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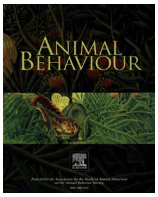

Behavioural responses to sound exposure in captivity by two fish species with different hearing ability

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Underwater sound generated by human activities is increasing in, on and near aquatic environments. Such anthropogenic noise can induce artificially elevated ambient sound levels and cause various detrimental effects in fish, such as temporary or permanent hearing loss, masking of relevant acoustic signals and cues or behavioural changes that may have fitness consequences. Also, captive fish are often exposed to noisy conditions, which may have consequences for production in aquaculture, biases in scientific results in laboratories or welfare in hobby aquaria. However, we still have limited insight into how fish cope with artificial sound exposure and how species differ in sensitivity. Here, we compared zebrafish, *Danio rerio*, and Lake Victoria cichlids, *Haplochromis piceatus*, the former being sensitive to lower absolute thresholds and wider spectral ranges than the latter. Experimental sound exposure induced a significant reduction in swimming speed in the first minute of exposure for both species in captive conditions. Furthermore, zebrafish showed clear startle response behaviour with the onset of the sound exposure leading to an initial, brief increase in swimming speed, which was not found for the cichlids. Neither species showed spatial shifts away from the active speaker in the horizontal plane, but cichlids shifted downwards to spend more time in the bottom area of the fish tank after the onset of sound exposure, while zebrafish retained their average swimming height during the same exposure levels. Our results show that sound exposure can cause both similar and species-specific responses in two fish species and that these responses are not obviously related to differences in their hearing ability.

Human activities have acoustically changed aquatic environments over the past decades and anthropogenic noise is now recognized as a ubiquitous pollutant (Radford, Kerridge, & Simpson, 2014; Slabbekoorn et al., 2010). Shipping activities, wind mills, pile driving, seismic surveys, naval sonars and fisheries activities are all accompanied by the introduction of both intended and unintended anthropogenic sounds in the water. Consequently, anthropogenic noise comes in many forms and can vary greatly in both temporal and spectral patterns. Although we know that sounds can play an important role for fish in natural habitats (Montgomery, Jeffs, Simpson, Meekan, & Tindle, 2006; Radford, Jeffs, & Montgomery, 2007; Radford, Jeffs, Tindle, & Montgomery, 2008), we still have little understanding of the potentially negative consequences of noise pollution for aquatic life. While field studies in open water conditions are challenging to implement (Slabbekoorn, 2016), studies in tanks have only just started to reveal, for example, the importance of temporal variation in sound exposure (Neo et al., 2014) and variation in disturbance tendency between species (Shafiei Sabet, Neo, & Slabbekoorn, 2015; Voellmy, Purser, Simpson, & Radford, 2014).

Although there is an increasing awareness of the potentially detrimental effects of anthropogenic noise on the behaviour of free-ranging fish, there still remains a paucity of empirical evidence on the subject. So far, a limited number of studies have reported on fish responses in the wild, for a limited number of anthropogenic noise sources, and these reports are often anecdotal or without replication. For instance, vessel noise was reported to change both the schooling structure and swimming behaviour of pelagic tuna, *Thunnus thynnus* (Sarà et al., 2007) and air gun shooting during a seismic survey made various fish species swim away from the sound source and down the water column (Engås & Løkkeberg, 1996; Slotte, Hansen, Dalen, & Ona, 2004). Moreover, short, impulsive pile driving-like sounds caused density changes in sprat, *Sprattus sprattus*, and depth changes in mackerel, *Scomber scombrus* (Hawkins, Roberts, & Cheesman,

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2014). A study on roach, *Rutilus rutilus*, and rudd, *Scardinius erythrophthalmus*, reported interruption of spawning activities by a fast-moving power-boat (Boussard, 1981), while boat noise also reduced outside-burrow activities of red-lip gobies, *Gobius cruentatus*, and disturbed nest care activities in damselfish, *Chromis chromis* (Picciulin, Sebastianutto, Codarin, Farina, & Ferrero, 2010). However, understanding the effect of noise on fish behaviour through studies in natural habitats is challenging as replication with fish of known background is hard to achieve and species may vary in their behavioural response (Slabbekoorn, 2016).

Noise impact studies in indoor conditions provide the opportunity to manipulate the experimental environment, to control the test group of subjects and to achieve sufficient replication. Studies on captive fish have revealed, for example, that acoustic overexposure can cause temporary or permanent hearing loss (Amoser, Wysocki, & Ladich, 2004; Popper et al., 2005; Smith, 2004). Also, more moderate but realistic anthropogenic noise levels have been tested in the laboratory and have been shown to mask relevant acoustic signals and cues (Codarin, Wysocki, Ladich, & Picciulin, 2009; Vasconcelos, Amorim, & Ladich, 2007), to affect antipredator behaviour (Bruintjes & Radford, 2013; Simpson, Purser, & Radford, 2015; Voellmy, Purser, Simpson, et al., 2014) and to reduce foraging performance (McLaughlin & Kunc, 2015; Purser & Radford, 2011; Shafiei Sabet et al., 2015; Voellmy, Purser, Flynn, et al., 2014). However, studies on noise-dependent spatial avoidance, such as those done on several terrestrial animals (Knutson & Bailey, 1974; MacKenzie, Foster, & Temple, 1993; McAdie, Foster, Temple, & Matthews, 1993; O'connor et al., 2011; Schaub, Ostwald, & Siemers, 2008), are difficult on captive fish. Fish tanks yield obvious limitations for escape behaviour and sound field conditions are complex and different from outdoor conditions (Slabbekoorn, 2016).

