

Noise impact in *Daphnia magna*

Claudia Cosio based on peer reviews by **Marie-Agnès Coutellec** and 1 anonymous reviewer

Loïc Prosnier, Emilie Rojas, Vincent Médoc (2023) No evidence for an effect of chronic boat noise on the fitness of reared water fleas. bioRxiv, ver. 4, peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry.

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Our ability to anticipate and estimate how pollution affects biota is of paramount importance in the field of ecotoxicology. Impact of chemical pollution by metals, drugs or pesticides was widely studied in different species using acute and chronic scenarios. While environmental factors such as temperature are also often considered, noise is largely ignored in these models despite the knowledge of its detrimental effects in vertebrates. Studies of noise impacts included behavior and fitness endpoints and showed no effect to death depending on intensity, frequency and the distance from the noise source (Peng et al., 2015). Nonetheless, the impact of noise in biota is not well-understood, which impairs its effective mitigation.

Noise or acoustic pollution due to boat traffic produce low-frequency stationary noise. It is a pervasive and ubiquitous pollutant found in aquatic ecosystems. In this context, Prosnier et al. (2023) addresses how intermittent and random noise impacted *Daphnia magna*, a representative of zooplankton model, widely used in ecotoxicology. Endpoints of lifespan and clonal offspring production were measured in the presence or absence of motorboat noises, in animals reared from birth to death. Noise consisted in a playlist of 15 sounds of motorboat recorded in the Grangent lake (Loire, France). Their intensity ranged from 0 to -25 dB Re 1 μ Pa by 5 dB to create 75 sounds from 103 to 150 dB RMS Re 1 μ Pa – a range of levels occurring in lakes. Treatment had no effect on analyzed endpoints, contrary to a continuous broadband noise (100-20,000 Hz) that caused higher survival and fecundity, and reduced speed of motion compared to control (Prosnier et al., 2022). Data point that temporal (continuous, regular, random) and frequency of noise are instrumental for its effects.

References:

Peng, C., X. Zhao and G. Liu (2015). "Noise in the Sea and Its Impacts on Marine Organisms." *Int J Environ Res Public Health* 12(10): 12304-12323. <https://doi.org/10.3390/ijerph121012304>

Prosnier, L., E. Rojas and V. Médoc (2023). "No evidence for an effect of chronic boat noise on the fitness of reared water fleas." bioRxiv: 2022. ver. 4 peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry. <https://doi.org/10.1101/2022.11.20.517267>

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

Reviews

Evaluation round #3

DOI or URL of the preprint: <https://doi.org/10.1101/2022.11.20.517267>

Version of the preprint: 4

Authors' reply, 18 July 2023

Dear recommender and reviewer.

Thank you for your opinion about the improving clarity of the article.

We appologize for these two confusing titles. The title in the response "Effects of acoustic pollution on *Daphnia magna* fitness: the importance of noise type." was a proposition by some co-authors. It was not approved by all the co-authors because it focus more on one (interesting) point of discussion, and not on the main result of the experiment. This title should be more convenient for a meta-analysis paper. In the present state there are only two papers (not yet peer-reviewed) for this discussion, thus this title would be an over-interpretation.

All the authors have approved that the title should more focus on the main results, but without overinterpretation contrary to the previous title - according to your previous comments. Thus, all the authors approved "No evidence for an effect of chronic boat noise on the fitness of reared water fleas"

Consequently, we would like to conserve the current title, on the preprint:

"No evidence for an effect of chronic boat noise on the fitness of reared water fleas"

Best regards,

Loïc Prosnier, Emilie Rojas and Vincent Médoc

Decision by [Claudia Cosio](#), posted 18 July 2023, validated 18 July 2023

minor revision

Please adapt title as suggested.

Reviewed by anonymous reviewer 1, 11 July 2023

I agree with the new title proposed by authors in their reply "Effects of acoustic pollution on *Daphnia magna* fitness: the importance of noise type."

However, this is not the title included in the change tracking files (wich is "No evidence for an effect of chronic boat noise on the 1 fitness of reared water fleas"). I think that "Effects of acoustic pollution on *Daphnia magna* fitness: the importance of noise type." should be kept.

Apart from that point, authors improved the clarity of their article, which doesn't deserve further modification.

Evaluation round #2

DOI or URL of the preprint: <https://doi.org/10.1101/2022.11.20.517267>

Version of the preprint: 3

Authors' reply, 16 June 2023

[Download author's reply](#)

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Decision by [Claudia Cosio](#), posted 01 June 2023, validated 02 June 2023

Major revision

Please address comments made by the reviewer.

Best regards,

Claudia

Reviewed by [Marie-Agnès Coutellec](#), 01 May 2023

Dear Editor,

This revised version is fully satisfying to me. I guess that the Morse running issue can be dealt with independently, and doesn't deserve further consideration in the context of the present article.

Reviewed by anonymous reviewer 1, 01 June 2023

For this 2nd round of review, authors have significantly taken into account the comments of the reviewers. The results description and discussion have been globally improved, and the representativity of these results have been slightly mitigated in light of the suboptimal (not to say bad) physiological condition of their daphnia used to respond to their problematic.

