PCI Ecotox Env Chem #103

Chronic boat noise does not alter the fitness of *Daphnia magna*

(A freshwater zooplankton in the face to boat noise pollution)

Loïc Prosnier, Emilie Rojas, Vincent Médoc

Responses to the Recommender, Claudia Cosio

- Please adress all points raised by the two reviewers

We thank you, and the reviewers, for your interest in this study and the useful comments. Following the reviewers' comments, we improved the M&M, the statistical analyses, and the Results and the Discussion sections.
Note that, for better readability, we clarified the title "Chronic boat noise does not alter the fitness of *Daphnia magna*"

Responses to the Reviewer 1

- 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.

We thank you for your interest in this article. We have taken into account your many useful comments to improve our manuscript: we added a schematic of the set up and some information in the M&M, described more the results and improved the discussion.

- 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?

We added a schematic of the set up (Fig. 1).

The 18 microcosms were at 20 cm from the speaker, mesocosms being the same room, relatively closed, the boat noise emitted during the noise treatment was detectable in the control mesocosm but at low intensity. Indeed, before the beginning of the experiment, we quantified the perceived sound level in each microcosm and we visualized the noise structure which we corrected to consider the disturbances. Figure 2 summarizes the noise during the experiments for the control and noise treatments (thus after the noise correction). In detail, the noise in the Figure 2 is the noise in half of microcosms in our symmetric setup (due to technical constraints we only took brief measurements in the other half to verify that there is no different/surprising noise structure): we saw that noise was similar in all control, and noise microcosms.

- There are some missing information to fully describe the methodology:

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

In the Zenodo data, we added the physiochemical parameters of tap water from public health agency (Agence Régionale de Santé). We added: "(physiochemical composition is available on Zenodo repository (Prosnier et al., 2023))" (L93)

- 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 alguae.

We could not determine the amount of carbon of dry algae. On the other hand, we have information of the energy and we have therefore added: "(i.e., 736 mJ)" (L95)

- 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…).

In the M&M we added that we replaced the dead newborns during "the eight first days of the experiment", and that, finally, "half of [individuals] (respectively 26 [in control] and 23 [in noise]) reached maturity". (L112-118)

Indeed, we were disappointed by the high mortality of newborn (compared to a previous experiment by some of the authors, but under different conditions), as you noted in a subsequent comment. You are correct that it was not clear that we only included newborn (<24h) in the experiment. Thus, we modified by: "we replaced dead *D. magna* by new newborns (we have maintained isolated mothers in 50 mL jars during this initial period to be able to initiate new replicates with newborns)" (L114). Therefore, there is only a difference in the birth date, but it should not affect the results because all individuals are under the same conditions throughout all the experiment (from birth of the first individual to death of the last). In the analyses we did not consider dates but ages.

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

We replaced "SPL" by "Sound-Pressure Level".

We wrote "The four colours (red, yellow, green, blue) correspond to four noise structure spectra that were visually determined (for instance red and yellow have low energy between 200 and 700 or 1000 Hz compared to green and blue boat noises)."

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

Coming from a bioacoustics laboratory, all the acoustic measurements and the calibration of noises in our configuration have been validated beforehand by researchers of the laboratory in order to provide the best quality of restitutions (playbacks). Following your comment, Jérémy Rouch and Joël Attia, bioacousticians from our lab, reviewed the M&M section. We corrected some details and added missing information: "using a one-third octave graphic equalizer (with Adobe Audition 2020)" (L143) and "Particle motion cannot be measured due to the absence of adequate equipment, despite its importance for non-hearing species (Nedelec et al., 2016).

However, Olivier et al. (2023) showed that results can still be qualitatively relevant when based solely on sound pressure level." (L151)

- 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.

We have supplemented the description of the results with data (L173-183).
We believe that the short lifespan of mature Daphnia is more likely to explain the low number of clutches because the death of Daphnia before maturity was not considered for the mean clutch number and size. Because of lack information, we added in the M&M section: "For the fecundity parameters, only individuals that clutched at least one time (i.e., that reached maturity) were considered in the analyses." (L162)

- 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.

