Annoying noise: effect of anthropogenic underwater noise on movement and feeding performance in the red cherry shrimp *Neocaridina davidi*

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Abstract

Acoustic pollution in aquatic environments has increased at a dramatic rate with adverse effects on many organisms. Benthic organisms, including many invertebrates, are capable of sensing underwater sounds, yet the responses they trigger on organisms have received little attention. This study investigates the impact of underwater sound on the behaviour of the red cherry shrimp *Neocaridina davidi* as a model of freshwater decapod. We assessed the effect of underwater sound exposure on movement behaviour and feeding performance of shrimps individually. The movement speed in sound and control treatments, showed a significant decrease as soon as opening the divider, but there were no significant changes between total minutes of control and sound treatments implying no sound-related initial changes for releasing movement. The spatial distribution of shrimps in response to the sound treatment showed significant changes; shrimps spent more time at the farthest point from the sound source. The time to find the food source (latency) also increased significantly in the sound treatment compared to control. Moreover, in terms of the number of success and failure in finding the food source in the control treatment, significantly more shrimps were succeeded to find the food source. Besides, the number of re-visiting the food source decreased in sound treatment compared to control and more shrimps were significantly distracted in sound treatment. Our study highlights the crustacean´s ability to receive human-made sound. Thus, they are prone to the impacts of anthropogenic noise causing negative impacts on their movement-swimming activities, feeding behaviour and exposing them to potential predator threat. Affecting foraging performance in this gregarious species may even

have detrimental impacts on their reproductive success and subsequently unexpected fitness consequences.

Keywords: Behaviour, Ornamental shrimp, Feeding performance, Noise pollution, Movement activities, Invasive species.

Introduction

Over recent decades, environmental pollution caused by human activities has altered terrestrial and aquatic habitats on a global scale (Kight and Swaddle, 2011). Aquatic animals use signals and exploit cues to explore their habitat and to perform a wide range of biological activities (Kight and Swaddle, 2011). In aquatic habitats, anthropogenic sensory pollutants such as chemical contaminants (Tornero and Hanke, 2016), artificial light at night (ALAN) (Gaston et al., 2015) and anthropogenic noise (Kunc et al., 2015, Peng et al., 2015) have quite diversely changed the chemical components, daylight luminance and acoustic characteristics of the environment globally, and subsequently encountered many species under threat of extinction. All these sources of pollutants are prominent and pervasive and can be perceived by aquatic animals via different sensory mechanisms, modalities, organs. Animals rely on their sensory organs in different modalities (e.g. smell, vision and hearing) to perceive and process a range of information (signals and cues) of their habitats (Ukaogo et al., 2020). The ability to exploit such habitat information is crucial and prerequisite for survival, and any interference with these sensory systems due to introducing novel stimuli may affect detrimentally their successfulness in feeding efficiency, communication with con-specifics, locating preys, avoiding predators, and courtship performances (Dominoni et al., 2020). In the aquatic environment, sound as an informative sensory stimulus, is a key signal due to the fact that it is not attenuated as fast as chemical or light pollutants, and can extremely be propagated over long distances (Hawkins and Myrberg, 1983).

Noise pollution is omnipresent aquatic habitats and may affect large areas therefore negatively affect individual species, as well as changing species interactions and animal communities (Kunc et al., 2016; Shannon et al., 2016; Slabbekoorn et al., 2010, 2019, De Jong et al., 2018). Although, the underwater realm has long been considered a “silent world” (Cousteau and

Dumas, 1987), studies performed in recent decades have revealed that the underwater environment is full of sounds and vibrations (see Williams et al., 2015), which can arise from natural sources such as waves, rain and currents or from biotic sources such as animal vocalization, displacement, hunting and feeding (Gordon and Tyack, 2001; Stocker, 2002; Scott, 2004). Moreover, anthropogenic activities also contribute to underwater sound and vibration through a variety of activities, including construction, resource extraction, transportation, aquaculture, fishing and recreation (Slabbekoorn et al., 2010). The marked increase in anthropogenic noise (Frisk, 2012) and associated vibration has consequently changed the underwater acoustic landscape (McDonald et al., 2006; Andrew et al., 2011; Duarte et al., 2021). Shipping noise, motorboat sound and recreational boating are the main source of anthropogenic noise in most of the world's oceans (Hildebrand, 2009) and freshwater habitats (Mickle & Higgs, 2018) with a typical continuous acoustic temporal pattern being the most dominant within the low frequency portion of the spectrum (propulsion systems of ships: <200 Hz). Moreover, pumping systems, aerators and feeding machines contribute in producing continuous sound patterns in fish facility, laboratory and ornamental pet shop centers (Forouharmajd et al., 2012).

Research on the impact of anthropogenic sound in the aquatic environment has focused historically on marine mammals (Androw et al., 2007; Payne and Webb, 1971; Richardson et al., 1995; Nowacek et al., 2007; Southall et al., 2008) and more recently on fishes (Popper, 2003; Hawkins et al., 2014; Bruintjes et al., 2015; Simpson et al., 2016; Herbert-Read et al., 2017), while the potential impacts on invertebrates have received less attention (Wale et al., 2013; Roberts et al., 2015, 2016; Williams et al., 2015). Although invertebrates are quite diverse, important for all trophic levels and have relatively huge abundance in both marine and freshwater environments (Morley et al., 2014; Solan et al., 2016), their capability to detect and potentially exploit sound is not relatively known. Marine decapods play an important role in top-down control pray populations and can shift the abundance and community structure in aquatic habitats (Reise, 1979, Bell and Coull, 1978) and the potential impacts of sound may cause cascading impacts on many species in a variety of taxa (Slabbekoorn et al., 2010; Shafiei Sabet et al., 2016a,b). In contrast to marine habitats, given the broad-ranging diet of the red cherry shrimp it is likely that this species also influences food webs in freshwater habitats. The potential effects of anthropogenic noise on freshwater decapods and their role to regulate top-down and button -top control of predator-prey interaction and feeding activities are largely unknown.

Elevated sound levels could affect foraging behaviour in three main ways, which are not mutually exclusive. First, noise could act as a stressor (Williams et al., 2015), decreasing feeding behaviour directly through reduced appetite (Charmandari et al., 2004), or indirectly through a reduction in activity and locomotion (Mendl, 1999) or alterations to the cognitive processes involved in food detection, classification and decision making (De Kloet et al., 1999; Lupien & McEwen, 1997). Second, noise could act as a distracting stimulus, diverting an individual's limited amount of attention from their primary tasks to the noise stimuli that have been added to the environment (Chan & Blumstein, 2011; Mendl, 1999). This could impair foraging success if, for instance, suitable food sources are detected less often or more slowly, are assessed less accurately, or if prey items are mishandled (Purser & Radford, 2011). Third, noise could mask crucial acoustic cues (Brumm & Slabbekoorn, 2005). If cues produced by prey are masked, feeding opportunities may be missed (Schaub et al., 2008; Siemers & Schaub, 2011). If acoustic predator cues are masked and animals compensate by relying on visual information to a greater extent (Quinn et al., 2006), then visually guided food searching and acquisition might be compromised.

The red cherry shrimp (*Neocaridina davidi*) is a tiny and gregarious benthic freshwater invertebrate species of shrimp with a wide distribution, occurring along the Southeast Asia to the rivers of Europe (Wowor et al., 2004; Liang, 2004; Klotz et al., 2013, Onuki & Fuke, 2022). This species is used as an ornamental crustacean and provides an important link between trophic levels and food webs (Kelly et al., 2012, Weber and Traunspurger, 2016); its behavioural responses to stress stimuli are still poorly understood. To our knowledge, no previous research has addressed behavioural changes of the red cherry shrimp in response to sound exposure.

In this study, two experiments were conducted to analyse the effect of sound exposure on locomotor behaviour and feeding activities of the red cherry shrimp under laboratory conditions. Experimental studies conducted by manipulating the system under laboratory conditions enhance control of other possible confounding factors and sources of unwanted variability. In the first experiment, we evaluated the initial releasing movement speed, spatial distribution, and food finding latency: the time to find the food source; the number of success and failure in finding the food source, the number of revisiting to the food source in response to ambient sound conditions as control and elevated generated sound level. In the second experiment, sound-related feeding distraction was investigated in response to ambient sound conditions as control and elevated

generated sound level. Red cherry shrimps were exposed to a 400-2000 Hz acoustic stimulus generated under laboratory conditions mimicking a frequency bandwidth range that they can detect and represents most of the frequencies produces by human activities and shrimps can be exposed in their natural habitats and under rearing facility centers (Lovell et al., 2005; Slater et al., 2020).

