A freshwater zooplankton in the face to boat noise pollution

Loïc Prosnier[*1,2], Emilie Rojas[1] and Vincent Médoc[1]

1. Equipe Neuro-Ethologie Sensorielle, ENES/CRNL, CNRS UMR 5292, Université de Lyon/Saint-Etienne, 23 rue du Dr Paul Michelon, 42023 Saint-Etienne Cedex 2, France
2. Pôle emploi, 42000 Saint-Etienne, France.

* Corresponding author. Loïc Prosnier, ENES, Université Jean Monnet - St-Etienne, Campus Métare, Bâtiment K. 21, rue du Dr Paul Michelon 42100 Saint-Etienne, FRANCE. lprosnier@gmail.com ORCID: 0000-0001-5576-3601

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 a key zooplankton species (used as bioindicator) and the effect on fitness (survival and fecundity). We exposed isolated water fleas, *Daphnia magna*, to chronic boat noise or to a silence broadcasted 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 previous acute noise exposure, but also opposite to other ones found with acute and chronic noise effect. 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 (ion, heavy metals, drugs), exposure durations (acute or chronic), intensity (e.g., EC50, LD50-respectively Half maximum Effective Concentration and Lethal Dose) 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 pollution. 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 lead 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 (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, sound (e.g., turbine, boat noise) 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). 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 obtain 75 noises from 103 to 150 dB, using the software Adobe Audition 2020 (13.0.0.519,

