A freshwater zooplankton in the face to boat noise pollution

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Abstract

Ecotoxicological studies mainly focus on chemical pollution, however, since past decades, there has been a growing interest for the acoustic pollution. Previous studies on underwater acoustic pollution showed that noise affects vertebrates' behaviour, like fish and marine mammals. However, little is known about other organisms. Consequently, we studied important lacking aspects, well known with chemical pollution: the effect on the fitness (survival and fecundity) of a key zooplankton species (used as bioindicator). We exposed isolated water fleas, *Daphnia magna*, to chronic boat noises or to a broadcasted silence as control, from birth to death. We measured effects on lifespan and clonal offspring production (e.g., clutch size, number of produced offspring along life). We did not observe any effect of the chronic boat noise exposition on *Daphnia*'s fitness. These results are consistent with results on some previous acute noise exposures, but also opposite to other ones found with some acute and chronic noises. Thus, we discuss how the noise structure and temporal pattern could affect its impacts on aquatic organisms. Our work highlights that noise pollution should be integrated in ecotoxicological studies, but also that some particular aspects of this pollutant should be considered differently than chemical pollutants.

Keyword: *Daphnia magna*, Acoustic pollution, Boat noise, Fitness, Survival, Reproduction.

1. Introduction

Freshwater ecosystems suffer seriously from all type of anthropogenic pollutions (e.g., chemicals, light, radioactivity, nanopollution, sound) (see for instance Longcore & Rich, 2004; André et al., 2011; Song et al., 2020; Jan et al., 2022) but the most documented to date remain chemical pollutions (e.g., industrial effluents, urban waste, pesticides, drugs) (Truhaut, 1977; Villeneuve & Garcia-Reyero, 2011). Toxicological studies have documented, in a comprehensive and accurate way, the effects of different type of pollutants (e.g., ion, heavy metals, drugs), exposure durations (acute or chronic), intensity (e.g., median Lethal Dose LD_{50}) and their interactions between them and with environmental parameters (temperature, acidity, humidity …). Those results contributed to the knowledge allowing the assessment of other types of pollutions. In our study, we were interested in acoustic, or noise, pollution, that is now described as pervasive and omnipresent in all ecosystems (terrestrials, marine, and freshwater) (Shannon et al., 2016; Popper & Hawkins, 2019; Kunc & Schmidt, 2019), and leads to an increasing amount of research (Williams et al., 2015). Specifically, we focused on the ship noise, which is the major noise threat to freshwater systems (Rountree et al., 2020; Duarte et al., 2021).

One particular threat of noise pollution is the different temporal patterns that it presents: chronic or acute exposure (Duarte et al., 2021). Chronic exposure means a continuous, also intermittent, regular, or random, sounds (e.g., turbines, boat noises) whereas acute exposure represents punctual sound (e.g., airgun) (Nichols et al., 2015; McCauley et al., 2017). The nature of acoustic pollution, affecting frequency and temporal pattern, is known to affect behaviour and physiology of organisms in different way. For instance in aquatic studies, fish are more affected by a random noise, than by a continuous or regular one (Nichols et al., 2015). These results are interpreted as an ability for vertebrates to habituate to some long-term noise

exposition (Rojas et al., 2021). However, there is a lack concerning questions about the effect of noise on survival and fecundity because it focuses substantially on behaviour (Richardson et al., 1985; Duarte et al., 2021). Thus, contrary to many ecotoxicological studies, since Truhaut (1977), there remains a gap in understanding how stress due to noise pollution affects individual fitness.

Noise pollution studies largely neglected lower trophic levels (small organisms, invertebrates, without hearing system) (Hawkins et al., 2015), yet generally used as bioindicators in ecotoxicology (Parmar et al., 2016). Although they did not possess hearing structures, they present mechanoreceptors that allow them to detect environmental vibration. For instance, Gassie et al. (1993) showed that marine copepods detect water vibrations, and Buskey et al. (2002) that vibrations lead to an individual acceleration. Consequently, acute or chronic noise expositions should be able to affect invertebrates. In fact, marine zooplankton (e.g., copepods) exposed to acute airguns induce negative effect of their survival (McCauley et al., 2017). Similarly, Fields et al. (2019) showed an reduction of copepods survival, but effects seem smaller than those of McCauley. Additionally, an important zooplankton predator (*Chaoborus flavicans*) increased anti-predatory defence behaviour when exposed to short-term exposure to boat noise (Rojas et al., 2021). These works highlight that noise could affect both fitness and behaviour of zooplankton species, however they studied only an acute noise exposition.

