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

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

[a] Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara,
Iran, P.O. box: 1144

Abstract:

Anthropogenic sound is currently recognized as a source of environmental pollution in terrestrial and aquatic habitats. Elevated sound levels may cause a broad range of impacts on aquatic organisms among taxa. 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 such as driving as a distracting stimulus, masking important relevant acoustic signals and cues in a range of marine and freshwater species. 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 prey, respectively. We also used four sound treatments with different temporal patterns (all in the same frequency range and moderate exposure level). Our results constitute strong evidence for clear sound-related effects on zebrafish behaviour. All sound treatments induced a significant increase in the number of startle response, brief and prolonged swimming speed for zebrafish ($P<0.05$). Zebrafish reached to the baseline swimming speed after 60 seconds in all treatments. We found partially brief and prolonged sound effects on spatial distribution of zebrafish; Although we did not find any significant sound-related behavioural changes for horizontal spatial displacement in all treatments ($P>0.05$), zebrafish swam significantly more in the lower layer of the fish tank except irregular intermittent 1:1-7 in brief sound exposure ($P<0.05$). The results of foraging performance showed that food discrimination error was low for the zebrafish and unaffected by sound treatments ($P>0.05$). However, food handling error was affected by sound treatments; all treatments caused a rise in handling error ($P<0.001$). This study highlights the impact of sound on zebrafish swimming activities, and that more attacks are needed to consume the same number of prey items 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 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. Environmental pollutions (including chemical, light, and sound) are introduced by human activities in different time, scale and space ranges and have elevated underwater ambient noise levels with alternating intensities which may affect aquatic organisms in their habitats. Among these, 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).

Sound sources in aquatic habitats, such as merchant shipping, recreational boating, wind turbines, pile-driving, underwater mining explorations, and explosions related to geological and research experiments, are frequent in the number of events 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. Moreover, the high speed of sound underwater, which is about 5 times faster than the speed of sound in air, shows the importance and priority of examining the role and applications of acoustic stimuli and their effects on aquatic organisms.

Recent studies have investigated impacts of anthropogenic sound on a wide variety of taxa and across a range 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 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).

Depending distance from the sound source, recent studies have shown dramatic effects of sound such as physical damages, sever injury or even death (Budelmann, 2011; Halvorsen et al., 2012; Keevin and Hempen, 1997). Further distance from the sound source, there may be physiological responses such as permanent and temporary hearing threshold shifts, fluctuations in physiological indices (André et al., 2011; Casper et al., 2013; McCauley et al., 2003; Popper et al., 2007; Popper et al., 2005; Scholik and Yan, 2002; Smith et al., 2004; Wysocki et al., 2007; Wysocki et al., 2006) such as elevated cortisol levels, the classical stress-related hormone (Johansson et al., 2016; Nichols et al., 2015; Santulli et al., 1999; Smith et al., 2004; Wysocki et al., 2006) and increased hear rates (Graham and Cooke, 2008; Simpson et al., 2015). Furthest distance from sound source, in a broader extent, behavioural effects are the most likely to occur and thus play as a stress driver (Popper and Hawkins, 2019) as a distracting stimulus (Popper and Carlson, 1998), 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 (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), much less is known across the current literature about these effects on the behaviour of freshwater fishes (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). 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 the Cyprinidae family and acclimates well in captivity (Detrich et al., 2011). This fish naturally lives in the tropical freshwater (Spence et al., 2008). Morphologically, the zebrafish's body is narrow and elongated, with golden and blue stripes that stretch along the body and tail (Detrich et al., 2004). Males and females are easily separable, so that females having a more prominent abdomen and body than males, and males have a spindle-shaped body (Spence et al., 2008). Zebrafish live in the temperature range of 24±2 ºC and in the pH range of 6.8 to 7.5 (Cortemeglia and Beitinger, 2005). The adult size of the zebra fish is approximately 4.5 to 6.5 cm (Gerhard et al., 2002; Spence et al., 2008). In recent years, zebrafish have been used as biological models in genetic, physiological, toxicological, behavioural, ecological, and other studies (Detrich et al., 2004; Gerlai, 2019; Gerlai et al., 2000; Kalueff and Cachat, 2011; Lieschke et al., 2009). The high genetic, physiological, and pharmacological similarities of this species with humans could be a reason for the use of this species as a biological model in research (Crawford et al., 2008).

Progress in behavioural biology 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). This invertebrate is the first crustacean to have its genome sequenced (Stollewerk, 2010) and because of features such as easy cultivation, small size and short generation time it is a popular model organism in various biological disciplines from aquatic ecology to biomedical sciences (Seda and Petrusek, 2011). Daphnia may also be a useful species to study behavioural studies such as sound impacts on invertebrates.

