Experimental sound exposure modifies swimming activities and increases food handling error in adult zebrafish (*Danio rerio*)

Reza Mohsenpour[a], Saeed Shafiei Sabet[a,*]

[a] Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Iran.

Abstract:

Anthropogenic sound is globally increasing and is recognized as a source of environmental pollution in terrestrial and aquatic habitats. Sound is an important sensory stimulus for aquatic organisms and it may cause fluctuations in stress-related physiological indices and in a broader extent induce behavioural effects in a range of marine and freshwater fishes. However sound exposure may also induce changes in swimming activities, feed efficiency and productivity of available food sources in fish. Here, we experimentally tested sound effects on swimming activities and foraging performance in thirty adult Zebrafish (*Danio rerio*) individually in captivity. We used adult zebrafish and water flea (*Daphnia magna*) as model predator and prey, respectively. In terms of acoustic stimuli, we used four sound treatments with different temporal patterns (all in the same frequency range and moderate exposure level). Our results constitute strong evidence for sound-related effects on zebrafish behaviour. All sound treatments led to a significant increase in the number of startle response, brief and prolonged swimming speed for zebrafish ($P<0.05$). We found partially brief and prolonged sound effects on the spatial distribution of zebrafish; Although there were not any significant sound-related changes for horizontal spatial displacement in all treatments ($P>0.05$), zebrafish swam significantly more in the lower layer of the tank except for irregular intermittent 1:1-7 in brief sound exposure ($P<0.05$). The results of foraging performance showed that food discrimination error unaffected by sound treatments and was low for the zebrafish ($P>0.05$). However, food handling error was affected by sound treatments; all treatments induced a significant rise in handling error ($P<0.001$). This study highlights the impact of sound on zebrafish swimming activities, and that more feeding bouts are needed to consume the same number of food items increasing energy demand under noisy conditions.

Keywords: Anthropogenic sound, Behaviour, Foraging performance, Sound impact, Zebrafish

1. Introduction

Nowadays, due to the increase in human activities and the advancement of technology globally since the Industrial Revolution, the living environment has undergone extensive changes (Normandeau Associates, 2012). These environmental changes can affect the planet and living organisms, and that can be a major threat to the biodiversity inhabit Earth (Kunc et al., 2016). The rapid growth of these changes poses many environmental challenges (Tuomainen and Candolin, 2011) in both terrestrial and aquatic habitats. One of the main sources of environmental pollution which may also can be recognized as an environmental stress stimulus is anthropogenic sound that in addition to affecting terrestrial animals, also have many consequences on aquatic organisms (Popper et al., 2020; Slabbekoorn et al., 2010; Slabbekoorn and Ripmeester, 2008). Anthropogenic sound sources in aquatic habitats, such as military sonars, merchant shipping, wind turbines, pile-driving, recreational boating, underwater mining explorations and explosions are quite frequent in the number of events, temporal patterns and widespread geographically. (McDonald et al., 2006; Normandeau Associates, 2012). Consequently, anthropogenic sound has changed underwater soundscapes worldwide and represents a very subtle driver of environmental change and novel challenge to aquatic organisms.

Recent studies have investigated impacts of anthropogenic sound on a wide range of taxa and across a variety of scales (Barber et al., 2009; Morley et al., 2014; Normandeau Associates, 2012; Slabbekoorn et al., 2010; Thomsen et al., 2021; Tyack, 2008). Anthropogenic sound can cause a range of physical, physiological, and behavioural disorders in aquatic organisms, including marine mammals (Erbe et al., 2018; Moore et al., 2012; Southall et al., 2008), seabirds (Bermúdez-Cuamatzin et al., 2018; Green et al., 2016; Hansen et al., 2020), reptiles (Injaian et al., 2020; Simmons and Narins, 2018), fish (Hastings and Popper, 2005; Hawkins, 1986; Mills et al., 2020; Popper et al., 2003), and invertebrates (Carroll et al., 2017; Coquereau et al., 2016; Murchy et al., 2019). Because of the high propagation rate and low attenuation over large distances in underwater environments acoustic stimuli play an important role in aquatic environments (Tyack, 1998; Popper and Hastings, 2009; Slabbekoorn et al., 2010).

Behavioural effects are the most likely to occur and thus play as a stress driver (Smith et al., 2004; Popper and Hawkins, 2019) as a distracting stimulus (Popper and Carlson, 1998; Chan et al., 2010), interfere with detecting prey and antipredator behaviour (Hawkins and Myrberg, 1983), compromise foraging performance (Neo et al., 2015; Purser and Radford, 2011; Shafiei

Sabet et al., 2015; Voellmy et al., 2016), disrupt reproductive behaviour (McCloskey et al., 2020) or mask important acoustic signals and cues for conspecific recognition and communication purposes (Codarin et al., 2009; Amorim et al., 2015; De Jong et al., 2018b; Hawkins and Picciulin, 2019) in a range of marine and freshwater species.

Many marine and freshwater fishes have well-developed hearing abilities that provide them a key biological privilege to detect sound and perceive a broad range of frequencies (Hawkins, 1986; Heath et al., 2021; Popper et al., 2019; Wahlberg and Westerberg, 2005; Wysocki et al., 2006). While there are well-documented studies regarding the effects of sound on the behaviour of marine fishes (de Jong et al., 2018a; Mortensen et al., 2021; Peng et al., 2015), and its adverse repercussions in many marine fish species at the individual and community level, yet much less is known across the current literature about these effects on the behaviour of freshwater fish and has not received sufficient interest so far (Fedoroff, 2021; Mickle and Higgs, 2018; Pieniazek et al., 2020). Moreover, sound exposure can also change spatial distribution and swimming behaviour of fish which may consequently affect ecologically on their avoiding to forage in noisy food areas and their navigations but also change biologically their swimming activities and foraging performance (de Vincenzi et al., 2021; Hanache et al., 2020; Hubert et al., 2021; Shafiei Sabet et al., 2016a). There is growing evidence that foraging performance is likely to be modulated by anthropogenic sound that can induce shift from successful feeding bouts to typical behavioural stress responses in fish (Purser and Radford, 2011; Shafiei Sabet et al., 2015; Voellmy et al., 2016. Currently, little is known about the effects of sound exposure on swimming activity and foraging performance of fish, although there are some well-documented studies. It has been shown that increased boating activity was associated with a reduction in activity rates, changed vertical distribution and compromised foraging success of free-ranging mulloway (*Argyrosomus japonicus*) (Payne et al., 2015) and Mediterranean Damselfish (*Chromis chromis*) (Bracciali et al., 2012).

Other studies have shown that experimental sound exposure increase performance errors and therefore displayed a negative impacts on foraging efficiency in both the three-spined stickle backs (*Gasterosteus aculeatus*) (Purser and Radford, 2011) and the European minnow, (*Phoxinus phoxinus*) (Voellmy et al., 2014a). More recently our previous study also have shown a clear sound impact on zebrafish foraging performance; more food handling errors under noisy conditions (Shafiei Sabet et al., 2015). A primary consequence of sound exposure would appear to be shifts in the spatial displacement. The resulting disturbance might induce modifications in allocated foraging time budget, foraging patterns and the relative abundance

of prey items and predatory species. Such changes in turn may increase foraging energy demand and the amount of time allocated by fish to foraging which, subsequently induce a number of major changes such as affect food searching, discriminating and handling.

In general, *Danio rerio* is known as a model fish species in behavioural studies and responding to environmental conditions (Cachat et al., 2010; Egan et al., 2009; Whitfield, 2002). Zebrafish is a member of the Cypriniformes order and acclimates well in captivity (Detrich et al., 2011) and naturally lives in the tropical freshwater (Spence et al., 2008). Zebrafish have specialized hearing structures, the Weberian ossicles, with an auditory system that has homologies to the mammalian auditory system (Weber, 1820; Alexander, 1964; Whitfield, 2002).

Progress in the field of behavioural biology over conservation ecology and findings about the potential impacts pollutants on organisms is also to a large extent linked with the study of invertebrates. Daphnia is a small crustacean and inhabits in open and light waters, also they are an important part of the food web in freshwater habitats and inhabits many types of shallow water bodies (Ebert, 2005; Parejko and Dodson, 1991; Reynolds, 2011).

In the present study, as a follow up of our recent study (Shafiei Sabet et al., 20215), we experimentally tested the hypothesis that whether experimental sound exposure ensonified by an underwater speaker affect the general swimming activities and foraging behaviour of zebrafish as predator upon water flea as prey item under laboratory conditions. Comparing to Shafiei Sabet er al., 2015 that we used an in-ear speaker, in this study we adopted an underwater speaker to increase sound pressure levels and potential gradient of it in the arena. We also extended the time of behavioural analysis to explore more long-term effects of sound on the zebrafish. Our specific goals were: firstly, to assess the effect of experimental sound exposure on zebrafish activities including swimming speed and spatial displacement. Secondly, to estimate whether the temporal pattern of sound exposure matters and affects differently zebrafish behaviour. And thirdly, to verify our recent laboratory-based findings of sound impacts on zebrafish swimming activity and foraging behaviour.

2. Materials and Methods

This study was performed in the ornamental fish breeding facility center at University of Guilan, Sowmeh Sara, Iran (37°17′39″N, 49°19′55″E), using an aquarium with dimensions of 50 ×15 ×20 cm in the period of 10:00 to 14:00 every day. Zebrafish (~ age of 45 days old and of the wild-type, short-fin variety weight (± s.d.) of 1.23 ± 0.02 g) were obtained from an ornamental fish breeding shop in Sowmeh Sara, Iran. Zebrafish were stored in a stock glass

tank with dimensions 50×30×40 cm for two weeks and adapted to environmental conditions to reduce possible stress and hormonal changes due to transportation, captivity conditions and animal welfare issues (Deakin et al., 2019). The fish were fed 0.8 mm commercial Biomar® feed until the day before the experiment (Neo et al., 2015). Waterfleas were caught every morning, during the whole experiment days (See Shafiei Sabet et al., 2015; Shafiei Sabet et al., 2019).

2.1. Sound treatments

Four sound treatments with different temporal patterns along with control treatment were used, including the first treatment as control treatment in which the fish were exposed to ambient noise (AN), regular intermittent sound (IN) with fast pulse rate (1:1) (Fig. 1 (a)), regular intermittent sound with slow pulse rate (1:4) (Fig. 1 (b)), irregular intermittent sound (1:1-7) (Fig. 1 (c)), and continuous sound (CS) (Fig. 1 (d)). All three intermittent treatments include one second of sound, but the difference between these sound treatments is the intervals between these sounds (silence time) (See also Shafiei Sabet et al., 2015). The sound treatments were generated and modified using Audacity® software (2.3.1) at the sound frequency that can be detected and heard for zebrafish (300-1500 Hz) (Higgs et al., 2002) as well as the bandwidth of anthropogenic sounds, including vehicles, pump systems, and similar pile driving that overlap (Slabbekoorn et al., 2010). The designed sound was produced by the software in the same sound frequency range of 400-2000 Hz based on our earlier study (Shafiei Sabet et al., 202).

2.2. Experiment Tank

The experimental tank with dimensions of 50×20×15 cm with black background was prepared to improve the contrast between Daphnia and fish in the video file. The zebrafish swimming activities were filmed by a video camera (Panasonic HC-V180 Full HD) at a distance of 50 cm from in front of the test tank. Sound treatments were played back as stereo WAV files using a Laptop (Sony Vaio SVF1421A4E) connected to an underwater speaker (custom-build speaker, 30 W, 10 Hz- 10 KHz). In this experiment, a divider plate was placed transversely in the tank and the tank length was halved (25×20×15 cm) in order to increase the enclosure on the zebrafish swimming environment and make the entire fish swimming space visible for video-recording (Fig. 2). The test tank was surrounded by black plastic so that the fish's behaviour was not affected by other visual factors and only the camcorder lens passed through the plastic and was the same for all treatments and repetitions. To isolate the experiments from external

sounds and reduce unwanted sounds, we placed and covered all walls at the entrance to the experimental room and also inside walls using egg boxes.

The underwater speaker used in the experiment was placed horizontally on the other side of the separator plate (See Fig. 2). Then, each test was performed as follow: after ten minutes, the sound treatment was played by a speaker and a sound player for 20 minutes, However, the food item (Daphnia) and non-food item (Duckweed) were added to the experiment tank after ten minutes from onset of each sound playback in all sound treatments with a 15-minute interval between treatments and the fish was exposed to all five acoustic treatments and repeated the next day for the next fish. The order of broadcast of sound treatments on a daily basis was randomly balanced. We used a similar methodological approach previously described to quantify behavioural indices of zebrafish and acoustic measurements of tank in this system (Shafiei Sabet et al., 2015). The photoperiod used in the experiment was 12:12 (Higgs et al., 2002; Villamizar et al., 2014) and the light intensity measured in this experiment by the light meter model (TES_1336A – TES Electrical Electronic Corp. Taiwan) averaged 62 lux. Water temperature and the amount of dissolved oxygen (DO) in the water were measured during the experiment and was maintained at 26±1 °C at 8±1 mg / L, respectively.