Although spatial avoidance or phonotaxis may not be expected from captive fish within the confinement and complex sound field of a fish tank (Akamatsu, Okumura, Novarini, & Yan, 2002; Parvulescu, 1967), a few studies have addressed this issue (see e.g. Febrina et al., 2015; Neo et al., 2015). Horizontal displacements have been used to infer fish's ability to localize sound sources under natural conditions in the wild (Fay & Popper, 2005; Popper & Fay, 1993; Tolimieri, Jeffs, & Montgomery, 2000), but several studies have also shown that captive fish can localize sound sources and reveal positive phonotaxis in the horizontal plane (Higgs, Rollo, Janssen, & Andraso, 2007; Rollo & Higgs, 2008; Verzijden, Ripmeester, Ohms, Snelderwaard, & Slabbekoorn, 2010). Vertical displacements may be another relevant spatial read-out that may indicate an anxiety-related response (Brown, Rive, Ferrari, & Chivers, 2006; Luca & Gerlai, 2012; Neo et al., 2014; Pearson, Skalski, & Malme, 1992), providing a tool to study the effects of temporal variety in sound exposure or differences between fish species.

In this study, we investigated how sound exposure affects two fish species with different swimming behaviour and different hearing abilities. We selected zebrafish, *Danio rerio*, and Lake Victoria cichlids, *Haplochromis piceatus*, as they represent fish with distinct swimming tendencies and hearing abilities and they were readily available. Zebrafish typically swim continuously, often with quick turns and frequent changes in speed, but always with a forward pace (see e.g. Cachat et al., 2010; Neo et al., 2015). Cichlids are much slower swimmers in general and alternate swimming bouts with periods of no movement (see e.g. Estramil et al., 2014; Heuts, 1999). Zebrafish have Weberian ossicles that provide a lower absolute threshold and a wider spectral range of auditory sensitivity compared to Lake Victoria cichlids (Higgs, Souza, Wilkins, Presson, & Popper, 2002; Kenyon, Ladich, & Yan, 1998; Ladich & Fay, 2013), which vary in hearing sensitivity due to variation in swim bladder size and position, but do not have the more advanced hearing aids of cyprinid fishes (Popper & Fay, 1993; Schulz-Mirbach, Metscher, & Ladich, 2012).

Our aims were to test how continuous and intermittent sound exposure changes swimming speed and spatial behaviour in a long fish tank in which sound is played from one or the other side. We compared baseline behaviour for individual fish of both species and tested differences in swimming speed in brief periods around sound onset (reflecting startle responses and sudden acceleration) as well as prolonged changes in swimming speed. In addition, we tested sound-related spatial variation by measurement of horizontal and vertical displacements. Moreover, we tested for internal consistency in swimming behaviour among behavioural measurements for which sound exposure had a significant impact. We expected no sound impact on horizontal displacement (cf Estramil et al., 2014; Neo et al., 2015), but we did expect anxiety-related vertical displacement (cf Gerlai, 2010; Voellmy, Purser, Simpson, et al., 2014) that could be correlated with an initial speeding response and with slowing down in the long term. We further expected that differences in the behavioural effects of sounds that are well within the audible range for both species would not necessarily be related to their relative hearing abilities.

METHODS

Study Species and Housing Condition

Thirty adult wild type zebrafish (sex ratio about 1:1) were obtained from our own breeding stock (Sylvius laboratory, Leiden University), which originated from fish stocks from Europet Bernina International BV (Gemert-Bakel, The Netherlands), bought at a local pet supplier (Selecta Aquarium Speciaalzaak). All zebrafish were housed in a 400-litre glass holding tank (200 × 40 cm and 50 cm deep; water depth: 40 cm; wall thickness: 4 mm) on a 14:10 h light:dark cycle (light switched on at 0600 and switched off at 2000) and with the water temperature kept at 24 °C. Zebrafish have their peak hearing sensitivity around 800 Hz (Higgs et al., 2002).

Thirty adult wild type Lake Victoria cichlids (sex ratio about 1:1) were taken from our own breeding stock (Sylvius laboratory, Leiden University, third generation in captivity), which originated from wild-caught fish imported from Tanzania. All cichlids were housed in a 300-litre glass holding tank (150 × 40 cm and 50 cm deep; water depth: ca. 40 cm; wall thickness: 4 mm), also on a 14:10 h light:dark cycle and with the water temperature kept at 24 °C. Fish holding tanks were connected to a central water recirculation system (Fleuren & Nooijen, Nederweert, The Netherlands). All individuals of both species were fed twice daily with dry food (DuplaRin M, Gelsdorf, Germany) and frozen Artemias (RUTO frozen fish food, The Netherlands).

Haplochromis piceatus has not been tested for hearing sensitivity, but cichlids with a range of swim bladder sizes and shapes varied in peak sensitivity between 200 and 500 Hz (Schulz-Mirbach et al., 2012). We inspected size and position of the swim bladder in a dead specimen of *H. piceatus*: no extreme morphology was observed and measures appeared well within the range of the three cichlid species tested by Schulz-Mirbach et al. (2012). Ambient noise conditions (around 95 dB re 1 uPa) were similar for both species as their holding tanks were on the same type of tables and in the same room.

Experimental Tank and Set-up

The experiments were conducted in a rectangular glass tank (200 × 35 cm and 45 cm deep; water depth: ca. 35 cm; wall thickness: 1 cm). The tank was placed on a steady table on top of a layer of