However, I don't understand the choice to change the title of the article for this second round from "A freshwater zooplankton in the face to boat noise pollution" to "Chronic boat noise does not alter the fitness of *Daphnia magna*"

I strongly disagree with the new proposed title - I don't consider that the main conclusion of this study can be that chronic boat noise does not alter the fitness of *Daphnia magna* - and this should not be the 'take home message' of this article. Due to the bad physiological condition of daphnia in this study (more than 50% mortality before maturity), I don't think that authors can generalize their observation at this point, this would be an overinterpretation. This is even more reinforced by the fact that the causes of this suboptimal physiological state are not identified and controlled by the authors. To my point of view, the message that can be derived from this study is that, at similar suboptimal physiological condition, impacts on fitness differs between two scenarios of noise exposure (from the current article and Prosnier, Rojas, et al. (2022)). But the lack of fitness impact of chronic boat noise on *D. magna* cannot be established as an absolute conclusion based on this single study.

Specific comments:

L95: mj is not a usual metrics to describe daphnia food intake. Could you provide some (at least one) references to estimate the adequacy of this diet/mj amount for *D. magna* ?

L193: "differed from previous results obtained" - to help reader, please provide the ref of the previous results you refer to.

L205: "Although noise had no statistical effect on the survival and fecundity..." – It is not clear that you refer to the current article in this beginning of sentence, as it directly follow a reference to Prosnier, Rojas, et al. (2022). Please clarify to help understanding of your argumentation.

L248 ("As a perspective"): As your data do not provide direct insight toward multi-stress, I think this paragraph isn't a perspective of your study, but an additional interesting consideration.

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2022.11.20.517267>

Version of the preprint: 2

Authors' reply, 02 April 2023

[Download author's reply](#)

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Decision by **Claudia Cosio**, posted 25 January 2023, validated 25 January 2023

Major revision

Please adress all points raised by the two reviewers.

Reviewed by anonymous reviewer 1, 24 January 2023

The article entitled "A freshwater zooplankton in the face to boat noise pollution", by L. Prosnier, E. Rojas and V. Medoc focuses on an interesting and understudied topic: the consequence of chronic irregular noise exposure on *Daphnia magna* survival and reproduction, as proxy for fitness. This study is based on valuable individual data of daily survival and reproduction under two conditions of noise exposure.

It is well written, and the context, the objectives and working hypotheses are well explained.

The experimental design is well described, even if it could be profitable to provide picture of the device (L96). This could allow to figure out some points: are all the 18 microcosms at the same distance of the loudspeaker? Is it possible that some microcosm disturbs the noise transmission to other ones?

There are some missing information to fully describe the methodology:

L. 88: the physicochemical parameters of the used tap water is not provided

L89: further than algae mass, it could be interesting/important to consider the amount of carbon, and thus to precise the amount of C in 0.05g of dry algae.

L105-106: Please provide the rate of mortality that caused the need of individuals replacement. Is it a normal phenomenon? Where came from the alternative individuals? (same brood? Which raising condition until integration in the experimental device?). This is a really important point, as this could lead to a major source of variability in the data (eg : missing window of exposure in some individuals...).

Fig. 1 caption: please define "SPL". "visuals category" should be clarified.

I am not qualified to judge the suitability of sound exposure setup and suitability of sound signal treatment.

The experimental design is really interesting, and seems suitable to provide interesting insight on chronic irregular noise exposure on *Daphnia magna*.

However, results are far too briefly described, and there is a lack of description of reproductive and survival parameters in control individuals – there is to few data and statistical description and details in the results section. Lethal times for 50% of individuals are represented on a scale with intervals much too large to be graphically read (fig 2a), and their numerical value isn't given in the text. This would really help the reader to consider this major element = the survival rate of control individuals drops down to 50% within only 5 days,

that is, I guess likely before the first reproductive cycle. This may explain the low total number of clutches during lifetime.

In addition, the number of individuals considered in each condition is provided only for the “number of clutches”, were it should be provided for all parameters.

These interesting individual data of daily survival and reproduction should also be further exploited (eg: providing age at first clutch). Given that the number of surviving individuals, and thus the number of individuals able to reproduce, decreases with time, it would be interesting to represent the cumulated production of neonates per daphnia in relation to age, and to include the number of considered individuals for each clutch. This would allow to figure out the representativity of mean values for each clutch, and the evolution of clutch size during lifetime which is supposed to be observed in *D. magna*.

The survival rate drops down to 50% within only 5 days, and the number and size of clutch seems really low compared to other studies (eg: Parisot et al, *Aquatic Toxicology* 163 (2015) 27–36). This raises major questions about the suitability of rearing conditions during the time of the experiment, and this could suggest that the considered individuals (whatever the noise exposure) are in suboptimal physiological condition. In my point of view, this should be one of the main points of discussion of the article, as this may limit, or at least open another perspective on the representativity of the results: are individuals unaffected because their physiological performances are already at their lower point? The lack of discussion on that point weakens the conclusion of the article. It is to note that this problem of physiological condition is also found on the article of the same authors (Prosnier et al 2022, non peer-reviewed preprint) that is used as the main comparison point in the discussion.

