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

Shafiei Sabet, Saeed

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/38704 holds various files of this Leiden University dissertation

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

Chapter 2

The effect of temporal variation in sound exposure on swimming and foraging behaviour of captive zebrafish

Abstract

Anthropogenic noise of variable temporal patterns is increasing in both marine and freshwater systems. Aquatic animals often rely on sounds for communication and orientation, which may therefore become more difficult. Predator-prey interactions may be affected by masking of auditory cues, sound-related disturbance or attentional interference. Here, we investigated the impact on both predator and prey for zebrafish (*Danio rerio*) preying on water fleas (*Daphnia magna*). We experimentally raised ambient sound levels in an aquarium and tested four sound conditions that varied in temporal pattern: continuous, fast and slow regular intermittent and irregular intermittent, which we compared to   ambient sound levels with no extra exposure. We found no effects on water flea swimming speed or depth but there was an increasing number of individual zebrafish with an increased number of startle responses, especially to the intermittent sound treatments, which was also reflected in a significant increase in zebrafish swimming speed, but not in any change in zebrafish swimming depth. Discrimination in attacking edible water fleas or inedible duckweed particles was low for the zebrafish and unaffected by sound exposure, but foraging was affected in two ways: intermittent sounds delayed initial acceleration response and all treatments caused a rise in handling error. These insights confirm that elevated sound levels, and especially intermittent conditions, may affect predator-prey interactions. Our results apply to laboratory conditions but call for outdoor studies that go beyond single-species effects. If acoustic impact of human activities extends to

multiple species and their interactions, natural sound conditions may turn out to be important for the stability and dynamics of aquatic ecosystems.

Introduction

A variety of human activities introduce anthropogenic noise in different temporal patterns above and below the water surface in marine and freshwater systems (Andrew et al. 2002; Amoser et al. 2004; McDonaldet al. 2006). Although empirical evidence confirming short-term and especially long-term effects is still scarce, aquatic animals can be negatively affected by anthropogenic noise in many ways (Richardson et al. 1995; Popper et al. 2003; Popper et al. 2014). Masking may for example cause interference with acoustic communication, soundscape orientation, or acoustically guided predator-prey interactions, while anthropogenic noise may also cause interruption or modification of group movements, migratory activities, and courtship or other reproductive behaviours (see reviews: Slabbekoorn et al. 2010; Radford et al. 2014; Hawkins & Popper 2014).

Different taxonomic groups such as marine mammals and fish can be part of the same community, but may be affected by anthropogenic noise in different ways and to a variable extent (Weilgart 2007; Slabbekoorn et al. 2010; Popper et al. 2014; Shafiei Sabet et al. 2016). In air, it has been shown that human-induced changes in ambient noise levels can have direct and

indirect effects and can lead to changes in abundance and diversity of animals and plants (Francis et al. 2009; 2011a; Francis et al. 2012a). We currently lack such insights for aquatic communities and it is clear that more data are needed that go beyond single-species effects.

There are several recent studies in various taxa which revealed an impact of artificial sound levels on predator-prey relationships. For example, Siemers & Schaub, (2010) showed that elevated sound levels may negatively affect foraging performance in bats (*Myotis myotis*) by masking auditory cues that are critical for catching invertebrate prey. Quinn et al. (2006) also reported sound-dependent changes in foraging efficiency in chaffinches (*Fringilla coelebs*) as higher ambient noise levels made them eat less and scan more. In crustaceans, Chan et al. (2010) found that boat sounds distracted hermit crabs (*Coenobita clypeatus*) in such a way that they responded less quickly to a visual stimulus indicating approaching danger. So, it appears that sound impact is widespread taxonomically, that acoustic masking or distraction can affect auditory as well as visual perception, and that anthropogenic noise may affect predator as well as prey species.

As far as we know, fish are also likely to be susceptible to the human-induced rise in underwater sound, as they are well-known to hear and use sounds for many aspects of their underwater life (Ladich 2004; Fay 2009; Slabbekoorn et al. 2010). Like in air, underwater masking effects are determined by the spectral overlap of ambient noise with biologically relevant sounds (Codarin et al. 2009; Vasconcelos et al. 2010; Gutscher et al. 2011). Independent of masking, several studies have also reported

behavioural changes in response to artificial tones or wide-band sounds. For example, Andersson et al. (2007) showed several different behavioural changes in captive roach (*Rutilus rutilus*) and sticklebacks (*Gasterosteus aculeatus*) which were interpreted as species-specific responses to perceived danger of predation risk. Picciulin et al. (2010) revealed a negative impact on the time budget spent on behaviours that are critical for reproductive success in red-mouthed gobies (*Gobius cruentatus*) in their natural habitat. Sebastianutto et al. (2011) also showed that the typical outcome of acoustically mediated territorial conflicts of this species was undermined under experimentally noisy conditions. Although these studies suggest that predator-prey interactions in fish may also be affected by artificial sound exposure, this phenomenon that has potential consequences across aquatic food webs, has received relatively little attention.

Recently, a study on sticklebacks experimentally explored the impact of artificial noise on predator-prey interactions in sticklebacks catching water fleas (*Daphnia magna*). Purser & Radford, (2011) were able to show that sound playback, compared to more quiet conditions, increased the amount of errors in food-particle discrimination and food handling. Voellmy et al. (2014) showed that different species may respond differently to playback of additional ship sounds as European minnows (*Phoxinus phoxinus*) differed from sticklebacks in becoming less active and more social. These experimental data clearly show an acoustic impact on a seemingly visual task with a direct impact on fish foraging efficiency. As masking is unlikely to be important, the performance decline may be due to attentional shifts (Dukas 2002; Mendl, 1999) as found in the studies on birds

and hermit crabs mentioned above (Quinn et al. 2006; Chan et al. 2010). In the experimental studies on fish (Purser & Radford 2011; Voellmy et al. 2014), it was assumed but not investigated that the effect of sound on foraging efficiency was caused by an impact on the predator and not on the prey and the relevance of temporal variation in sound characteristics (c.f. Neo et al. 2014; Neo et al. 2015) remained unexplored.

Zebrafish (*Danio rerio*) are a very suitable model system to assess behavioural changes in response to environmental conditions in general (e.g. Cachat et al. 2010; Egan et al. 2009; Gaikwad et al. 2011; Gerlai et al. 2006) and to tackle questions of sound impact on predator-prey interactions in particular. Neo et al. (2015) exposed adult zebrafish to different sound patterns and showed initial startle responses, relatively brief anxiety-related response behaviours, but no longer-lasting effects or spatial avoidance. They reported sound exposure related changes in swimming speed and group coherence, while fish moved upward in response to moderate sound levels (112 dB re 1 µPa) and downward (for brief periods) in response to higher sound levels (120-140 dB re 1 µPa). We have no insight yet into whether and how foraging behaviour in this species is affected by sound exposure (c.f. Purser & Radford 2011; Voellmy et al. 2014), but also zebrafish readily feed on live prey and provide a perfect model system to assess the impact of temporal variation in sound exposure on foraging efficiency.

Water fleas (*Daphnia* spp) are small crustaceans and important food items for many fish species in freshwater systems (e.g. Ebert 2005; Gulati 1990). They show predictable spatial behaviour by avoiding darker water

areas and preferring open space (negative scototaxis and negative thigmotaxis), which probably reduces exposure to predators that may hide in the dark and in vegetation (e.g. Van Gool & Ringelberg 1995; Dodson et al. 1997). Although sensory systems for aquatic invertebrates may vary, both short-term sound effects on response behaviour to approaching predators (Chan et al. 2010) and long-term sound effects on growth and reproduction (Lagardère 1982) have been reported for example in crustaceans. Furthermore, at a larval stage, marine crustaceans have been reported to respond phonotactically to reef sounds (e.g. Radford et al. 2007; Stanley et al. 2011). Also larvae of aquatic invertebrates, of similar size as water fleas, have been shown to either increase or decrease their swimming activity in response to natural and anthropogenic sound exposure Therefore, we believe it is important to check whether or not anthropogenic noise has any effect on water flea behaviour that may have consequences for predation risk (c.f. Morley et al. 2014).