Material and methods

Ethical Note

We adhered to the Guidelines for the treatment of animals in behavioural research and teaching (ASAB, 2020). There are no legal requirements for studies involving crustacean in Iran.

Study Animals and Husbandry

All shrimps were purchased from an aquatic pets local dealer in Rasht city, Iran. For acclimation to the new tank conditions, the animals were transferred to a glass holding tank (Figure 1) (48 × 28 × 32 cm and 4 mm wall thickness) for one week equipped with a 150 watts heater, sponge biofilter, Java moss, natural plant, 12 watt LED light (Model: 2330, Made in China), sandy bed and plant soil Ista® company premium model. Water temperature was kept at 26±1°C, aeration 5L/min and the light period was also (14 D:10 L) while. Water quality was maintained within safe parameters. Shrimps were fed every 12 hours with *Spirulina* algae tablets with any excess cleared from the holding tank during tank maintenance no more than 4 h after feeding. Although there was a constant water change within the holding tank, 25% of water was removed by siphon with excess food and waste; this water was replaced by normal tank flow-through. Water changes and the flow-through system ensured maintenance of constant oxygen levels and the removal of any metabolic waste materials. It should be noted that the physical and chemical conditions such as ambient acoustics, water temperature, water changes, and lack of stress hormones for both tanks (holding and experiment tanks) were similar. The sex ratio of shrimp was (1:1), and all mature shrimps had a body length within the range 2-2.5cm).

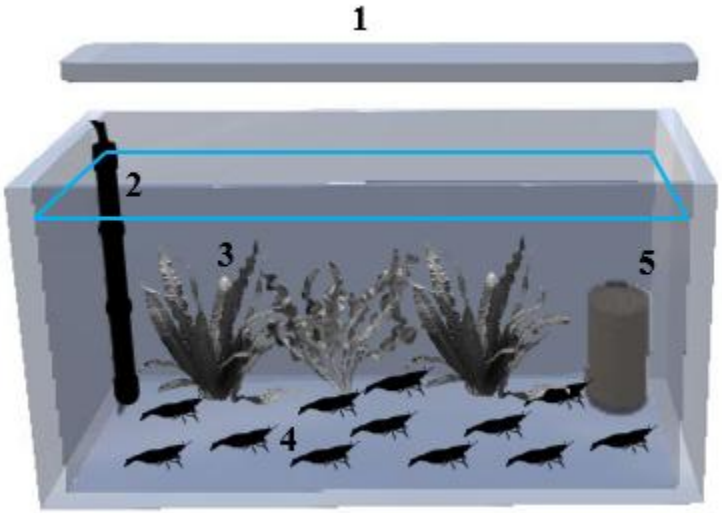


Fig. 1. Experimental configuration to maintain *Neocaridina davidi* in the holding aquarium. Set up of the holding tank: 48 × 28 × 32 cm and 4 mm wall thickness. 1) LED; 2) heater; 3) aquatic plant; 4) shrimps and 5) sponge filter.

General Experimental Design

Both experiments were conducted in a tank (40 × 30 × 30 cm) with walls 4 mm thick, equipped with two divider plates and dewatering surface of 20 cm. The following chemical and physical parameters of water were measured: Water temperature, pH, total dissolved solids (TDS), light intensity and also NO_3^--, NO_2^- , NH_4^- and pH = 7.2±2 was measured by pH meter (model: SANA SL-901), TDS (Total Dissolved Solids) = 160±1 (ppm) and was measured by TDS meter (model: TDS-3, made in China), light intensity= 113.8±2 lux was measured by a Lux meter (model: Digital Lux Meter-GM1010), temperature= 26±1 °C was measured by mercury thermometers, and NO_3^--, NO_2^- and NH_4^- were respectively; <2, 0 and 0.21 mg/l were measured by, Bante321-NO3 Portable Nitrate Ion Meter, JBL Nitrite Test Kit, Bante321-NH4 Portable Ammonium Ion Meter, respectively. The light intensity was recorded at the beginning and end of each treatment. Other parameters were measured before each treatment. The exact location of light intensity was measured daily before and after each experiment in a fixed place at a height of 20 cm above the water level and also on the bottom of the aquarium in the middle of the

aquarium. At each of two experiments, 35 shrimps were used individually for each treatment with alternation of both males and females. Also, in order to eliminate the effects of test time on the treatments, the order of control and sound treatments were applied one by one. The playback speaker was placed behind a vertical partition situated 6 cm from one short side. Experimental tank was placed on 5 cm thick foam pipe lagging, to minimize sound transfer, and it had the sides covered with opaque black paint to all the height, to minimize distraction from visual reflections.

Sound characteristics

The sounds used in this study were made by specialized software (Audacity-win-vr 2.3.1), based on Shafiei Sabet et al. (2015). The ambient sound frequency was 400 to 2000 Hz, intensity of 96.54±1 dB in WAV format. For playback sound treatment, a type of sound with a continuous pattern and frequency of 400 to 2000 Hz, sound intensity of 110.40 ±1 and WAV format was used. The reason for choosing continuous sound with this frequency range is the similarity of this continuous sound pattern with the sound of motor boats, aeration pumps and filtration systems that are commonly used in farms (Peng et al., 2015; Forouharmajd et al., 2012). A 30-watt hand-built underwater speaker with a frequency of zero to 3,000 Hz, a model laptop (Lenovo-V110), an amplifier, and a 12 V sealed battery were used to supply the required power.

Sound measurement and analysis

The sound emitted in the experimental aquarium environment was measured by a sound measuring device model (TASCAM-DR-100MK2) equipped with a calibrated hydrophone by a specialized standard method (Shafiei Sabet et al., 2015). Then, the sounds data were transferred to the software (Rstudio - vr-3.6.3) for analysis under specialized acoustic programming and the sound intensity (dB ref 1µPa), frequency (Hz), sensitivity and sample rate were measured. Finally, graphs of sound spectrum that indicates the amount of sound energy (Figure 2) and sound pressure level or SPL per unit (dB ref 1µPa2 / Hz) were drawn by the software (Figure 3).

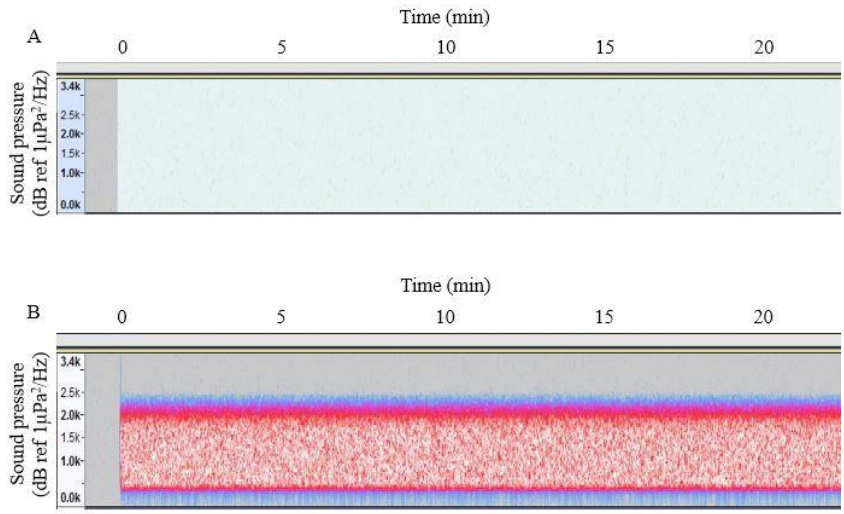


Fig. 2. Spectrogram (for 20 s time slot) magnification of A) Control and B) Sound wave (Sound treatments) used in the experiment: frequency (kHz) vs. time (min). The intensity is reflected by the colour scale (dB re 1 lPa ^{2}/Hz).