2.3. Underwater sound measurement in the tank

Sound file were played back using a sound player connected to a custom-build underwater speaker which a custom-build sound tuning amplifier was attached (See Shafiei Sabet et al., 2015). The level of sound pressure was recorded by a hydrophone model (Aquarian Scientific AS-1) which connected to the amplifier model (PA-4) and a Tascam linear PCM recorder model (DR-100MKII). Sound pressure levels and power spectral density of the sound for the continuous treatment were evaluated in R studio software (Vr. 1.1.456). The sound pressure of continuous sound treatment during playback was average 121 dB re 1 $\mu Pa^2/Hz$ for 5 seconds and the ambient sound pressure was average 96 dB re 1 $\mu Pa^2/Hz$ for 5 seconds (Fig. 3).

2.4. Effect of sound on Zebrafish swimming behaviour

To investigate the effect of sound on the behaviour of Zebrafish, 30 fishes (15 F: 15 M) were introduced individually in the experimental tank. The fish were introduced to the test tank in the day before (Overnighting) and the fish were given the opportunity to get used to the environment so that they could clearly use the entire space of the tank for their swimming and display normal behaviour (Neo et al., 2015; Shafiei Sabet et al., 2015). Also, all fish were given

30 minutes to acclimate in the experiment tank and since then, the fish has undergone five sound treatments.

Zebrafish behavioural responses were video recorded for a maximum of 30 minutes for each treatment (10 mins before exposure and 20 mins during exposure). Swimming behaviour parameters such as startle response (which is the peak of swimming speed of fish more than 10 cm per second that occurs immediately after the sound was played for one minute (See Shafiei Sabet et al. (2015)), brief swimming speed (5 seconds before and 5 seconds during sound) and prolonged swimming speed (one minute before and one minute during sound) were evaluated for all treatments. Also, to explore spatial distribution of fish in the tank, in the vertical/column profile, according to the dewatering height of 15 cm of the test tank, tank height during inspection and analysis behavioural data was divided into two parts: zero to 7.5 cm and 7.5 to 15 cm. To check the distance from the sound source in the horizontal profile, the length of the tank was divided into three parts: zero to 8.33 cm, 8.33 to 16.66 cm and 16.66 to 25 cm.

2.5. Effect of sound on foraging behaviour of zebrafish on water fleas

The effect of sound on the foraging behaviour of zebrafish was investigated in such a way that 5 waterfleas (~3 mm) as a prey species (target) and 5 non-food substances as non-food item in the same size as Daphnia (~ 3mm) was mixed in 25 ml beaker container and added gently to the fish tank in the same manner for all treatments. The waterfleas were in the same sizes that caught with plastic Pasteur pipettes to decrease damaging water, which is suitable for feeding this species of fish at puberty and can be received by the mouth of the fish (Shafiei Sabet et al., 2015). Naturally, both of food and non-food items are present in the habitat of this fish. Foraging performance was assessed as described in Shafiei Sabet et al., 2015.

2.6. Behavioural information processing and statistical analysis

Recorded videos of zebrafish behaviour were converted to 10 frames per second by Xilisoft Video Converter Ultimate software to reduce the magnification of time, in order to increase the accuracy of the fish swimming survey and also to reduce the fish speed for spatial inspection per second. Logger Pro® video tracking software (Vernier Software, Beaverton, OR, USA, version 3.10.1) was used to examine behavioural responses including the number of startle responses, swimming speed, and spatial distribution of the fish. Data analysis was performed using SPSS 25 software and Excel 2016. The normality of the data was evaluated by Kolmogorov-Smirnov test and the homogeneity of the data by Levene test. Then, the presence

or absence of significant differences between the mean of the data was assessed by repeated measures ANOVA analysis and using Tukey multi-range test. A HuynheFeldt correction was performed when sphericity could not be assumed in the repeated measures ANOVA. Bonferroni corrected post hoc tests were performed when ANOVA test results were significant. The level of significance in this study was considered $P<0.05$. A custom-written acoustic calibration script in R studio software (Version 1.1.456) was also used to evaluate sound pressure levels and power spectral density that were played by the underwater speaker.

3. Ethical note

All housing, handling and experimental conditions were in accordance with the guidelines for the treatment of animals in behavioural research and teaching (ASAB, 2020). Water fleas and zebrafish were allowed to acclimatize gradually to the laboratory conditions before they were used in any of the experiments and showed no signs of adverse effects of the experimental conditions. Zebrafish showed only a brief startle response with the onset of the moderate sound playbacks and no mortalities or physical damages were observed during experiments (Neo et al., 2015; Shafiei Sabet et al., 2015). There are no legal requirements for studies involving invertebrates such as waterfleas (Daphnia) in Iran.

4. Result

4.1. Impact of sound on swimming behaviour of Zebrafish

Experimental sound exposure has changed zebrafish swimming activities in different ways. Sound treatments significantly increased the parameters of startle response and swimming speed of fish (Fig. 4a, b, c). The number of startle response instantly increased in exposure to sound treatments so that all sound treatments showed a significant difference compared to the ambient condition (repeated measures ANOVA: $F_{3.23,93.68}=6.31$, $P=0.000435$), But no significant differences were observed in terms of temporal patterns between sound treatments ($P>0.05$) (Fig 4a). Also, in the brief swimming speed difference (5 seconds before the sound and 5 seconds during the sound) there was a significant difference compared to the ambient condition (repeated measures ANOVA: $F_{3.06,88.70}=11.17$, $P=0.000002$), although there was no significant difference between the sound treatments with different temporal patterns in this time period ($P>0.05$) (Fig 4b). This difference in swimming speed in exposure to sound treatments was also true in prolonged (60 seconds before the sound and 60 seconds during the sound), so that there was a significant affect between sound treatments compared to ambient conditions

(repeated measures ANOVA: $F_{3.39,98.34}$=7.72, P=0.000054), however again there was nonsignificant difference between sound treatments (P>0.05) (Fig 4C).

According to Figure 5, in four sound treatments (CS, 1:1, 1:4, 1:1-7), a sudden increase in fish swimming speed was observed once sound treatments were played at 60 seconds. Observations also showed that in all sound treatments, fish reached to the baseline after 60 seconds during sound. In ambient condition (Fig 5a), no significant difference in swimming speed was observed in any of the time periods. While in continuous sound and regular intermittent 1:1 treatment (Fig 5b, c), a significant difference was observed in the comparison of 5 seconds before sound playback and the first 5 seconds of sound playback (CS= repeated measure ANOVA: $F_{4.54,131.66}$=9.53, P≤0.001, 1:1= repeated measure ANOVA: $F_{3.85,111.72}$=11.72, P≤0.001). This is while in this treatment, compared to the last 5 seconds before the sound and the last 5 seconds of the sound, fish reached the base line and there was no difference (P>0.05). Also, this difference was observed in the two treatments regular intermittent 1:4 and irregular intermittent 1:1-7 (Fig 5d, e) with more intensity compared to before and during the sound (1:4= repeated measure ANOVA: $F_{6.24,181.01}$=14.17, P≤0.0001, 1:1-7= repeated measure ANOVA: $F_{7.14,207.15}$=15.01, P≤0.0001). But in comparing to the baseline time in these two treatments, no significant difference was observed and the fish reached the baseline (P>0.05).

Changes in the spatial distribution of zebrafish were investigated when exposed to sound treatments in vertical scale (lower layer) (Fig. 6a, b) and horizontal scale (X position) (Fig. 7a, b). According to Figure 6a, the average percentage of fish time spent in the lower layer of the tank during sound treatments exposure over a brief time (15 seconds before sound and 15 seconds during sound exposure) there was no treatment effect ($F_{3.77,109.47}$=1.486, P=0.214) or interaction for treatment*times ($F_{3.55,102.92}$=0.634, P=0.621). But there was effect of times ($F_{1,29}$=28.274, P=0.000011). In two treatments of ambient (AN) and irregular intermittent sound (1:1-7) with before sound exposure was not significant effect (AN= $F_{1,29}$=28.274, P=0.104, 1:1-7= $F_{1,29}$=28.274, P=0.051). But there was a significant difference between two sound treatments: continuous sound (CS) and 1:4 during and before exposure to sound (CS= $F_{1,29}$=28.274, P=0.007, 1:4= $F_{1,29}$=28.274, P=0.004) and also 1:1 treatment was a significant difference with before the sound exposure ($F_{1,29}$=28.274, P=0.011). According to Figure 6b, the average percentage of fish time spent in the lower layer of the tank during sound treatments over a prolonged time (60 seconds before sound and 60 seconds during sound exposure), there was no treatment effect ($F_{3.65,105.92}$=1.837, P=0.133) or interaction for treatment*times ($F_{4,116}$=1.780, P=0.137). But there was effect of times ($F_{1,29}$=35.398,

P=0.000002). In control treatment (AN) between before and during sound exposure do not show a significant difference ($F_{1,29}$=35.398, P=0.975). While in continuous treatment (CS) and 1:4 between before and during sound exposure showed a significant difference (CS= $F_{1,29}$=35.398, P=0.000411, 1:4= $F_{1,29}$=35.398, P=0.000360) and 1:1 treatment was significant difference between during exposure and before time ($F_{1,29}$=35.398, P=0.008). Also, irregular intermittent sound treatment (1:1-7) between during and before sound exposure was significant difference ($F_{1,29}$=35.398, P=0.042). But in the horizontal profile (X position), there was no significant difference between sound treatments and control conditions, both in the brief and prolonged time ($F_{4,116}$=1.369, P=0.249) (Fig 7a), ($F_{3.78,109.71}$=1.810, P=0.136) (Fig 7b). Also, according to Figure 8a, b, the effects of different sound patterns on the spatial distribution of fish can be seen as a heat map in brief and prolonged duration. Acoustic treatments affected changes in swimming pattern from the top layer to the bottom layer but had no effect on the distance from the sound source (right side of the tank).

4.2. Impact of sound on foraging performance of Zebrafish

According to Figure 9a, none of the acoustic treatments showed a significant difference compared to the silent treatment on zebrafish food discrimination error (repeated measure ANOVA: F4,116=1.339, P=0.260). In fact, there was no food discrimination error between food and non-food item by broadcasting sound treatments compared to control treatment. However, all acoustic treatments showed a significant difference compared to the control treatment (repeated measure ANOVA: F2.82,81.91=26.023, P≤0.001) but no significant difference was observed between sound treatments (P>0.05) (Fig. 9b). This means that with the broadcast of acoustic treatments compared to the control treatment, there was a significant handling error in food intake in the food.

5. Discussion

Our results unequivocally demonstrate that acoustic stimuli affect zebrafish behaviour in different ways. In this experiment, zebrafish swimming behaviour indices such as the number of startle response, the difference in brief swimming speed (within 5 seconds), the difference in prolonged swimming speed (within 60 seconds) and the spatial distribution of zebrafish such as the percentage of fish in the lower layer of the tank and horizontal spatial distribution in response to continuous and intermittent sound patterns were examined. The results showed that the number of startle response indicating anxiety in zebrafish and other aquatic species (Blaser et al., 2010; Maximino et al., 2010), in different sound treatments, showed a significant

difference compared to control condition. However, these changes did not show a significant difference between sound treatments. Also, in another part of the results, the difference between brief and prolonged swimming speeds in all sound treatments compared to the control condition was significant. However, these changes did not show a significant difference between sound treatments. Moreover, interestingly like what we have shown in our earlier study (Shafiei Sabet et al., 2015), we have seen the same impact of sound exposure on foraging performance in zebrafish as all will be discussed further as follows in the next sections.

5.1. Startle responses as a specific indicator of moderate anxiety in zebrafish?

Startle response is an involuntary action that is controlled by a pair of brain neurons in the Mauthner (M-) cells in the mesencephalon and play a major role in the decision-making process (Eaton et al., 1977; Eaton et al., 1991; Mirjany et al., 2011; Zottoli, 1977). Increasing the intensity of the sound triggers a response by involuntary Mauthner cells in the mesencephalon, leading to an involuntary escape response in fish (Eaton et al., 1977; Eaton et al., 1991). Increasing the number of startle response and brief swimming speed of fish by playing sound treatments, causes behavioural responses related to fear and anxiety in this species. Previous studies have shown that sounds increase motor acceleration and startle responses in zebrafish (Neo et al., 2015; Shafiei Sabet et al., 2015). However, Shafiei Sabet et al. (2015) using an in-air speaker as a sound source reported that the difference in the number of startle response between continuous and intermittent (1:1) treatments with the ambient treatment was not significant which is not consistent with the results of this study. The reason of this difference in the occurrence of stress-related swimming behaviour could be due to differences in the background sound intensity before the test, differences in the method of ensonifiying of the fish tank; the sound source (speaker) under water or in-air, different storage conditions of fish and also differences Genetics and individual characteristics in zebrafish.