In the current study, we tested the impact of temporal variation in artificial noise exposure, mimicking temporal and spectral patterns of man-made sounds that exist in natural environments, on: 1) behaviour of water fleas (*D. magna*); 2) behaviour of zebrafish; and 3) on zebra fish preying on water fleas. We measured startle responses, swimming speed, and spatial distribution in water fleas and zebrafish. Sound treatments varied in being continuous or intermittent and the latter category in being fast or slow and in having regular or irregular intervals. We aimed for answers to the following questions: Does exposure to artificial noise reduce foraging efficiency of zebrafish hunting for water fleas? And is this impact attributable to a

behavioural impact on prey, predator, or both? Furthermore, does variation in temporal patterns matter or not? We expected water flea swimming behaviour to change with the onset of sound exposure and foraging efficiency of zebrafish to be negatively affected by sound exposure through an impact on foraging performance, discrimination and handling (c.f. Purser & Radford 2011; Voellmy et al. 2014). We also expected less impact from continuous sound than from intermittent sound and less impact from regular than from irregular sound exposure.

Methods

Animal maintenance and housing

Zebrafish (adult, 4-6 month old and of the wild-type, short fin variety) were obtained from a local pet supplier in Leiden (Selecta Aquarium Speciaalzaak, who obtains stock from Europet Bernina International BV; Gemert-Bakel, The Netherlands). The fish were housed in a long stock tank (50 x 40 x 200 cm) connected to a water circulation system before being transferred individually and sequentially to the experimental set up. The fish stock was kept at 24±1ºC on a 14/10 h light/dark cycle (light switched on from 06:00–20:00) and was fed on dry food twice a day (DuplaRin M, Gelsdorf, Germany). After the experiment, the exposed fish were transferred to a stock tank. Water fleas were captured in the morning (around 7:00) on the day of the experiment in which they were used. They were always captured in shallow water bodies in the

southern part of Leiden ($23^0 9^{'}27^{"}$ N, $48^0 5^{'}18^{"}$ E) by gentle pulling of a net (mesh size: 2 mm) through the water at a depth of about 30 cm. The outside water temperature ranged from 14-18°C and water fleas were allowed to acclimatize gradually to the indoor water temperature of 24°C over the period of one hour before use in any of the experiments. Water fleas appeared to handle the transfer to indoor conditions well and individuals compared among different sound treatments always had the same environmental background and procedural experience.

Artificial noise stimulus preparation

Four sound treatments were used with varying temporal patterns: continuous sound (CS), intermittent regular with a fast pulse rate, intermittent regular with a slow pulse rate and intermittent irregular sound, and ambient noise (AN) as a control (Fig. 1). All three intermittent sound treatments consisted of one-second pulses but differed from each other in terms of the length of the intervals without extra sound exposure. Intermittent regular noise with a fast pulse rate (1-1) consisted of 1s pulses interspersed with 1s intervals and intermittent regular noise with a slow pulse rate (1-4) consisted of 1s pulses interspersed with 4s intervals, irregular noise (1-7) consisted of 1s pulses interspersed with 1, 2, 3, 4, 5, 6 or 7s intervals in randomized sequence (using an online random number generator: http://www.random.org/), leading to a mean interval of 4s.

Continuous sound as well as sound pulses were created in Audacity (2.0.3) software, using band-filtered white noise (band-passed between 300-1500 Hz), which matches the frequency range of best hearing for zebrafish

(Higgs et al. 2002; Popper et al. 2001) and also matches in general terms with the typical wide-band sound characteristics of anthropogenic sources, such as vessels, pumping systems or pile driving (Slabbekoorn et al. 2010; Wysocki et al. 2006). The frequency range of auditory sensitivity for invertebrates varies (Morley et al. 2014) and there are no data for water fleas. However, we expect that their sensitivity could be overlapping the frequency range of fish hearing and our current stimuli. Our behavioural test will reveal whether we can exclude an impact of prey behaviour on sound-dependent foraging efficiency of the predator, but not the underlying mechanism of a potential lack of response to exposure. We used 5 ms ramps to fade in and fade out pulses for smooth transitions in the intermittent sound patterns.
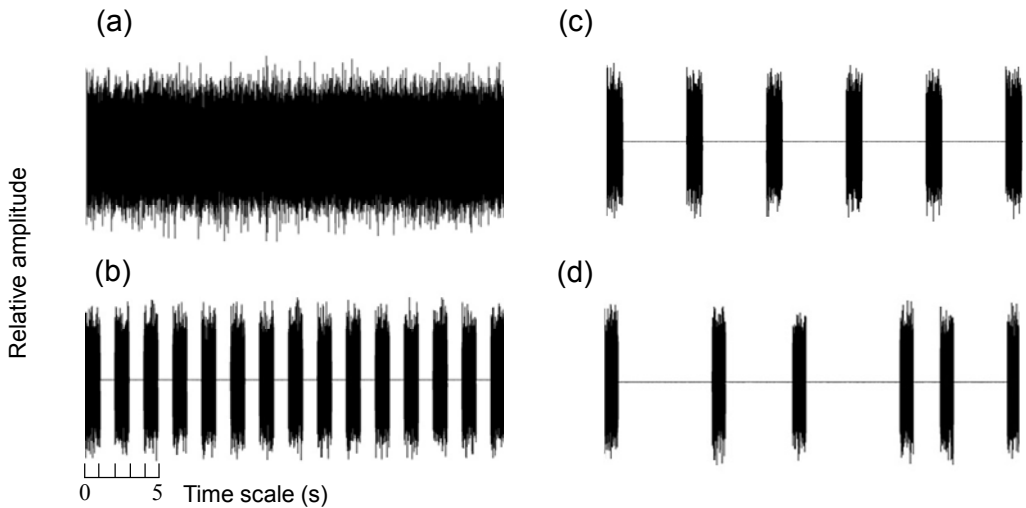
Fig. 1. Amplitude waves showing temporal variation in the four sound treatments used in the exposure experiments: (a) Continuous sound (CS). (b), Intermittent regular (1-1) with a high pulse rate of 1s sound and 1s interval. (c), intermittent regular (1-4) with a low pulse rate of 1s sound and 4s interval and (d) intermittent irregular (1-7) with 1s sound and variable intervals randomly selected from the range of 1-7s (7 different whole-second durations, on average 4s).

Experimental tank conditions

The experimental trials were conducted in a narrow subdivision (25cm×15cm×20 cm) of a larger fish tank (50cm×20cm×20cm). We reduced the swimming space by using Styrofoam dividers and we scored animal movement in two dimensions: vertical and horizontal. A black sheet of plastic covered the background of the tank to increase the contrast for the water fleas and zebrafish on video files, recorded using a 1080 P AIPTEK full HD camcorder (model H 500). The water was disconnected from the

water recirculation system during the experiments and the water temperature was kept at 24.0 °C during all trials. The sounds (WAV format, 44.1-kHz sampling rate) for all treatments were played back with a portable Tascam digital recorder (model DR-07) connected to an in-air HARMAN speaker (model EON JBL 500), which was placed at 1.5 m from the fish tank wall (long end) at the same height from the floor as the fish tank (on a separate table and on top of a Styrofoam layer to reduce transfer of sound vibrations into the floor).

In our experiments, the test animals experience variable and complex near-field conditions inherent to the fact that they are able to hear low-frequency sound of long wave lengths and that they swim in an indoor fish tank (Parvulescu 1967; Akamatsu et al. 2002). Zebrafish are cyprinids that are sensitive to the sound pressure as well as the particle motion component of sound (Fay & Popper 1974; and see Higgs et al. 2002; Bretschneider et al. 2013), while water fleas are likely only sensitive to the latter (e.g. Patek 2001; Stocks et al. 2012; Wale et al. 2013). It is therefore important for our test that both sound pressure and particle motion are elevated during experimental exposure and we therefore assessed both (definitions for our acoustic terminology follow ANSI/ASA S1.1-2013). The underwater sound pressure levels (SPL) were determined by using a High Tech hydrophone (model HTI 96 min), connected to a Marantz Solid state audio recorder (model PMD620). The hydrophone was placed in four different positions at each of which we took three measurements. We calculated the cumulative SPL within the 300-1500 Hz frequency range (rms), using a Matlab script (R2013a) calibrated for the recording set. The ambient SPL of 95 dB re 1

μPa was elevated during sound playback (continuous and intermittent treatments) to 122 dB re 1 μPa. Sound pressure levels at the bottom and close to the walls were slightly higher than in middle strata and center of the tank: mean ± SE at the bottom: 126.3 ± 0.7; in the middle: 122.4 ± 0.7; and at the surface: 121.0 ± 0.5. Spectrum levels varied due to speaker output characteristics and propagation through air into the fish tank, but sound levels were well-elevated throughout the relevant hearing range of zebrafish (see Fig. 2a and b).