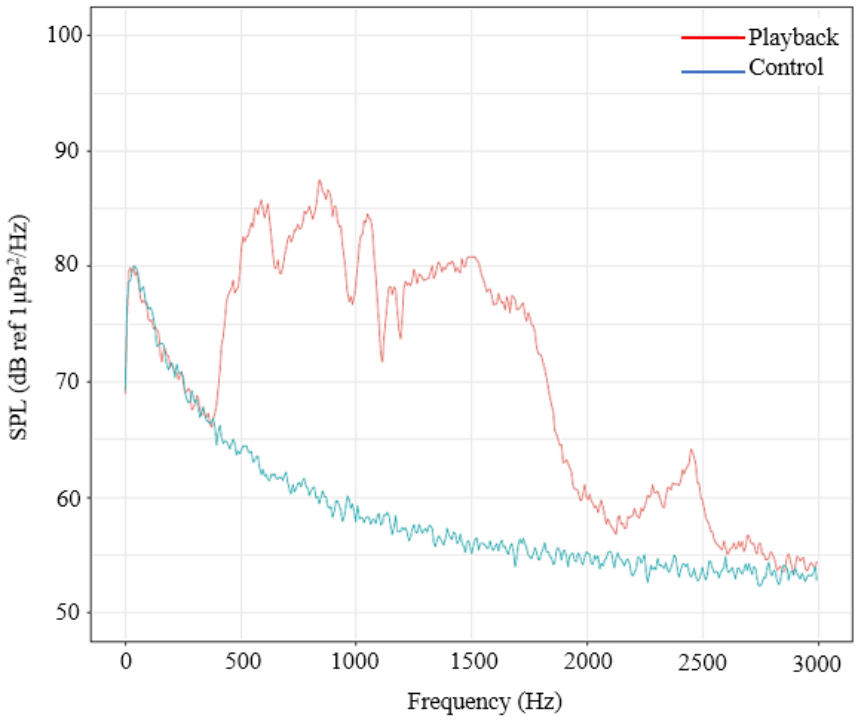


Fig. 3. Mean power spectrum of the sound stimuli (red line) and of the experimental tank background noise (blue line). Sound Pressure Level expressed in dB re 1 µPa^2/Hz (Welch's method, Hann window, FFT length 1024).

The first experiment: movement speed, spatial distribution, food finding latency, number of revisiting and food-finding

In this experiment, before starting the main experiments, shrimps (35 individuals per treatment and a total of 70 individuals) were starved for 48 hours in the holding tank. Then, in order to acclimation to the experiment tank (Figure 4), 1 hour before the test, they were individually transferred to the starting area behind a divider plate. All transferring procedures were gently and same for trials. At this time the food source was added to the other side of the divider plate (see figure 4). In the control treatment, at the same time with the divider plate being removed (2 cm upwards from the bottom of the aquarium, with minimal physical movement of the divider plate so that less stress is applied to the shrimp and it can pass through it), the control playback was played for 20 minutes (Figure 5a). Each shrimp was given a maximum of 10 minutes to find food source. Also in the sound playback, it played for 20 minutes (the same exposure duration just like ambient treatment as control) at the same time as the divider plate was removed (in a completely similar way) and each shrimp was given a maximum of 10 minutes to find the food source (Figure 5b). The behavioural indicators (Table 1) measured in this section are: the initial releasing movement speed, spatial distribution, food finding latency: the time took to find the food source, finding-food as: The number of success and failure in finding the food source and the number of revisiting to the food source in both treatments.

Table 1 Description of behavioural actions by the Red cherry shrimp (*Neocaridina davidi*) registered during the experimental tests, based on videotapes visualizations.

Behaviour	Description	Unit	Application test
Movement speed	Mean distance moved by the center point of the subject per unit time	Centimetres/seconds	Single
Spatial distribution	The mean attendance of individuals in a specific area	Seconds & Centimetres	Single
Food-finding	Number of individuals that succeed to find the food source	Number of individuals	Single
Feeding latency	Mean time to find the food source	Seconds	Single
Number of revisiting	Number of revisiting to food source for a certain period of time	Number of individuals	Single
Feeding distraction	Number of individuals were complete freezing and movement away from the food after sound playback	Number of individuals	Single

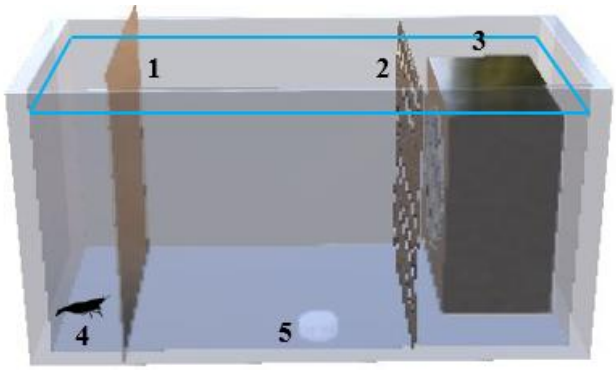


Fig. 4. First experiment set up (40 × 30 × 30 cm), 1) divider, 2) speaker holder, 3) underwater speaker, 4) the shrimp, 5) food source.

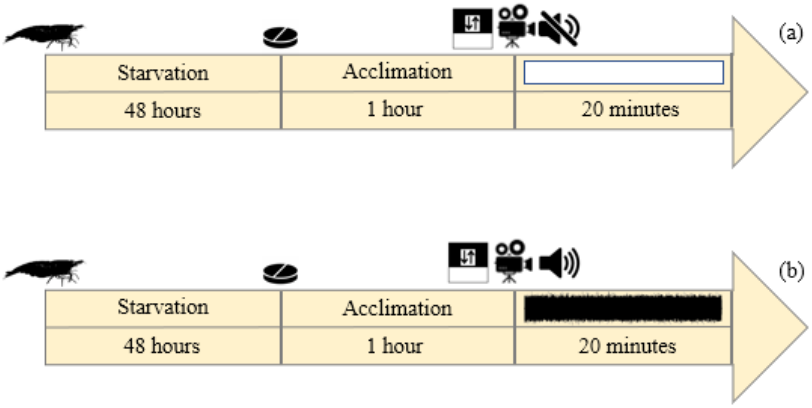


Fig. 5. Timeline view of the first experiment in a) control and b) sound treatments with *Neocaridina davidi*.) the shrimp,) food source,) opening divider,) start filming,) ambient noise,) sound.

The second experiment: feeding distraction

In this experiment, shrimps (35 individuals per treatment and a total of 70 individuals) were starved for 48 hours in the holding tank. The size and sex ratio, feeding regime and starvation period, for both experiments were the same to avoid any confounding factor affecting their behaviour unintentionally. In the control treatment, after 1hour acclimation behind the divider plate in the experiment tank (Figure 6), the shrimp were allowed to search for food without playing any sound by removing the divider plate. As soon as the shrimp found the food and started feeding, the control treatment was played for 20 minutes and each reaction was recorded

by the observer in real time (Figure 7a). In the sound treatment, after 1 hour of acclimation behind the divider plate in the experimental aquarium (the same procedure adopted just like ambient conditions as control), then, the divider plate was removed and the shrimp were allowed to search for food without any sound (Figure 7b). As soon as the shrimp found the food and started feeding, the sound treatment was played for 20 minutes and each reaction was recorded in real time by the observer and the camera. A feeding distraction is defined as a stop eating, freezing and moving away from the food source (within 10 minutes). Therefore, in this experiment, the number of shrimps whose feeding function was disturbed as well as those whose feeding was not disturbed was recorded in each of the control and sound treatments.

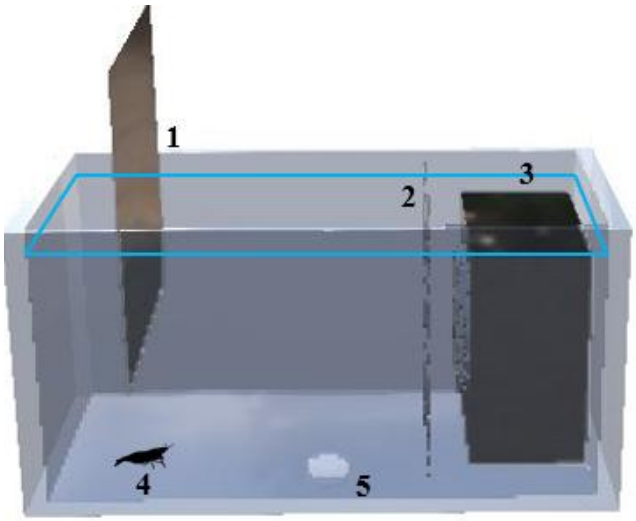


Fig. 6. Second experiment set up (40 × 30 × 30 cm). 1) divider, 2) speaker holder, 3) underwater speaker, 4) the shrimp, 5) food source.