In addition, it has been reported that in the larval stage, marine crustaceans respond to reef sounds (Radford et al., 2008). Also, the aquatic invertebrate larvae, which were the same size as the Daphnia, reacted to natural sounds and sounds from human activities, and their swimming activities have changed accordingly (Morley et al., 2014). Daphnia is used as a model in ecological (Stollewerk, 2010), physiological (Altshuler et al., 2011), genetic (Harris et al., 2012; Miner et al., 2012), toxicological (Shaw et al., 2008), and parasitological (Ebert, 2008) studies.

In the present study, we investigated whether experimental sound exposure ensonified by an underwater speaker affect the general swimming activities and foraging behaviour of zebrafish under laboratory conditions. Our specific goals were: firstly, to access the effect of experimental sound exposure on zebrafish 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 Fisheries Department, Faculty of Natural Resources, University of Guilan, located in Sowmeh Sara city, Guilan province, Iran (37°17′39″N, 49°19′55″E), using an aquarium with dimensions of 50×15×20 cm with a volume of water intake of 112.5 liters in the period of 1000 to 1400 every day. Zebrafish (approximate age of 45 days old and of the wild-type, short-fin variety weight (± standard deviation) of 1.23 ± 0.02 g) were obtained from an ornamental fish breeding center located in Bazar-Jomeh in Sowmeh-Sara county, Guilan province, Iran.

Zebrafish were stored in a stock 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, from the surface layer of the pool of the faculty, by a plankton net with a net mesh size of 0.2 mm. This was done by horizontal twisting at a depth of 10 cm and a length of 30 cm and was kept overnight in a separate tank at the same temperature as the test tank to adapt to the temperature conditions. According to the previous study of Shafiei Sabet et al. (2019) as well as the same sampling location and depth in the same time period with present study and the identification key used in the previous Daphnia species study, *Daphnia magna* was identified (Shafiei Sabet et al., 2019).

2.1. Sound treatments

In the present experiment, 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). Second treatment, Continuous sound (CS) (Fig. 1 (a)), third treatment, regular intermittent noise (IN) with fast pulse rate (1:1), fourth treatment, regular intermittent sound with slow pulse rate (1:4) and Fifth treatment is the irregular intermittent sound (1:1-7). All three intermittent treatments include one second of sound, but the difference between these sound treatments is the intervals between these sounds (silence time), which are described in detail below.

Regular intermittent sound with a fast pulse rate (1:1) involves one second of sound and one second of silence (Fig. 1 (b)). Regular intermittent sound with slow pulse rate (1:4) consists of one second of sound and four seconds of silence (Fig. 1 (c)) and irregular intermittent sound (1: 1-7) includes one second of sound and interval 1, 2, 3,4 ,5, 6 or 7 seconds of silence is random (Fig. 1 (d)). Also, the sound treatments that are broadcast for different fish on different days were identified quite randomly.

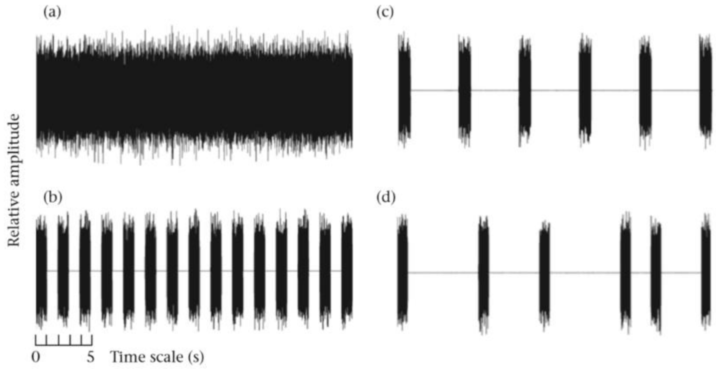

Figure 1: Continuous and intermittent sound patterns used in the experiment. (a) Continuous sound pattern, (b) Regular intermittent sound treatment with fast rotation speed (1:1), (c) Regular intermittent sound treatment with slow rotation speed (1: 4), (d) Irregular intermittent sound treatment (1:1-7).

The sound treatments were performed with 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 software in the same sound range of 400-2000 Hz.

2.2. Experiment Tank

The experimental tank with dimensions of 50×20×15 cm with black background was prepared to increase the contrast between Daphnia and fish in the video file. During the broadcast of the sound treatments, the experiment tank was filmed by a video camera (Panasonic HC-V180 Full HD 28 mm Wide Lens Camcorder) at a distance of about 50 cm from in front of the test tank. After production of sound treatments, they were played by a player connected (Sony Vaio SVF1421A4E Laptop) to an underwater speaker (custom-build speaker in Iran, 8Ω, 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 (Fig. 2). In order to reduce the stress of the fish and also to reduce the effects of people moving to the test site, the test tank was surrounded by black plastic so that the fish's behaviour was not affected by other factors and only the camcorder lens passed through the plastic and was the same for all treatments and repetitions. In such a way that when disconnecting and connecting the camcorder between sound treatments, the fish is not seen and is not affected.