Maybe it was not clear enough, but all parameters are for the same individual, so the number of individuals is identical. We also wrote in the legend "Numbers in d) are the numbers of *D. magna* for the two treatments." and we added "for b, c and e". But the different numbers in the M&M section should be problematic, so we added in the M&M: "almost half of the juveniles in each condition (26 in control and 23 in noise) reached maturity" (L117)

- 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.

You are right that we did not provide any information about the age at maturity, although we have tested it. We detailed: “age at maturity around 8 days” (L176). We also added data in the results section as per your previous comment, but we have not detailed in the initial text the exact value for both treatments because the difference was not significant, and writing two values could mislead readers.
In Fig. 3, we replaced the unnecessary subfigure d) according to your suggestion of a figure of cumulative reproduction close to the subfigure e) where data for total offspring productions were statistically analysed. We also added analysis of daily clutch size (where no effect of the noise treatment was found). Additionally, we added “The effect of both noise and age on daily clutch size was analysed by a type II analysis of variance, completed with a pairwise Wilcoxon test between the treatments within each age.” (L166) and “Daily clutch size was not influenced by noise (noise: p-value = 0.89, noise x age: p-value = 0.35, pairwise: p-values > 0.38; Fig. A1), but changed with age (p-value = 0.003) with larger clutches at intermediate ages.” (L177). Finally, we added the resulting Fig A1 in the Appendix section.

- 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 weaken the conclusion of the article. It is to note than 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.

We agree with you that the suboptimal conditions should affect our results. We added in a new paragraph to the discussion: “It could be also useful to investigate whether all the unwanted noises produced by many experimental setups (to control light, temperature, oxygenation, and food) interact with the stressors studied and influence the results. For instance, in the present study there was a very high mortality in D. magna juveniles compared to similar studies (Parisot et al., 2015; Prosnier, Loeuille, et al., 2022) that suggests suboptimal conditions (i.e., other stressor than noise) that might have affected the outcomes through the masking of effects for example. However, on the other side, suboptimal conditions could make individuals more prone to be affected by an additional stress like noise. Note that, with the same suboptimal conditions, Prosnier, Rojas, et al. (2022) obtained a significant difference between the control and noise treatments. The recent Larvosonic system, developed by Olivier et al. (2023), could be useful to study the impact of noise on zooplankton with a better control of the environmental conditions.” (L256-267)

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

In accordance with your suggestions and those of the other reviewer, we hope that the changes to the ‘results and discussion’ sections will add value to our paper and encourage further work on this interesting issue.

<u>Responses to the Reviewer 2, Marie-Agnès Coutellec</u>

- 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 sofar 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.

We thank you for your interest and for the present study and its thematic. We improved the manuscript based on your suggestions; therefore, we improved our statistical analyses using the Euler-Lotka equation, and we added/modified three paragraphs in the discussion section.

- 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.

We omitted to write that "– a naturally-occurring range of noise levels found in lakes (V. Médoc, pers. obs.)" (L131). In fact, V. Médoc measured the noise level in the field as we did in the experiment.
Probably by "vibrations" you were thinking of "turbulence" near boats (because sound is a vibration), which could be of great importance for aquatic food webs (Blottière et al. 2017 – Effects of mixing on the pelagic food web in shallow lakes)? It might be interesting to consider (the effect as well, as the multiple stress of passing boats) but we think that vibrations concern organisms very close to boats, while noises are perceptible at several meters.

- 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 avoided, as much as possible).

We added "the eight first days of the experiment" (L112).

We started the exposure on day 1 because (1) in nature, individuals should be exposed from birth, (2) we wanted to know if noises could affect juvenile mortality and age at maturity, and (3) because there is no difference with and without noise, we should have this high mortality in the first days despite we started exposure on day 10. Since replacing individuals is far from ideal, this should not affect the results because all individuals were under the same conditions (noise, temperature, light, water composition).

- 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 mentionned. If so, this should be clearly specified.

We added: “The experiment lasted 46 days, from the birth of the first individual to the death of the last one (the oldest *D. magna* survived 39 days).” (L118)

- 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).