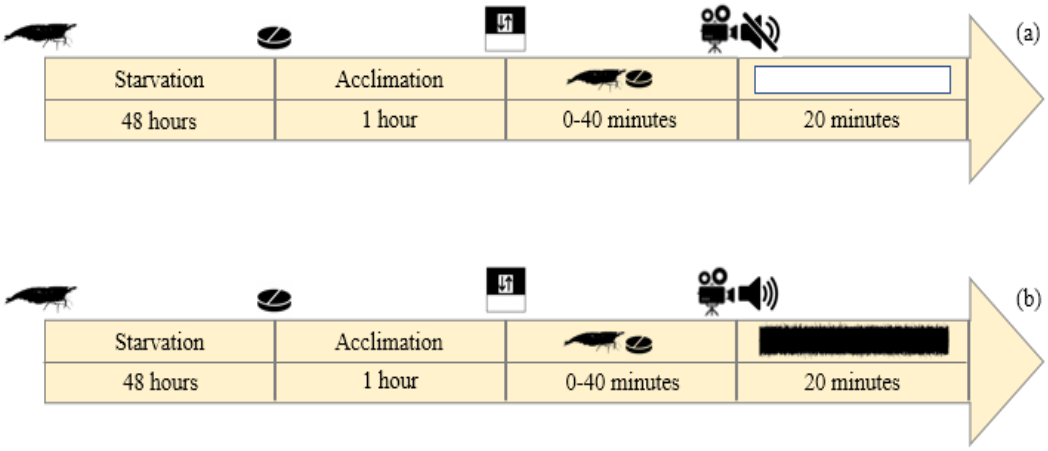


Fig. 7. Timeline view of second experiment in a) control and b) sound treatments.) the shrimp,) food source,) opening divider,) start filming,) ambient noise,) sound.

Statistical Analysis

Statistical analysis of data was performed using SPSS-25 and Excel-2019 softwares. Kolmogorov-Smirnov test and Levene test were used to test the normality of the data. Also, to determine the level of significance of the data for the parameter of movement speed, one-way t-test and one-way repeated measures, and for the parameters of spatial distribution, revisiting and latency, independent t-test was used at the level of 0.05. Chi-square test was also used for the two-state parameters of food-finding and feeding distraction. To analyze behavioural observations in all trials, the recorded videos were also reduced to 1 fps magnification (due to the slow movement of the shrimp and the ease of recording the movement points) and the movement speed and spatial distribution of the shrimps were analyzed using Logger Pro 3.14.1 software.

Results

Movement speed

There were no significant changes between total duration of control and sound treatments (Figure 8) ($P>0.05$). However, the initial movement speed in sound and control treatments as soon as opening the divider, showed a significant decrease ($P<0.001$) (Figure 9).

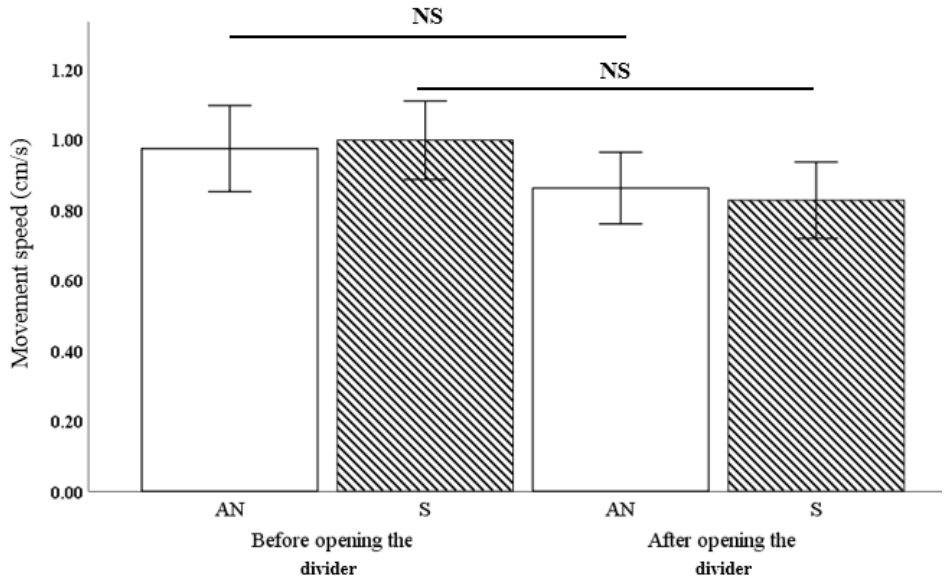


Fig. 8. Mean ± SE movement speed (cm/s) for each sound treatment; ambient sound condition as control (AN) and sound treatment (S) for 35 red cherry shrimps. Red cherry shrimp movement speed did not change with sound conditions among treatments. NS: Not Significant.

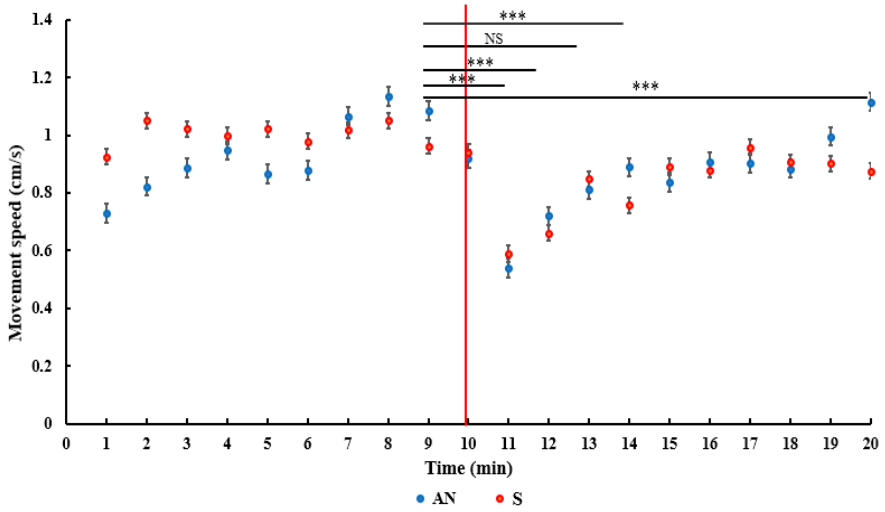


Fig. 9. Movement speed in control (AN) and sound (S) treatments changed as soon as opening the divider. As soon as opening the divider significantly movement speed reduced. NS: Not Significant, ***P<0.001.

Spatial distribution

Shrimps in the control treatment had almost the same distribution in all available space before opening the divider, and after opening the divider with the shrimp entering the new environment, almost equally in the whole aquarium space and more in one-third of the right and one-third of the left side of the aquarium was distributed (Figure 10, A and B). In the sound treatment before

opening the divider, the shrimps had almost the same distribution in the available space, but when the divider was opened, the shrimp were exposed to the sound, and dispersed significantly to the left of the tank (farthest point from the sound source) (Figure 10, C and D).

(A) Before exposure (10 mins)

Visit counts						
15<Y<20	10.18	0	103	0	0	0
10<Y<15	15.2	32.3	73.21	0	0	0
5<Y<10	20.73	37.3	65	0	0	0
0<Y<5	111.06	58.92	73.1	0	0	0
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24
Attendance percentage						
15<Y<20	1.696667	0	17.16667	0	0	0
10<Y<15	2.533333	5.383333	12.20167	0	0	0
5<Y<10	3.455	6.216667	10.83333	0	0	0
0<Y<5	18.51	9.82	12.18333	0	0	0
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24

(B) After exposure (10 mins)

Visit counts						
15<Y<20	63.62	21.92	15.61	8.76	13.73	59.31
10<Y<15	36.2	12.03	6.32	3.3	21.36	47.2
5<Y<10	26.3	11.01	4	4.21	13.95	48.31
0<Y<5	43.2	21.63	31.03	14.59	19.31	53.1
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24
Attendance percentage						
15<Y<20	10.60333	3.653333	2.601667	1.46	2.288333	9.885
10<Y<15	6.033333	2.005	1.053333	0.55	3.56	7.866667
5<Y<10	4.383333	1.835	0.666667	0.701667	2.325	8.051667
0<Y<5	7.2	3.605	5.171667	2.431667	3.218333	8.85
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24

(C) Before exposure (10 mins)

Visit counts						
15<Y<20	62.22	25.3	50.3	0	0	0
10<Y<15	33.18	15.2	30	0	0	0
5<Y<10	81.62	14.24	41.71	0	0	0
0<Y<5	101.21	60.7	84.32	0	0	0
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24
Attendance percentage						
15<Y<20	10.37	4.216667	8.383333	0	0	0
10<Y<15	5.53	2.533333	5	0	0	0
5<Y<10	13.60333	2.373333	6.951667	0	0	0
0<Y<5	16.86833	10.11667	14.05333	0	0	0
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24

(D) After exposure (10 mins)

Visit counts						
15<Y<20	101	25.34	14.32	17.3	27.03	16.36
10<Y<15	62.96	18.1	16.03	15.35	12.2	7.21
5<Y<10	44.36	5	4.82	17.1	27.02	18.26
0<Y<5	51.32	18.23	21.02	19.36	21.35	18.96
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24
Attendance percentage						
15<Y<20	16.83333	4.223333	2.386667	2.883333	4.505	2.726667
10<Y<15	10.49333	3.016667	2.671667	2.558333	2.033333	1.201667
5<Y<10	7.393333	0.833333	0.803333	2.85	4.503333	3.043333
0<Y<5	8.553333	3.038333	3.503333	3.226667	3.558333	3.16
Y/X	0<X<4	4<X<8	8<X<12	12<X<16	16<X<20	20<X<24

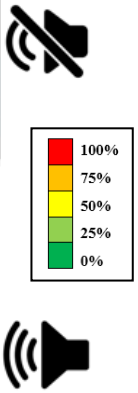


Fig. 10. Cluster heatmap showing the spatial occupancy in terms of time spent in movement (expressed in seconds) by the shrimps among the aquarium cells. On the right wall, the location of the underwater sound speaker is presented. A,B) control condition C,D) sound condition.