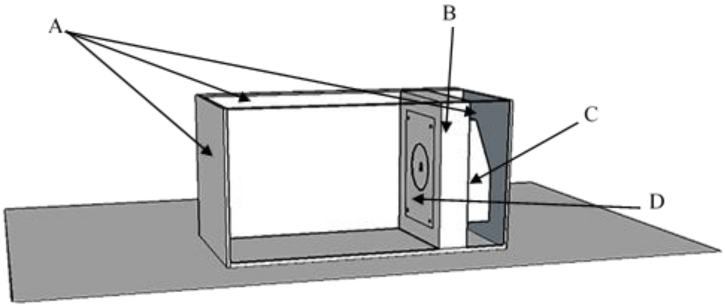

Figure 2: Schematic view of the test tank. A: The obscured pages of the test tank to enhance the fish's visual contrast in the film. B: Underwater speaker space separator screen with fish swimming space. C: Underwater speaker holder box. D: Underwater speaker.

The physicochemical properties of the water were the same and monitored regularly on a daily basis. The photoperiod used in the experiment was 12 hours of light and 12 hours of darkness (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 was measured during the experiment 26±1 °C and also the amount of dissolved oxygen in the water was measured 8±1 mg / L.

The underwater speaker used in the experiment was placed horizontally on the other side of the separator plate (See Fig. 2). In this experiment, after introducing the fish to the test tank during the night for about 20 hours, the fish was given the opportunity to adapt to the environment so that it could use the entire tank space for swimming and display natural swimming behaviour (Shafiei Sabet et al., 2015) and have the normal conditions (Neo et al., 2015; Shafiei Sabet et al., 2015). Then, test was performed with a video camera located in front of the tank. 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) added to the experiment tank after ten minutes from sound playback. The nutrition of the predatory species was investigated. This same process was performed for the other 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 (Fig. 3). The order of broadcast of sound treatments on a daily basis was randomly balanced.

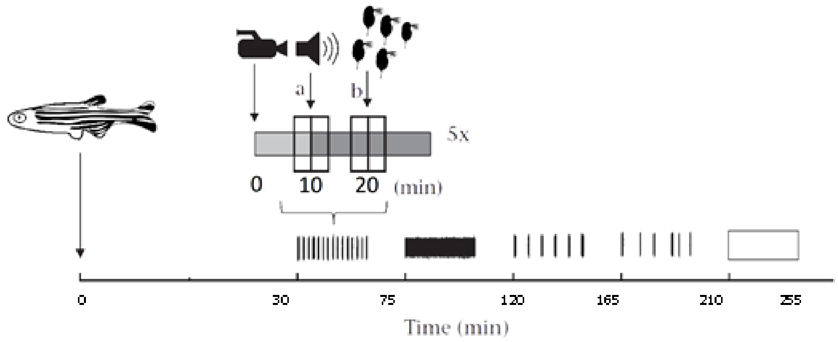

Figure 3: Schematic of the time scale of exposure to the sound of each Zebrafish. Each fish underwent five sound treatments during the experiment: continuous sound (CN), 1:1, 1:4, 1: 1-7, and ambient sound (AN) as control evidence. Each sound treatment was played for 20 minutes on an underwater speaker, and 10 minutes before the sound treatment was filmed by the camera. In this experiment, we examined behavioural changes by comparing two time periods in two moments: 1. Up to 10 minutes before and after exposure to sound treatments to check the effect of sound and 2. Up to 10 minutes before and after the introduction of Daphnia to examine the effect Sound on foraging behaviour.

2.3. Underwater sound measurement

In order to check the intensity of the sound that can be played by the underwater speaker and to understand whether the tested fish was able to detect and perceive the sound treatments or not, first the continuous treatment sound file was played back using a laptop sound player (Sony Vaio SVF1421A4E) connected to a custom-build underwater speaker which a custom-build sound tuning amplifier was attached. The level of sound intensity under water 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). The recorded sound file was evaluated in Rstudio software (Version 1.1.456 - © 2009-2018 RStudio, Inc.).

According to the Fig. 4, during continuous sound playback, the frequency range emitted from the underwater speaker was completely in the zebrafish's hearing range in the range of 300-1500 Hz (Higgs et al., 2002) and far above the ambient noise playback. The sound pressure of continuous sound treatment during playback was average 121 dB ref 1 $\mu Pa^2/Hz$ for 5 seconds and the ambient sound pressure was average 96 dB ref 1 $\mu Pa^2/Hz$ for 5 seconds.