Other species of fish respond similar sudden swimming behaviour in response to sound exposure. European minnow (*Phoxinus phoxinus*) and sticklebacks (*Gasterosteus aculeatus*) also showed a significant increase in the number of startle response in the face of sound, which is similar to the results of this study (Purser and Radford, 2011; Voellmy et al., 2014a). Startle response at the onset of sudden sound exposure is a common behavioural feature in fish kept in captivity and in the laboratory conditions. Of course, fish in the open and natural conditions can also show behavioural responses related to fear and anxiety (Neo et al., 2016; Staaterman et al., 2020). Spiga et al. (2017) stated that European seabass (*Dicentrarchus labrax*) also

showed a higher number of startle response in continuous and intermittent sound treatment against ambient (control) treatment.

Startle response by prey fish is a behavioural response to enhance survival rate in predator-prey relationships (Webb, 1986). By hearing the sounds of predator fish and receiving sound signals related to the attack, the prey fish starts swimming at high speed and explosively in the opposite direction of the perceived sound in order to increase the success rate of escaping and staying away from the predatory species. Sounds can affect the prey fish's decision-making power against sound sources or danger, the way in which prey assesses risk (Dukas, 2004), and the loss of focus and lack of appropriate response to danger (Chan et al., 2010; Simpson et al., 2015). It has been suggested that increasing sound levels can potentially impair the perception of danger by predatory fish species (Slabbekoorn et al., 2010). Involuntary and acquired behavioural responses related to fear and anxiety are associated with the potential for the presence of danger (Blaser et al., 2010; Maximino et al., 2010). The quality and quantity of behavioural responses of fish in captivity and in vitro to brief and severe stress stimuli are different from those of fish living in the habitat, reducing the behavioural responses in the habitat and in the wild (Malavasi et al., 2004). One of the reasons for these differences could be due to the most ability of fish to respond in the wild before reaching the stimulus threshold, so that these fish have a longer time to make decisions and escape from the danger zone by hearing and perceiving closely the sounds associated with the predator species and they have more space available than controlled laboratory environments. Another reason could be the high level of basal stress potential in controlled laboratory environments, which with additional stress due to the perception of the predator species leads to an increase and intensification of total stress in the prey species and more intense responses are shown.

In addition to behavioural responses, increasing sound levels can also affect physiological responses in the laboratory and in the natural habitat of fish. The study of Spiga et al. (2017) and Radford et al. (2016) showed that sound exposure had a significant effect on the number of opening and closing of gills and thus on the gill ventilation of European seabass (*Dicentrarchus labrax*) compared to the control treatment. This increase in oxygen demand by European bass (*Dicentrarchus labrax*), which is accompanied by an increase in gill ventilation and the opening and closing of gill operculum, indicates an increase in stress levels. Santulli et al. (1999) showed that blood biochemical parameters including cortisol and glucose in European seabass (*Dicentrarchus labrax*) increased in sound treatments compared to the ambient treatment. Staaterman et al. (2020) stated that anthropogenic sound treatments in the

natural environment also have the potential to affect stress-related physiology in coral reef fish, so that the amount of cortisol in sound treatments was significantly increased compared to the control treatment.

5.2. Sound impacts on the behavioural tolerance in swimming activities

In the experiment performed on zebrafish at groups and individual level, different sound patterns had a significant effect on zebrafish swimming activities such as the brief and prolonged swimming speed of the fish compared to the ambient treatment, which is consistent with the observations of the present study (Neo et al., 2015; Shafiei Sabet et al., 2015; Shafiei Sabet et al., 2016a). In the experiment of Neo et al. (2015) with a group of 5 zebrafish in each treatment, in comparison with increasing the swimming speed of fish with the ambient treatment, as in the results of this study, the intermittent (1:1) treatment had a higher mean speed and significant difference than other treatments. Also, with onset of sound exposure, a significant difference in swimming speed was observed in cod fish (*Gadus morhua*), which was consistent with the results of this study (Handegard et al., 2003). There was also a significant difference in the swimming speed of European seabass (*Dicentrarchus labrax*) compared to the silent conditions (Neo et al., 2018). The prolonged swimming speed of the zebrafish weakened with repeated exposure suggesting a habituation process. Zebrafish might experience negative consequences if they are disturbed and do not habituate contrary to sound exposure. Shafiei Sabet et al. (2016b) by comparing the effect of sound on the swimming behaviour of two species of The Lake Victoria cichlids (*Haplochromis piceatus*) and zebrafish (*Danio rerio*) have showed that the application of sound treatments reduced the swimming speed of cichlids (*Haplochromis piceatus*) and increased the swimming speed of zebrafish (Shafiei Sabet et al., 2016b). The reason of the difference in the swimming speed of cichlids (*Haplochromis piceatus*) can be related to species-specific behaviouraltreats in response to acoustic stimuli, genetic characteristics and habitat conditions.

5.3. The effect of sound on the spatial distribution of zebrafish

Studies on the stress indices of zebrafish in the face of different sound patterns showed that with the onset of sound treatments, the spatial distribution of fish changes and the fish shows a greater tendency to swim in the lower layer of the aquarium environment. Neo et al. (2015) revealed that zebrafish with the beginning of broadcasting sound treatments showed startle response and increased brief swimming speed and the spatial distribution of zebrafish changed so that fish was more inclined to and they swam in the top and surface layers of the test tank.

Also, there were no observations of the freezing and standing of the fish in the lower layer of the tank (Neo et al. 2015). However, the results of the present experiment in the spatial distribution of zebrafish, the percentage of time fish staying in the lower layer of the test tank was higher, which is contrary to the report of Neo et al. (2015). One of the reasons for this difference in the results of vertical spatial distribution could be the amount of sound intensity emitted in the treatments used in these two studies. The intensity of sound emitted in acoustic treatments was equal to 112 dB re 1µ Pa, which is less than the intensity of sound in this study (121 dB re 1µ Pa). Therefore, a significant increase in sound intensity and high sound level difference between sound treatments and ambient treatments can lead to different responses in fish.

It is also important to consider the potential impacts of acoustic stimuli in real field conditions. Other fishes have also shown spatial distribution changes in response to acoustic stimuli in field conditions. Neo et al. (2018) found that anthropogenic sounds increase the swimming depth of European seabass and distance from the sound source, which results in behavioural responses is consistent with present study in lower layer results. In a field study (Kok et al., 2021) have shown that bottom-moored echosounders, representative of a high intensity impulsive intermittent anthropogenic sound, affect the abundance, schooling cohesion behaviour and swimming depth of pelagic fish. Two recent telemetry tagging studies demonstrated the effects of another intermittent source of sound, seismic surveys, on free-ranging benthic fish species. Bruce et al. (2018) showed shift of diurnal activity patterns and general swimming speed in eight tiger flatheads (*Neoplatycephalus richardsoni*). van der Knaap et al. (2021) demonstrated in a field study that Atlantic cod (*Gadus morhua)* left noisy area more quickly and decrease their swimming activity rather than ambient sound treatment. They also have shown that seismic surveys can disrupt diurnal activity cycles which may have long term negative consequences on this species foraging success and fitness. Although to date, there is a gap of knowledge and very few studies have investigated soundscapes in freshwater environments and the effects of elevated sound levels on fish in freshwater habitats (Bolgan et al., 2016; Linke et al., 2018; Rountree et al., 2020; Putland and Mensinger, 2020).

The swimming of zebrafish towards the upper layer at the beginning of the sound transmission has been interpreted as curiosity and searching behaviour, as the authors' experimental observations have shown that by opening the door of the zebrafish test saloon and walking the staff to perform feeding fish usually produce low-pitched sounds, which attract the attention and curiosity of zebrafish and show the highest distribution at the water column level for

feeding activities (See Shafiei Sabet et al., 2015), However, the response of changing the spatial distribution of fish to the depth and lower layer with the beginning of sound treatments indicates the occurrence of stress and fear in fish, which is similar and expressed in studies of other researchers on other fish species (Neo et al., 2018; Sarà et al., 2007). Examination of fish behaviour to other stimuli including chemicals and fear extract has also shown that with the release of chemicals and fear extract, fish move to the lower layer (deep) column and this pattern of spatial distribution is a behavioural indicator of fear in many interpreted fish species (Gerlai et al., 2000; Gerlai et al., 2006). In addition, another reason for the difference in the vertical distribution behavioural results observed in the study of Neo et al. (2015) and the present study could be the difference in the use of speakers, such as the use of in air speaker in the previous study (Neo et al., 2015) and the use of underwater speaker in the present study. The use of speakers in air to broadcast sound treatments leads to the production of more sound intensity in the deeper parts than in the middle and the surface of the water in the aquarium tank, which may cause the fish to move and escape towards the upper and surface layer where less sound intensity is felt (Shafiei Sabet et al., 2015).

In the study of Shafiei Sabet et al. (2015), they investigated the effect of acoustic treatments with sound intensity almost similar to this study (122 dB re 1µ Pa) on the spatial distribution of zebrafish showed that it is not consistent with the present study. The reason for this difference in spatial distribution behavioural response could be the use of in air speaker in the study by Shafiei Sabet et al. (2015) and underwater speaker in this study, as well as the complexity of sound distribution patterns and sound gradients in aquarium environments (Campbell et al., 2019). Other factors such as fish tank size and available arena, life cycle and location of the fish storage tank, as well as differences in the species and genetics, personality of the fish. Also no significant differences were observed in the x position horizontally by Shafiei Sabet et al. (2015) and the current study.

According to the available sources for measuring sound intensity and scattering patterns in aquarium environments, an experiment was designed by Parvulescu (1967) and Akamatsu et al. (2002) which shows the complexity and variability of sound scattering patterns and sound gradients in enclosed aquarium tank environments. This fact indicates the limitations of studying the spatial distribution of aquatic animals in enclosed and controlled environments that must be considered. Therefore, in order to study the distribution patterns of fish and other aquatic species more accurately, it is recommended to conduct field studies in natural

environments of animal species in order to obtain a more accurate and complete understanding of the manner and patterns of sound-dependent distribution in aquatic species.

5.4. The importance of particle motion in fish tanks; behavioural observations for future works

In order to understand the behavioural changes of zebrafish in response to acoustic stimuli, first of all, it is very important to understand the mechanisms behind and that how the species detects and processes, and how it behaviourally responds to sound (Hawkins and Popper, 2020). Because well-documented studies already showed that the auditory system of fishes evolved primarily to detect particle motion, many fishes are most sensitive to particle motion and they can use it to determine the direction of a sound source (Hawkins and Popper, 2018; Popper and Hawkins, 2018; Sand and Bleckmann, 2008; Sisneros and Rogers, 2016). Underwater sound consists of both sound pressure and particle motion (Popper and Hawkins, 2018). In fact, all fishes and all invertebrates primarily detect particle motion but not all detect sound pressure. Only some of fishes, including the zebrafish, are sensitive to sound pressure as well as the particle motion (Popper and Fay, 2011; Popper and Hawkins, 2018). There are some studies revealing directional hearing and sound source localization in fish under laboratory conditions and in free sound fields. Schuijf (1975) proposed that the cod (*Gadus morhua*) are able to detect sound directionality by monitoring the particle motion of the sound field, presumably employing the directional orientation of the inner ear sensory cells (Dale, 1976). Although, Schuijf (1975) also concluded that to determine the direction of a sound source, the direction of only particle motion may not be sufficient. It has already been shown that cod could discriminate between signals coming towards the head as compared to those coming towards the tail (Buwalda et al., 1983; Schuijf and Buwalda, 1975). They argued that to eliminate any remaining 180^0 ambiguities, directional hearing might involve both comparing the responses of hear cells oriented in different directions and also analysis of the phase relationship between the sound pressure and particle motion components (Schuijf, 1976).

5.5. How important it is particle motion to fishes and invertebrates

We did not mention the levels and direction of the particle motion that is generated within the fish tank because of our restriction in acquiring the particle motion measurements devices and the COVID-19 pandemic. Therefore, we believe it is premature to conclude that zebrafish cannot localize sound source in our experimental set up. One might be because we know very little about hearing in fishes only over 120 species of the more than 33000 known fish species (Ladich and Fay, 2013) and that the empirical and theoretical work on sound source localization

and directional hearing in fishes have been contradictory and obscure for decades (Sisneros and Rogers, 2016). Moreover, some explanations would be that practically because it is difficult to monitor particle motion in fish tank, the lack of easily used and reasonably priced instrumentation to measure particle motion, lack of sound exposure criteria for particle motion and finally lack of particle motion measurement standards (Popper et al., 2014).