Figure 11(A and B), shows the spatial distribution time of 70 shrimps in the right third of the tank (near the sound source) and the left third of the tank (away from the sound source). In the control treatment, the distribution of shrimp on both sides of the tank was the same and no significant changes were observed ($P > 0.05$). While in sound treatment, the horizontal distribution of shrimp on the left side of the tank (the farthest distance from the sound production source) significantly increased ($P < 0.01$).

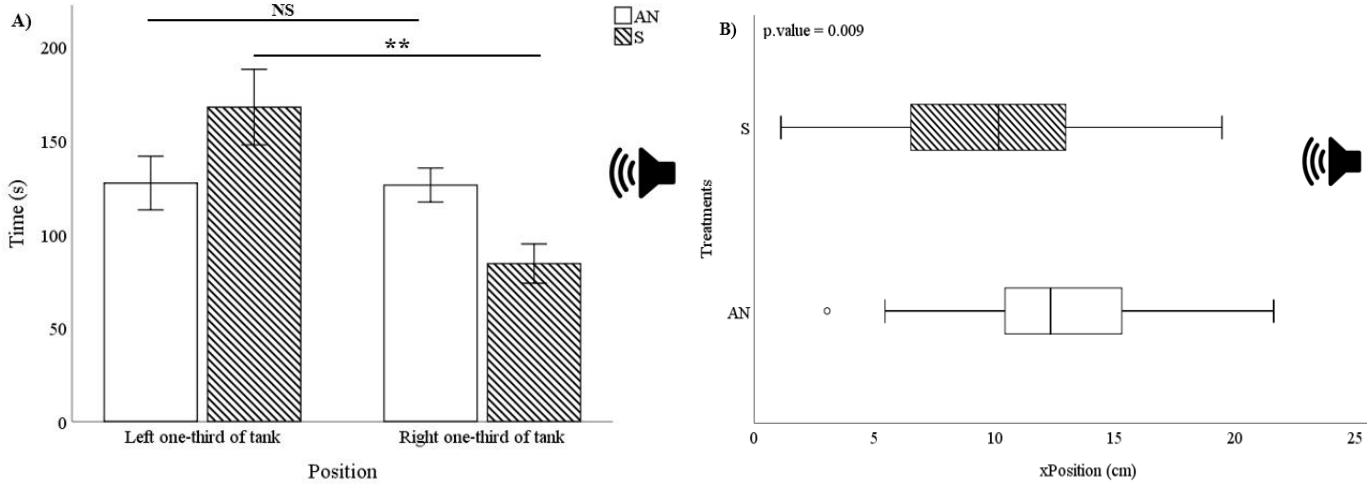
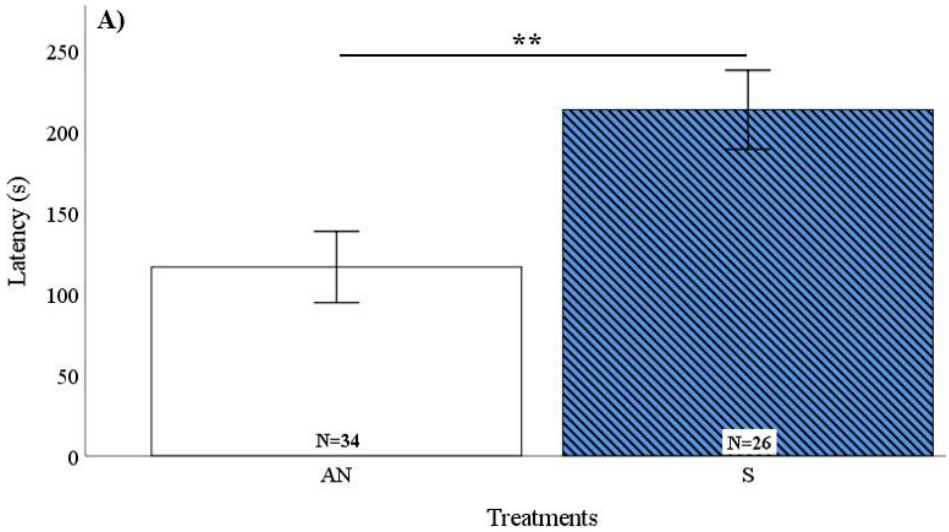
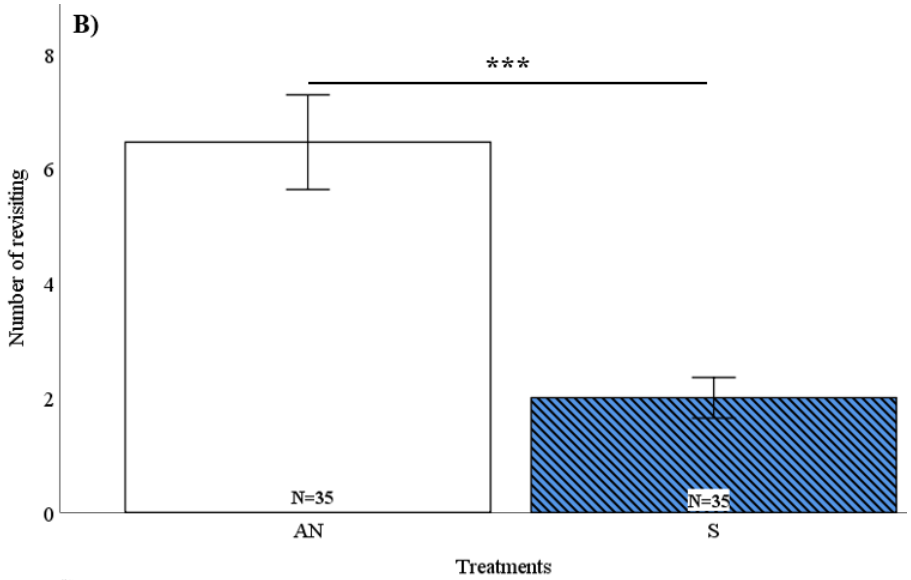


Fig. 11. A) Mean ± SE time-place budget distribution of *Neocaridina davidi* . In sound condition the distribution of shrimp significantly increased in the left one-third of the tank (the point farthest from the speaker). B) Mean ± SE spatial distribution in horizontal (x) position in AN: Ambient Noise and S: Sound conditions) position of underwater speaker in tank. NS: Not Significant, **P<0.01

Feeding activities

In sound treatment individuals significantly found food source later than control treatment (Figure 12 A, N=35, P <0.01). Also, in the control treatment, one individual and in the sound treatment 9 individuals could not find food source during 10 minutes. The number of revisiting of shrimp to the food source was significantly reduced in sound treatment (Figure 12 B, N=35, P <0.001), and significantly more individuals were succeeded to find food source in the control treatment compared to the sound treatment (Figure 12 C, N=35, P <0.001). In sound treatment, significantly fewer shrimps were succeeded to find food source than the control treatment (P <0.001).