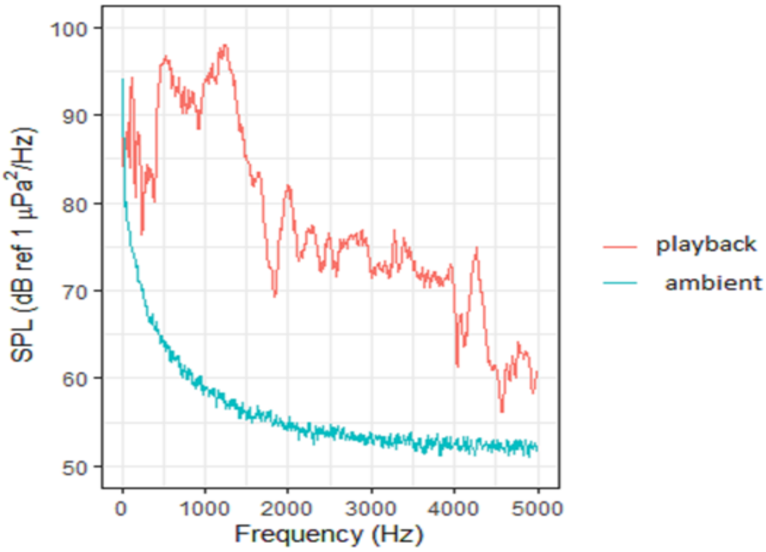

Figure 4: Spectral distribution of continuous sound pressure level compared to silent treatment (dB ref 1 µPa^2/Hz). Silence conditions (blue) and continuous sound playback (red). The diagram shows that the sound intensity level has increased significantly in the range of hearing frequencies of zebrafish.

2.4. Effect of sound on Zebrafish swimming behaviour

To investigate the effect of sound on the behaviour of Zebrafish, 30 fishes (15 males and 15 females) were introduced individually in the experimental tank after biometrics. The fish were introduced to the test tank in last day (Overnighting) and the fish were given the opportunity to get used to the environment so that they could use the entire space of the tank for their swimming and have normal conditions. Also, all fish were given 30 minutes to relax. Since then, the fish has undergone five sound treatments.

Zebrafish behavioural response to five sound treatments was video recorded for a maximum of 30 minutes for each treatment (maximum 20 minutes for exposure and a maximum of 10 minutes before exposure) (Fig. 3). 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

As mentioned earlier (Fig. 3), the effect of sound on the foraging behaviour of zebrafish was investigated in such a way that 5 waterfleas (about three millimeters) as a prey species (target) and 5 non-food substances as non-food item in the same size as Daphnia (about three millimeters) was mixed in 25 ml beaker and added 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.

To investigate the effect of sound treatments on the foraging power of Zebrafish, the parameters of food discrimination error (Formula 1) and food handling error (Formula 2) were measured.

$$\text{Food Discrimination Error} = \frac{\text{number of non-food item attacks}}{\text{total number of food and non-food attacks}} \qquad \text{(Formula 1)}$$

$$\text{Food Handling Error} = \frac{\text{number of unsuccessful attacks on water fleas}}{\text{total number of successful and unsuccessful attacks on water fleas}} \qquad \text{(Formula 2)}$$

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 software (Vernier Software & Technology, Beaverton, OR, U.S.A., version 3.6.0) was used to examine behavioural responses including the number of explosive movements, swimming speed, and spatial distribution of the fish. Entering information and data in M.S. Excel 2016 and data analysis was performed using SPSS 25 software. 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 - ©

2009-2018 RStudio, Inc.) was also used to evaluate sound pressure levels and power spectral density that were played by the underwater speaker.

3. Ethical note

We considered the 3Rs in behavioural research, the guidelines with respect to Good Laboratory Practice (GLP). 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 waterfleas (Daphnia) in Iran. The principal investigator (S.SH.S) passed the exam for the course on laboratory animal science at Leiden University, the Netherlands and holds animal testing act certificate “as an Article 9 researcher”.

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. 5a, 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 5a). 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 5b). 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 5b).

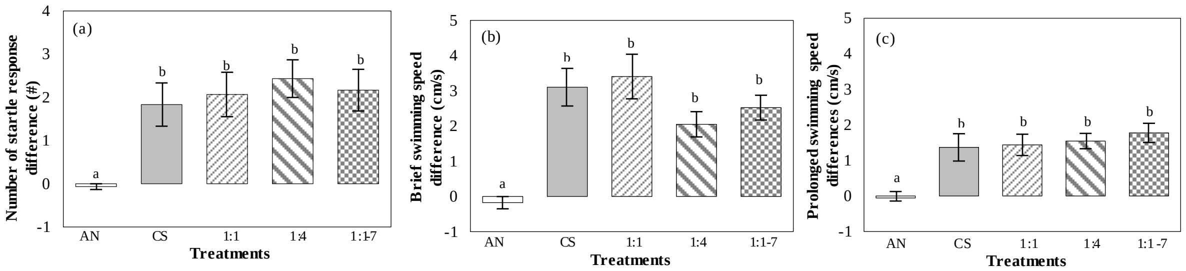

Figure 5. Effect of sound exposure treatment on swimming behaviour of Zebrafish. (a) Number of startle responses expressed as the difference between the first 60 seconds during sound and the last 60 seconds before sound exposure onset: Continuous sound (CS) and three intermittent sound (1:1, 1:4, 1:1-7) 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)).

