frontiers | Research Topics

Marine invertebrates and sound

Edited by
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Published in
Frontiers in Marine Science

ISSN 1664-8714
ISBN 978-2-8325-3217-1
DOI 10.3389/978-2-8325-3217-1

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Marine invertebrates and sound

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Citation

Solé, M., André, M., eds. (2023). *Marine invertebrates and sound*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3217-1

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frontiers | Frontiers in Marine Science

TYPE Editorial
PUBLISHED 27 July 2023
DOI 10.3389/fmars.2023.1257952

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 13 July 2023
ACCEPTED 20 July 2023
PUBLISHED 27 July 2023

CITATION
Solé M and André M (2023) Editorial: Marine invertebrates and sound.
Front. Mar. Sci. 10:1257952.
doi: 10.3389/fmars.2023.1257952

Editorial: Marine invertebrates and sound

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KEYWORDS

marine invertebrates, marine noise pollution, sound production, sound detection, noise effects, statocyst, sound pressure, particle motion

Editorial on the Research Topic
Marine invertebrates and sound

The growing pressure from anthropogenic activities impacts organisms at their communities and ecosystems levels. Among these stressors, the recent introduction of artificial noises in the oceans affects their inhabitants, altering their metabolism, deriving in malfunctions of physiological processes, or in behavioural disruptions. These dramatic changes may lead to significant transformations at population levels and negatively influence the whole oceanic ecosystem.

Sound is an important sensory modality for marine organisms, especially because other senses (vision, smell or taste) may be limited due to information loss in aquatic habitats. While marine mammals and fishes have received a great scientific attention in the last three decades, our knowledge of the biological significance of sound perception and production in marine invertebrates is scarce. Most of them are able either to produce and/or detect sounds through specialised hearing organs, or mechanoreceptors, which respond to the kinetic component of the sound. In some species, sounds can have various ecological functions (e.g. communication, territorial, social and sexual behaviour, species recognition), but it is generally considered that they are produced as a reaction to environmental stressors (predators – prey, alarm or stress reactions). Similarly, hearing sensitivity and its related behavioural patterns are little known.

Marine invertebrates play a central role in food webs and ecosystem services, as well as represent an important economical resource. Recent findings have shown that invertebrates are sensitive to anthropogenic noise. Noise can cause physical injuries, physiological stress, alterations of embryonic and larval development, changes in behaviour, reduction of growth and reproduction, increase of mortality and decrease of ecological success. These effects can have long-term consequences for the survival and adaptation of marine invertebrates in an increasingly noisy ocean, and indicate that this sensitivity may have a direct consequence on ocean biodiversity, placing them as direct indicators of ocean health. There is a clear need for more research to progressively assess the risks generated by noise exposure and to identify the gaps in knowledge on the potential effects that noise exposure may trigger in marine invertebrates.

This Research Topic aims at contributing to the advancement of our scientific knowledge on marine invertebrate bioacoustics and their implications for biodiversity and the functioning of marine ecosystems. The papers under this Research Topic show the

complexity of effects caused by acoustic pollution on these understudied taxa. The results indicate the importance of multi-level research on the effects of noise as stressor on marine invertebrates and identify the existing gaps, proposing future lines of research that will allow improving the assessment and mitigation of the impacts of anthropogenic noise on marine invertebrates and on the whole oceanic ecosystem.

This Research Topic collects a series of studies regarding marine invertebrate bioacoustics. Solé et al. summarise the current scientific knowledge on sound production, reception and sensitivity and review how marine invertebrates are affected by anthropogenic noises, identifying gaps that will frame future research for the assessment of the tolerance to noise of marine ecosystems.

Another review (Pysanczyn et al.) analyses the role of acoustics in the sensory landscape of coral larval settlement, to first provide an updated overview of the abiotic and biotic cues used by coral larvae to guide settlement, highlighting the potential for incorporation of acoustic enrichment techniques in coral reef conservation and restoration interventions. The snapping shrimp contribution on the Southern China coastal soundscape is analysed in Song et al., indicating that snaps are important communication means in light-limited conditions, which improves our understanding on the correlation of snapping behaviour and ecological environments.