We assessed the experimental elevation of the particle velocity level (PVL) by adding up the vectorial measures from 3 accelerometers, (one for each direction: X-, Y- and Z-coordinate). The accelerometers were fixed inside a custom-made transparent Plexiglas sphere (9.5 cm in diameter) with a hydrophone in the middle and suspended into the water with thin nylon wires (c.f. Bretschneider et al. 2013; van den Berg & Schuijf 1985). Accelerometers and hydrophone were connected to a digital oscilloscope: PicoScope model 3425, using a resolution of 12 bits at 20 ms/s, bandwidth 5MHz (Pico Technology, St. Neots, United Kingdom). We measured at 7 cm height from the bottom at a replicate set of seven positions in the fish tank. The ambient PVL was 165 dB ref 1 nm/s, which was elevated to 200 dB ref 1 nm/s during exposure. The spectral distribution of particle motion levels was also not flat, but PVL was elevated throughout the 300-1500 Hz range (as we were unable to calibrate absolute levels, we reported the relative levels of elevation above ambient, see Fig. 2).
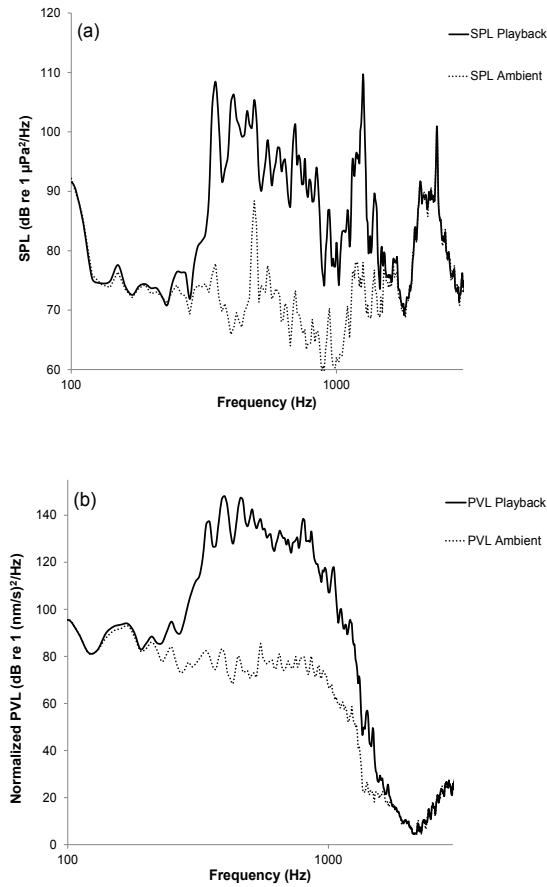
Fig. 2. (a) Spectral distribution of continuous sound pressure level in dB (re 1 $\mu Pa^2/Hz$) (dotted line) ambient condition and (continuous line) sound playback) and (b) the normalized particle velocity level in dB (re 1 $(nm/s)^2/Hz$) in ambient condition (dotted line) and sound playback (continuous line) of continuous sound exposure as measured within the fish tank in the laboratory. The graphs show that both SPL and normalized PVL increased considerably in the same frequency range. Note that the particle velocity level concerns the sum of the root mean square averages from the vector sensors in all three X, Y and Z directions measured in the center of the tank (both SPL and PVL measurements were averaged over 10 sec and were measured in the same position at the center of the tank; 7 cm from the bottom and 10 cm from the side wall of the tank).
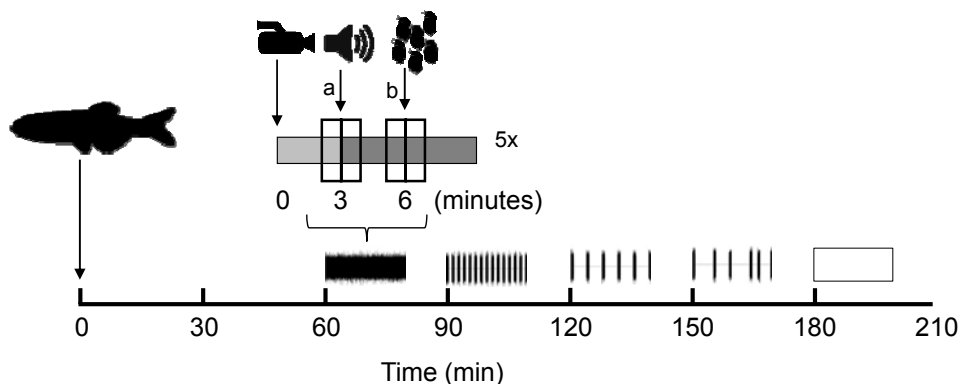
47

Fig. 3. Schematic representation of the exposure timeline for a single trial of an individual zebrafish. Each individual in an experimental trial was exposed to five subsequent sound treatments in randomized sequence: CS, 1-1, 1-4, 1-7, and ambient level with no extra exposure as a control (AN). Each treatment lasted for 6 min of experimental exposure through playback with our in-air speaker (preceded by 3 min of video period and followed by 18 min of interval to the video-onset of the next treatment). We assessed the behavioural impact by making comparisons between two time periods at two moments: (a) 1 min before versus 1 min after onset of sound exposure to measure variation in initial sound impact and (b) 5 seconds before and 5 seconds after introducing the 10 individual water fleas to measure variation in sound impact during foraging.

Sound impact on water fleas

We investigated sound impact on water flea swimming behaviour by tracing individuals during sound treatments in two separate tests. In the first test, a group of 10 water fleas was introduced in the experimental tank and analysed for non-targeted swimming speed difference (before sound exposure versus during sound exposure) and swimming depth difference (before sound exposure versus during sound exposure; distance to the

48

bottom) using averages per group. In the second test, individual water fleas were introduced on the right side of a dark tank to measure the impact of sound treatment on targeted swimming speed towards the lightened left side of the tank. We randomized the order of five trials per group or individual, for the four sound treatments and one control to avoid the effect of treatment being confounded by an order effect.

In the first test, we selected 10 groups of 10 individual water fleas of equal size (~3 mm), which entered the experimental tank per group (using a pipet) after at least 1 hour of acclimatization to indoor conditions. The exposure to each of the four sound treatments (continuous and three pulsed treatments: CS, 1-1, 1-4 and 1-7) and the ambient control (AN) was recorded for 9 min per treatment (each time: 3 min before sound on-set and 6 min during sound exposure) (see Fig. 3). We analyzed swimming behaviour for all 10 individuals and compared among treatments (1 min just after sound on-set subtracted from 1 min just before sound on-set; the longer recording periods avoid an impact of observer presence during the start of the video on the selected periods for analyses). Full-tank illumination let to a range in light illuminance from 750 to 1100 lux from bottom to surface as measured by a LUNASIX F light meter (P. Gossen & Co, Erlangen made in Germany). This light condition provides sufficient visibility to allow continuous tracing of water fleas on video throughout the entire tank.

In the second test, we selected 12 water fleas which entered the experiment individually by gently pouring them into the water on the right side of the tank in dark conditions (using a pipet and after at least 1 hour of

acclimatization). The top and sides of the tank were covered by a plastic black sheet for 20 cm, leaving 5 cm open on the left side as the light source to trigger positive phototaxis. We assessed targeted swimming speed by timing the duration it took each individual to cross the approximately 19 cm from the location of introduction on the right to the 5 cm lit-up area on the left.

Sound impact on zebrafish

We investigated sound impact on swimming behaviour of zebrafish by introducing 14 fish (7 males and 7 females) individually on different days into the same experimental tank as used for the water flea trials (full-tank illumination conditions). We determined zebrafish sex by coloration patterns and belly shape (Schilling 2002). After introduction into the experimental tank, each individual was allowed one hour of acclimatization before the on-set of video recording and subsequent sound exposure to the four treatments (CS, 1-1,1-4 and 1-7) and the ambient noise level (AN) as a control. All individuals were tested between 9:00 and 13:00.