You are right that it is an interesting method that we were not aware of. We did this analysis and added in the M&M section: “Based on daily survival and daily clutch, populational data was analysed using the Euler-Lotka equation ($\sum f_x m_x e^{-rx} = 1$), with f_x the fecundity at age *x*, m_x the survival at age *x*, and *r* the intrinsic rate of increase. This equation allows to calculate the reproductive output R_0 ($R_0 = \sum f_x m_x$), the generation time G_t $\left(G_t = \frac{\sum x f_x m_x}{\sum f_x m_x}\right)$, and the intrinsic rate of increase *r* $\left(r = \frac{\log R_0}{G_T}\right)$ (Leung et al., 2007; Starke et al., 2021).” (L120-124)

And we also added in the result section a last paragraph: “The populational analysis done with the Lotka-Euler equation confirmed the tendancy on total offspring production with a reproductive output (R0) higher for the noise treatment with 63 offspring compared to the 54 offspring in control. Generation time (GT) was longer in the noise treatment, with 16.6 days, compared to the 15.2 days in the control. The combination of both led to an intrinsic rate of increase of 0.25 day^{-1} in the noise treatment compared to 0.26 day^{-1} in the control.” (L184-189)

Note that we did the same analysis on our other not-yet-reviewed paper (with broadband noise), where there is a clear effect with a higher offspring production with noise exposure. We find a higher intrinsic rate in the noise treatment (despite a higher generation time, as here) – thus, it does not change our conclusion thus the discussion about it within the current manuscript.

- 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.

First, concerning the relationship between reproduction and mortality data, we considered the reproduction data in two variables: the fecundity itself (clutch size and clutch interval, and now daily clutch size), independent from mortality, and the offspring production along life (our fitness-proxy, affected by both the survival and the fecundity). We clarified: "Data allowed to analyse separately the effects on mortality (death age and adult survival) and fecundity (age at maturity, clutch frequency, mean clutch size, and daily clutch size). The combination of mortality and fecundity was used as a proxy of fitness and quantified through total offspring production. Data was also described at the population scale using the Euler-Lotka equation (but without statistical analysis due to absence of populational replicates)" (L156-160)
We tested morse/mosaic, but it seemed for population data (with replicates) for few concentrations and not individual data (we tried considering all individuals in a treatment as a population, or two populations, one per tank) with only one "concentration" in addition to the control. It has seemed to not work with our data (the mosaic website indicated an error with no details or explanation), and R error was unclear to us.

- 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 appearant, and they authors should better explain why they view discrepancies between the two studies as contradictory.

It was probably not clear that we were not considering contradictions between the two studies, but we highlighted differences that could be explained by the differences in the temporal and spectral structure of noises (continuous and regular vs intermittent and irregular). We replaced the term “contradictory” by “differ from” and “difference”. We also put more emphasis on this aspect in the introduction and in the discussion by adding the term “predictable” for instance.

- 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 wether fitness is affected or not.

You are right that the fitness of individuals considers the viability of offspring. That is why we added a new paragraph: “Despite there was no effect on fecundity, it would be necessary to focus on the offspring coming from mothers exposed to noise. Here, there was no qualitative effect reported during the current experiments, i.e., all the water fleas produced seemed viable and mobile. More, there was no increase of mortality of newborns due to noise, and no effect was reported on size of D. magna exposed to chronic noise (Prosnier, Rojas, et al., 2022). This would be consistent with the study of Day et al. (2016) where exposure to air gun did not affect the embryonic development of the spiny lobster Jasus edwardsii (Decapodae). However, airgun exposition reduces growth and development stage of Acartia tonsa nauplii (Vereide et al., 2023). This aspect seems important as it is known that stress on mother and early stages can affect daphnia’s development (Mittmann et al., 2014; Mushegian et al., 2016) and that effects can differ across generations (Campos et al., 2016). Consequently, impact studies on noise should focus on embryonic development and perform multigenerational experiments to determine the long-term effects of chronic exposure resulting from embryonic misdevelopment (Mushegian et al., 2016), maternal effects (Radersma et al., 2018), and acclimatation or adaptation (Ringot et al., 2018; Abdullahi et al., 2022).” (L233-247)