Therefore, we investigated the effect of chronic exposure to motorboat noise (intermittent and irregular noises) on the fitness of the water flea *Daphnia magna*. Recent works showed contradictory results concerning their response to noise. It was found no change in their mobility when exposed to acute noise (Sabet et al., 2015, 2019), whereas prior experiment on broadband noise chronic exposure (i.e., a continuous noise) showed that noise was able to alter

both fitness and behaviour (Prosnier et al., 2022). Consequently, we expected that motorboat noise stresses *D. magna*, and thus negatively affects their fitness, by reducing survival or fecundity or both, according to McCauley et al. (2017). However, note that Prosnier et al. (2022) surprisingly showed a higher survival of individuals exposed to noise.

2. Material and Methods

2.1. Collection and maintenance of organisms

Daphnia magna had been purchased from Aqualiment (Grand Est, France) and stored in 20 L aquarium, filled with aged tap water, for one month. They were reared at 18°C under a 12:12 light:dark cycle. *D. magna* were fed, each two days, with 0.05g of algae, a mix with 80% of *Arthrospira platensis* and 20% of *Aphanizomenon flos-aquae* (Algo'nergy® Spiruline + Klamath).

2.2. Fecundity and mortality

We measured reproductive success and survival during an experiment (similar as done in Prosnier et al., 2022). We collected gravid *D. magna* from aquarium and isolated them in 50 mL jars containing Volvic® water. Newborns (<24h) were transferred individually into 150 mL glass-microcosms, closed with an X-mesh tissue allowing water flows and noise transmission.

We used four 150L rectangular tanks (75 x 60 x 35 cm), filled with 90L of aged tap water, at 20-22°C and under a 12:12 light:dark cycle where 18 glass-microcosms were disposed at 20 cm of a underwater loudspeaker. We broadcasted silence in two control tanks and a boat noise playlist (see below) as treatment in the two other tanks. For each *D. magna* mother, we exposed half of the newborns to the control treatment, and the other half to the noise treatment, thus in the two conditions individuals are clones. Each day, survival and newborns production were controlled – if *D. magna* spawned, we counted and took off offspring. Each two days, we fed individuals with 2 mL of algae (1g/L), and each week water changes were performed. During

the first days of the experiment (i.e., before the first hatchings), we replaced dead *D. magna* by new individuals to increase the number of replicates. Experiments were performed with 115 juveniles (58 exposed to control and 57 to noise treatment) coming from 25 mothers.

2.3. Acoustic treatments

We exposed *D. magna* to two acoustic treatments (see Rojas et al., 2021, for more details): either silence, a repeated 1-h playlist without sound, or boat noise, a playlist with 2 x 75 boat noises, from 15 recorded boat noises in Grangent lake broadcasted from 9 a.m. to 6 p.m. (Fig. 1a). We modified the intensity of the 15 boat noises, from 0 to -25 dB Re 1 µPa by 5 dB, to