Like for the water fleas, the response by the zebrafish to exposure to each of the four sound treatments (CS, 1-1, 1-4 and 1-7) and the control (AN) was recorded on video for 9 min per treatment (each time: 3 min before sound on-set and 6 min during sound exposure)
(see Fig. 3). We analyzed swimming behaviour with and without sound exposure by comparing the difference between 1 min just after sound on-set

and 1 min just before sound on-set. We determined the number of startle response differences (here defined as sudden peaks in swimming speed that were above 10 cm/s and associated with a distinct change in swimming direction), the swimming speed differences and the swimming depth differences (distance to the bottom).

Sound impact on zebra fish preying on water fleas

We investigated sound impact on foraging behaviour of zebrafish by following the test animals further during the sound exposure periods as described above. We introduced a group of 10 water fleas, again selected for equal size (~ 3 mm), together with 10 particles of duckweed leaves (~ 6-10 mm) as inedible targets, by gently pouring some water from a petri dish which contained the animals and plant particles. The visual presence of the investigator was obstructed by the non-transparent back of the experimental tank and only part of the hand was briefly in sight for the fish for all treatments. We first analyzed the initial response to water flea introduction by comparing swimming speed difference with and without sound exposure among treatments (measured by subtraction of swimming speed in the last 5 seconds before introduction from the first 5 seconds after introduction (see Fig. 3).

We subsequently measured sound impact on foraging efficiency by assessing two behavioural measures: food discrimination error and food handling error. The food discrimination error was determined by subdividing the number of attacks to inedible particles of duckweed by the total number of attacks to both the edible water fleas and the inedible

particles of duckweed. The food handling error was determined by subdividing the number of unsuccessful attacks to water fleas by the total number of unsuccessful and successful attacks to water fleas. Attacks could be unsuccessful because a zebrafish pursuit and bite missed the target or because a zebrafish lost control of a captured water flea that was released again.

As we were uncertain about whether zebrafish would get saturated and less eager to forage after sequential feeding bouts, we exposed individuals to period of sounds on two subsequent days and avoided the introduction of too many water fleas on a single day. We pseudo-randomly assigned whether or not a treatment was associated with the introduction of water fleas so that two or three of the treatments received water fleas on the first day. On the second day, we introduced water fleas during treatments which had not been associated with the introduction of water fleas on the first day yet. For assessing sound impact on foraging efficiency, we analyzed only those exposure periods in which we introduced water fleas, which were sometimes on day 1 and sometimes on day 2.

Processing behavioural data

We always started video recording (Fig. 3 - shaded light grey) well before automatic sound onset and continued sound exposure and video recording (Fig. 3 - shaded dark grey) well beyond the last period used for measurements (see Fig. 3). We converted all video files of water flea and zebrafish trials by reducing the temporal resolution to 5 frames per second. Video recordings were analyzed with Logger *Pro* (Vernier Software &

Technology, Beaverton, OR, USA, version 3.6.0), quantifying startle responses and measuring swimming speed and swimming depth. We quantified startle responses as the number of sudden peaks in swimming speed that were above 10 cm/s and associated with a distinct change in swimming direction. Video recordings were also used to assess food discrimination error and food handling error. All video analyses were done without audio track and therefore blind to the treatment sequence for the observer (SSS). A portion of the data was independently scored by a second observer (YYN), which confirmed inter-observer reliability.

Statistical analyses

We compared fish behaviour al changes caused by different sound treatments (CS, 1-1, 1-4, 1-7 and AN) by testing the difference between before and during sound exposure and before and after the introduction of water fleas using one-way repeated-measures ANOVA, with sound treatment as a fixed factor. The treatment exposure sequence was randomized to avoid order effects. Although the statistical power is low due to limited sampling of each treatment in each position in the sequence, we also checked statistically for an order effect by including the position of the treatment in the trial sequence as a random factor. We did not find an order effect in any of our test results (all $P > 0.1$). Data fitted the assumptions of normality and homoscedasticity for parametric testing for all measurements (if not immediately, after the data were log-transformed), except for the number of startle responses. When sphericity could not be assumed, we used Huynh-Feldt corrections. Whenever the outcome of the repeated-measures

ANOVA was significant, Bonferroni corrected post-hoc tests were performed for pairwise comparisons among the four different sound treatments and the ambient noise control. For the difference in number of startle responses the data fitted to a Poisson distribution and we therefore used a non-parametric test (Friedman test). All tests were done by SPSS statistics for windows, version 21.0. (Armonk, NY. IBM Corp.)

Ethical note

Water fleas were allowed to acclimatize gradually to the laboratory conditions before using them 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 sound playbacks and did not show any sign of anxiety or unusual swimming behaviours in their holding tanks after the experiments (c.f. Neo et al. 2015). All housing and experimental conditions were in accordance with the ethical guidelines of the Association for the Study of Animal Behaviour. The experiments were only carried out after evaluation and approval of the experimental procedure (DEC no: 10060) by the Animal Experiments Committee of Leiden University (UDEC).

Results

Sound impact on water fleas

Water flea swimming behaviour appeared to slow down for the ambient noise control and to speed up slightly during the sound treatments (Fig. 4). However, individual variability was high and this pattern did not result in an effect of sound treatment, as we did not find a significant treatment effect of sound exposure on non-targeted swimming speed (repeated- measures ANOVA: $F_{4, 36}=0.919$, $P= 0.464$) or swimming depth (repeated-measures ANOVA: $F_{4,36}=0.208$, $P= 0.849$). Water flea swimming speed showed highly variable patterns among and within individuals, but targeted swimming speed was twice as high compared to the non-targeted swimming speed (See table 1). However, there was also no significant effect of elevated sound levels on the targeted swimming speed for any of the treatments (repeated-measures ANOVA: $F_{4, 44}=0.624$, $P= 0.648$).
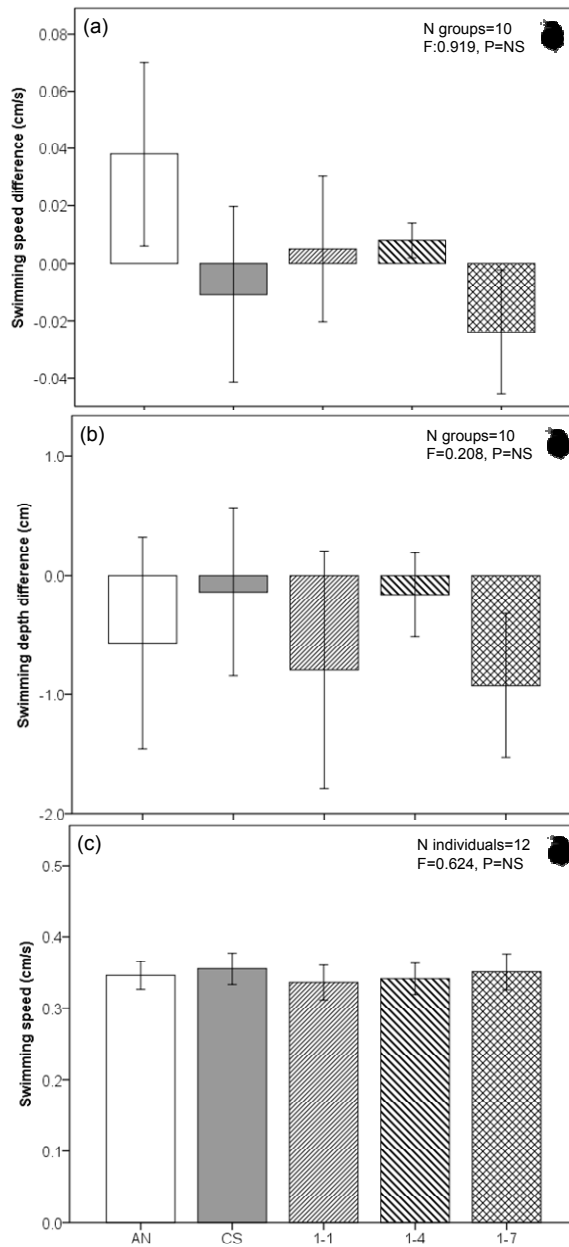
Fig. 4. Effect of sound exposure treatments on water flea behaviour: (a) water flea non-targeted swimming speed difference from the last min before to the first min during different treatments: continuous sound (CS), three intermittent treatments (1-1, 1-4 and 1-7) and ambient (AN) levels as control, (b) water flea swimming depth difference from the last

min before to the first min during the different treatments in the same test as the swimming speed score in (a). And (c) water flea swimming speed during the targeted-swimming mode (check Table 1 for absolute numbers) induced in a separate test by a light source on one side of a dark fish tank. Sample sizes were 5 x 10 = 50 individuals for (a) and (b) and 5 x 12 = 60 individuals for (c). Bars show means ± S.E.M. We found no significant effect of any sound exposure on either type of swimming speed or on swimming depth.