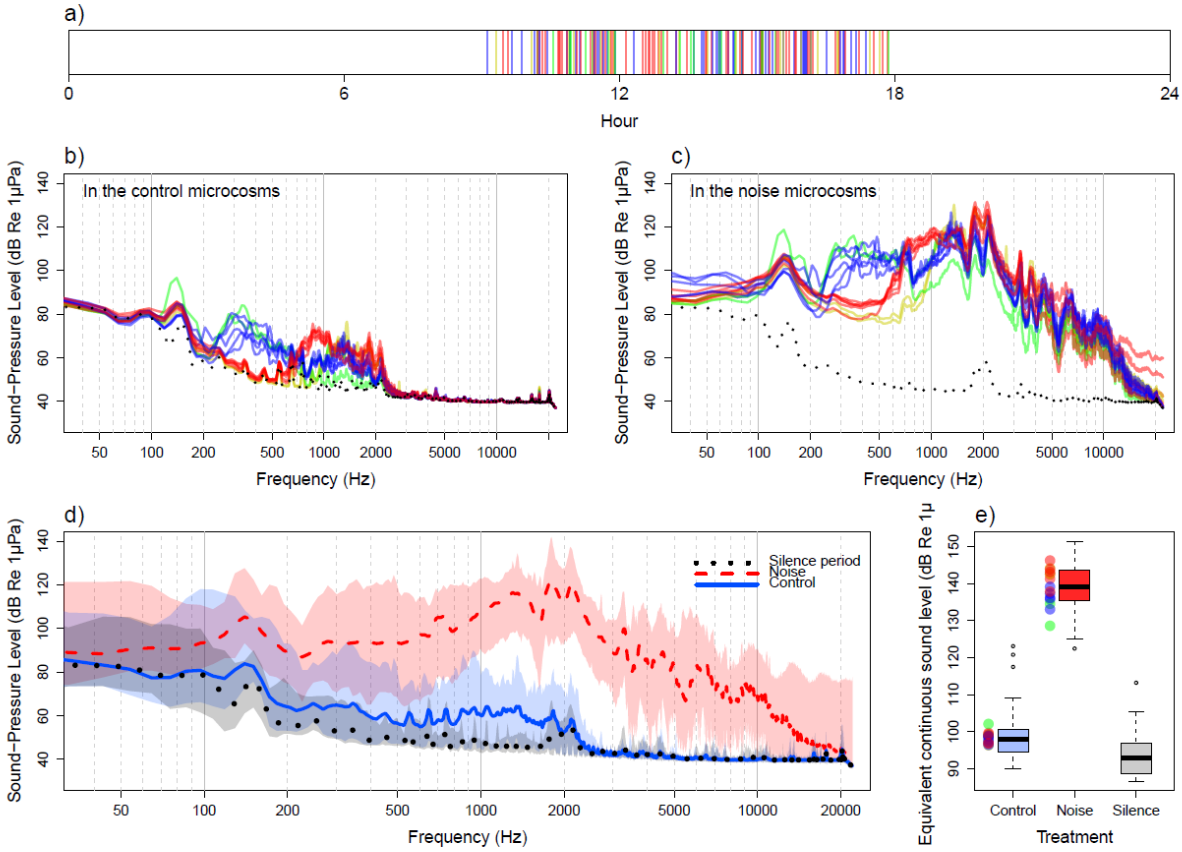

Figure 1. Acoustic treatments. a) 24h temporal sequence of the broadcasted motor boat noises, from 9 a.m. to 6 p.m. Each vertical line represents a boat. b) Sound measured in half of the microcosms in control tank, during the 15 boat broadcasts at their maximal intensity (solid lines) and silence broadcast (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.

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 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), 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.