Within an aquarium tank the levels of particle motion are often highest at the water surface, and close to the tank walls, when an underwater loudspeaker is used (Jones et al., 2019). Although, resonant frequencies and reverberation may influence propagation and spectro-temporal structure of received acoustic stimuli in fish ranks (Jones et al., 2019). Our fish moved towards the lower levels of the tank, which may be because the particle motion levels were highest close to the water surface, and lower at the bottom of the tank. It is always important to monitor the particle motion when examining the effects of sounds upon fishes and invertebrates (Nedelec et al., 2016; Popper and Hawkins, 2018). Moreover, Invertebrates are especially sensitive to substrate vibration (Aimon et al., 2021; Hawkins et al., 2021; Morley et al., 2014; Roberts et al., 2016), and some fish are too. Particle motion measurement may play an important role in answering crucial biological and ecological questions relating to fishes and other species among taxa (Nedelec et al., 2016). Thus, in doing future experiments to explore anthropogenic sound impacts on the behaviour of fishes or invertebrates under laboratory conditions it is necessary to develop open source and accessible protocols for monitor both particle motion on three axes and sound pressure.

5.6. Acoustic stimuli trigger foraging performance modifications negatively in zebrafish

In the present study, the parameters of fish foraging behaviour such as food discrimination error and food handling error were examined. The results of this experiment showed that the zebrafish did not show any significant difference in the food discrimination error when exposed to sound treatments compared to the ambient treatment. Also, the results of food handling error showed that all sound treatments showed a significant difference compared to the ambient treatment, but these sound treatments did not show a significant difference compared to each other. Here we confirmed our earlier findings (Shafiei Sabet et al., 2015) that sound impacts may goes beyond single species. Experimental sound exposure causes more food handling errors and foraging in zebrafish as predator, which led to more survival in waterflea as prey and avoiding from being eaten by a predator in noisy conditions.

In a study by Purser and Radford (2011), they found that the broadcasting of sound treatments significantly affected the foraging performance of stickleback and food discrimination error and food handling error increased significantly compared to the ambient treatment and reduced foraging performance, which in the food discrimination error, it is not consistent with the results of the present experiment, and one of the reasons for this difference is the difference in the physiology of fish and its diet. Also, the possible difference in the physiology of fish visual sense can be one of the factors influencing these differences, but in the food handling error, a similar result was observed with the results of the present experiment. In other studies, by Voellmy et al. (2014b), the results showed a significant difference in the number of unsuccessful takes of Daphnia in stickleback and no significant difference in minnow fish, which can generally indicate the physiological difference between the two species and possibly the difference in visual sense between the two species. The minnow fish, which belongs to the Cyprinidae family which similar to zebrafish family, did not show a significant difference in the unsuccessful takes of Daphnia, but in another species, a significant difference was observed.

In a study almost similar to the present experiment, Shafiei Sabet et al. (2015) designed an experiment to investigate the effect of different sound patterns on the swimming and foraging behaviour of zebrafish and found that the application of sound treatments caused a significant difference in fish handling error. There was no significant difference in food discrimination error that was consistent with the results of this experiment. Sound playback can be effective in identifying a substance as food and making decisions and attacking it. Since zebrafish are among the fish that have strong visual sense and use this sense to catch and hunt, so sounds can affect the ability to perceive potential vision. Other studies under field conditions have also focused on the effects of sound on fish species. In a comparative study both in a field study in the Laurentian Great Lakes also under laboratory conditions Pieniazek et al., 2020 shown that experimental generated white noise and boat noise have changed swimming patterns and foraging behaviour of freshwater fish species in different ways and with highly variable patterns by family. They demonstrated that captive black bullhead, *Ameiurus melas*, foraged less and startled more when exposed to both white noise and boat noise treatments. Our laboratory experiments were conducted to verify that acoustic stimuli affect swimming behaviour and foraging performance of zebrafish and establish hypothesis for future exploration to be conducted on other freshwater fish species and in the field.

6. Conclusion

The results of this study highlighted impacts of acoustic stimuli on a freshwater fish species and confirmed our earlier study on the same fish species (zebrafish) under laboratory conditions. Our findings show that the parameters of zebrafish swimming and foraging behaviour in different sound patterns were significantly different compared to control or ambient treatment. Sound pollution as a stressor in the brief and prolonged time can cause behavioural changes and disturbances in the individual levels of aquatic species and have broad and important repercussions on the communities of an ecosystem. It should be noted that the results of this study are obtained in captivity and under laboratory conditions therefore the interpretation of the results should be done with caution and attention on the conditions of natural environments in the various habitat and behavioural limitations of any species.

Funding:

The current research was funded by the Iranian Ministry of Science, Research and Technology, University of Guilan with the grant number: 971.511.210004.

Appendices

Data accessibility:

All data used for the analyses reported in this article and some videos are available from the figshare. Moreover, the data and some videos that support the findings of this article are available from the first author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments:

Thanks to Jeroen Hubert and Ozkan Sertlek for their guide and advice on hydrophone calibrations, editing R script versions and acoustic measurements. Special tanks to Hans Slabbekoorn for providing accessibility to acoustic measurement devices. We also thank Narjes Karimi for helping us in reviewing the results and graphs.

References:

Aimon, C., Simpson, S.D., Hazelwood, R.A., Bruintjes, R., Urbina, M.A., 2021. Anthropogenic underwater vibrations are sensed and stressful for the shore crab Carcinus maenas. Environmental Pollution 285, 117148. https://doi.org/10.1016/j.envpol.2021.117148.

Akamatsu, T., Okumura, T., Novarini, N., Yan, H.Y., 2002. Empirical refinements applicable to the recording of fish sounds in small tanks. The Journal of the Acoustical Society of America 112, 3073-3082. https://doi.org/10.1121/1.1515799.

Alexander, R. 1964. The structure of the Weberian apparatus in the Siluri, in Proceedings of the Zoological Society ofLondon, Wiley Online Library, pp. 419–440. https://doi.org/10.1111/j.1469-7998.1964.tb04507.x.

Amorim, M.C.P., Vasconcelos, R.O., Fonseca, P.J., 2015. Fish sounds and mate choice, Sound communication in fishes. Springer, pp. 1-33. https://doi.org/10.1007/978-3-7091-1846-7_1

Animal Behaviour. 2020. Guidelines for the treatment of animals in behavioural research and teaching. Animal Behaviour. 159:1–11. https://doi.org/10.1006/anbe.1996.0293.

Barber, J.R., Fristrup, K.M., Brown, C.L., Hardy, A.R., Angeloni, L.M., Crooks, K.R., 2009. Conserving the wild life therein: Protecting park fauna from anthropogenic noise. 26, 26-31. http://www.nature.nps.gov/ParkScience/index.cfm?ArticleID=370&Page=1

Bermúdez-Cuamatzin, E., López-Hernández, M., Campbell, J., Zuria, I., Slabbekoorn, H., 2018. The role of singing style in song adjustments to fluctuating sound conditions: a comparative study on Mexican birds. Behavioural processes 157, 645-655. https://doi.org/10.1016/j.beproc.2018.04.005.

Blaser, R., Chadwick, L., McGinnis, G., 2010. Behavioral measures of anxiety in zebrafish (*Danio rerio*). Behavioural brain research 208, 56-62. https://doi.org/10.1016/j.bbr.2009.11.009.

Bolgan, M., Chorazyczewska, E., Winfield, I.J., Codarin, A., O'Brien, J. and Gammell, M., 2016. First observations of anthropogenic underwater noise in a large multi-use lake. Journal of Limnology, 75(3), pp.644-651. doi: 10.4081/jlimnol.2016.1405.

Bracciali, C., Campobello, D., Giacoma, C., Sara, G., 2012. Effects of nautical traffic and noise on foraging patterns of Mediterranean damselfish (*Chromis chromis*). PloS one 7, e40582. https://doi.org/10.1371/journal.pone.0040582.

Bruce, B., Bradford, R., Foster, S., Lee, K., Lansdell, M., Cooper, S., Przeslawski, R., 2018. Quantifying fish behaviour and commercial catch rates in relation to a marine seismic survey. Marine environmental research 140, 18-30. https://doi.org/10.1016/j.marenvres.2018.05.005.

Buwalda, R., Schuijf, A., Hawkins, A., 1983. Discrimination by the cod of sounds from opposing directions. Journal of Comparative Physiology 150, 175-184. https://doi.org/10.1007/BF00606367.

Cachat, J., Canavello, P., Elegante, M., Bartels, B., Hart, P., Bergner, C., Egan, R., Duncan, A., Tien, D., Chung, A., 2010. Modeling withdrawal syndrome in zebrafish. Behavioural brain research 208, 371-376. https://doi.org/10.1016/j.bbr.2009.12.004.

Campbell, J., Shafiei Sabet, S., Slabbekoorn, H., 2019. Particle motion and sound pressure in fish tanks: a behavioural exploration of acoustic sensitivity in the zebrafish. Behavioural processes 164, 38-47. https://doi.org/10.1016/j.beproc.2019.04.001.

Carroll, A., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B., 2017. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Marine Pollution Bulletin 114, 9-24. https://doi.org/10.1016/j.marpolbul.2016.11.038.

Chan, A.A.Y.-H., Giraldo-Perez, P., Smith, S., Blumstein, D.T., 2010. Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biology letters 6, 458-461. https://doi.org/10.1098/rsbl.2009.1081.

Coquereau, L., Grall, J., Chauvaud, L., Gervaise, C., Clavier, J., Jolivet, A., Di Iorio, L., 2016. Sound production and associated behaviours of benthic invertebrates from a coastal habitat in the north-east Atlantic. Marine biology 163, 127. https://doi.org/10.1007/s00227-016-2902-2.

Dale, T., 1976. The labyrinthine mechanoreceptor organs of the cod *Gadus morhua* l-(teleostei: gadidae). a scanning electron microscopical study, with special reference to the morphological polarization of the macular sensory cells. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL7650248453.

de Jong, K., Amorim, M., Fonseca, P.J., Heubel, K.U., 2018a. Noise affects multimodal communication during courtship in a marine fish. Frontiers in Ecology and Evolution 6, 113. https://doi.org/10.3389/fevo.2018.00113.

De Jong, K., Amorim, M.C.P., Fonseca, P.J., Fox, C.J., Heubel, K.U., 2018b. Noise can affect acoustic communication and subsequent spawning success in fish. Environmental Pollution 237, 814-823. https://doi.org/10.1016/j.envpol.2017.11.003.

de Vincenzi, G., Micarelli, P., Viola, S., Buffa, G., Sciacca, V., Maccarrone, V., Corrias, V., Reinero, F.R., Giacoma, C., Filiciotto, F., 2021. Biological sound vs. anthropogenic noise: assessment of behavioural changes in *Scyliorhinus canicula* exposed to boats noise. Animals 11, 174. https://doi.org/10.3390/ani11010174.

Deakin, A.G., Spencer, J.W., Cossins, A.R., Young, I.S., Sneddon, L.U., 2019. Welfare challenges influence the complexity of movement: fractal analysis of behaviour in zebrafish. Fishes 4, 8. https://doi.org/10.3390/fishes4010008.

Detrich, H.W., Detrich III, H.W., Westerfield, M., Zon, L.I., 2011. The zebrafish: genetics, genomics and informatics. Academic Press.

Detrich, H.W., Westerfield, M., Zon, L.I., 2004. The zebrafish: Biology. ACADEMIC PRESS.

Dukas, R., 2004. Causes and consequences of limited attention. Brain, Behavior and Evolution 63, 197-210. https://doi.org/10.1159/000076781.

Eaton, R.C., Bombardieri, R.A., Meyer, D.L., 1977. The Mauthner-initiated startle response in teleost fish. Journal of Experimental Biology 66, 65-81. https://doi.org/10.1242/jeb.66.1.65.

Eaton, R.C., Didomenico, R., Nissanov, J., 1991. Role of the Mauthner cell in sensorimotor integration by the brain stem escape network. Brain, Behavior and Evolution 37, 272-285. https://doi.org/10.1159/000114365.

Ebert, D., 2005. Ecology, epidemiology, and evolution of parasitism in Daphnia. National Library of Medicine. https://edoc.unibas.ch/13505/1/Ebert-Parasitism_in_Daphnia-2005-A4-print.pdf.

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioural brain research 205, 38-44. https://doi.org/10.1016/j.bbr.2009.06.022.

Erbe, C., Dunlop, R., Dolman, S., 2018. Effects of noise on marine mammals, Effects of anthropogenic noise on animals. Springer, pp. 277-309. https://doi.org/10.1007/978-1-4939-8574-6_10.