Sound impact on zebrafish

Sound exposure often led to an increase in the number of startle responses, sometimes in an increase in swimming speed and occasionally fish moved up in the water column (Fig. 5 a, b and c). The increase in number of startle responses upon exposure was significantly affected by treatment (Friedman chi-squared test: X^2_4=10.465, P =0.033). The sound-induced increase in startle responses was especially found for the intermittent exposures due to a growing number of individuals that exhibited increasingly more startle responses from CS, to 1-1, 1-4 and 1-7 (up to 19 startles, see table 1 for variation in the absolute number of startle responses among treatments). Post-hoc comparisons revealed that there was a significant difference between AN versus 1-4 (P=0.012) and AN versus 1-7 (P=0.003), and also a non-significant trend for CS versus 1-7 (P=0.058). There was also a significant effect of sound exposure treatment on swimming speed difference (repeated-measures ANOVA: $F_{4, 52}$=3.193, P= 0.020). Post–hoc comparisons revealed significant differences among treatments; for AN versus 1-1 (P=0.025), and 1-7 (P=0.032), and for 1-4 versus 1-7 (P=0.044). There was also a non-significant trend for a difference between CS and 1-7 (P=0.080). Swimming depth turned out to be quite

variable for the trials with sound exposures and the ambient noise control (AN) and we found no treatment effects for the difference in swimming depth between before and during sound exposure (repeated-measures ANOVA: $F_{4, 52}=0.869$, $P= 0.489$).
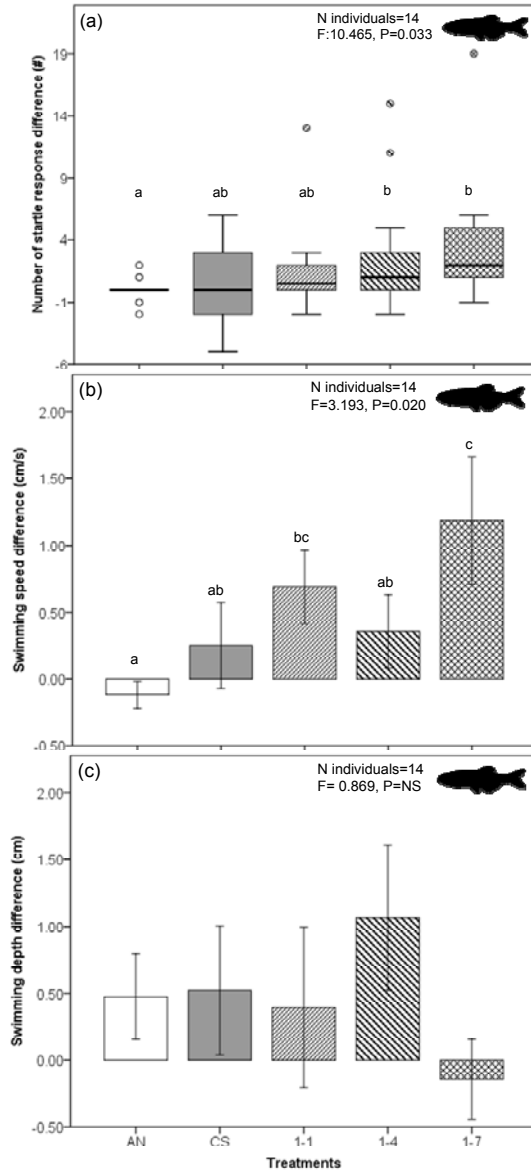
Fig. 5. Effect of sound exposure treatments on zebrafish behaviour: (a) Number of startle responses expressed as the difference between the first min during and the last min before exposure on-set: continuous sound (CS), three intermittent treatments (1-1, 1-4 and 1-7) and ambient (AN) levels as control. These count data are not normally distributed and box-

whisker plots show the median, lower and upper quartiles, extreme values and outliers (b) zebrafish swimming speed difference and (c) zebrafish swimming depth difference, both expressed as the difference between the first min during and the last min before exposure onset for the different treatments. The sample size was 14 individuals for repeated measurements on each of the four treatments and the control. Bars show means ± S.E.M. Treatments that differ significantly from one another are labeled with different letters a, b, and c (P<0.05). Non-significant trends (P<0.1) are not indicated, but just mentioned in the main text.

Sound impact on zebra fish preying on water fleas

Especially the intermittent sound exposure treatments seemed to affect zebrafish swimming in the initial response to the introduction of water fleas into the water, while there was no sound impact on food item discrimination. However, all sound treatments clearly affected food item handling (Fig. 6 a, b and c). For the initial swimming speed difference, we found a significant effect of treatments (repeated-measures ANOVA: $F_{4, 52}$=4.563, P= 0.003). All zebrafish hunted for water fleas and increased their swimming speed with the introduction of water fleas in all treatments and the control, but this was very rapid especially for CS and to a lesser extent for AN. Post-hoc comparisons revealed significant differences among treatments for swimming speed difference between the first 5 sec after introduction and the last 5 sec before introduction of the waterfleas: CS versus 1-1(P=0.010), 1-4 (P=0.002) and 1-7 (P=0.002), but not for CS versus AN (P= 0.136). There was no significant impact of sound exposure on food discrimination error for any of the sound treatments (repeated-measures ANOVA: $F_{4, 48}$=0.622, P=0.649). However, there was a clear and

significant effect of sound treatment on food handling error (repeated-measures ANOVA: $F_{4, 52}$=4.159, P=0.005). Post-hoc comparisons showed a significant and indiscriminant impact for all treatments in comparison to AN as the control group; CS (P=0.004); 1-1 (P=0.006), 1-4 (P=0.022), and 1-7 (P=0.009).
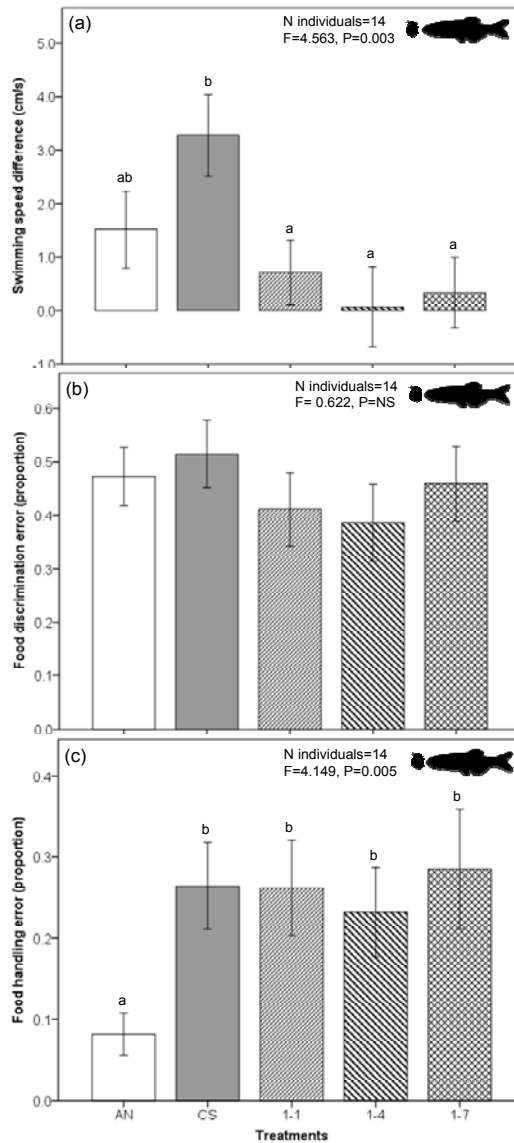
Fig. 6. Effect of sound exposure on zebrafish foraging behaviour: (a) difference in zebrafish swimming speed during sound exposure in the first 5 seconds after the introduction of water fleas subtracted from the swimming speed in the last 5 seconds before the introduction of water fleas for all treatments: continuous sound (CS), three intermittent treatments (1-1, 1-4 and 1-7) and ambient (AN) levels as control, (b) food discrimination

error as the proportion of duckweed particles attacked relative to the total number of attacks to both duckweed particles and water fleas with introduction of food items until the end of sound exposure in sequence for each zebrafish individual, and (c) food handling error as the proportion of the total of water fleas attacked that are missed or released again after initial grasping with onset of food introduction until the end of sound exposure in sequence for each zebrafish individual. Sample sizes were 14 individuals for repeated measurements on each of the four treatments and the control. Bars show means ± S.E.M. and treatments that differ significantly from one another are labeled with different letters a and b (P<0.05).