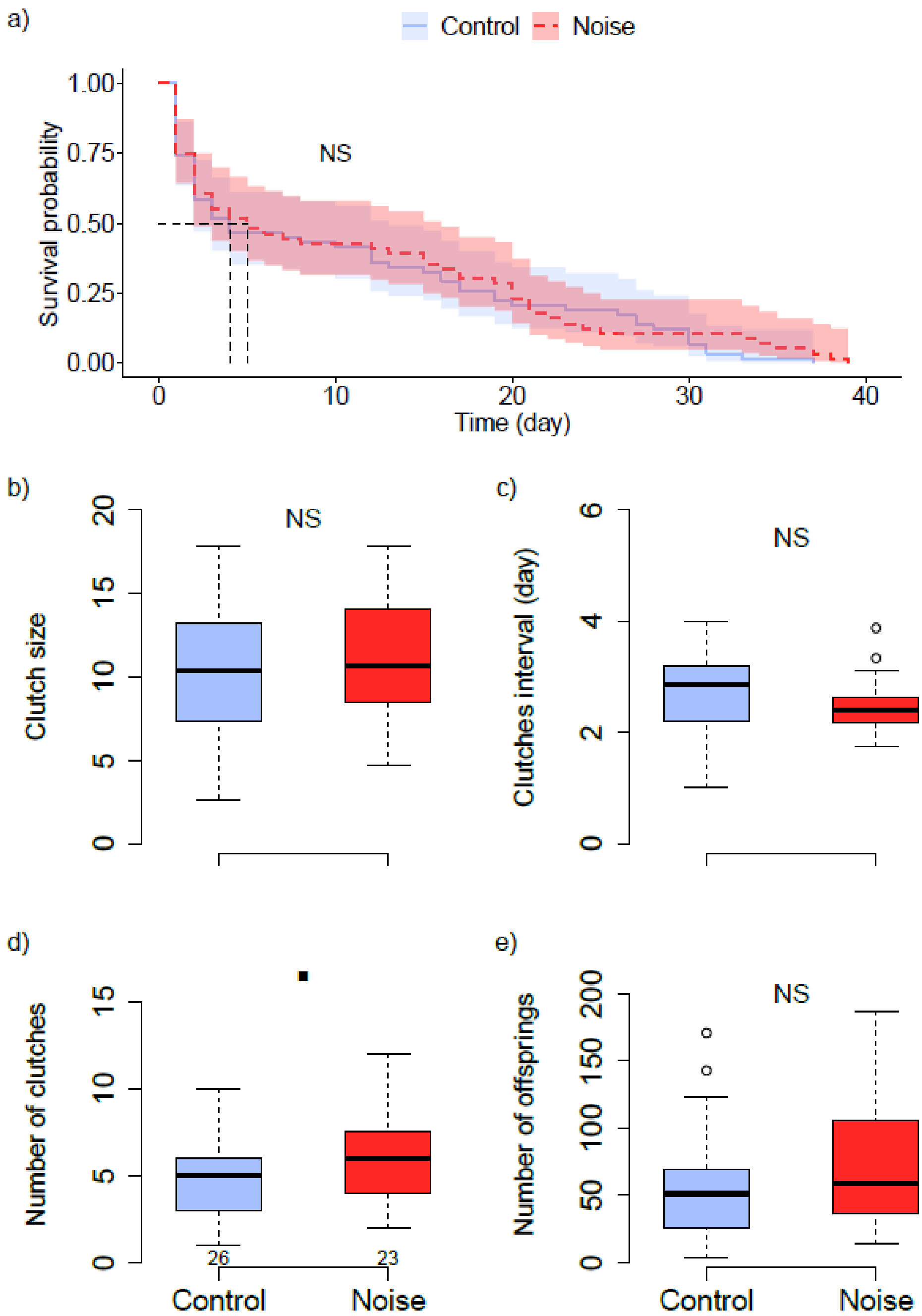

Figure 2. Effects of noise treatments on *Daphnia magna* survival and fecundity. a) Survival of *D. magna*; b) clutch size; c) clutch frequency; d) total number of clutches during lifetime; and e) total number of offspring during lifetime. Numbers in d) are the numbers of *D. magna* for the two treatments. 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.

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. This contradiction suggested that the effect of chronic noise pollution, on zooplankton species, could depend on the structure of noise. Indeed, in the previous experiment 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 noise could affect ecosystems.

Acknowledgment

The authors would like to thank all the people who contributed to the success of this experiment: Nicolas Boyer and Aurélie Pradeau for *Daphnia* rearing and providing material, Léo Papet and Joël Attia for the acoustic treatments and analysis and for providing material, Olivier Valéro for sounds recording, Marilyn Beauchaud and Paolo Fonseca for the acoustic calibration, and the EYD (ENES Young Discussion) for the useful discussions.

Conflict of interest disclosure

The authors declare they have no conflict of interest relating to the content of this article.

Data, script and code availability

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

References

André M, Solé M, Lenoir M, Durfort M, Quero C, Mas A, Lombarte A, van der Schaar M, López-Bejar M, Morell M, Zaugg S, Houégnigan L (2011) Low-frequency sounds induce acoustic trauma in cephalopods. *Frontiers in Ecology and the Environment*, **9**, 489–493. https://doi.org/10.1890/100124

Buskey EJ, Lenz PH, Hartline DK (2002) Escape behavior of planktonic copepods in response to hydrodynamic disturbances: high speed video analysis. *Marine Ecology Progress Series*, **235**, 135–146. https://doi.org/10.3354/meps235135

Duarte CM, Chapuis L, Collin SP, Costa DP, Devassy RP, Eguiluz VM, Erbe C, Gordon TAC, Halpern BS, Harding HR, Havlik MN, Meekan M, Merchant ND, Miksis-Olds JL, Parsons M, Predragovic M, Radford AN, Radford CA, Simpson SD, Slabbekoorn H, Staaterman E, Van Opzeeland IC, Winderen J, Zhang X, Juanes F (2021) The soundscape of the Anthropocene ocean. *Science*, **371**, eaba4658. https://doi.org/10.1126/science.aba4658

Gassie D V., Lenz PH, Jeannette Y, Hartline DK (1993) Mechanoreception in zooplankton first antennae: electrophysiological techniques. *Bulletin of Marine Science*, **53**, 96–105.

Hawkins AD, Pembroke AE, Popper AN (2015) Information gaps in understanding the effects of noise on fishes and invertebrates. *Reviews in Fish Biology and Fisheries*, **25**, 39–64. https://doi.org/10.1007/s11160-014-9369-3

Jan N, Majeed N, Ahmad M, Ahmad Lone W, John R (2022) Nano-pollution: Why it should worry us. *Chemosphere*, **302**, 134746. https://doi.org/10.1016/j.chemosphere.2022.134746

de Jong K, Forland TN, Amorim MCP, Rieucau G, Slabbekoorn H, Sivle LD (2020) Predicting the effects of anthropogenic noise on fish reproduction. *Reviews in Fish Biology and Fisheries*, **30**, 245–268. https://doi.org/10.1007/s11160-020-09598-9

Kunc HP, Schmidt R (2019) The effects of anthropogenic noise on animals: a meta-analysis. *Biology Letters*, **15**, 20190649. https://doi.org/10.1098/rsbl.2019.0649

Lara RA, Vasconcelos RO (2021) Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. *Scientific Reports*, **11**, 6615. https://doi.org/10.1038/s41598-021-85296-1

Longcore T, Rich C (2004) Ecological light pollution. *Frontiers in Ecology and the Environment*, **2**, 191–198. https://doi.org/10.1890/1540-9295(2004)002[0191:ELP]2.0.CO;2

McCauley RD, Day RD, Swadling KM, Fitzgibbon QP, Watson RA, Semmens JM (2017) Widely used marine seismic survey air gun operations negatively impact zooplankton. *Nature Ecology & Evolution*, **1**, 0195. https://doi.org/10.1038/s41559-017-0195