According to Figure 6, 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 the baseline after 60 seconds during sound. In ambient condition (Fig 6a), 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 6b, 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: $F_{4.54,131.66}=9.53$, $P\leq0.001$, 1:1= repeated measure ANOVA: $F_{3.85,111.72}=11.72$, $P\leq0.001$). 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 6d, e) with more intensity compared to before and during the sound (1:4= repeated measure ANOVA: $F_{6.24,181.01}=14.17$, $P\leq0.0001$, 1:1-7= repeated measure ANOVA: $F_{7.14,207.15}=15.01$, $P\leq0.0001$). But in comparing the baseline time in these two treatments, no significant difference was observed and the fish reached the baseline ($P>0.05$).

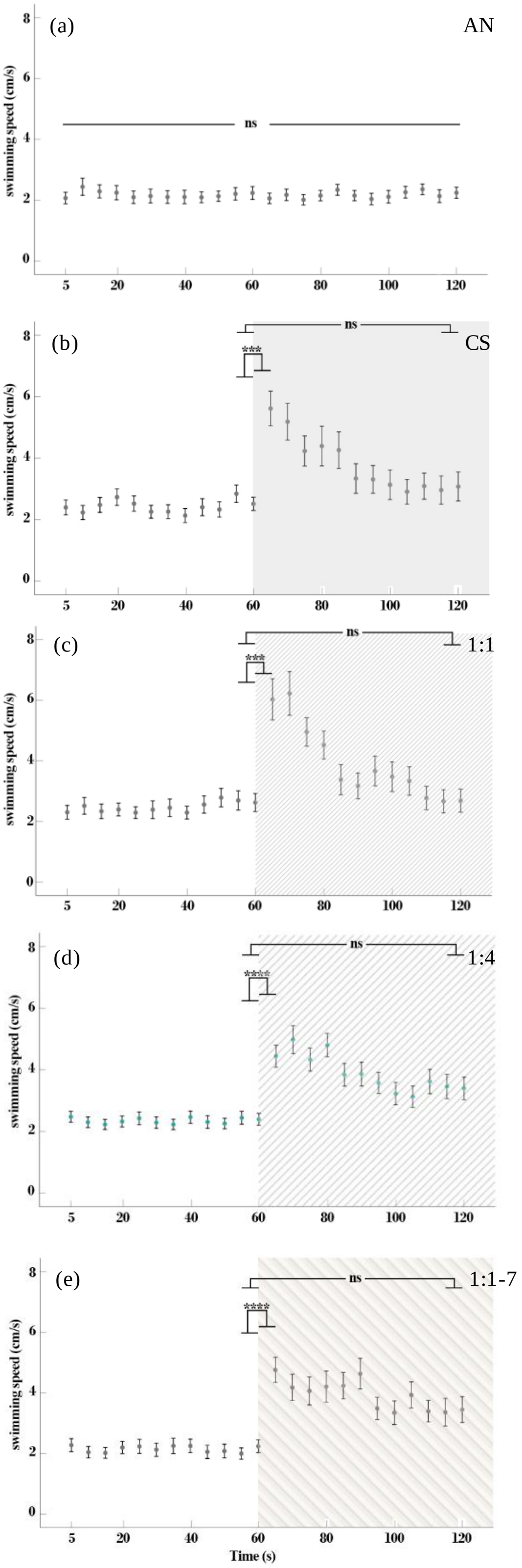

Figure 6. Effect of sound treatments on zebrafish swimming speed. (a) Ambient condition. (b) Continuous sound. (c) regular intermittent 1:1. (d) regular intermittent 1:4. (e) irregular intermittent 1:1-7. The time was divided into three period bins for formal statistical analysis: the last 5 seconds before sound exposure, the first 5 seconds during sound exposure and the last 5 seconds during sound exposure. (NS= no significance, ***= $P \leq 0.001$, ****= $P \leq 0.0001$).

Changes in the spatial distribution of Zebrafish were investigated when exposed to sound treatments in vertical scale (lower layer) (Fig. 7a, b) and horizontal scale (X position) (Fig. 8a, b). According to Figure 7a, 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 7b, 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 8a) ($F_{3.78,109.71}$=1.810, P=0.136) (Fig 8b). Also, according to Figure 9a, 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).