Discussion

We investigated potential effects of artificial noise exposure on underwater predator-prey interactions through testing the effect of temporal variation in experimental exposure on zebrafish hunting for water fleas under laboratory conditions. We were unable to detect effects of sound exposure on water flea swimming speed or depth but we found several significant effects on zebrafish. The zebrafish showed significantly more startle responses especially for two of the three intermittent sound exposures. This pattern was also reflected in an increased swimming speed for two (not the same two) of the three intermittent treatments. In contrast, there were no significant changes in zebrafish swimming depth in response to any of the treatments. Discrimination error in attacking edible water fleas or inedible duckweed particles was high and unaffected by sound exposure. However, foraging was affected in two ways: intermittent treatments significantly delayed initial acceleration response in swimming speed

relative to the continuous sound exposure and all sound exposure treatments caused a significant rise in handling error.

Sound impact on foraging fish

In comparison to earlier studies on sound impact on foraging fish, some of our data are confirmative; some are contrasting, and some concern new findings. The food handling error significantly increased in all sound treatments compared to the control: like the sticklebacks, the zebrafish often missed prey in the first strike and often had problems with handling the prey item before they could swallow under noisy conditions (c.f. Purser & Radford 2011; Voellmy et al. 2014), irrespective of the temporal pattern of exposure. In the earlier studies, sticklebacks also had a problem under noisy conditions in discrimination of water fleas from other small particles that happened to float in the water, which was less under more quiet conditions. In contrast, our zebrafish were indiscriminant in all of the treatment and control conditions in attacking both edible water fleas and inedible pieces of duckweed. This species discrepancy might be due to the fact that zebrafish seem much more active, explorative, and opportunistic foragers (e.g. Grant & Kramer 1992) than the more considerate and maybe more selective sticklebacks (e.g. Matthews et al. 2010).

A new finding in our study was the immediate increase in swimming speed with the introduction of water fleas in the continuous sound treatment and to a lesser extent in the ambient noise control, while the foraging onset seemed delayed in the intermittent sound treatments. This effect may be due to masking as the introduction of water fleas may be accompanied by an

auditory cue (sound associated with water fleas entering the water) that will be less easy to detect against the background of intermittent sounds than against a background of continuous sound levels. Alternatively, the intermittent sounds may have reduced the focus of the fish on foraging opportunities as they could be more aversive and may be perceived as potential danger for increased predation risk. Oswald & Robinson (2008) recently showed that aversive stimuli of mechanical, visual and chemical nature slow down foraging in zebrafish, which may also be true for acoustic stimuli depending on the sound level (Neo et al. 2015) as has also been shown for European minnows, which slow down their activities dramatically under experimental sound exposure (Voellmy et al. 2014).

We believe that the most likely explanation that can apply to both types of sound impact (the few seconds of response delay and the attack and handling problems) may be a general performance drop due to attentional shifts. This explanation has been suggested for sound-impact on non-auditory tasks in several different taxa (Chan et al. 2010; Purser & Radford 2011; Wale et al. 2013). In addition, sound exposure not only affected response latency to the water flea introduction and foraging efficiency, but also altered their swimming behaviour immediately after the on-set of the sound exposure (c.f. Neo et al. 2015). This behavioural change, which was true for relatively brief increases in swimming speed during two of the intermittent sound treatments, likely reflects the startle responses in the initial period of exposure. It seems that showing up to about five distinct startle responses in response to sudden on-set of sound exposure is a shared feature among different fish species tested in captivity. European minnows

and sticklebacks had very similar amounts of startle responses compared to our zebrafish and also showed a significant increase in number from exposure to white noise to a more variable exposure type of boat noise recordings (Purser & Radford 2011; Voellmy et al. 2014).

The zebrafish moved up towards the surface during earlier sound exposure experiments (Neo et al. 2015), which was occasionally seen again but did not lead to consistent and significant treatment effects on swimming depth in the current experiments. Using an in-air speaker results in slightly higher sound levels at the bottom of the tank than in the middle and upper layers, which could trigger the fish to move upward to escape high exposure levels. However, upward swimming responses may also suggest that the on-set of sounds from experimental exposure draws attention and may induce explorative behaviour (c.f. Neo et al. 2015). However, as we did not confirm this effect in the current data-set, the intermittent treatments may here actually be responsible for missing the initial cue for the introduction of waterfleas to the water. Masking or habituation to short sound pulses may be the mechanistic explanation of this effect, while general distraction may be the explanation for an effect on general performance level from continued presence of any sound pattern (c.f. Chan et al. 2010; Purser & Radford 2011; Wale et al. 2013).

Are water fleas not affected by sound?

Our results showed that water fleas do not change their swimming behaviour in response to the current sound exposure conditions and suggest that they are not sensitive to the elevated sound levels within the target

spectrum that do affect fish behaviour. This may seem surprising as there is considerable evidence that invertebrates perceive sound and use sound in social interactions, habitat defense, conspecific communication and directional orientation (Patek 2001; Popper et al. 2001; Stocks, 2012; Sueur et al. 2011; Vermeij et al. 2010). Invertebrate species are also known to be able to detect acoustic stimuli in variable ranges of the spectrum (Hughes et al. 2014; Popper et al. 2001; Stocks 2012). For example, free swimming larvae of coral reefs (*Montastraea faveolata*) were reported to exhibit both horizontal and vertical movements specifically towards playbacks of sounds recorded at reefs (Vermeij et al. 2010).

It could be that water flea behaviour is not affected by the sound exposure level that we created in our laboratory test condition, but that it would be affected at higher exposure levels. Further experiments are needed to exclude this possibility. Based on our particle motion measurements, we believe that using an in-air speaker to ensonify the experimental fish tank (as we used in our experiment) is a sufficient tool to generate high sound velocity levels. However, we may have to explore the impact of higher exposure levels in terms of particle motion by using under-water speakers. An alternative interpretation for the lack of a response in water fleas could be that the frequency range of our experimental exposure was outside their detection range. Although there are invertebrates sensitive to a wide frequency range that covers the current experimental spectrum, they may typically be more sensitive to lower frequencies (Packard et al. 1990; Lovell et al. 2005; Kaifu et al. 2008; Mooney et al. 2010). Important for our study

here is that the prey is not likely to have contributed to the pattern of sound-dependent foraging efficiency of the predator.

Sound impact beyond single-species effects

It is important to realize that our results are based on tests in laboratory settings and do not allow direct extrapolation to outdoor conditions in the field (c.f. Slabbekoorn, 2016). More applied insights for outdoor conditions and data on more long-term effects that amount to fitness consequences require more and different studies (Hawkins & Popper 2014; Radford et al. 2014; Slabbekoorn et al. 2010). However, the accumulating evidence for a possible impact of sound exposure on predator foraging performance means that consequences of sound pollution in the natural environment are also likely to go beyond single-species effects (Francis et al. 2009; Francis et al. 2011b; Francis et al. 2012a; Francis et al. 2012b; Shafiei Sabet et al. 2016; Slabbekoorn & Halfwerk 2009).

Changes in foraging tendency and efficiency may directly affect relative species abundance of both predator and prey and induce changes at the community level in a similar way as with underwater light pollution (e.g. Becker et al. 2013), changes in water turbulence or flow (e.g. Powers & Kittinger 2002) or chemical pollution (reviewed in Fleeger et al. 2003). Studies on the impact of acoustic changes in air in the terrestrial environment have already confirmed such effects of anthropogenic noise at the community level (Bayne et al. 2008; Francis et al. 2009, 2011a; 2011b; Francis et al. 2012a; 2012b). Consequently, we need to be on the look-out

for negative effects of anthropogenic noise on underwater food web dynamics and stability in both freshwater and marine environments.