Thus, I think that this article deserves more work on results description and discussion to provide valuable insight into anthropogenic noise impact on *Daphnia magna*.

Reviewed by **Marie-Agnès Coutellec**, 20 January 2023

As indicated in its title « A freshwater zooplankton in the face to boat noise pollution », the preprint submitted by Loïc Prosnier and his collaborators to PCI-Ecotoxicology, reports on a study of the impact of anthropogenic noise to aquatic invertebrate life. Like light, noise is an emerging environmental issue of great concern, which has been so far totally neglected in ecotoxicology. Given that such insidious pollutions are expected to amplify with the human population growth and associated activities and to combine with other stressors and pollutants, it is urgent to start to assess their impacts to natural ecosystems. In this respect, the present report, by addressing this issue in *Daphnia magna* (a zooplanktonic species widely used as model in standard ecotoxicity testing) experimentally exposed to boat noises, is of strong intrinsic value.

Not being an expert in noise treatment and effects, I focus my review on general characteristics of the design, and on result analysis and discussion. My only request on noise pollution is the extent that the intensity used in the laboratory is representative or not of real levels of environmental exposure. Did the authors check for it and how did they do it? It might also be important to recall that in the « field », noise may not be uncoupled from vibrations, which was not simulated in the experiment.

The experimental design is globally well described, yet additional details would be helpful to the reader.

Mortality is said to be corrected at the beginning, by replacing dead juveniles. Please indicate the exact period concerned by this treatment. It seems that mortality was quite high at start (50 % by day 5, whatever the treatment), which may lead to ask why not delaying exposure to day 10 or so, as it would have allowed to avoid replacing dead juveniles during the course of the experiment (which should be avoided, as much as possible).

The experiment duration is not specified, and only accessible on Fig dealing with survival. It is a pity, because apparently data are not censored (traits measured until the death of all animals), which is rare enough (in ecotoxicity testing) to be mentioned. If so, this should be clearly specified.

Next, the dataset (individual-level survival and reproduction) is appropriate to the estimation of population growth (using Euler-Lotka's equation), and is advocated to have a better idea of fitness, as fitness impact was targeted by the authors (not only fitness-related traits taken separately).

Also, it is not clear if maternal mortality was accounted for in reproduction data. I suggest using a model suited to deal with toxicity over time (as implemented in MORSE package, see Baudrot & Charles, 2021). I am quite curious to know about the findings based on such analysis.

Results do not indicate fitness effects of the noise treatment applied. The authors consider them as contradictory to those obtained in their previous study (Prosnier et al. 2022), yet noise conditions differed (constant vs intermittent application, intensity). Thus, to me, the contradiction may only be apparent, and they authors should better explain why they view discrepancies between the two studies as contradictory.

Next, discussion should extend to development, for two reasons : development is a particularly sensitive period in an organism life and noise and it would be interesting to know if newborns were well developed, if aborted embryos occurred, etc. Even if only qualitatively noted throughout the experiment, this information is critical to assess whether fitness is affected or not.

Finally, as perspective, the authors mention foodweb-level experiments, as a way to go further into noise effect assessment, yet I would rather suggest that functional approaches dealing with mechanoreceptors and genes potentially involved in noise perception, would be more relevant in a « non hearing » organism. At least, this would deserve mention. It is surprising that the following reference was lacking (Popper, A., Salmon, M. & Horch, K. Acoustic detection and communication by decapod crustaceans. *J Comp Physiol A* 187, 83–89 (2001). <https://doi.org/10.1007/s003590100184/>). I also believe that discussed could be enriched by considering terrestrial invertebrates, among which several groups are acutely sensitive to noise. (see e.g., Morley, E.L., Jones, G., Radford, A.N., 2014. The importance of invertebrates when considering the impacts of anthropogenic noise. *Proceedings of the Royal Society B: Biological Sciences* 281, 20132683. <https://doi.org/10.1098/rspb.2013.2683>)

Another issue that might be mentioned as perspective is that noise ability to act synergistically with other stressors present in daphnid's environment could be worth testing.

Other minor comments :

X-mesh : specify mesh-size

L71 : « Additionally, an important zooplankton predator (*Chaoborus flavicans*) increased anti-predatory defence behaviour when exposed ». This sentence is a little confusing. Is the behaviour observed on *Chaoborus*'s prey ? If yes, specify species (or organism).

L97-99. Four tanks were used, each containing 18 individual 150ml vessels. Please specify that this was per treatment (otherwise $18 \times 4 = 72$ microcosms are not sufficient to get 57+58 experimental units). Note also that the tank effect should be included as random factor in the test design. Instead of a t-test, please use a linear mixed effect model (lmer if normal, glmer if not, with lme4 package).

Fig.1. Please indicate why you measured noise only on half of the tanks.

Discussion: I am not sure to understand what "hearing vertebrates" really means.