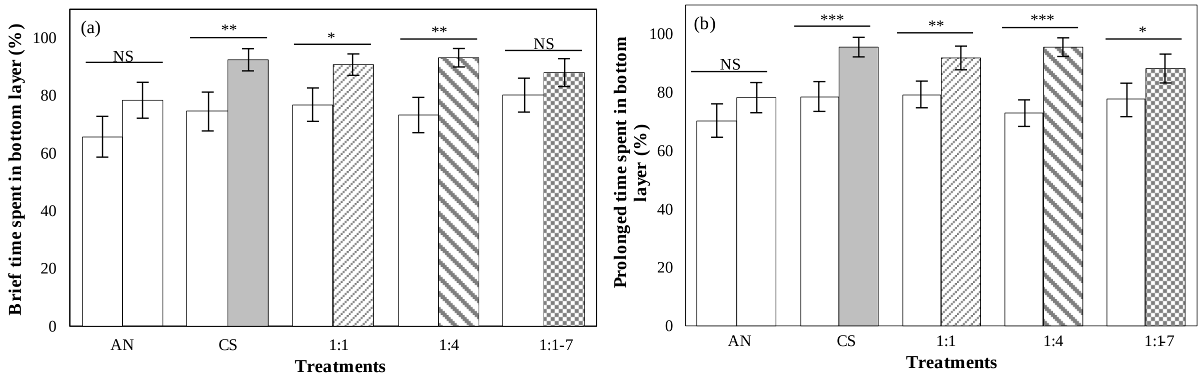

Figure 7. Average percentage of fish time spent in bottom layer of tank (N=30). (a) Brief time (15 seconds before and 15 seconds exposed to sound) (NS= no significance, *= *P*≤0.05, **= *P*≤0.01). (b) Prolonged time (60 seconds before and 60 seconds exposed to sound) (NS= no significance, *= *P*≤0.05, **= *P*≤0.01, ***= *P*≤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).

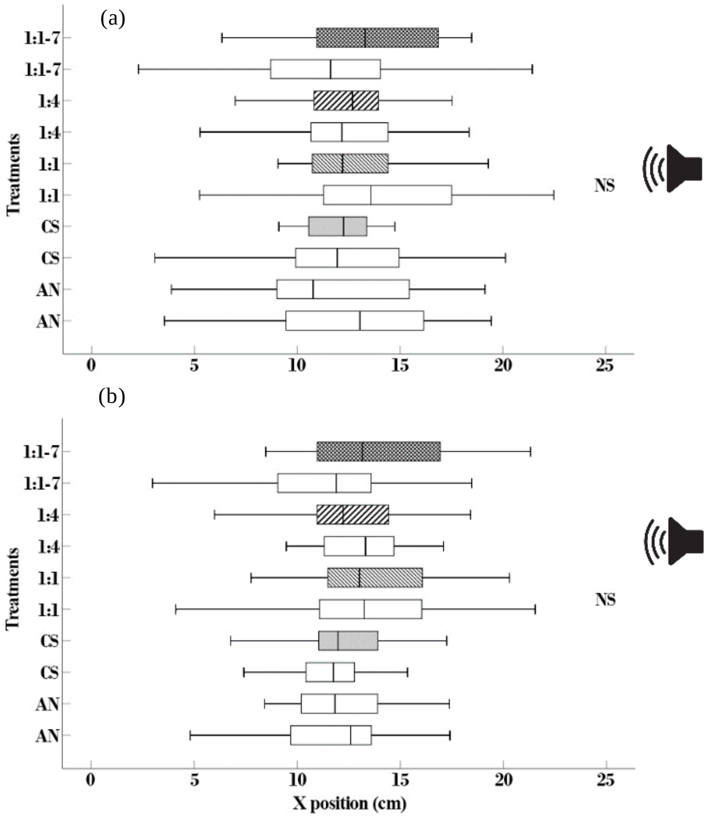

Figure 8. Effect of sound exposure on horizontal spatial distribution of Zebrafish. (a) Brief time (15 seconds before and 15 seconds exposed to sound). (b) Prolonged time (60 seconds before and 60 seconds exposed to sound). The underwater speaker played back from the right tank. Bars show Means ± SE.