Conclusion

Our current study does not provide evidence for an effect of artificial noise on water flea swimming behaviour, but clearly reveals an effect of experimental sound exposure on individual zebrafish swimming behaviour and foraging efficiency while hunting for water fleas. It seems that several fish species are affected by sound exposure in terms of foraging and that the impact is due to effects on the vertebrate predator instead of the invertebrate prey (Purser & Radford 2011; Voellmy et al. 2014). Furthermore, our study also reveals significant effects in terms of temporal variation, as intermittent sound treatments had stronger and different effects than continuous sound. We believe this is a relevant finding as the 'natural' occurrence of anthropogenic noise is characterized by highly variable conditions and intermittent sounds are almost omnipresent. Our laboratory study should not be extrapolated directly to outdoor conditions, but calls for investigation of behavioural responses of free-ranging fish to sound exposures of different temporal patterns. Also under natural conditions, anthropogenic noise may affect species interactions and may have community level consequences that are important to the stability and dynamics of aquatic ecosystems.

Acknowledgements

References

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(6), 3073-3082.

Amoser, S., Wysocki, L. E., & Ladich, F. (2004). Noise emission during the first powerboat race in an Alpine lake and potential impact on fish communities. The Journal of the Acoustical Society of America, 116(6), 3789-3797.

Andersson, M. H., Dock-Åkerman, E., Ubral-Hedneberg, R., Ohman, M. C., & Sigray, P. (2007). Swimming behavior of Roach (*Rutilus rutilus*) and three-spined stick- leback (*Gasterosteus aculeatus*) in response to wind power noise and single-tone frequencies.

Andrew, R. K., Howe, B. M., Mercer, J. A., & Dzieciuch, M. A. (2002). Ocean ambient sound: comparing the 1960s with the 1990s for a receiver off the California coast. Acoustics Research Letters Online, 3(2), 65-70.

ANSI/ASA S1.1. (2013). American National Standards Institute. Acoustical terminology. Melville, NY: Acoustical Society of America. https://global.ihs.com/home_page_ asa.cfm?&rid¼ASA

Bayne, E. M., Habib, L., & Boutin, S. (2008). Impacts of chronic anthropogenic noise from energy-sector activity on abundance of songbirds in the boreal forest. Conservation Biology, 22(5), 1186-1193.

Becker, A., Whitfield, A. K., Cowley, P. D., Järnegren, J., & Næsje, T. F. (2013). Potential effects of artificial light associated with anthropogenic infrastructure on the abundance and foraging behaviour of estuary-associated fishes. Journal of Applied Ecology, 50(1), 43-50.

Bretschneider, F., van Veen, H., Teunis, P. F., Peters, R. C., & van den Berg, A. V. (2013). Zebrafish can hear sound pressure and particle motion in a synthesized sound field. Animal Biology, 63(2), 199-215.

Cachat, J., Canavello, P., Elegante, M., Bartels, B., Hart, P., Bergner, C., Egan, R., Duncan, A., Tien, D., Chung, A. & Wong, K., Wong, K. (2010). Modeling withdrawal syndrome in zebrafish. Behavioural Brain Research, 208(2), 371-376.

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(4), 458-461.

Codarin, A., Wysocki, L. E., Ladich, F., & Picciulin, M. (2009). Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Marine pollution bulletin,58(12), 1880-1887.

Dodson, S. I., Tollrian, R., & Lampert, W. (1997). Daphnia swimming behavior during vertical migration. Journal of Plankton Research, 19(8), 969-978.

Dukas, R. (2002). Behavioural and ecological consequences of limited attention. Philosophical Transactions of the Royal Society B: Biological Sciences, 357(1427), 1539-1547.

Ebert, D. (2005). Ecology, epidemiology, and evolution of parasitism in Daphnia. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information.

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., & Mohnot, S. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioural brain research, 205(1), 38-44.

Fay, R. (2009). Soundscapes and the sense of hearing of fishes. Integrative Zoology, 4(1), 26-32.

Fay, R. R., & Popper, A. N. (1974). Acoustic stimulation of the ear of the goldfish (*Carassius auratus*). Journal of Experimental Biology, 61(1), 243-260.

Fleeger, J. W., Carman, K. R., & Nisbet, R. M. (2003). Indirect effects of contaminants in aquatic ecosystems. Science of the Total Environment, 317(1), 207-233.

Francis, C. D., Ortega, C. P., & Cruz, A. (2009). Noise pollution changes avian communities and species interactions. Current biology, 19(16), 1415-1419.

Francis, C. D., Ortega, C. P., & Cruz, A. (2011a). Noise pollution filters bird communities based on vocal frequency. PLoS ONE 6(11): e27052. doi:10.1371/journal.pone.0027052.

Francis, C. D., Ortega, C. P., & Cruz, A. (2011b). Vocal frequency change reflects different responses to anthropogenic noise in two suboscine tyrant flycatchers. Proceedings of the Royal Society of London B: Biological Sciences, 278(1714), 2025–2031.

Francis, C. D., Kleist, N. J., Ortega, C. P., & Cruz, A. (2012a). Noise pollution alters ecological services: enhanced pollination and disrupted seed dispersal. Proceedings of the Royal Society of London B: Biological Sciences, 279(1739), 2727-2735.

Francis, C. D., Kleist, N. J., Davidson, B. J., Ortega, C. P., & Cruz, A. (2012b). Behavioral responses by two songbirds to natural-gas-well compressor noise. Ornithological Monographs, 2012(74), 36-46.

Gaikwad, S., Stewart, A., Hart, P., Wong, K., Piet, V., Cachat, J., & Kalueff, A. V. (2011). Acute stress disrupts performance of zebrafish in the cued and spatial memory tests: The utility of fish models to study stress–memory interplay. Behavioural processes, 87(2), 224-230.

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(4), 752-761.

Grant, J. W., & Kramer, D. L. (1992). Temporal clumping of food arrival reduces its monopolization and defence by zebrafish, *Brachydanio rerio*.Animal Behaviour, 44(1), 101-110.

Gulati, R. D. (1990). Structural and grazing responses of zooplankton community to biomanipulation of some Dutch water bodies. Hydrobiologia, 200(1), 99-118.

Gutscher, M., Wysocki, L. E., & Ladich, F. (2011). Effects of aquarium and pond noise on hearing sensitivity in an otophysine fish. Bioacoustics, 20(2), 117-136.

Hawkins, A., & Popper, A. (2014). Assessing the impact of underwater sounds on fishes and other forms of marine life. Acoustics Today, 10,30e41.

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(2), 174-184.

Hughes, A. R., Mann, D. A., & Kimbro, D. L. (2014). Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. Proceedings of the Royal Society of London B: Biological Sciences, 281(1788), 20140715.

Kaifu, K., Akamatsu, T., & Segawa, S. (2008). Underwater sound detection by cephalopod statocyst. Fisheries Science, 74(4), 781-786.

Lagardère, J. P. (1982). Effects of noise on growth and reproduction of *Crangon crangon* in rearing tanks. Marine Biology, 71(2), 177-185.

Ladich, F. (2004). Sound production and acoustic communication. In The Senses of Fish: Adaptations for the Reception of Natural Stimuli (Von der Emde, G., Mogdans, J. & Kapoor, B. G., eds), 210–230. New Delhi: Narosa Publishing House.

Lovell, J. M., Findlay, M. M., Moate, R. M., & Yan, H. Y. (2005). The hearing abilities of the prawn Palaemon serratus. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 140(1), 89-100.

Matthews, B., Marchinko, K. B., Bolnick, D. I., & Mazumder, A. (2010). Specialization of trophic position and habitat use by sticklebacks in an adaptive radiation. Ecology, 91(4), 1025-1034.

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(2), 711-718.

Mendl, M. (1999). Performing under pressure: stress and cognitive function.Applied Animal Behaviour Science, 65(3), 221-244.

Mooney, T. A., Hanlon, R. T., Christensen-Dalsgaard, J., Madsen, P. T., Ketten, D. R., & Nachtigall, P. E. (2010). Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. The Journal of experimental biology, 213(21), 3748-3759.

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 of London B: Biological Sciences,281(1776), 20132683.

Neo, Y. Y., Seitz, J., Kastelein, R. A., Winter, H. V., Ten Cate, C., & Slabbekoorn, H. (2014). Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biological Conservation, 178, 65-73.

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.