Fedoroff, K.J., 2021. Sound bites: the impact of boat noise on freshwater fish. University of Saskatchewan. http://hdl.handle.net/10388/13300.

Gerlai, R., Lahav, M., Guo, S., Rosenthal, A., 2000. Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. Pharmacology Biochemistry and Behavior 67, 773-782. https://doi.org/10.1016/S0091-3057(00)00422-6.

Gerlai, R., Lee, V., Blaser, R., 2006. Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*). Pharmacology Biochemistry and Behavior 85, 752-761. https://doi.org/10.1016/j.pbb.2006.11.010.

Green, R.E., Langston, R.H., McCluskie, A., Sutherland, R., Wilson, J.D., 2016. Lack of sound science in assessing wind farm impacts on seabirds. Journal of Applied Ecology 53, 1635-1641. doi: 10.1111/1365-2664.12731.

Hanache, P., Spataro, T., Firmat, C., Boyer, N., Fonseca, P., Médoc, V., 2020. Noise-induced reduction in the attack rate of a planktivorous freshwater fish revealed by functional response analysis. Freshwater Biology 65, 75-85. https://doi.org/10.1111/fwb.13271.

Handegard, N.O., Michalsen, K., Tjøstheim, D., 2003. Avoidance behaviour in cod (Gadus morhua) to a bottom-trawling vessel. Aquatic Living Resources 16, 265-270. https://doi.org/10.1016/S0990-7440(03)00020-2.

Hansen, K.A., Hernandez, A., Mooney, T.A., Rasmussen, M.H., Sørensen, K., Wahlberg, M., 2020. The common murre (*Uria aalge*), an auk seabird, reacts to underwater sound. J. Acoust. Soc. Am 147, 4069-4074. https://doi.org/10.1121/10.0001400.

Hastings, M.C., Popper, A.N., 2005. Effects of sound on fish. California Department of Transportation. https://www.researchgate.net/profile/Arthur-Popper/publication/253413309_Update_on_exposure_metrics_for_evaluation_of_effects_of_sound_on_fish/links/0c9605295e4f7183f7000000/Update-on-exposure-metrics-for-evaluation-of-effects-of-sound-on-fish.pdf.

Hawkins, A., Myrberg, A., 1983. Hearing and sound communication under water. Bioacoustics, a comparative approach. Academic Press, London, 347-405.

Hawkins, A.D., 1986. Underwater sound and fish behaviour, The behaviour of teleost fishes. Springer, pp. 114-151. https://doi.org/10.1007/978-1-4684-8261-4_5.

Hawkins, A.D., Hazelwood, R.A., Popper, A.N., Macey, P.C., 2021. Substrate vibrations and their potential effects upon fishes and invertebrates. The Journal of the Acoustical Society of America 149, 2782-2790. https://doi.org/10.1121/10.0004773.

Hawkins, A.D., Picciulin, M., 2019. The importance of underwater sounds to gadoid fishes. The Journal of the Acoustical Society of America 146, 3536-3551. https://doi.org/10.1121/1.5134683.

Hawkins, A.D., Popper, A.N., 2018. Directional hearing and sound source localization by fishes. The Journal of the Acoustical Society of America 144, 3329-3350. https://doi.org/10.1121/1.5082306.

Hawkins, A.D., Popper, A.N., 2020. Sound detection by Atlantic cod: An overview. The Journal of the Acoustical Society of America 148, 3027-3041. https://doi.org/10.1121/10.0002363.

Heath, V.L., Miehls, S., Johnson, N., Higgs, D.M., 2021. Behavioural response of sea lamprey (*Petromyzon marinus*) to acoustic stimuli in a small stream. Canadian Journal of Fisheries and Aquatic Sciences 78, 341-348. https://doi.org/10.1139/cjfas-2020-0301.

Higgs, D.M., Souza, M.J., Wilkins, H.R., Presson, J.C., Popper, A.N., 2002. Age-and size-related changes in the inner ear and hearing ability of the adult zebrafish (*Danio rerio*). JARO: Journal of the Association for Research in Otolaryngology 3, 174. 10.1007/s101620020035.

Hubert, J., van Bemmelen, J., Slabbekoorn, H., 2021. No negative effects of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs in a T-maze. Environmental Pollution 270, 116184. https://doi.org/10.1016/j.envpol.2020.116184.

Injaian, A.S., Francis, C.D., Ouyang, J.Q., Dominoni, D.M., Donald, J.W., Fuxjager, M.J., Goymann, W., Hau, M., Husak, J.F., Johnson, M.A., 2020. Baseline and stress-induced corticosterone levels across birds and reptiles do not reflect urbanication levels. Conservation Physiology 8, 1. 10.1093/conphys/coz110.

Jones, I.T., Stanley, J.A., Bonnel, J., Mooney, T.A., 2019. Complexities of tank acoustics warrant direct, careful measurement of particle motion and pressure for bioacoustic studies, Proceedings of Meetings on Acoustics 5ENAL. Acoustical Society of America, p. 010005. https://doi.org/10.1121/2.0001073.

Kok, A.C., Bruil, L., Berges, B., Sakinan, S., Debusschere, E., Reubens, J., de Haan, D., Norro, A., Slabbekoorn, H., 2021. An echosounder view on the potential effects of impulsive noise pollution on pelagic fish around windfarms in the North Sea. Environmental Pollution, 118063. https://doi.org/10.1016/j.envpol.2021.118063.

Kunc, H.P., McLaughlin, K.E., Schmidt, R., 2016. Aquatic noise pollution: implications for individuals, populations, and ecosystems. Proceedings of the Royal Society B: Biological Sciences 283, 20160839. https://doi.org/10.1098/rspb.2016.0839.

Ladich, F., Fay, R.R., 2013. Auditory evoked potential audiometry in fish. Reviews in Fish Biology and Fisheries 23, 317-364. 10.1007/s11160-012-9297-z.

Linke, S., Gifford, T., Desjonquères, C., Tonolla, D., Aubin, T., Barclay, L., Karaconstantis, C., Kennard, M.J., Rybak, F. and Sueur, J., 2018. Freshwater ecoacoustics as a tool for continuous ecosystem monitoring. Frontiers in Ecology and the Environment, 16(4), pp.231-238. https://doi.org/10.1002/fee.1779.

Malavasi, S., Georgalas, V., Lugli, M., Torricelli, P., Mainardi, D., 2004. Differences in the pattern of antipredator behaviour between hatchery-reared and wild European sea bass juveniles. Journal of fish biology 65, 143-155. https://doi.org/10.1111/j.0022-1112.2004.00545.x.

Maximino, C., de Brito, T.M., da Silva Batista, A.W., Herculano, A.M., Morato, S., Gouveia Jr, A., 2010. Measuring anxiety in zebrafish: a critical review. Behavioural brain research 214, 157-171. https://doi.org/10.1016/j.bbr.2010.05.031.

McCloskey, K.P., Chapman, K.E., Chapuis, L., McCormick, M.I., Radford, A.N., Simpson, S.D., 2020. Assessing and mitigating impacts of motorboat noise on nesting damselfish. Environmental Pollution 266, 115376. https://doi.org/10.1016/j.envpol.2020.115376.

McDonald, M.A., Hildebrand, J.A., Wiggins, S.M., 2006. Increases in deep ocean ambient noise in the Northeast Pacific west of San Nicolas Island, California. The Journal of the Acoustical Society of America 120, 711-718. https://doi.org/10.1121/1.2216565.

Mickle, M.F., Higgs, D.M., 2018. Integrating techniques: a review of the effects of anthropogenic noise on freshwater fish. Canadian Journal of Fisheries and Aquatic Sciences 75, 1534-1541. https://doi.org/10.1139/cjfas-2017-0245.

Mills, S.C., Beldade, R., Henry, L., Laverty, D., Nedelec, S.L., Simpson, S.D., Radford, A.N., 2020. Hormonal and behavioural effects of motorboat noise on wild coral reef fish. Environmental Pollution 262, 114250. https://doi.org/10.1016/j.envpol.2020.114250.

Mirjany, M., Preuss, T., Faber, D.S., 2011. Role of the lateral line mechanosensory system in directionality of goldfish auditory evoked escape response. Journal of Experimental Biology 214, 3358-3367. https://doi.org/10.1242/jeb.052894.

Moore, S.E., Reeves, R.R., Southall, B.L., Ragen, T.J., Suydam, R.S., Clark, C.W., 2012. A new framework for assessing the effects of anthropogenic sound on marine mammals in a rapidly changing Arctic. BioScience 62, 289-295. https://doi.org/10.1525/bio.2012.62.3.10.

Morley, E.L., Jones, G., Radford, A.N., 2014. The importance of invertebrates when considering the impacts of anthropogenic noise. Proceedings of the Royal Society B: Biological Sciences 281, 20132683. https://doi.org/10.1098/rspb.2013.2683.

Mortensen, L.O., Chudzinska, M.E., Slabbekoorn, H., Thomsen, F., 2021. Agent-based models to investigate sound impact on marine animals: bridging the gap between effects on individual behaviour and population level consequences. Oikos. https://doi.org/10.1111/oik.08078.

Murchy, K.A., Davies, H., Shafer, H., Cox, K., Nikolich, K., Juanes, F., 2019. Impacts of noise on the behavior and physiology of marine invertebrates: A meta-analysis. Proceedings of Meetings on Acoustics 5ENAL. Acoustical Society of America, p. 040002. https://doi.org/10.1121/2.0001217.

Nedelec, S.L., Campbell, J., Radford, A.N., Simpson, S.D., Merchant, N.D., 2016. Particle motion: the missing link in underwater acoustic ecology. Methods in Ecology and Evolution 7, 836-842.

https://doi.org/10.1111/2041-210X.12544.

Neo, Y., Hubert, J., Bolle, L., Winter, H., Slabbekoorn, H., 2018. European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environmental Pollution 239, 367-374. https://doi.org/10.1016/j.envpol.2018.04.018.

Neo, Y., Hubert, J., Bolle, L., Winter, H.V., Ten Cate, C., Slabbekoorn, H., 2016. Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure. Environmental Pollution 214, 26-34. https://doi.org/10.1016/j.envpol.2016.03.075.

Neo, Y.Y., Parie, L., Bakker, F., Snelderwaard, P., Tudorache, C., Schaaf, M., Slabbekoorn, H., 2015. Behavioral changes in response to sound exposure and no spatial avoidance of noisy conditions in captive zebrafish. Frontiers in behavioral neuroscience 9, 28. https://doi.org/10.3389/fnbeh.2015.00028.

Normandeau Associates, I., 2012. Effects of Noise on Fish, Fisheries, and Invertebrates in the U.S. Atlantic and Arctic from Energy Industry Sound-Generating Activities., in: Interior, A.L.S.f.t.U.S.D.o.t. (Ed.), p. 135. https://www.cbd.int/doc/meetings/mar/mcbem-2014-01/other/mcbem-2014-01-submission-boem-04-en.pdf.

Parejko, K., Dodson, S.I., 1991. The evolutionary ecology of an antipredator reaction norm: *Daphnia pulex* and *Chaoborus americanus*. Evolution 45, 1665-1674. doi: 10.1111/j.1558-5646.1991.tb02671.x..

Parvulescu, A., 1967. The acoustics of small tanks. Marine Bio Acoustics 2, 7-13. https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=55337.

Payne, N.L., van der Meulen, D.E., Suthers, I.M., Gray, C.A., Taylor, M.D., 2015. Foraging intensity of wild mulloway *Argyrosomus japonicus* decreases with increasing anthropogenic disturbance. Marine biology 162, 539-546. https://doi.org/10.1007/s00227-014-2603-7.

Peng, C., Zhao, X., Liu, G., 2015. Noise in the sea and its impacts on marine organisms. International journal of environmental research and public health 12, 12304-12323. https://doi.org/10.3390/ijerph121012304.

Pieniazek, R.H., Mickle, M.F., Higgs, D.M., 2020. Comparative analysis of noise effects on wild and captive freshwater fish behaviour. Animal Behaviour 168, 129-135. https://doi.org/10.1016/j.anbehav.2020.08.004.

Popper, A.N., Carlson, T.J., 1998. Application of sound and other stimuli to control fish behavior. Transactions of the American Fisheries Society 127, 673-707. https://doi.org/10.1577/1548-8659(1998)127<0673:AOSAOS>2.0.CO;2.

Popper, A.N., Fay, R.R., 2011. Rethinking sound detection by fishes. Hearing research 273, 25-36. https://doi.org/10.1016/j.heares.2009.12.023.

Popper, A.N., Fewtrell, J., Smith, M.E., McCauley, R.D., 2003. Anthropogenic sound: effects on the behavior and physiology of fishes. Marine Technology Society Journal 37, 35-40. https://doi.org/10.4031/002533203787537050.