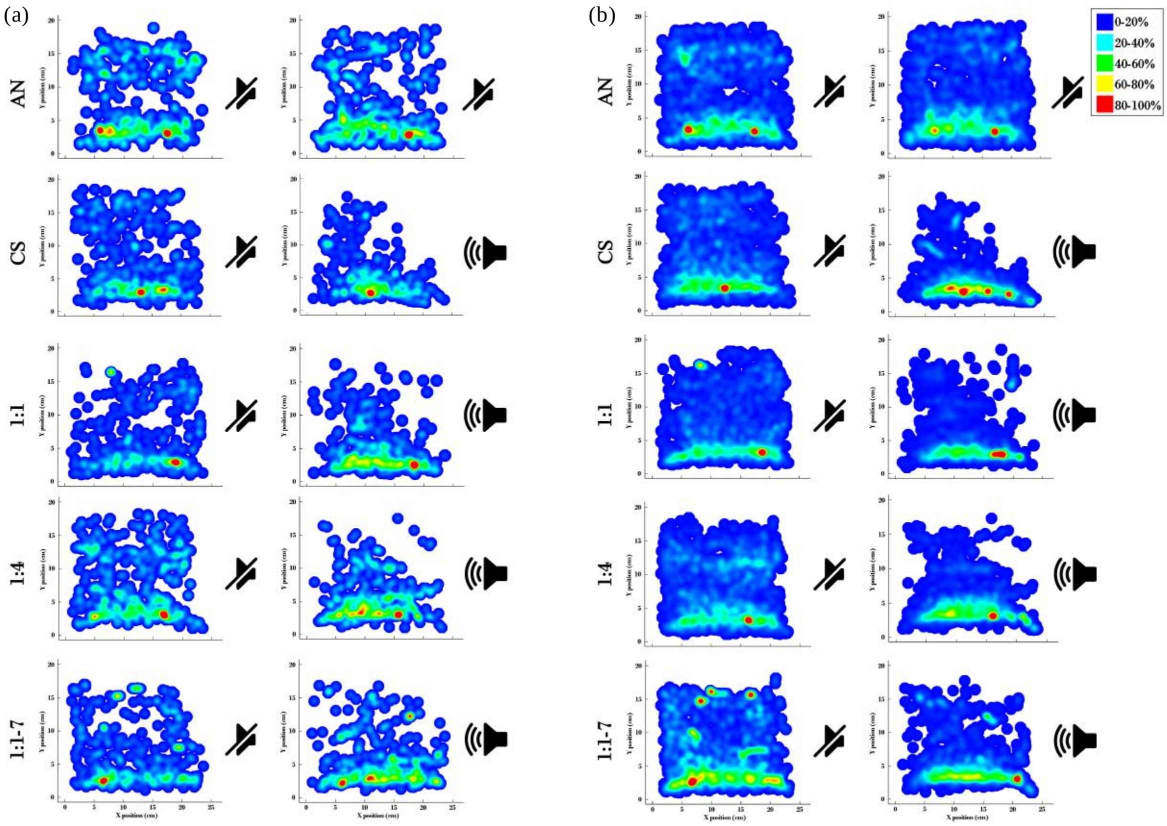

Figure 9. Heat map of fish swimming in the tank environment. (a) Brief time (15 seconds before and 15 seconds exposed to sound). (b) Prolonged time (60 seconds before and 60 seconds exposed to sound). The blue color (#0000FE) indicates the 0-20 % of the fish in the tank. The aqua color (#01FFFF) indicates 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.

4.2. Impact of sound on foraging performance of Zebrafish

According to Figure 10a, none of the acoustic treatments showed a significant difference compared to the silent treatment on zebrafish food discrimination error (repeated measure ANOVA: $F_{4,116}$=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: $F_{2.82,81.91}$=26.023, $P \leq 0.001$) but no significant difference was observed between sound treatments (P>0.05) (Fig. 10b). 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.

