounds are relatively low in frequency, within the range of hearing sensitivity for most fish species, but also overlapping dramatically with the typical spectrum of anthropogenic noise. Therefore, the widespread occurrence of artificially elevated noise levels due to human activities has the potential to mask these biological sounds and affect the behaviour of many acoustically dependent fish. Besides masking, a large impact may also result from interruption of natural

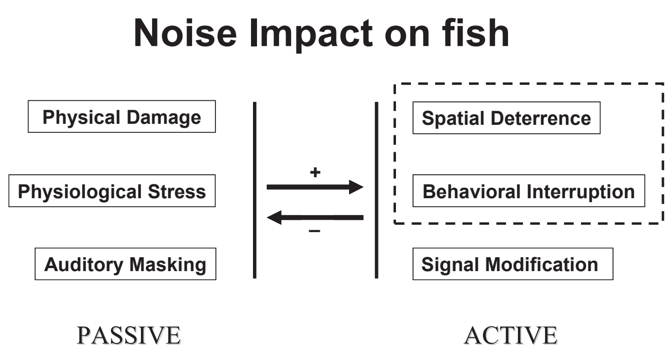

Figure 1. Schematic overview of six dominant factors that may play a role in assessing the impact of anthropogenic noise on fish welfare and fitness. The arrows indicate the relationship between factors. The ‘passive’ factors on the left reflect consequences of undergoing a particular exposure, which typically also increase the probability of occurrence of the ‘active’ factors on the right. The ‘active’ factors, in contrast, involve behavioural decisions by the individual that are likely to reduce the severity of the ‘passive’ factors. The purpose of this subdivision into categories is just to emphasize the complexity of noise impact assessments as, although the overall representation is generally true, the relative importance of factors and the magnitude and even direction of the relationships will vary per exposure level and duration, per species, and with physiological and environmental conditions. The dashed line block demarcates the focus of the reported zebrafish studies.

behaviours due to startle responses, attentional disruption, and lower efficiency of acoustically guided behaviours. Such impact is depending on the nature of fluctuations in the noise background and the nature of noise events. Although there are still only few data papers, all of these noise effects may negatively affect growth, reproductive success, and survival or cause fish to escape noisy areas.

Behaviour and stress

Behavioural changes related to artificial noise exposure may have detrimental effects by themselves but may also be associated with stress: physiological changes that yield energetic costs and have negative effects on growth, reproduction, or survival. Several studies have shown an impact of extreme anthropogenic noise exposure on, for example, increased heart rate and elevated cortisol levels [3]. However, although common and more moderate exposures that lead to behavioural changes will be accompanied by underlying physiological changes, these should not all necessarily be regarded as stress. There are general physiological changes related to the energetic demands and the state of mind required for making and executing adequate behavioural decisions to external stimuli. Different kinds of stimuli may for example raise interest, arousal, fear, or anxiety [4]. The associated physiological changes are an integral part of the natural regulatory capacity of healthy fish and are not necessarily different between stimuli or states that we would intuitively regard as positive or negative. Nevertheless, especially exposure to unpredictable and uncontrollable noise events and biologically relevant sounds that are critical for survival and become unpredictable and uncontrollable due to masking noise may yield physiological changes that deserve the label of stress [5].

Zebrafish as a high-throughput model organism

The zebrafish (*Danio rerio*) is emerging as a new model organism and rapidly gains popularity in a variety of disciplines [6]. They earn this popularity by practical, financial, and ethical advantages over other vertebrates, such as rodents and primates. They are relatively easy and cheap in maintenance and reproduce readily in captivity. Importantly, zebrafish also share many physiological similarities with terrestrial vertebrates. They are highly similar and comparable in the structure and function of neuro-chemical and behavioural systems as well as in the general organization of their stress-regulating systems. They have proven to be suitable for laboratory studies on behavioural assessments of anxiety, while technological advances now allow high-throughput drug screening and discovery.