Popper, A.N., Hawkins, A.D., 2018. The importance of particle motion to fishes and invertebrates. The Journal of the Acoustical Society of America 143, 470-488. https://doi.org/10.1121/1.5021594.

Popper, A.N., Hawkins, A.D., 2019. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. Journal of fish biology 94, 692-713. https://doi.org/10.1111/jfb.13948.

Popper, A.N., Hawkins, A.D., Fay, R.R., Mann, D.A., Bartol, S., Carlson, T.J., Coombs, S., Ellison, W.T., Gentry, R.L., Halvorsen, M.B., 2014. Sound exposure guidelines, ASA S3/SC1. 4 TR-2014 Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report prepared by ANSI-Accredited Standards Committee S3/SC1 and registered with ANSI. Springer, pp. 33-51. https://doi.org/10.1007/978-3-319-06659-2_7.

Popper, A.N., Hawkins, A.D., Sand, O., Sisneros, J.A., 2019. Examining the hearing abilities of fishes. The Journal of the Acoustical Society of America 146, 948-955. https://doi.org/10.1121/1.5120185.

Popper, A.N., Hawkins, A.D., Thomsen, F., 2020. Taking the animals' perspective regarding anthropogenic underwater sound. Trends in ecology & evolution 35, 787-794. https://doi.org/10.1016/j.tree.2020.05.002.

Popper, A.N., Smith, M.E., Cott, P.A., Hanna, B.W., MacGillivray, A.O., Austin, M.E., Mann, D.A., 2005. Effects of exposure to seismic airgun use on hearing of three fish species. The Journal of the Acoustical Society of America 117, 3958-3971. https://doi.org/10.1121/1.1904386.

Purser, J., Radford, A.N., 2011. Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PloS one 6, e17478. https://doi.org/10.1371/journal.pone.0017478.

Putland, R.L. and Mensinger, A.F., 2020. Exploring the soundscape of small freshwater lakes. Ecological Informatics, 55, p.101018. https://doi.org/10.1016/j.ecoinf.2019.101018.

Radford, A.N., Lèbre, L., Lecaillon, G., Nedelec, S.L., Simpson, S.D., 2016. Repeated exposure reduces the response to impulsive noise in European seabass. Global change biology 22, 3349-3360. https://doi.org/10.1111/gcb.13352.

Reynolds, C., 2011. Daphnia: Development of Model Organism in Ecology and Evolution-2011. Freshwater Reviews 4, 85-87. https://doi.org/10.1608/FRJ-4.1.425.

Roberts, L., Harding, H.R., Voellmy, I., Bruintjes, R., Simpson, S.D., Radford, A.N., Breithaupt, T., Elliott, M., 2016. Exposure of benthic invertebrates to sediment vibration: from laboratory experiments to outdoor simulated pile-driving, Proceedings of Meetings on Acoustics 4ENAL. Acoustical Society of America, p. 010029. https://doi.org/10.1121/2.0000324.

Rountree, R.A., Juanes, F. and Bolgan, M., 2020. Temperate freshwater soundscapes: A cacophony of undescribed biological sounds now threatened by anthropogenic noise. Plos one, 15(3), p.e0221842. https://doi.org/10.1371/journal.pone.0221842.

Sand, O., Bleckmann, H., 2008. Orientation to auditory and lateral line stimuli, Fish bioacoustics. Springer, pp. 183-231. https://doi.org/10.1007/978-0-387-73029-5_6.

Santulli, A., Modica, A., Messina, C., Ceffa, L., Curatolo, A., Rivas, G., Fabi, G., D'amelio, V., 1999. Biochemical responses of European sea bass (*Dicentrarchus labrax* L.) to the stress induced by off shore experimental seismic prospecting. Marine Pollution Bulletin 38, 1105-1114. https://doi.org/10.1016/S0025-326X(99)00136-8.

Sarà, G., Dean, J., d'Amato, D., Buscaino, G., Oliveri, A., Genovese, S., Ferro, S., Buffa, G., Martire, M.L., Mazzola, S., 2007. Effect of boat noise on the behaviour of bluefin tuna Thunnus thynnus in the Mediterranean Sea. Marine Ecology Progress Series 331, 243-253. https://www.int-res.com/abstracts/meps/v331/p243-253/.

Schuijf, A., 1975. Directional hearing of cod (*Gadus morhua*) under approximate free field conditions. Journal of Comparative Physiology 98, 307-332. https://doi.org/10.1007/BF00709803.

Schuijf, A., 1976. The phase model of directional hearing in fish. Sound reception in fish, 63-86.

Schuijf, A., Buwalda, R., 1975. On the mechanism of directional hearing in cod (*Gadus morhua* L.). Journal of Comparative Physiology 98, 333-343. https://doi.org/10.1007/BF00709804.

Shafiei Sabet, S., Karnagh, S.A., Azbari, F.Z., 2019. Experimental test of sound and light exposure on water flea swimming behaviour, Proceedings of Meetings on Acoustics 5ENAL. Acoustical Society of America, p. 010015. https://doi.org/10.1121/2.0001270.

Shafiei Sabet, S., Neo, Y.Y., Slabbekoorn, H., 2015. The effect of temporal variation in sound exposure on swimming and foraging behaviour of captive zebrafish. Animal Behaviour 107, 49-60. https://doi.org/10.1016/j.anbehav.2015.05.022.

Shafiei Sabet, S., Van Dooren, D., Slabbekoorn, H., 2016a. Son et lumière: Sound and light effects on spatial distribution and swimming behavior in captive zebrafish. Environmental Pollution 212, 480-488. https://doi.org/10.1016/j.envpol.2016.02.046.

Shafiei Sabet, S., Wesdorp, K., Campbell, J., Snelderwaard, P., Slabbekoorn, H., 2016b. Behavioural responses to sound exposure in captivity by two fish species with different hearing ability. Animal Behaviour 116, 1-11. https://doi.org/10.1016/j.anbehav.2016.03.027.

Simmons, A.M., Narins, P.M., 2018. Effects of anthropogenic noise on amphibians and reptiles, Effects of anthropogenic noise on animals. Springer, pp. 179-208. https://doi.org/10.1007/978-1-4939-8574-6_7.

Simpson, S.D., Purser, J., Radford, A.N., 2015. Anthropogenic noise compromises antipredator behaviour in European eels. Global change biology 21, 586-593. https://doi.org/10.1111/gcb.12685.

Sisneros, J.A., Rogers, P.H., 2016. Directional hearing and sound source localization in fishes. Fish hearing and bioacoustics, 121-155. https://doi.org/10.1007/978-3-319-21059-9_7.

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, 419-427. https://doi.org/10.1016/j.tree.2010.04.005.

Slabbekoorn, H., Ripmeester, E.A.P., 2008. Birdsong and anthropogenic noise: implications and applications for conservation. Molecular ecology 17, 72-83. https://doi.org/10.1111/j.1365-294X.2007.03487.x.

Southall, B.L., Bowles, A.E., Ellison, W.T., Finneran, J.J., Gentry, R.L., Greene Jr, C.R., Kastak, D., Ketten, D.R., Miller, J.H., Nachtigall, P.E., 2008. Marine mammal noise-exposure criteria: initial scientific recommendations. Bioacoustics 17, 273-275. https://doi.org/10.1080/09524622.2008.9753846.

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish, *Danio rerio*. Biological Reviews 83, 13-34. https://doi.org/10.1111/j.1469-185X.2007.00030.x.

Spiga, I., Aldred, N., Caldwell, G.S., 2017. Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.). Marine Pollution Bulletin 122, 297-305. https://doi.org/10.1016/j.marpolbul.2017.06.067.

Staaterman, E., Gallagher, A., Holder, P., Reid, C., Altieri, A., Ogburn, M., Rummer, J., Cooke, S., 2020. Exposure to boat noise in the field yields minimal stress response in wild reef fish. Aquatic Biology 29, 93-103. https://doi.org/10.3354/ab00728.

Thomsen, F., Popper, A.N., Hawkins, A.D., 2021. Sound Impact Studies: A Reply to Risch et al. Trends in ecology & evolution 36, 382-384. https://doi.org/10.1016/j.tree.2021.01.012.

Tuomainen, U., Candolin, U., 2011. Behavioural responses to human-induced environmental change. Biological Reviews 86, 640-657. https://doi.org/10.1111/j.1469-185X.2010.00164.x.

Tyack, P.L., 1998. Acoustic communication under the sea. Animal acoustic communication, pp.163-220. https://doi.org/10.1007/978-3-642-76220-8_6.

Tyack, P.L., 2008. Implications for marine mammals of large-scale changes in the marine acoustic environment. Journal of Mammalogy 89, 549-558. https://doi.org/10.1644/07-MAMM-S-307R.1.

van der Knaap, I., Reubens, J., Thomas, L., Ainslie, M.A., Winter, H.V., Hubert, J., Martin, B., Slabbekoorn, H., 2021. Effects of a seismic survey on movement of free-ranging Atlantic cod. Current Biology 31, 1555-1562. e1554. https://doi.org/10.1016/j.cub.2021.01.050.

Villamizar, N., Vera, L.M., Foulkes, N.S., Sánchez-Vázquez, F.J., 2014. Effect of lighting conditions on zebrafish growth and development. Zebrafish 11, 173-181. https://doi.org/10.1089/zeb.2013.0926.

Voellmy, I.K., Purser, J., Flynn, D., Kennedy, P., Simpson, S.D., Radford, A.N., 2014a. Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Animal Behaviour 89, 191-198. https://doi.org/10.1016/j.anbehav.2013.12.029.

Voellmy, I.K., Purser, J., Simpson, S.D., Radford, A.N., 2014b. Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. PloS one 9, e102946. https://doi.org/10.1371/journal.pone.0102946.

Voellmy, I.K., Purser, J., Simpson, S.D., Radford, A.N., 2016. Effects of previous acoustic experience on behavioral responses to experimental sound stimuli and implications for research, The effects of noise on aquatic life II. Springer, pp. 1191-1196. https://doi.org/10.1007/978-1-4939-2981-8_149.

Wahlberg, M., Westerberg, H., 2005. Hearing in fish and their reactions to sounds from offshore wind farms. Marine Ecology Progress Series 288, 295-309. 10.3354/meps288295.

Webb, P., 1986. Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). Canadian Journal of Fisheries and Aquatic Sciences 43, 763-771. https://doi.org/10.1139/f86-094.

Whitfield, T.T., 2002. Zebrafish as a model for hearing and deafness. Journal of neurobiology 53, 157-171. https://doi.org/10.1002/neu.10123.

Weber, E. H. 1820. De aure et auditu hominis et animalium. Pars I (The Ear and Hearing ofHumans and Animals) (Gerhard Fleischer, Leipzig).

Wysocki, L.E., Dittami, J.P., Ladich, F., 2006. Ship noise and cortisol secretion in European freshwater fishes. Biological conservation 128, 501-508. https://doi.org/10.1016/j.biocon.2005.10.020.

Zottoli, S.J., 1977. Correlation of the startle reflex and Mauthner cell auditory responses in unrestrained goldfish. Journal of Experimental Biology 66, 243-254. https://doi.org/10.1242/jeb.66.1.243.

Figure legends

Figure 1: Waveforms and spectrograms (for 20 s stime slot) of all intermittent and continuous sound patterns used in the experiment (see also Shafiei Sabet et al., 2015). (a) Regular intermittent sound treatment with fast pulse rate (1:1), (b) Regular intermittent sound treatment with slow pulse rate (1: 4), (c) Irregular intermittent sound (1:1-7), (d) Continuous sound treatments accordingly.

Figure 2: Experimental set-up. Schematic view of the test tank. A: The obscured pages of the test tank to enhance the fish's visual contrast in the film. B: Underwater speaker space separator screen with fish swimming space. C: Underwater speaker holder box. D: Underwater speaker.

Figure 3: Spectral distribution of continuous sound pressure level compared to silent treatment (dB ref 1 $\mu Pa^2/Hz$). Silence conditions (blue) and continuous sound playback (red). The diagram shows that the sound intensity level has increased significantly in the range of hearing frequencies of zebrafish. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

Figure 4. Effect of sound exposure treatment on swimming behaviour of Zebrafish. (a) Number of startle responses expressed as the difference between the first 60 seconds during sound and the last 60 seconds before sound exposure onset: Continuous sound (CS) and three intermittent sound (1:1, 1:4, 1:1-7) and Ambient noises (AN) as control treatment (N=30, F=6.312, P=0.000435, Standard error changes (±1)). (b) Brief swimming speed difference of Zebrafish between the first 5 seconds during sound and the last 5 seconds before sound exposure on each four sound treatments and the ambient (N=30, F=11.172, P=0.000002, Standard error changes (±1)). (c) Prolonged swimming speed difference of Zebrafish between the last 60 seconds before sound and the first 60 seconds during sound exposure on five treatments (N=30, F=7.725, P=0.000054 Standard error changes (±1)).