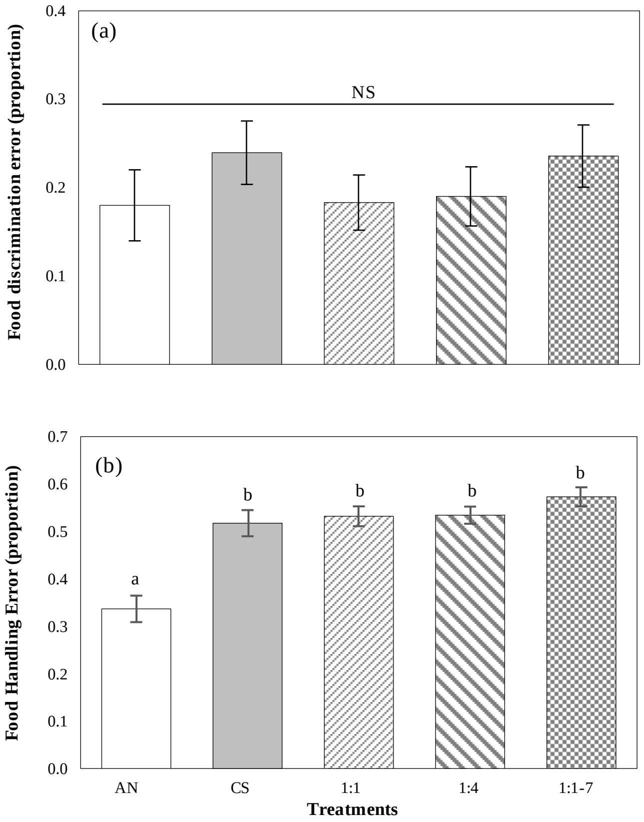

Figure 10. Effect of sound treatment on foraging behaviour of Zebrafish. (a) Food discrimination error as the proportion of duckweed particles attacked relative to the total number of attacks to both duckweed particles and water fleas from the introduction of food items until the end of sound exposure in sequence for each zebrafish individual. (N=30, df=3.756, *F*=1.339, *P*=0.226) (b) Food handling error 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. (N=30, df=2.825, *F*=26.023, *P*=0.000019). Standard error changes (±1)

5. Discussion

In this experiment, our results unequivocally demonstrate that acoustic stimuli affect zebrafish behaviour and swimming activities under laboratory conditions. 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.

Studies by other researchers have shown that other species of fish respond similarly to 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 increase survival 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, different sound patterns had a significant effect on the 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 compared to the silent conditions (Neo et al., 2018). 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 behavioural responses in response to acoustic stimuli, genetic characteristics and habitat conditions.

5.3. The effect of sound on the spatial distribution of zebrafish (Vertical/Horizontal)

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. Also, the study on the percentage of fish in the lower layer of the test tank showed that in some sound treatments in the brief time there was a significant difference and in the prolonged time in all sound treatments except the ambient treatment, this means Which had a greater tendency to be present in the lower layer when playing the sound of fish. In another part of the results of this study and the study of the spatial distribution of the presence of fish in the horizontal profile (X position) also showed that in none of the sound treatments the fish did not tend to distance from the sound source and did not show significant differences.

Neo et al. (2015) in the study of the effect of sound on the spatial distribution of zebrafish, found 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, in this study (Neo et al. 2015), there were no observations of the freezing and standing of the fish in the lower layer of the tank. 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 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. In another study, Neo et al. (2018) designed an experiment to investigate the effect of different sound patterns on the behaviour of European seabass and 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.

Other fishes have also shown spatial distribution changes in response to acoustic stimuli. 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) revealed .....

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 (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) and other factors include the size of the test tank, the life cycle and location of the fish storage tank, as well as differences in the species and genetics of the fish species. Also in the study of Shafiei Sabet et al. (2015) in the spatial distribution of fish horizontally, a similar result was shown with the results of the present study and no significant difference was observed in the x position.

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 sound, first of all, it is very important to understand how the species detects and processes, and how it behaviourally responds to sound (Hawkins and Popper, 2020). Because 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). Only some of them, 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 determined sound direction 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 the direction of only particle motion may be insufficient to determine the direction of a sound source. 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 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 to eliminate any remaining 180^0 ambiguities (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. 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 boadcasting 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 Ciprinidae 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.

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. The results showed that sound treatments compared to the ambient treatment caused a significant difference in the parameters of swimming behaviour including the number of startle response, swimming speed of fish in the brief and prolonged time, spatial distribution of fish in vertical and horizontal profiles. In general, the observed behavioural patterns in response to sound treatments as a stressor, especially in the brief time of this study have been observed in natural environments and in other fish species. 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.

Also, the results obtained in the foraging behaviour showed that different sound patterns in comparison with the ambient treatment caused a significant difference in food handling error but no significant difference was observed in food discrimination error. Depending on the species characteristics, these behavioural responses include escaping the predator species, hiding in a shelter to avoid being hunted, forming crowded clusters, and even approaching the predator species aggressively. The movement towards the lower layer observed in the experimental tank by zebrafish can be due to the stress caused by the potential for the presence of a predatory species, that in their natural habitats, where clear water is permeable to light, to the deeper parts of their habitat, which have less light and have vegetation that reduces the visibility of the predator species, and thus ensure its survival. In general, due to the simultaneous presence of the species used in this experiment in their main habitat (zebrafish, daphnia and duckweed) and the results obtained can be understood that sound causes overlap in the water particles motion, which is one of the factors in the perception of the prey species by the predator species, and the zebrafish cannot have a proper understanding of the presence of the prey species. On the other hand, creating a state of distress and anxiety after playing sound treatments has caused a lack of proper vision of the prey species and the zebrafish, which is an omnivorous and visually impaired fish in prey, cannot see and hunt well.

Increased background sound levels by human activities can be recognized as a stressor and lead to a series of changes in the activities and swimming patterns of aquatic species. 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.

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Credit author statement

Reza Mohsenpour: Conceptualization, Methodology, Software, Formal analysis, Supervision, Visualization, Data curation, writing - original draft.

Saeed Shafiei Sabet: Conceptualization, Methodology, Supervision, Project administration, Writing - review & editing.

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.

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