One of the most commonly used paradigms to test behavioural changes in adult zebrafish is the novel environment test [7]. A single or a small group of individual fish is transferred to a new tank in which the behavioural response to the novel environment (and novel social conditions) can be recorded on video and monitored manually or automatically. Typical behavioural assessments include: startle responses and erratic swimming movements, swimming speed, accumulated swimming distance, group cohesion, freezing bouts, delay to first entry of upper half or upper third of the fish tank, and number of entries and total time spent in that same area relatively close to the surface. The impact of a particular treatment is evaluated by whether it yields an incline or a decline in the anxiety-related responses. Stronger anxiety is for example related to faster swimming, stronger group cohesion, and staying away from the surface and closer to the bottom for longer [8].

Noise impact assessment in zebrafish

Although it does not apply to all noise exposure related to human activities, there is certainly potential for unpredictable and uncontrollable sound events that could yield physiological stress. The occurrence of anthropogenic noise in the natural environment is characterized by variety in time at various scales. There are more or less continuous noises from vessels, pumping systems, windmill farms, and gas extraction platforms. There are also repetitive sounds from pile driving, sonar use, and seismic surveys, and there are very brief but loud noise events related to explosions. Furthermore, there are many noise types that are in between these temporal extremes, such as sounds generated by dredging, water scooters, boats changing gear, and general construction activities in or close to the water. Very few noise impact studies have addressed such temporal variety, although it likely plays a critical role in the potentially negative effects of noise on fish. Neo et al. [9]

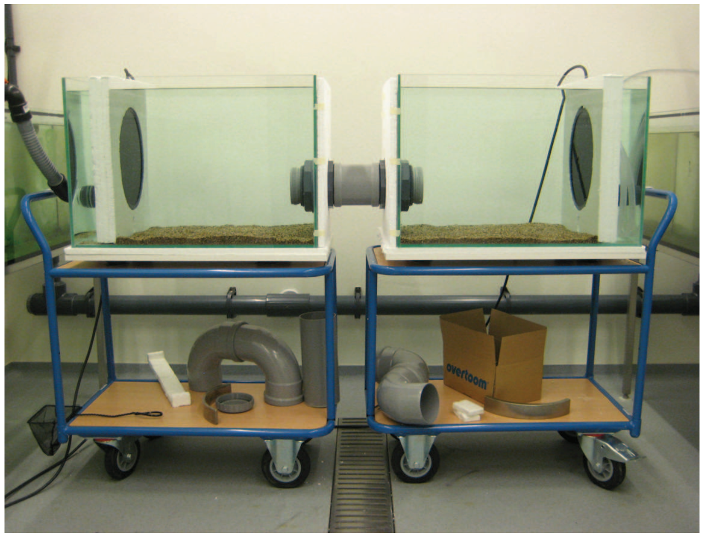

Figure 2. Picture of the two adjacent fish tanks connected by a pvc-pipe swim tunnel for the second experiment (see main text) of Neo et al. (unpublished). Both fish tanks are acoustically insulated from each other and their surroundings of the office building by air, four rubber shock pads each, and a layer of Styrofoam between the fish tanks and two separate trolley tables on rubber wheels. Water flows into the right tank through the tunnel to the left tank and out of the system on the left, but the circulation is stopped during noise exposure trials. There is an underwater speaker on the end of each fish tank opposite the tunnel for passage. Both speakers were used in alternating sequence: one speaker broadcasting artificial noise in one trial and the other in the next trial to avoid side effects.

investigated the disruptive effects of continuous noise as well as noise pulses on the behaviour of captive zebrafish and also compared the effects of slow and fast pulse rates, and predictable and unpredictable pulse intervals.

Neo et al. [9] assessed the impact of temporal variety on fish behaviour in two different experiments. In the first experiment, they used moderate exposure levels of about 112 dB re 1 μPa in a single tank without acoustic escape possibility. In the second experiment, they used higher exposure levels up to 140 dB re 1 μPa in a double-tank system with acoustic escape possibility. The first experiment revealed that noise pulses of moderate noise level could already alter behaviour of a small group of five zebrafish. Noise exposure through an in-air external speaker generated a relatively homogenous underwater field of sound pressure conditions, which resulted in changes for several behavioural measures such as swimming speed, group cohesion, and tendency to move up to the surface. Furthermore, the impact turned out to vary significantly among exposure regimes. The second experiment revealed that noise conditions can be made distinct in two adjacent fish tanks connected by a pvc-pipe swim tunnel of 35.0 cm in length and 12.5 cm in diameter (see Figure 2). Noise exposure through one of two underwater speakers generated sound pressure gradients in the left or right fish tank, while leaving the sound levels in the other close to baseline levels. Frequent tunnel passages by six individual zebrafish allowed testing of spatial avoidance of the noisy fish tank. The experiments were only carried out after ethical evaluation and approval by the Animal Experiments Committee of Leiden University (DEC# 10069).