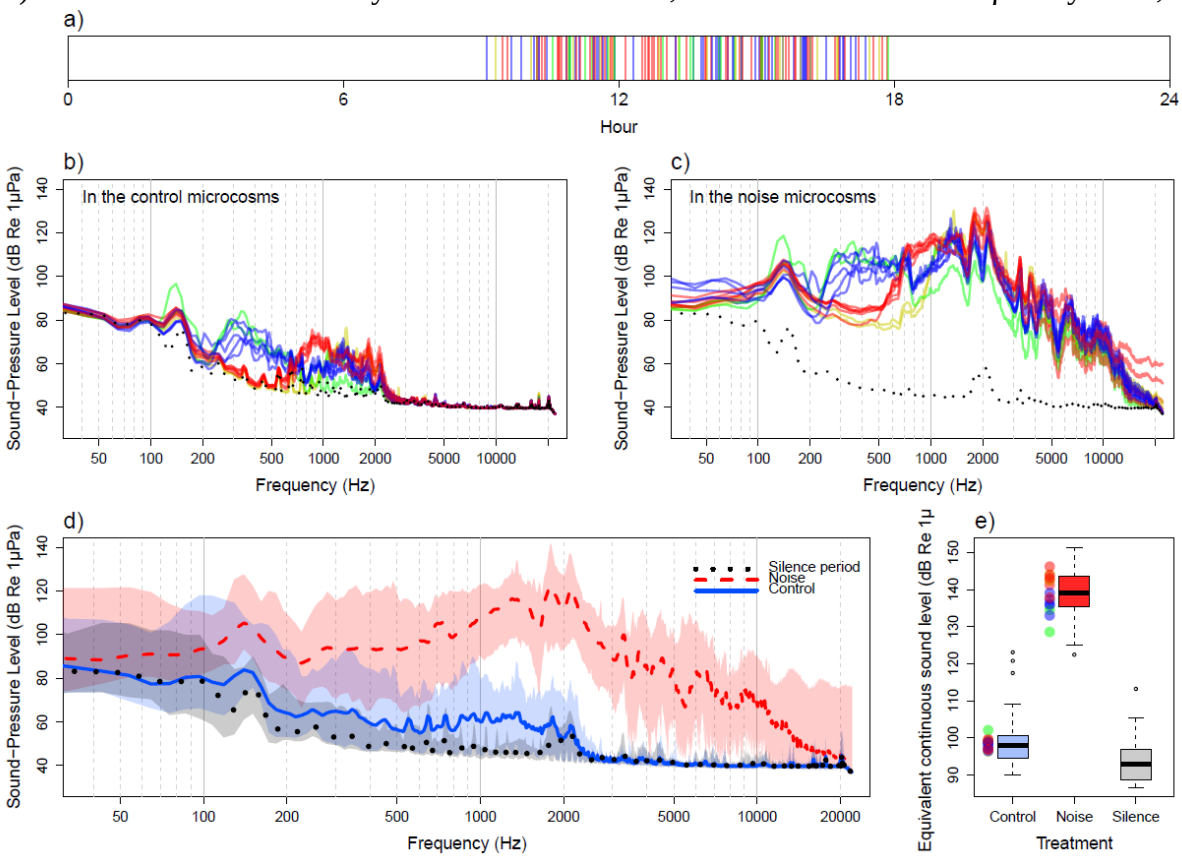

Figure 1. Acoustic treatments. a) 24h temporal sequence of the broadcasted motor boat noises, from 9 a.m. to 6 p.m. Each vertical line represents a boat. b) Sound measured in half of the microcosms in control tank, during the 15 boat broadcasts at their maximal intensity (solid lines) and silence broadcast (dotted line). c) Sound measured in half of the microcosms in noise tank, during the 15 boat broadcasts at their maximal intensity (solid lines) and silence broadcast (dotted line). d) Sound measures in half of the microcosms. Thick lines are means for control (full blue line) and noise treatment (dashed red line) during the 15 boat broadcasts at their maximal intensity, and during the silence period (dotted black line). Shaded areas delimit the min and max SPL. e) Sound levels in half microcosms. Central bars represent the median, boxes the interquartile range, and dots the outliers (> 1.5 times the interquartile range). Coloured dots are the sound level in control and noise microcosms during the 15 boat broadcasts at their maximal intensity. The four colours (red, yellow, green, blue) correspond to the four visuals category used for the noise correction.

obtain 75 noises from 103 to 150 dB, using the software Adobe Audition 2020 (13.0.0.519, Adobe Systems Inc., Mountain View, CA, USA). Sounds (stereo WAV files) have been played back using a Zoom® H4n recorder connected to an amplifier (DynaVox® CS-PA 1MK), and an underwater loudspeaker UW30 (Electro Voice®). To check the sound spectrum and intensity in both control and noise microcosms we re-recorded noise (Fig. 1b-e) with a Zoom® H4n coupled to a hydrophone (Aquarian Audio H2A-HLR Hydrophone, frequency response from 10 to 100 kHz), previously calibrated with a hydrophone (8104, Brüel & Kjær, Naerum, Denmark; sensitivity –205 dB re. 1 V μPa−1; frequency response from 0.1 Hz to 180 kHz) connected to a sound level meter (Bruël & Kjaer 2238 Mediator, Naerum, Denmark). Noises were corrected to be closer to the 15 real boat noise spectrums (visually regrouped in four categories to optimise the correction, Fig. 1) before the intensity modification previously described. Note that the noise in control was measured to less than 100 dB during the noise exposition in the treatment, little more than during the silence periods (Fig. 1e); it indicates that boat noise broadcasted in noise treatment tanks are almost not perceived in the control tanks.

2.4. Statistical analyses

Statistical analyses were performed using R (version 4.0.3) with a significant threshold at 5%. We performed a survival analysis (Log-Rank test) to compare survival (death age) and age at maturity (first clutch age) between control and noise treatment. Due to the normality of data, according to Shapiro test, we used t-test on clutch frequency (i.e., mean time between two clutches) and mean clutch size. To test noise effect on the total number of clutches and offspring along life we used a GLM with log function as the link function for quasi-Poisson distribution.