A)
**
Latency (s)
250
200
150
100
50
0
N=34
N=26
AN
S
Treatments


B)
***
Number of revisiting
8
6
4
2
0
N=35
N=35
AN
S
Treatments

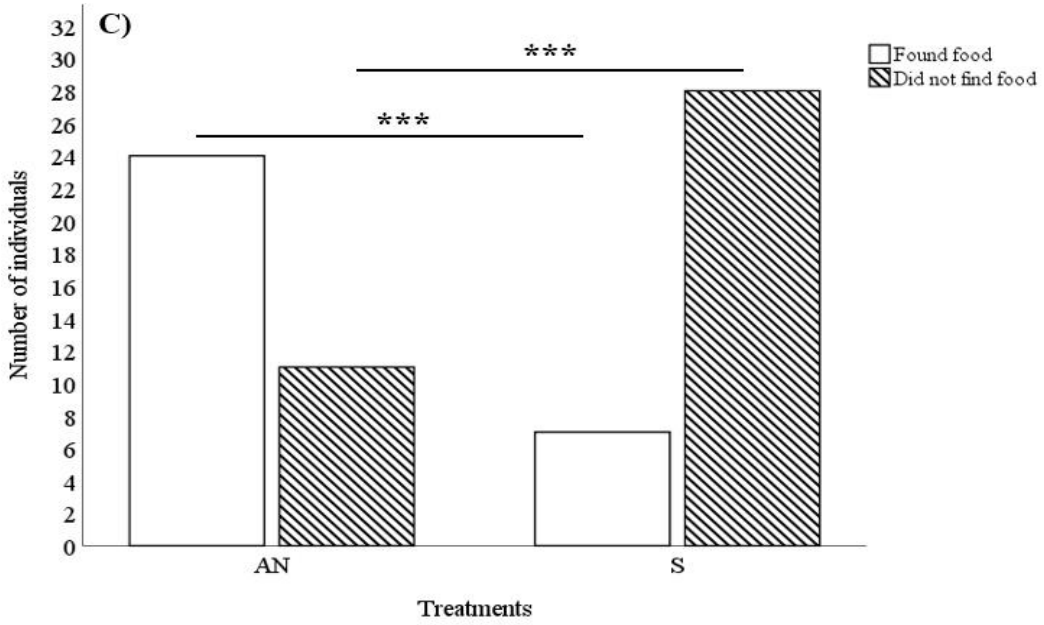

C)
***
***
Found food
Did not find food
Number of individuals
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
AN
S
Treatments

Fig. 12. A) Mean ± SE of the time of finding food source in AN: Ambient Noise and S: Sound conditions (N=35). In sound condition the time of finding food significantly increased. B) Mean ± SE of the number of revisiting to food source in AN: Ambient Noise and S: Sound conditions (N=35). In sound condition the number of revisiting to food source significantly decreased. C) The number of individuals that found/not found food source for 10 minutes in AN: Ambient Noise and S: Sound conditions (N=35). In sound condition significantly more shrimp did not find food source. NS: Not Significant, **P<0.01, ***P<0.001.

The second experiment: feeding distraction

In sound treatment, more individuals (P<0.001) were distracted in feeding than in the control treatment (Figure 13).

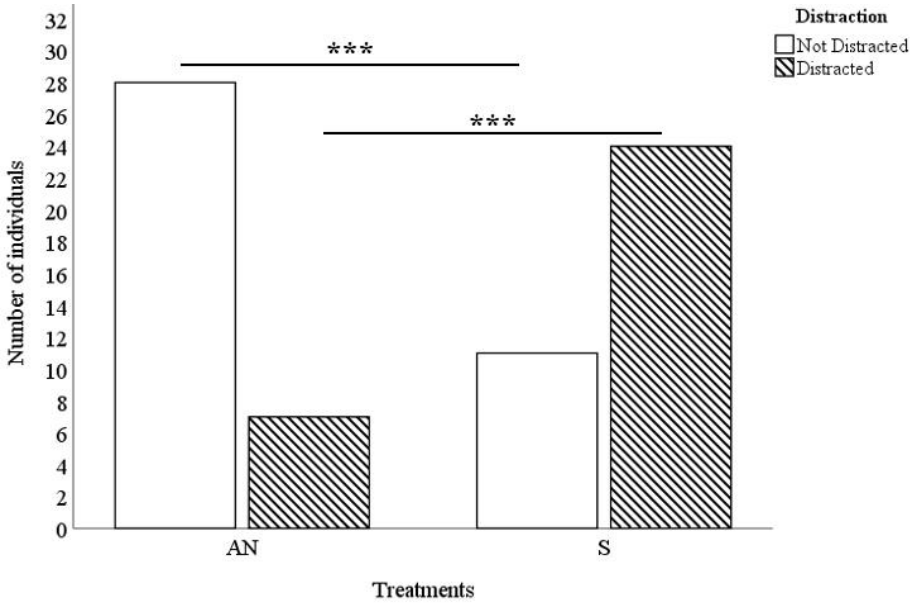


Fig. 13. The number of *Neocaridina davidi* that distracted/not distracted from food source for 10 minutes in AN: Ambient Noise and S: Sound conditions (N=35). In sound condition significantly more shrimp distracted in feeding. ***P<0.001.

Discussion

The impacts of underwater sound on aquatic animals in freshwater habitats have been largely ignored, and the few studies conducted to date showed significant impacts on invertebrates in sea and freshwater habitats (Breithaupt and Tautz, 1988; Popper et al., 2001; Roberts and Elliott, 2017; Roberts and Laidre, 2019; Ruiz-Ruiz et al., 2020). Although, the direct cascading impacts

and indirect consequences of aquatic invertebrates on food domains are quite diverse and important, little is known how anthropogenic noise may affect these process.

The present study is part of a series of studies with the same general intention about the analysis of the impact of underwater anthropogenic noise on fish and crustaceans (Shafiei Sabet et al., 2020; Mohsenpour and Shafiei Sabet, 2021). This study tested the behavioural effects of acoustic stimuli on the red cherry shrimp, *N. davidi* which, despite of its important ecological and commercial role, has been poorly investigated in this respect. Here, we highlight that invertebrates are likely to perceive sound via their mechanoreceptor organs and to be susceptible to the impact of sound as a source of stress, and they are potential valuable targets for further and detailed investigations into the effects of anthropogenic noise pollution.

The playback of experimental sound exposure resulted in a variety of behavioural changes in the red cherry shrimp in which some of those changes have been reported in other invertebrates and some seem to be species-specific. Our results demonstrate the first indication that freshwater decapods can be affected by acoustic stimuli. Sound treatment compromised movement and feeding activity and this is consistent with previous research on *Palinurus elephas*, *Cyrtograpsus angulatus*, *C. altimanus*, *Neohelice granulata* and *Leptuca uruguayensis (*Filiciotto et al., 2014; Sal Moyano et al., 2021). In a series of controlled tank-based experiments it has been shown that sound can affect the behaviour of feeding crabs and disrupt their feeding performance (Wale et al., 2013). Moreover, in other taxa, recent studies have shown that acoustic stimuli disrupted drastically feeding performance (Veollmy et al., 2014, Purser and Radford, 2011, Shafiei Sabet et al., 2015; Magnhagen et al., 2017, Mickle and Higgs, 2018)

First experiment

Sound impacts on displacement and locomotion behaviour of shrimp

The movement speed in sound and control treatments as soon as opening the divider, showed a significant decrease, but there were no significant changes between total minutes of control and sound treatments. This change may be due to the stress of facing the new environment (Shafiei Sabet et al., 2019) as shrimps in both control and sound treatments displayed the same pattern in a way reducing their movement speed. Although there are studies that have shown acoustic stimuli decrease locomotion activities in invertebrates. Roberts et al. (2016) shown that

experimental sound exposure similar to blasting and pile driving, anthropogenic substrate-borne vibrations, reduces locomotion in another invertebrate, adult hermit crab *Pagurus bernhardus*. Solan et al. (2016), also reported a reduction of locomotor activity when exposed the Norwegian lobster *Nephrops norvegicus* to continuous and impulsive sound. Also effects of anthropogenic noise on movement and swimming behaviour of crustacean and fishes were reported in several studies (De Vincenzi et al., 2015, Filiciotto et al., 2016, Sal Moyano et al., 2021, Sarà et al., 2007, Shafiei Sabet 2016a, b, Jimenez et al., 2020). As well, Snitman et al. (2022) reported that key crab species (*Neohelice granulata*) locomotion activity diminished when exposed to diverse sound. We argue that the reduced locomotion and movement speed could be related to a distraction or confusion effect. It has been shown that Caribbean hermit crabs (*Coenobita clypeatus*) in response to motor boat sound playback stayed more inactive and allowed a simulated predator to approach closer before their retracting movement. Therefore, crabs reallocated finite attention to acoustic stimuli and therefore efficiently distract them and thus preventing them from responding to an approaching threat (Chan et al., 2010). In another invertebrate species, earthworms, it has been shown that it is possible vibrational sound masks the vibrational cues of approaching foraging moles, making earthworms more prone to predation risks in ensonified areas (Dominoni et al., 2020). Moreover, earthworms may not be able to discriminate between the subterranean waves from anthropogenic sources and vibrations coming from approaching predators (Dominoni et al., 2020). Thus, potentially noise pollution can cause a multiple of impacts on locomotor activities and threat assessment in invertebrates. In other studies on invertebrates have been shown that acoustic stimuli have multiple negative effects (Edmonds et al., 2016; Carroll et al., 2017). Finding mate or detecting a predator has direct effects on reproductive success and survival in animals (Dall et al., 2005; Dominoni et al., 2020). If shrimps spend more time budget to find their food or shift their attention from foraging areas because of anthropogenic noise, therefore it may detrimentally alter their other important biological activities such as courtship and finding mate to increase their reproductive success or detecting approaching predator to survive in their habitats. These impacts can induce alterations not only on individuals but also can extend on populations and ecosystems especially when anthropogenic noise impacts keynote predators and habitat forming species that are functionally important species. This disruption potentially may have knock-on effects on important ecological and biological processes such as species abundance, survivorship and reproductive success of

aquatic organisms (Slabbekoorn et al., 2010; Williams et al., 2015; Shafiei Sabet et al., 2016 a,b).