Implications and extrapolations

Variable impact of different temporal patterns of experimental noise exposure on captive fish may have implications for noise impact assessments in natural environments, although one has to be cautious with extrapolations. I believe there is for example value in the relative differences of impact on anxiety-like behaviour for different noise exposure regimes. The variation in e.g. startle threshold levels, duration required for habituation, or effects on spatial avoidance, as assessed in captivity in response to different temporal patterns of artificial noise exposure, may also all apply to natural conditions. However, studies comparing such behavioural measures during experimental noise exposure in captivity and in natural water bodies are needed to confirm this statement. Absolute values for certain thresholds from inside noise exposure testing are almost certainly not likely to be very useful outside. This is due to the fact that inside and outside conditions are inherently very different, both with respect to the acoustics and with respect to the behavioural and physiological state of the fish.

Noise impact conditions in the wild differ from those in captivity as the participation in natural activities such as migration, feeding, or spawning, may dramatically alter perceptual threshold levels as well as the physical impact. The same is true for the continuous possibility of a predator attack in the wild compared to the complete safety in the laboratory fish tank. Furthermore, differences in absolute values and even in relative effects of different temporal patterns may vary by species, age, sex, and season. Neo et al. [9] focussed for example on sound pressure levels without assessing the exposure characteristic in the fish tank in terms of the particle motion component of sound. This may be right for adult zebrafish, which are sound pressure sensitive through the pressure-to-motion transduction by the so-called Weberian ossicles, which are connecting the swim bladder to the inner ear. However, juvenile zebrafish and many other species without these specialized adaptations for hearing are more motion sensitive and will experience noise conditions differently. Particle motion is more difficult to assess than sound pressure and also a more complex feature of sound close to the source and in the confined area of a fish tank.

In addition to the possibilities to extrapolate indoor insights to the reality of the outside world, it is also important to realize that for many fish the reality is inside. Many fish are kept in captivity by hobbyists at home, by zoo keepers in public aquaria, by professional breeders in aquaculture, and by scientists in research facilities. Typically, good health conditions are key to the purpose of keeping fish by these very divergent groups of people. They are often interested in the beauty of colour, body forms, and behavioural display, optimal growth and reproduction, or repeatable patterns of semi-natural behaviours or stereotypic responses to artificial stimuli. Nevertheless, noise conditions in fish tanks are customarily not consciously controlled and can be very high (e.g. through pumping systems) and unpredictable (e.g. due to knocking visitors). Habituation to continuous presence or habituation to repeated and predictable exposure can bring some relief, but still we are currently most often ignorant of the potentially detrimental effects of anthropogenic noise on the well-being of fish and on reaching the specific targets of the fish keepers in terms of the body condition and behavioural state of their fish.

In conclusion, there is a huge potential for fundamental studies on noise impact in fish with lots of opportunities for applied value. Fish tank studies in the laboratory are currently most accessible and they benefit significantly from the advantages of having the zebrafish as an ideal model organism and from the technological advances of measuring and processing behaviour automatically [6]. However, in parallel to the indoor studies we also need to go outside. We need to learn more about the role of sound in natural activities of fish and investigate the potentially negative effects of anthropogenic noise [1]. We can gain important insights through observational studies at noisy locations as well as through experimental exposure studies at locations that are still relatively unaffected acoustically by human activities. I hope future studies will be able to integrate studies from the laboratory and from the field to exploit the best of both worlds: the possibilities to assess effects of anthropogenic noise on fish in great detail and in large numbers in captivity with the ecological relevance and acoustic reality of the natural world.

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