Nichols TA, Anderson TW, Širović A (2015) Intermittent noise induces physiological stress in a coastal marine fish (CA Radford, Ed,). *PLoS ONE*, **10**, e0139157. https://doi.org/10.1371/journal.pone.0139157

Parmar TK, Rawtani D, Agrawal YK (2016) Bioindicators: the natural indicator of environmental pollution. *Frontiers in Life Science*, **9**, 110–118. https://doi.org/10.1080/21553769.2016.1162753

Popper AN, Hawkins AD (2019) An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. *Journal of Fish Biology*, **94**, 692–713. https://doi.org/10.1111/jfb.13948

Prosnier L, Rojas E, Valéro O, Médoc V (2022) Chronic noise unexpectedly increases fitness of a freshwater zooplankton. *bioRxiv*, 2022.11.19.517212. https://doi.org/10.1101/2022.11.19.517212

Prosnier L, Rojas E, Médoc V (2022). Datasets and R source code of manuscript "A freshwater zooplankton in the face to boat noise pollution" [Data set]. Zenodo. https://doi.org/10.5281/zenodo.7339911

Richardson WJ, Fraker MA, Würsig B, Wells RS (1985) Behaviour of Bowhead Whales Balaena mysticetus summering in the Beaufort Sea: Reactions to industrial activities. *Biological Conservation*, **32**, 195–230. https://doi.org/10.1016/0006-3207(85)90111-9

Rojas E, Gouret M, Agostini S, Fiorini S, Fonseca P, Lacroix G, Médoc V (2022) From behaviour to complex communities: Resilience to anthropogenic noise in a fish-induced trophic cascade. *bioRxiv*, 2022.07.05.498792. https://doi.org/10.1101/2022.07.05.498792

Rojas E, Thévenin S, Montes G, Boyer N, Médoc V (2021) From distraction to habituation: Ecological and behavioural responses of invasive fish to anthropogenic noise. *Freshwater Biology*, **66**, 1606–1618. https://doi.org/10.1111/fwb.13778

Sabet SS, Karnagh SA, Azbari FZ (2019) Experimental test of sound and light exposure on water flea swimming behaviour. *Proceedings of Meetings on Acoustics*, **37**, 010015. https://doi.org/10.1121/2.0001270

Sabet SS, Neo YY, Slabbekoorn H (2015) The effect of temporal variation in sound exposure on swimming and foraging behaviour of captive zebrafish. *Animal Behaviour*, **107**, 49–60. https://doi.org/10.1016/j.anbehav.2015.05.022

Shannon G, McKenna MF, Angeloni LM, Crooks KR, Fristrup KM, Brown E, Warner KA, Nelson MD, White C, Briggs J, McFarland S, Wittemyer G (2016) A synthesis of two decades of research documenting the effects of noise on wildlife. *Biological Reviews*, **91**, 982–1005. https://doi.org/10.1111/brv.12207

Song Y, Xie L, Lee Y, Brede DA, Lyne F, Kassaye Y, Thaulow J, Caldwell G, Salbu B, Tollefsen KE (2020) Integrative assessment of low-dose gamma radiation effects on Daphnia magna reproduction: Toxicity pathway assembly and AOP development. *Science of The Total Environment*, **705**, 135912. https://doi.org/10.1016/j.scitotenv.2019.135912

Truhaut R (1977) Ecotoxicology: Objectives, Principles and Perspectives. *Ecotoxicology and Environmental Safety*, **1**, 373–413. https://doi.org/10.1016/B978-0-08-021998-1.50038-5

Villeneuve DL, Garcia-Reyero N (2011) Vision & strategy: Predictive ecotoxicology in the 21st century. *Environmental Toxicology and Chemistry*, **30**, 1–8. https://doi.org/10.1002/etc.396

Williams R, Wright AJ, Ashe E, Blight LK, Bruintjes R, Canessa R, Clark CW, Cullis-Suzuki S, Dakin DT, Erbe C, Hammond PS, Merchant ND, O'Hara PD, Purser J, Radford AN, Simpson SD, Thomas L, Wale MA (2015) Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. *Ocean & Coastal Management*, **115**, 17–24. https://doi.org/10.1016/j.ocecoaman.2015.05.021