- 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

You are right that we failed to mention sources of information on other invertebrates. So, we added in the introduction: "Although the effects of noise on large invertebrates, such as decapods or bivalves, have recently received substantial interest (see the reviews of Popper et al. (2001) and Solé et al. (2023)), research largely neglected zooplanktonic invertebrates (Hawkins et al., 2015; Prosnier, 2022), despite their ecological importance and general use as bioindicators in ecotoxicology (Parmar et al., 2016)." (L49-53)
In the previous version, we never mentioned terrestrial invertebrates, although our question was also of interest to these organisms, or answers could be found with them. So, we added "Understanding the various reaction of vertebrates and invertebrates in term of behavior, but also in term of fitness is mandatory to study how noises could affect complex communities (Francis et al., 2009; Slabbekoorn & Halfwerk, 2009; Slabbekoorn, 2019). […] Moreover, in a community, pollutants can alter fitness directly (as in this study) but also indirectly through modification of vulnerability to natural enemies for instance (Read et al., 2014). For instance, noise do not affect frog abundance but reduce their parasite's one (McMahon et al., 2017). The need for more research on invertebrates and fitness impacts, particularly in arthropods, is also true for terrestrial communities (Morley et al., 2014). Thus, a more general overview on the response of invertebrates to anthropogenic noises should be beneficial to mitigate the impacts (Francis & Barber, 2013). (L273-285)

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

This is a common issue in ecotoxicology, so noise must be considered in it. So, we added: "An interesting perspective is to consider the effect of noise as part of cocktails of pollutants. It is now a common question in ecotoxicology to ask whether stressors (e.g., chemical pollution, temperature, food quality) act synergistically (Altshuler et al., 2011). For instance, Starke et al.

(2021) showed that food quality impacts more D. pulicaria at some higher temperature due to the increased metabolism. Prosnier et al. (2015) modelled the antagonistic effect of copper and nutrient enrichment on the Daphnia - algae interaction. Regarding noise, McMahon et al. (2017) studied the interactive effects of light and noise pollutions on a frog-parasite interaction. They showed that light reduced frog-biting midge (Corethrella spp.) abundance at low noise level, whereas there was no midge at high noise level. It could be also useful to investigate whether all the unwanted noises produced by many experimental setups (to control light, temperature, oxygenation, and food) interact with the stressors studied and influence the results. For instance, in the present study there was a very high mortality in D. magna juveniles compared to similar studies (Parisot et al., 2015; Prosnier, Loeuille, et al., 2022) that suggests suboptimal conditions (i.e., other stressor than noise) that might have affected the outcomes through the masking of effects for example. However, on the other side, suboptimal conditions could make individuals more prone to be affected by an additional stress like noise. Note that, with the same suboptimal conditions, Prosnier, Rojas, et al. (2022) obtained a significant difference between the control and noise treatments. The recent Larvosonic system, developed by Olivier et al. (2023), could be useful to study the impact of noise on zooplankton with a better control of the environmental conditions." (L248-276)

- Other minor comments :

  X-mesh : specify mesh-size

We specified: "0.3-mm mesh tissue" (L102)

- 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).

You are right that it was unclear due to the lack of the word "their" anti-predatory defence.

- L97-99. Four tanks were used, each containing 18 individual 150ml vessels. Please specify that this was per treatment (otherwise 18x4 = 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).

We had more than 18x4 Daphnia because we replaced them during the first eight days.
We updated the statistical analysis section by adding the tank as random effects.

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

We added: "Boat noises were re-recorded only in half of the microcosms (in each tank) given that they were qualitatively and quantitatively similar due to the symmetry of the setup and after controlling with a broadband noise (Prosnier, Rojas, et al., 2022)" (L145-148)

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

We added: "(i.e., with dedicated organs to detect as inner ear sound pressure variation)" (L216). Non-hearing species are only able to detect particle acceleration – we also added "Particle motion cannot be measured due to the absence of adequate equipment, despite its importance for non-hearing species (Nedelec et al., 2016). However, Olivier et al. (2023) showed that results can still be qualitatively relevant when based solely on sound pressure level." (L151)