Figure 5. Effect of sound treatments on zebrafish prolonged swimming speed. (a) Ambient condition. (b) Continuous sound. (c) regular intermittent 1:1. (d) regular intermittent 1:4. (e) irregular intermittent 1:1-7. The time was divided into three period bins for formal statistical analysis: the last 5 seconds before sound exposure, the first 5 seconds during sound exposure and the last 5 seconds during sound exposure. Prolonged swimming speed weakened with repeated exposure in all sound treatments. (NS= no significance, ***= $P \leq 0.001$, ****= $P \leq 0.0001$).

Figure 6. Average percentage of fish time spent in bottom layer of tank (N=30). (a) Brief time (15 seconds before and 15 seconds exposed to sound) (NS= no significance, *= $P \leq 0.05$, **= $P \leq 0.01$). (b) Prolonged time (60 seconds before and 60 seconds exposed to sound) (NS= no significance, *= $P \leq 0.05$, **= $P \leq 0.01$, ***= $P \leq 0.001$). Bottom layer area for spatial displacement was defined as the bottom layer with a vertical distance of 10 cm from the bottom of the tank. (df= 1) Standard error changes (±1).

Figure 7. Effect of sound exposure on horizontal spatial distribution of Zebrafish. (a) Brief time (15 seconds before and 15 seconds exposed to sound). (b) Prolonged time (60 seconds before and 60 seconds exposed to sound). The underwater speaker played back from the right tank. Bars show Means ± SE.

Figure 8. Heat map of fish swimming in the tank environment. (a) Brief time (15 seconds before and 15 seconds exposed to sound). (b) Prolonged time (60 seconds before and 60 seconds exposed to sound). The blue color (#0000FE) indicates the 0-20 % of the fish in the tank. The aqua color (#01FFFF) indicates the 20-40 % of the fish in the tank. The lime color (#00FF01) indicates the 40-60 % of the fish in the tank. The yellow color (#FFFF01) indicates the 60-80 % of the fish in the tank. The red color (#FE0000) indicates the 80-100 % of the fish in the tank. The underwater speaker played back from the right tank. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

Figure 9. Effect of acoustic stimulus on foraging activities of Zebrafish. (a) Food discrimination error; it described as the proportion of duckweed particles attacked relative to the total number of attacks to both duckweed particles and water fleas from the introduction of food items until the end of sound exposure in sequence for each zebrafish individual (Shafiei Sabet et al., 2015). (N=30, df=3.756, F=1.339, P=0.226) (b) Food handling error; It described as the proportion of the total of water fleas attacked that were missed or released again after initial grasping from onset of food introduction until the end of sound exposure in sequence for each zebrafish individual (Shafiei Sabet et al., 2015). (N=30, df=2.825, F=26.023, P=0.000019). Standard error changes (±1)

Figure 1

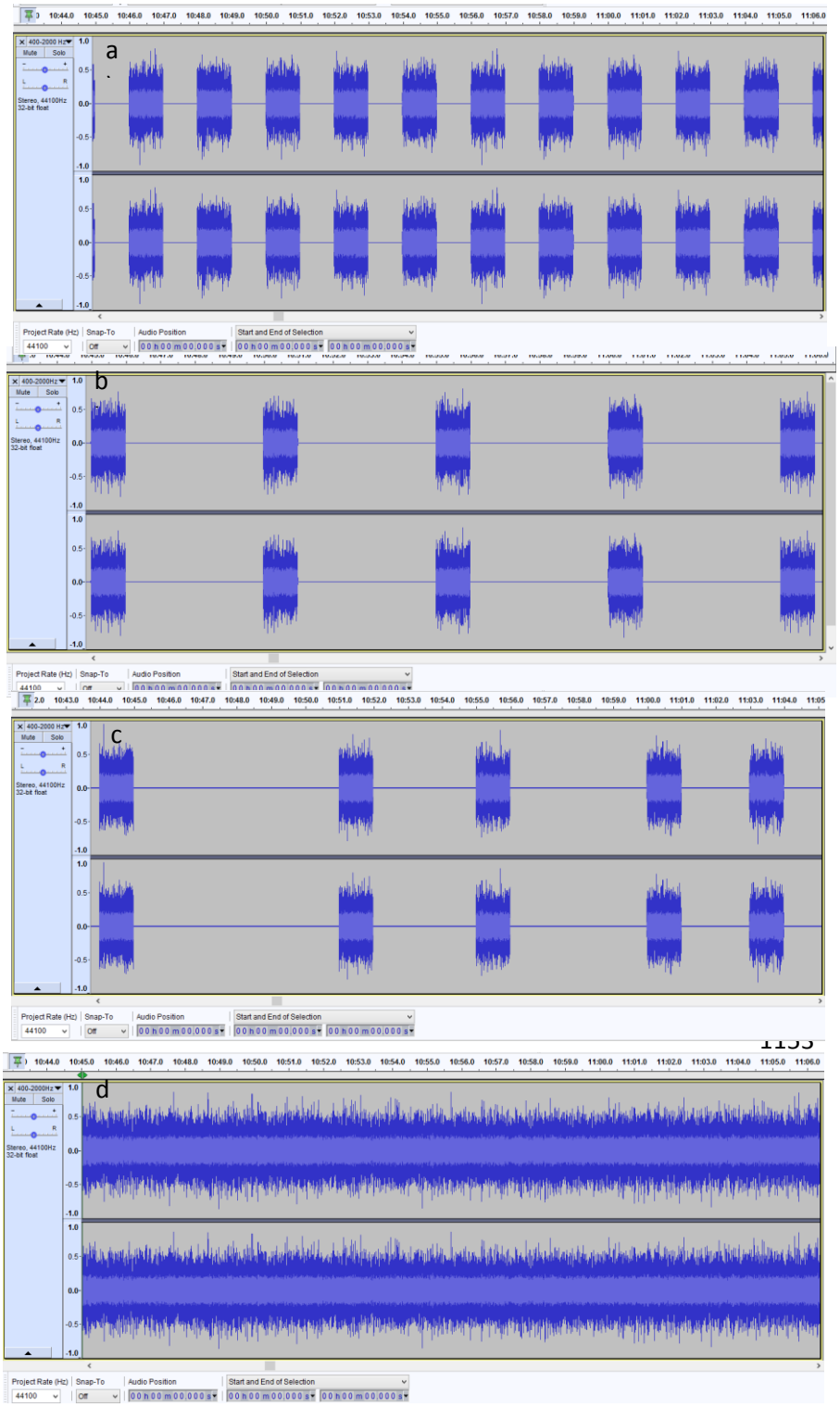


Figure 1

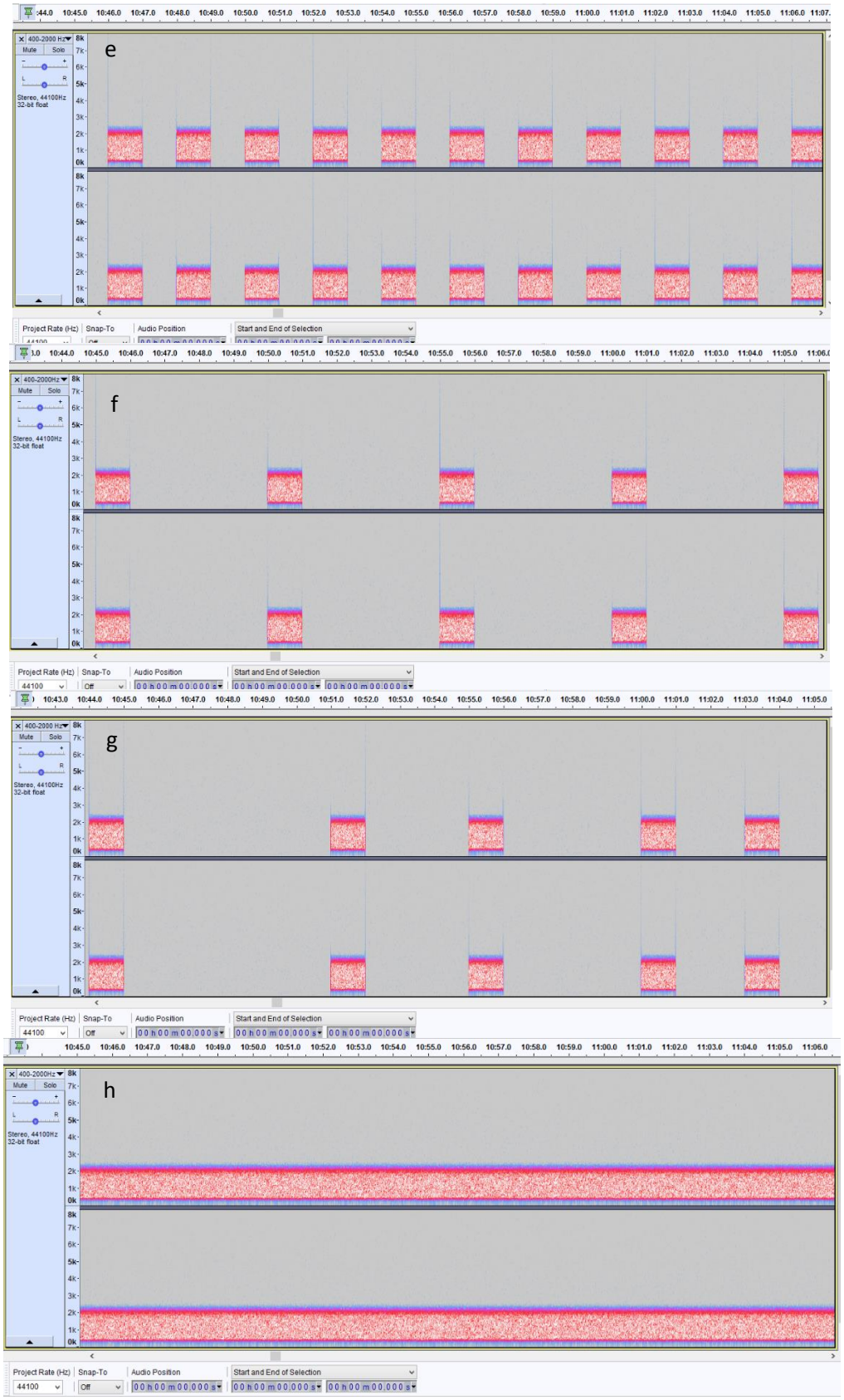

e
f
g
h
Project Rate (Hz)
Snap-To
Audio Position
Start and End of Selection
Stereo, 44100Hz
32-bit float

Figure 2

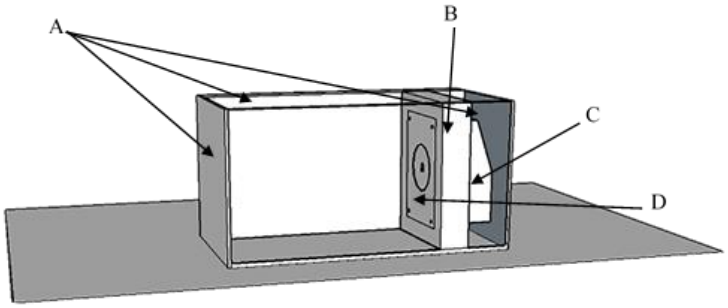


Figure 3

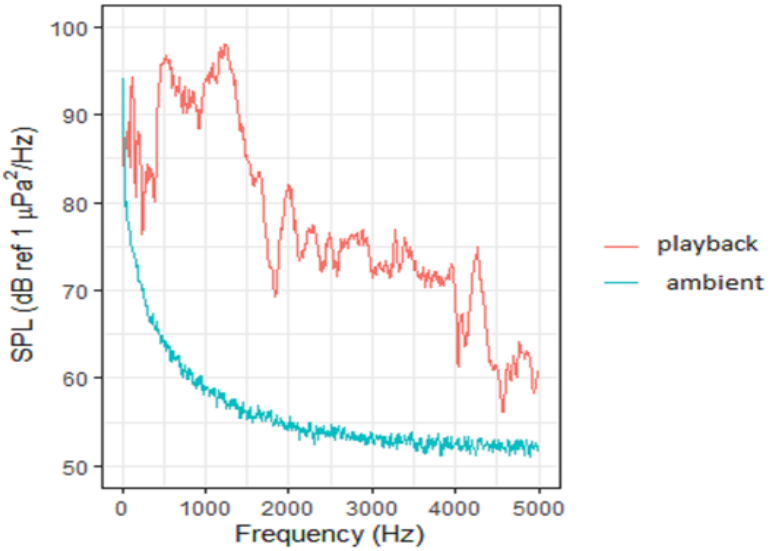


Figure 4

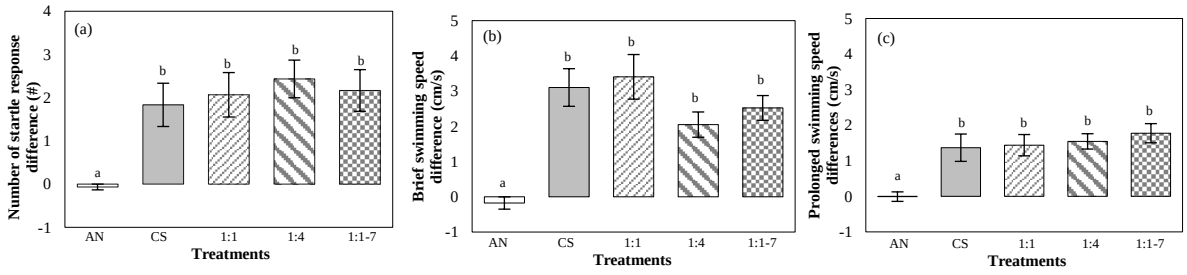

(a)
Number of startle response difference (#)
4
3
2
1
0
-1
a
b
b
b
b
AN
CS
1:1
1:4
1:1-7
Treatments
(b)
Brief swimming speed difference (cm/s)
5
4
3
2
1
0
-1
a
b
b
b
b
AN
CS
1:1
1:4
1:1-7
Treatments
(c)
Prolonged swimming speed differences (cm/s)
5
4
3
2
1
0
-1
a
b
b
b
b
AN
CS
1:1
1:4
1:1-7
Treatments