In the control treatment, no significant difference was observed in the horizontal spatial distribution, but in the sound treatment, the horizontal distribution of shrimp significantly increased when comparing the nearest point proximity of the underwater sound source with the furthest away. This spatial displacement because of sound exposure may have potentially negative impacts on the abundance of invertebrates (Velilla et al., 2021). They reported that earthworm abundance showed a strong decrease with increasing vibratory noise. Sound has acted as an annoying and disturbing factor that similar behavior has been reported in other species (De Vincenzi et al., 2021, Shafiei Sabet et al., 2016a, b). In mobile species such as the red cherry shrimp, locomotor movement is an important link between the behaviour of individuals and ecological processes (Herrnkind, 1983; Spanier et al., 1988; Lawton and Lavalli, 1995). Therefore, any impacts of anthropogenic noise on their locomotor activities may have detrimental consequences on their other biological activities such as foraging or anti-predator behaviour. Density-dependent mechanisms underlying the structure and dynamics of populations and communities are often sensitive to short-term changes in the spatial distribution of individuals due to movement (Milinski and Parker, 1991). De Vincenzi et al. (2021) showed that, small-spotted cat shark (*Scyliorhinus canicula*) exposed to anthropogenic noise made a different use of the left (close to the underwater speaker) and the right (away from the underwater speaker) sections of tank (about 36.3% and 63.7%, respectively), although was not statistically significant. Although, there are no well-studies impacts of acoustic stimuli on spatial distribution of the red cherry shrimps, in other species there are indications of anthropogenic noise effects on swimming activates and spatial displacement. Shafiei Sabet et al. (2016a) reported sound treatment caused various behavioral changes in both spatial distribution and swimming behavior of zebrafish (*Danio rerio*) within the treatment tank; sound exposure led to more freezing and less time spent near the active speaker. However, this behavioural response to sound exposure is not in line with and similar observation in another study on zebrafish under laboratory conditions (Neo et al., 2015). Shafiei Sabet et al., (2016b) compared zebrafish, *Danio rerio*, and Lake Victoria cichlids, *Haplochromis piceatus*, the former being sensitive to lower absolute thresholds and wider spectral ranges than the latter and consistent with present study, experimental sound exposure induced a significant reduction in swimming speed in the first minute of exposure for

both species in captive conditions. Also neither species showed spatial shifts away from the active speaker in the horizontal plane. Furthermore, zebrafish showed clear startle response behaviour with the onset of the sound exposure leading to an initial, brief increase in swimming speed, which was not found for the cichlids. Here we emphasize that our experimental set up were successful and able to explore direct assessment of the effects of acoustic stimuli on the horizontal movement of the shrimp. Even though, we raise attention that these parts of results and behavioural observations may suffer from the bias introduced by experimental and acoustics artifacts (Rogers et al., 2016; Parvulescu, 1967; Akamatsu et al., 2002). Moreover, because of logistic limitations and technical issues we were not able to measure particle motion components of our experimental set up which it is more likely that our behavioural observations are in response to this sound component rather than sound pressure (Popper and Hawkins, 2018; Hawkins et al., 2021).

Acoustic stimuli compromised feeding performance in shrimp

Anthropogenic noise was found to cause detectable effects on foraging performance in fish and in invertebrates. Feeding latency in shrimps was significantly increased in sound treatment compared to the control treatment. Delay in finding food source seems to be due to stress and confusion caused by sound playback, which this behavioral disorder has been observed in other studies o in response to sound in crustacean and fishes (Hughes et al., 2014, Gendron et al., 2020, Voellmy et al., 2013). Elevated levels of sound here in this study caused negative effects on feeding activities and performance in shrimps under laboratory conditions. Although it is also necessary to define clearly methodological procedures following different techniques, procedures, and instrumentation of feeding activities *per se* in experiments that might affect nutritional condition estimations (Lopeztegui-Castillo, 2021).

Significantly more individuals were successful to find food source in the control treatment than the sound treatment. This result is consistent with the findings of Voellmy et al. (2013), which confirms the effect of sound on feeding distraction in fish. These observations suggest that anthropogenic noise may cause similar impacts on foraging activities in animals among taxa under laboratory conditions. Although it is important to consider that our findings under laboratory based experiments cannot be interpreted for wild animals and fish in real field

conditions. While, Wale et al. (2013) and Hubert et al. (2021) reported that ship and boat noise did not have negative effects on food finding of shore crab (*Carcinus maenas*). Various studies have shown that food-deprivation influences behaviour including foraging; food-deprived individual of the three-spined sticklebacks (*Gasterosteus aculeatus*) were more likely to initiate predator inspection visits and had higher feeding rates than well-fed shoal mates (Godin and Crossman, 1994). Hungry fifteen-spined sticklebacks (*Spinachia spinachia*) were more likely than partially satiated individuals to feed at a food source associated with predator threat (so hungry sticklebacks were shown to be less risk-averse) (Croy and Hughes, 1991).

The number of revisiting to the food source also significantly decreased when exposed to sound. Such behavior changes in feeding have not been reported in other studies. This study, for the first time, showed that anthropogenic noise pollution influences the behavioural activity of the red cherry shrimp. Although no data on sensitivity to acoustic signals are currently available for this species, our results indicate that *N.davidi* may perceive all or part of the acoustic stimuli projected within the wider band-width of their underwater audible rasps characterized by signals with most of energy concentrated in the 4-2000 Hz range, with the peak frequency of 700 Hz and peak amplitude of 120 dB re 1 lPa (Buscaino et al., 2011b). Filiciotto et al. (2014) found in lobsters exposed to boat sound higher values of the mobility and moving state with respect to lobsters in the control condition. Shafiei Sabet et al. (2019) did not found significant changes in behavioural parameters of *Daphnia magna* while Sal Moyano et al. (2021) evaluated biological and anthropogenic acoustic signals on the orientation response of different stages (megalopae and juveniles) of 4 brachyuran crabs species. *C. angulatus* megalopae and juveniles responded positively towards crustacean signals, while juveniles responded negatively towards fish sounds. *N. granulata* juveniles orientated negatively towards crustacean, motorboat and fish signals while *C. altimanus* and *L. uruguayensis* juveniles did not respond to fish signals (Sal Moyano et al. 2021). The results support the idea that invertebrates can discriminate among conspecific signals and highlight the role of sound on prey-predator relationships. The behavioral orientation response to the motorboat sound evidences a presumably negative effect of anthropogenic noise on the biological interactions of species.

There is mounting experimental evidence that anthropogenic noise can have a variety of negative physiological and behavioural effects on individual animals, ultimately affecting their survival

and reproductive success (Kight and Swaddle, 2011; Shannon et al., 2015; Kunc et al., 2016; Morley et al., 2014). For example, sound from human activities can directly cause injury or hearing loss in some species (Halvorsen et al., 2012; Popper et al., 2005), or as a consequence of masking biologically important sound; the sound that the fish make themselves and the sound the predators make (i.e. reducing the signal-to-noise ratio). It reduces the range over fishes can detect the sounds of biological importance and subsequently impose them to negative fitness output (Popper et al., 2005). Elevated ambient sound can also impair the ability of animals to communicate (Slabbekoorn and Ripmeester, 2008, Kunc et al., 2015; Lampe et al., 2012). Further, sound can be perceived as a threat, be a distraction, or can cause increased stress, in turn impairing an animal's ability to forage efficiently (Wale et al., 2013; Voellmy et al., 2014), respond appropriately to information about predation risk (Morris-Drake et al., 2016; Simpson et al., 2016; Purser et al., 2016), perform adaptive behaviours during habitat-selection (Holles et al., 2013; Simpson et al., 2016), or reproduce successfully (Blickley et al., 2012; Nedelec, 2017).