3. Results

The boat noises did not affect (Appendix A) neither the survival of *D. magna* (p-value = 0.51, Fig. 2a) neither the fecundity parameters, i.e., clutch frequency (p-value = 0.27, Fig. 2b),

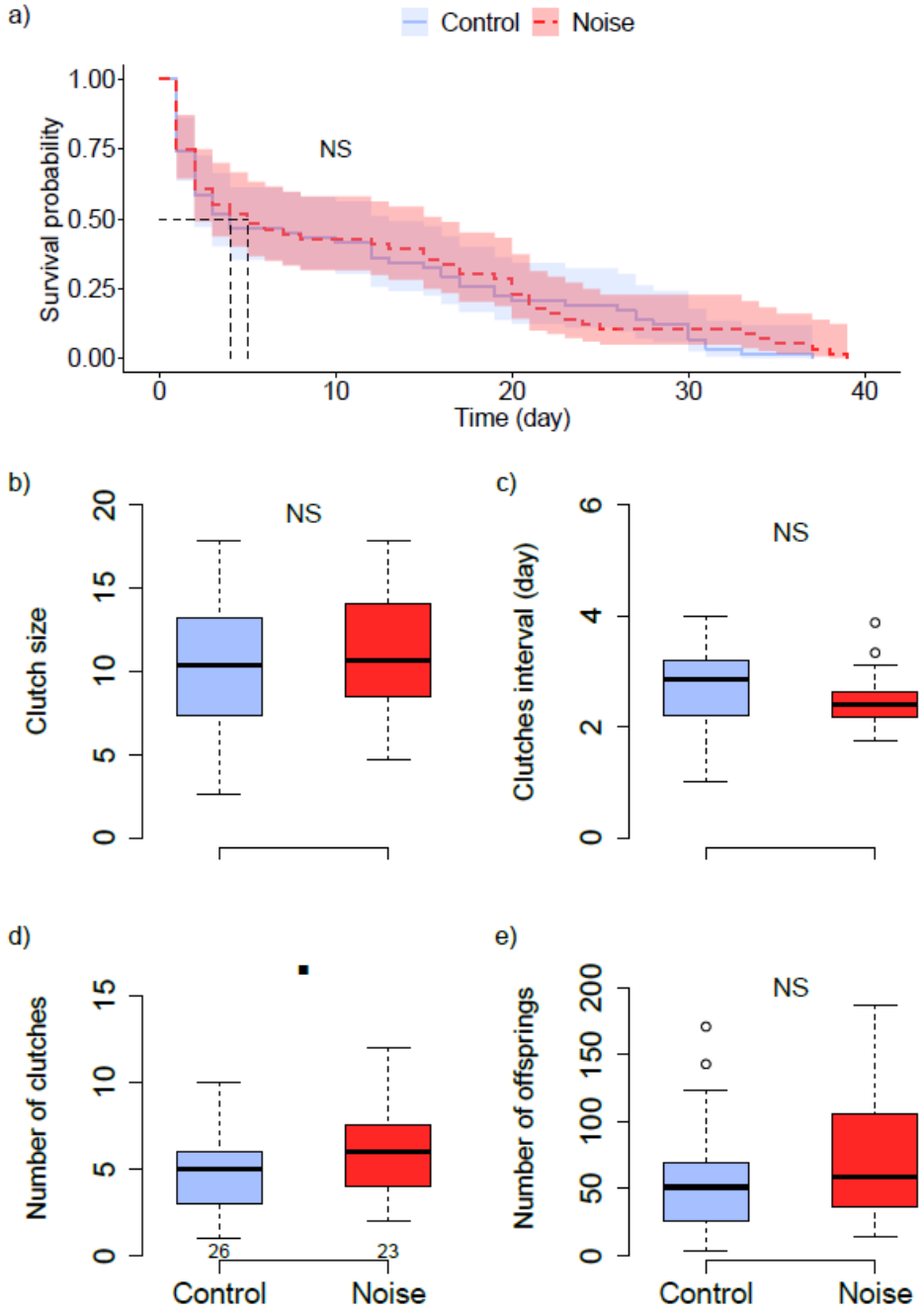

Figure 2. Effects of noise treatments on *Daphnia magna* survival and fecundity. a) Survival of *D. magna*; b) clutch size; c) clutch frequency; d) total number of clutches during lifetime; and e) total number of offspring during lifetime. Numbers in d) are the numbers of *D. magna* for the two treatments. a) Representation according to the Kaplan-Meier method; b-e) central bars represent the median, boxes the interquartile range, and dots the outliers (> 1.5 times the interquartile range). Statistical analysis: dot P < 0.1, * P < 0.05; ** P < 0.01; NS P>0.1. See Table A1 for statistical values.

clutch size (p-value = 0.72, Fig. 2c) and age at maturity (p-value = 0.65). The number of clutches (p-value = 0.06, Fig. 2d) and the total offspring production (p-value = 0.22, Fig. 2e), i.e., a proxy of individual fitness, were also not affected by boat noise treatment.