Figure 5

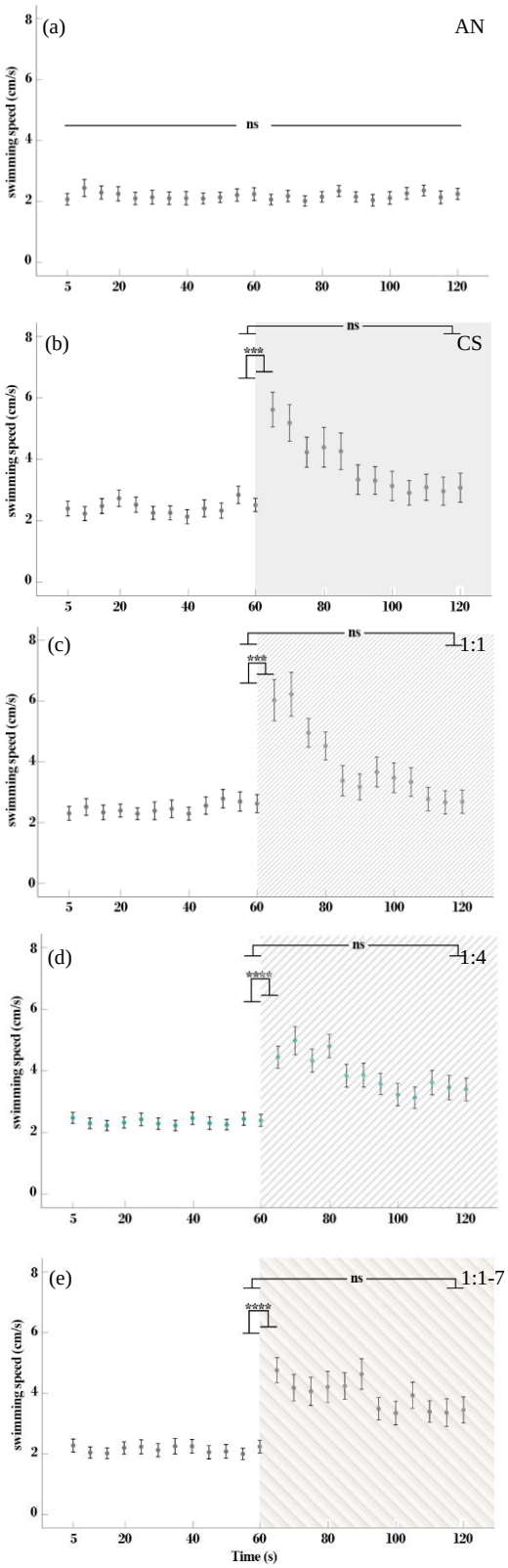


Figure 6

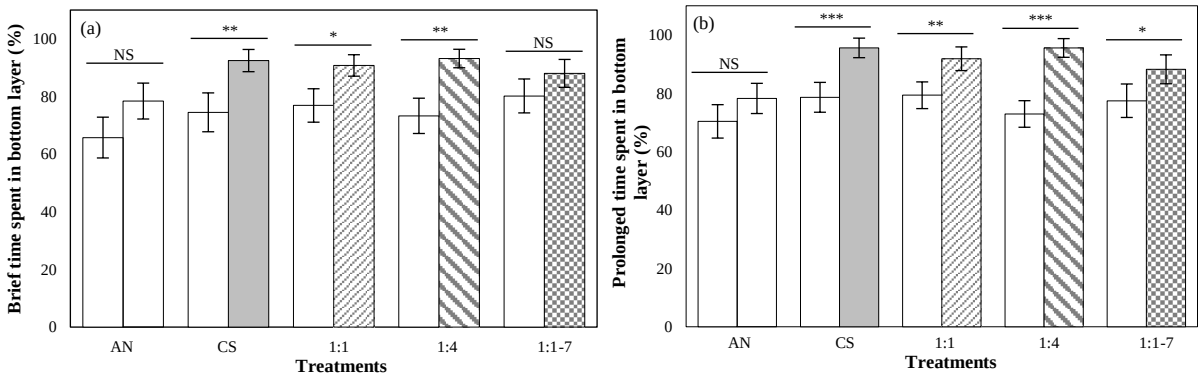


Figure 7

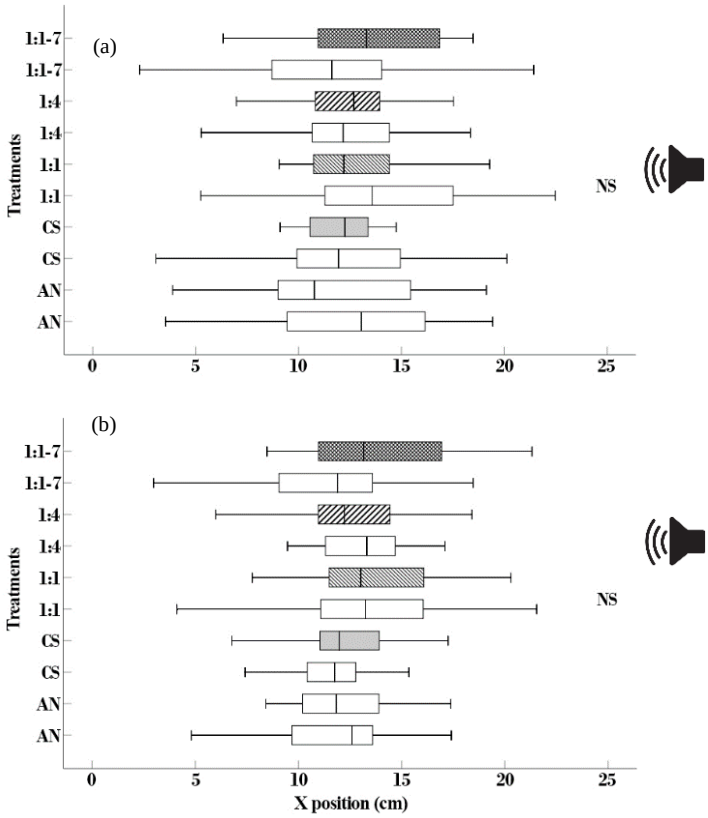


Figure 8

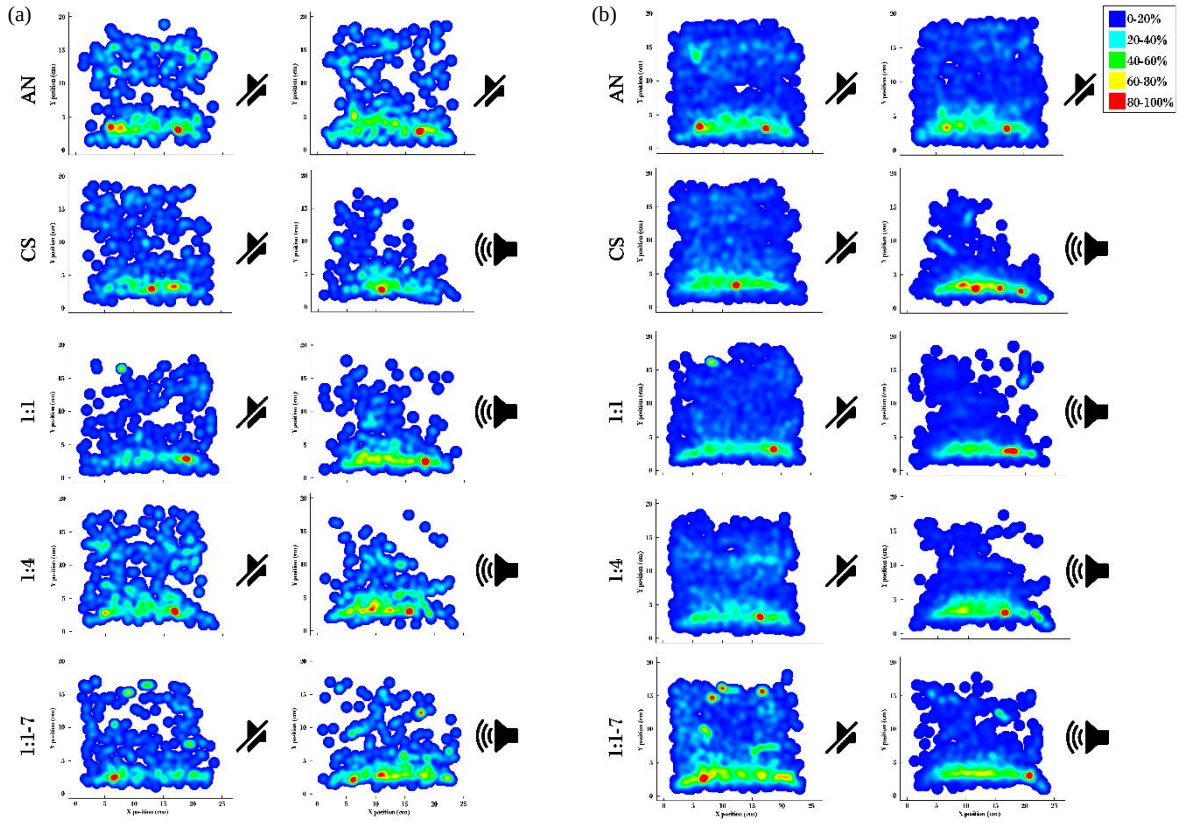
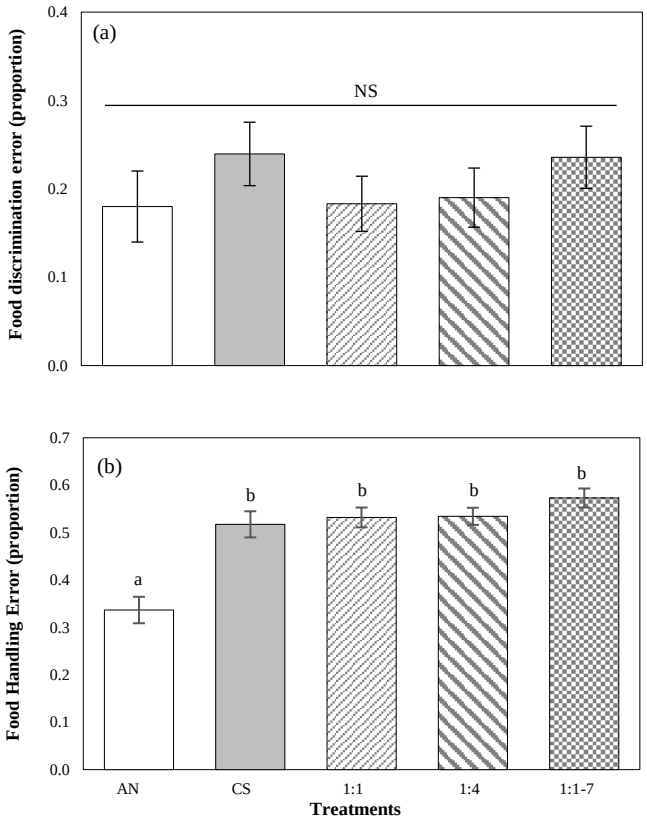


Figure 9


(a)
NS
Food discrimination error (proportion)
0.0
0.1
0.2
0.3
0.4
(b)
a
b
b
b
b
Food Handling Error (proportion)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
AN
CS
1:1
1:4
1:1-7
Treatments