There are studies on other taxa revealing sound effects on swimming activities and spatial distribution. Filiciotto et al., (2016) have shown that the common prawn (*Palaemon serratus*) in the boat noise condition had clear behavioural responses to acoustic stimuli exposure. In particular, those exposed to sounds had significantly lower values of encounters between subjects, but higher values of time spent outside the shelter and resting. Sound exposure caused behavioural modifications and negative effects on feeding frequencies in the mediterranean damselfish (*Chromis chromis*) (Bracciali et al., 2012). Sarà et al. (2007) found that in the absence of boat noise, tuna assumed a concentrated coordinated school structure with unidirectional swimming and without a precise shape. When a car ferry approached, tuna changed swimming direction and increased their vertical movement toward surface or bottom; the school exhibited an unconcentrated structure and uncoordinated swimming behaviour. Shafiei Sabet et al. (2016b) compared zebrafish, *Danio rerio*, and Lake Victoria cichlids, *Haplochromis piceatus*, the former being sensitive to lower absolute thresholds and wider spectral ranges than the latter. Experimental sound exposure induced a significant reduction in swimming speed in the first minute of exposure for both species in captive conditions. Furthermore, zebrafish showed clear startle response behaviour with the onset of the sound exposure leading to an initial, brief increase in swimming speed, which was not found for the cichlids. Neither species showed spatial shifts away from the active speaker in the horizontal plane. Blom et al., (2019) experimentally tested the impact of broadband sound exposure (added either continuously or intermittently), compared to a control, on the behaviour and reproductive success of the common goby (*Pomatoschistus microps*). Compared to the intermittent noise and control treatments, the continuous noise treatment increased latency to female nest inspection.

Second experiment

Does acoustic stimuli food source assessment and attention in shrimp?

In this experiment, the measured behavioural parameter was the feeding distraction in both sound and control treatments. According to the results, in sound treatment significantly more individuals were distracted in feeding compared to the control treatment. Similar results have been reported in previous studies on another species of this order (Decapoda) and other species (Wale et al., 2013, Hastie et al., 2021, Gendron et al., 2020, Hughes et al., 2014, Voellmy et al.,

2013). Wale et al., (2013) argued that, crabs *Carcinus maenas*, exposed to ship noise playback were more likely to suspend feeding than were those experiencing ambient-noise playback. Gendron et al., (2020) found the presence of boat noise had a significant effect on the hunting behaviour of *Pseudopleuronectes americanus* larvae. Larvae exposed to boat noise spent less time hunting and had smaller stomach volumes compared to those with no sound treatment. This suggests that more preys were consumed in the absence of boat noise. Purser and Radford (2011) noted a decrease in foraging performance in three-spined stickleback (*Gasterosteus aculeatus* L. 1758) exposed to noise; this decrease resulted from (1) the misidentification of food versus non-food items, as shown by an increased number of attacks on the latter, and (2) fewer successful attacks on food items under noisy conditions. Hanache et al. (2018) reported, boat noise significantly reduced attack rate, resulting in a functional response curve of the same height but with a less steep initial slope. European minnows (*Phoxinus phoxinus*) exhibited a stress-related response to noise including increased swimming distance, more social interactions, and altered spatial distribution. McLaughlin and Kunc. (2015), found that exposure to anthropogenic noise affected a couple of behaviours: an increase in sheltering was accompanied by a decrease in cichlid *Amititlania nigrofasciata* foraging. Their results highlight the multiple negative effects of an environmental stressor on an individual's behaviour. Hubert et al. (2021) in an experiment in which crabs could find food based on olfaction only, they found that there was no overall effect of boat sound on food finding success and efficacy, foraging duration or walking distance in shore crabs (*Carcinus maenas*). There are several mechanisms in explaining how anthropogenic noise affects behaviour: increasing stress (Smith et al., 2004), masking biological relevant cues (Hawkins and Chapman, 1975), distracting of attention (Chan et al., 2010; Dukas, 2004) and physical damaging on sensory system and internal organs (McCauley et al., 2003). These could be operating separately or simultaneously, and in many cases, it is difficult to unpick which of these is most relevant, especially in the present case study.

Wale et al., (2013) showed that the two cohorts of crabs used for each of the foraging experiments did not differ significantly in their likelihood of finding the food source. Their search times or their likelihood of being disrupted from feeding; cohorts were therefore combined for an assessment of the effect of ship noise playback. The ability of crabs to find the food item was not impaired by the playback of ship noise compared to ambient noise. Individuals exposed to ship noise playback were more likely to suspend feeding than were those

experiencing ambient-noise playback. Hughes et al. (2014) showed that the sounds made by predatory fish can affect the feeding behavior of crabs (*Panopeus* Spp.*)*. In the presence of the sound of predatory fish in the frequency range of 10 to 1600 Hz and the sound intensity of 146 to 116 dB, the amount of crab feeding from bivalves, is significantly reduced.

It has also been shown that sound exposure can affect feeding activities in other taxa. Gendron et al.(2020) investigated anthropogenic boat noise on feeding success in winter flounder larvae (*Pseudopleuronectes americanus*). Also the feeding behaviour of two sympatric fish species: the three-spined stickleback, *Gasterosteus aculeatus*, and the European minnow, *Phoxinus phoxinus* (Voellmy et al., 2014). Hastie et al. (2021) found that sound can negatively affect the foraging activity of gray seals (*Halichoerus grypus*): in the control treatment, the success of seal foraging was higher than the sound treatment. Goldbogen et al. (2013) demonstrated that sound can significantly affect blue whale during deep feeding behaviour. These sound related effects on foraging activates that have been observed in other species are in line with the current study.

Although it needs further research, shrimps that do not feed properly because of acute and/or chronic exposition to sound could be affected in their growth, size at sexual maturity, mate selection and brooding eggs behavior among other, thus affecting the whole life cycle and the species fitness and this role in the trophic webs. Sound generating human activities are interfering with biological relevance acoustic information of aquatic animals/ species across taxa with important population and community level consequences (Derryberry et al., 2020; Dominoni et al., 2020).

Our results provide important insights into the effects of sound on this species at individual level under laboratory conditions. First, we observed that opening the divider caused a no-auditory behavioural response in a way reducing instant movement of shrimps which could be an antipredator behaviour response and probably improves their survival by lowering visual visibility. Second, we showed that shrimps spent more time away active speaker explaining that they were able to detect our acoustic stimuli; probably particle motion components but because we were not able to measure this therefore not approved yet and further research measuring both sound pressure and particle motion components are recommended. Third, we demonstrated anthropogenic noise simultaneously could decrease food finding success and the number of revisiting to food, increase feeding latency and distraction. All together our finding highlights

negative impacts of anthropogenic noise on the behaviour of shrimps that could impair their foraging performance and potentially have cascading effects on their survival.

Conclusions

In conclusion, in this study, it was clearly observed how short-term exposure to sound changes some behavioural patterns in *N. davidi*. The simulated sound in laboratory condition influenced the behaviours of specimens, mainly in terms of spatial distribution, feeding latency, finding-food and number of revisiting to the food source and feeding distraction. As we acquired new knowledge we need to change the sound exposure criteria and implement updates of invertebrates' perception with regard to the acoustic environment. The metrics are often inappropriate in particular professionally may be expressed in terms of sound pressure and potential impacts on invertebrates. Whereas, we know that all fishes and most invertebrates are able to perceive and detect particle motion components (Popper and Hawkins, 2019; Hawkins et al., 2021). We suggest the need to explore anthropogenic noise effects on invertebrates both sound pressure levels and particle motion components need to be addressed.

The actual impacts of sound on populations of invertebrates and other taxa are often unknown and difficult to access. We argue that longer term studies under laboratory conditions are necessary to explore the potential chronic effects of sound to look at on populations and on ecological communities reverberating among food webs. However, we do not know what impact that will have on much larger populations of the animals and that is really important in accessing sound-related impacts and assess the ecological effects. Future experiments should be performed in order to assess the animal responses in nature where the acoustic field is not influenced by laboratory conditions. Moreover, further long-term field studies should be carried out to evaluate the effects of noise from human activity on some ecological and conservation factors in this species, providing and implementing innovative tools for the measure of the acoustic pollution phenomenon.

Credit author statement

Sasan Azarm-Karnagh: Investigation, Data curation, Visualization, Formal analysis, writing - original draft preparation.

Laura López Greco: Conceptualization, Methodology, Supervision, Writing- Reviewing and Editing.

Saeed Shafiei Sabet: Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing- Reviewing and Editing.

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