4. Discussion

We investigated the effect of chronic motorboat noise exposure on the fitness of the water flea *Daphnia magna*. Contrary to our expectations we did not find any effect of chronic exposure of boat noise on survival and fecundity parameters. Our findings contradicted previous results obtained with another type of chronic noise exposition. Therefore, our results raise the question of effects related to temporal and structural variations in noise.

We did not observe any effect of chronic boat noise exposition on *Daphnia magna* fecundity or survival. These results are opposite to Prosnier et al.'s one (2022), where an exposition to a continuous broadband noise leads to a surprising increase of individuals' fitness. These results are also opposite to those of acute exposition of copepods to airgun (McCauley et al., 2017; Fields et al., 2019). This contradiction suggested that the effect of chronic noise pollution, on zooplankton species, could depend on the structure of noise. Indeed, in the previous chronic noise exposure, broadband noise was continuous, at high level (130 dB re 1 µPa) for all frequencies (from 0.1 to 20 kHz), whereas, here, we used irregular sounds (a total of 2h of noise between 9 a.m. and 9 p.m.), with various levels (from 108 to 136 dB) and various frequency (some boat noises with low intensity between 0 and 1 kHz). It is already known, in hearing vertebrates, that animals respond differently to chronic noise pollution if there are, or not, temporal variations (continuous, regular, irregular) or structural variations (i.e., variation in the frequencies). Nichols et al. (2015) showed that fish are more stressed (higher cortisol concentration) with higher noise exposition (from 126 to 141 dB), and with an intermittent random noise compared to a continuous one. However, the review of de Jong et al. (2020) on

noise effects on fecundity revealed that continuous noise with irregular frequencies, such as boat noise, have a greater negative impact. Another study, on larvae of zebrafish using white noise, showed a higher negative effect of a continuous noise on survival and a higher cortisol concentration (Lara & Vasconcelos, 2021). Thus, it seems that, for hearing vertebrates, depending of the developmental stage and the considered characteristic, both temporal and structural sound pattern should be considered to understand how noise affect organism. For the smaller organisms, like zooplankton, little is known about the important of sound pattern. Here, the comparison with Lara & Vasconcelos (2021) and Prosnier et al. (2022) suggest that patterns should also be considered in understanding the noise effects, with higher effect with a continuous noise.

Current studies on noise exposition suggest that it is important to consider, as for other pollutants, the many ways in which organisms could be exposed to acoustic pollution (chronic vs acute), and the many organisms that could be affected by this pollution (e.g., fish vs zooplankton). Next step should be in two directions. The first one is to continue to investigate how noise affects organisms: which noise (acute or chronic, continuous or not, various frequency …) affect which organism (hearing species, zooplankton) in which way (behaviour and fitness) through which mechanism (physiology, environmental perception, ability to prey) (Duarte et al., 2021). The second one is the impact on more complex system, as a food web, with various organisms that are not similarly impacted by a type of noise (see for instance Rojas et al., 2022). As for other pollutants, these studies seem mandatory to understand how anthropogenic noises could affect ecosystems.

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Conflict of interest disclosure

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Data, script and code availability

Data, script and code are available on Zenodo. DOI: 10.5281/zenodo.7339911 (Prosnier *et al.* 2022)

Supplementary information

Supplementary information is available after the references:

- Appendix: Table of statistics

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Appendix: Tables of statistics

Table A1. Statistical results of chronic boat noises effects on fecundity and mortality of *Daphnia magna* (Fig. 2)

	Mortality	Reproduction			Fitness	
	Survival	Age at maturity	Clutch interval	Mean clutch size	Number of clutches	Number of offspring
df	1	1	40.475	46.722	1	1
χ^2/W	0.4	0.2	1.1302	-0.36206	3.5813	1.4824
p-value	0.5	0.65	0.27	0.72	0.06	0.22
R2	0.004	0.005	0.026	0.003	NA	NA