Oswald, M., & Robison, B. D. (2008). Strain-specific alteration of zebrafish feeding behavior in response to aversive stimuli. Canadian Journal of Zoology, 86, 1085e1094.

Packard, A., Karlsen, H. E., & Sand, O. (1990). Low frequency hearing in cephalopods. Journal of Comparative Physiology A, 166(4), 501-505.

Patek, S. N. (2001). Spiny lobsters stick and slip to make sound. Nature, 411(6834), 153-154.

Parvulescu, A. (1967). The acoustics of small tanks. In: Marine Bio-Acoustics, Vol. II (Ed. by W. N. Tavolga). Oxford: Pergamon Press, 7-13

Picciulin, M., Sebastianutto, L., Codarin, A., Farina, A., & Ferrero, E. (2010). In situ behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacen- tridae) living in a Marine Protected Area. Journal of Experimental Marine Biology and Ecology, 386,125e132.

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(4), 35-40.

Popper, A. N., Salmon, M., & Horch, K. W. (2001). Acoustic detection and communication by decapod crustaceans. Journal of Comparative Physiology A, 187(2), 83-89.

Popper, A. N., Hawkins, A. D., Fay, R. R., Mann, D., Bartol, S., Carlson, T., Coombs, S., Ellison, W.T., Gentry, R., Halvorsen, M.B. & Lokkeborg, S., (2014). 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.

Powers, S. P., & Kittinger, J. N. (2002). Hydrodynamic mediation of predator–prey interactions: differential patterns of prey susceptibility and predator success explained by variation in water flow. Journal of Experimental Marine Biology and Ecology, 273(2), 171-187.

Purser, J., & Radford, A. N. (2011). Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS ONE 6(2): e17478. doi:10.1371/journal.pone.0017478.

Quinn, J. L., Whittingham, M. J., Butler, S. J., & Cresswell, W. (2006). Noise, predation risk compensation and vigilance in the chaffinch Fringilla coelebs. Journal of Avian biology, 37(6), 601-608.

Radford, A. N., Kerridge, E., & Simpson, S. D. (2014). Acoustic communication in a noisy world: can fish compete with anthropogenic noise?. Behavioral Ecology, 25(5), 1022-1030.

Radford, C. A., Jeffs, A. G., & Montgomery, J. C. (2007). Directional swimming behavior by five species of crab postlarvae in response to reef sound. Bulletin of Marine Science, 80(2), 369-378.

Richardson, W. J., Greene, C. R., Jr., Malme, C. I., & Thomson, D. H. (1995). Marine mammals and noise. New York, NY: Academic Press.

Schilling, T. F. (2002). The morphology of larval and adult zebrafish. In S. Schulte- Merker, & C. Nusslein-Volhard (Eds.), Zebrafish: A practical approach (pp. 59e94). Oxford, U.K.: Oxford University Press.

Sebastianutto, L., Picciulin, M., Costantini, M., & Ferrero, E. A. (2011). How boat noise affects an ecologically crucial behaviour: the case of territoriality in *Gobius cruentatus* (Gobiidae). Environmental Biology of Fishes, 92, 207e215.

Shafiei Sabet, S., Neo, Y. Y., & Slabbekoorn, H. (2016). Impact of Anthropogenic Noise on Aquatic Animals: From Single Species to Community-Level Effects. In The Effects of Noise on Aquatic Life II (pp. 957-961). Springer New York.

Siemers, B. M., & Schaub, A. (2010). Hunting at the highway: traffic noise reduces foraging efficiency in acoustic predators. Proceedings of the Royal Society B: Biological Sciences. rspb20102262.

Slabbekoorn, H. (2016). Aiming for progress in understanding underwater noise impact on fish: complementary need for indoor and outdoor studies. InThe Effects of Noise on Aquatic Life II (pp. 1057-1065). Springer New York.

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(7), 419-427.

Slabbekoorn, H., & Halfwerk, W. (2009). Behavioural ecology: noise annoys at community level. Current Biology, 19(16), 693-695.

Stanley, J. A., Radford, C. A., & Jeffs, A. G. (2011). Behavioural response thresholds in New Zealand crab megalopae to ambient underwater sound. PLoS One, 6(12), e28572. doi:10.1371/journal.pone.0028572.

Stocks, J. R., Broad, A., Radford, C., Minchinton, T. E., & Davis, A. R. (2012). Response of marine invertebrate larvae to natural and anthropogenic sound: A Pilot Study. The Open Marine Biology Journal, 6(1), 57–61.

Sueur, J., Mackie, D., & Windmill, J. F. (2011). So small, so loud: Extremely high sound pressure level from a pygmy aquatic insect (Corixidae, Micronectinae). PloS one, 6(6), e21089. doi:10.1371/journal.pone.0021089.

Van den Berg, A. V., & Schuijf, A. (1985). Acoustics of a standing wave tank for studying the hearing capacity of fish. The Journal of the Acoustical Society of America, 78(1), 12-16.

Van Gool, E., & Ringelberg, J. (1995). Swimming of Daphnia galeata x hyalina in response to changing light intensities: influence of food availability and predator kairomone. Marine & Freshwater Behaviour & Phy, 26(2-4), 259-265.

Vasconcelos, R. O., Simões, J. M., Almada, V. C., Fonseca, P. J., & Amorim, M. (2010). Vocal Behavior During Territorial Intrusions in the Lusitanian Toadfish: Boatwhistles Also Function as Territorial 'Keep-Out'Signals. Ethology, 116(2), 155-165.

Vermeij, M. J., Marhaver, K. L., Huijbers, C. M., Nagelkerken, I., & Simpson, S. D. (2010). Coral larvae move toward reef sounds. PLoS ONE 5(5): e10660. doi:10.1371/journal.pone.0010660.

Voellmy, I. K., Purser, J., Flynn, D., Kennedy, P., Simpson, S. D., & Radford, A. N. (2014). Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Animal Behaviour, 89, 191-198.

Wale, M. A., Simpson, S. D., & Radford, A. N. (2013). Noise negatively affects foraging and antipredator behaviour in shore crabs. Animal Behaviour, 86(1), 111-118.

Weilgart, L. S. (2007). A brief review of known effects of noise on marine mammals. International Journal of Comparative Psychology, 20(2), 159-168.

Wysocki, L. E., Dittami, J. P., & Ladich, F. (2006). Ship noise and cortisol secretion in European freshwater fishes. Biological Conservation, 128(4), 501-508.

Treatment:	AN	CS	1-1	1-4	1-7	Fig.
Waterflea non-targeted swimming speed (cm/s)	0.22±0.05	0.17±0.03	0.20 ± 0.03	0.14±0.02	0.14±0.02	4a
Waterflea swimming depth (cm)	7.38±4.28	3.43±2.23	6.42±1.22	7.69±1.80	8.28±1.35	4b
Waterflea targeted swimming speed (cm/s)	0.35±0.02	0.36±0.02	0.34±0.02	0.34±0.02	0.35±0.03	4c
Zebrafish number of startle response (#)	0.93±0.67	2.57±0.93	2.71±1.20	3.57±1.80	4.43±1.58	5a
Zebrafish swimming speed (cm/s)	3.16±0.33	4.06±0.30	4.01±0.24	4.16±0.481	4.49±0.57	5b
Zebrafish swimming depth (cm)	5.94±0.94	6.09±0.70	5.75±0.88	5.73±0.83	4.33±0.62	5c
Zebrafish swimming speed response to food (cm/s)	5.31±0.65	6.96±0.75	5.40±0.44	5.26±0.60	4.85±0.53	6a
Zebrafish food discrimination error (%)	0.47±0.06	0.51±0.07	0.41±0.08	0.42±0.08	0.46±0.08	6b
Zebrafish food handling error (%)	0.08±0.03	0.26±0.06	0.26±0.07	0.23±0.06	0.29±0.09	6c

Table 1. Absolute values for mean (±SE.M) behavioural measurements of waterfleas and zebrafish. Swimming speed and depth , as well as the number of zebrafish startle responses and the food discrimination and handling error, during the ambient noise control (AN) and during the different sound treatments (CS=continuous sound; 1-1=intermittent, regular, fast; 1-4= intermittent, regular, slow; 1-7=intermittent, irregular, slow). The absolute numbers correspond with the relative numbers on changes, related to the periods before and during sound on-set or before and during waterflea introduction, reported in the respective figures indicated in the column on the far right