This Research Topic on Marine Invertebrates and Sound also includes the response of invertebrates to sound as an anthropogenic stressor. In that context, a wide range of physiological, behavioural and ultrastructural responses from invertebrates to noise pollution are introduced. These studies deal with (i) the most representative groups of invertebrates: bivalves (Ledoux et al.; Gigot et al.), crustaceans (Sal et al.; McCloskey et al.) and cephalopods (Cones et al.) and (ii) a wide range of effects: feeding behaviour (Aspirault et al., Kühn et al.), metabolism (Gigot et al.; Ledoux et al.), development (Aspirault et al.; Cervello et al.), reproduction (Sal et al.), locomotion (Cones et al.), survival and community structure (McCloskey et al.; Kühn et al.).

Ledoux et al. assess the valve gape velocity and the physiology effects under pile driving, drilling and boat sound exposure. The study of Sal et al. is the first to contribute to assess the effect of different sound sources on the maternal care behaviour of a crustacean species. The results of Cones et al. demonstrate that pile driving disrupts squid fine-scale movements, but these impacts are short-lived, suggesting that offshore windfarm construction may minimally affect the energetics of this ecologically key taxon. McCloskey et al. experimentally demonstrate that SCUBA noise can have at least some negative impacts on reef organisms at community level, confirming this sound source as an ecologically relevant pollutant.

Interestingly, a high proportion of the articles in the Research Topic are dedicated to the study of the noise impact on planktonic species. Aspirault et al. assess the vessel noise impact on the feeding behaviour of blue mussel (*Mytilus edulis*) veligers and of the copepod *Eurytemora herdmani* as well as on the growth of the rotifer *Brachionus plicatilis* determining different results depending on the species. In a similar way, Kühn et al. show decreasing feeding rates of copepod *Acartia tonsa* exposed to harbour traffic noise. *Venus verrucose* larvae response to pile driving and drilling is modulated by their physiological condition and the noise could reduce compensatory mechanisms to balance the temperature increase (Gigot et al.). Also pile driving, drilling and vessel sounds are used in Cervello et al. to assess their effects on larvae of model species involved in marine biofouling. The results of these works suggest that effects of noise on plankton are complex and more research needs to be devoted to these initial live stadia.

We thank all the authors and reviewers who have participated in this Research Topic for their valuable contribution to this emerging field of marine acoustic ecology and we hope that this Research Topic will stimulate further investigation and innovation.

Author contributions

MS: Writing – original draft, Writing – review & editing. MA: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 15 December 2022
DOI 10.3389/fmars.2022.1070290

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

RECEIVED 14 October 2022
ACCEPTED 23 November 2022
PUBLISHED 15 December 2022

CITATION
Cones SF, Jézéquel Y, Ferguson S, Aoki N and Mooney TA (2022) Pile driving noise induces transient gait disruptions in the longfin squid (*Doryteuthis pealeii*). *Front. Mar. Sci.* 9:1070290. doi: 10.3389/fmars.2022.1070290

Pile driving noise induces transient gait disruptions in the longfin squid (*Doryteuthis pealeii*)

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Anthropogenic noise is now a prominent pollutant increasing in both terrestrial and marine environments. In the ocean, proliferating offshore windfarms, a key renewable energy source, are a prominent noise concern, as their pile driving construction is among the most intense anthropogenic sound sources. Yet, across taxa, there is little information of pile driving noise impacts on organismal fine-scale movement despite its key link to individual fitness. Here, we experimentally quantified the swimming behavior of an abundant squid species (*Doryteuthis pealeii*) of vital commercial and ecological importance in response to *in situ* pile driving activity on multiple temporal and spatial scales (thus exposed to differing received levels, or noise-doses). Pile driving induced energetically costly alarm-jetting behaviors in most (69%) individuals at received sound levels (in zero to peak) of 112-123 dB re 1 μm s^{-2}, levels similar to those measured at the kilometer scale from some wind farm construction areas. No responses were found at a comparison site with lower received sound levels. Persistence of swimming pattern changes during noise-induced alarm responses, a key metric addressing energetic effects, lasted up to 14 s and were significantly shorter in duration than similar movement changes caused by natural conspecific interactions. Despite observing dramatic behavioral changes in response to initial pile driving noise, there was no evidence of gait changes over an experiment day. These results demonstrate that pile driving disrupts squid fine-scale movements, but impacts are short-lived suggesting that offshore windfarm construction may minimally impact the energetics of this ecologically key taxon. However, further work is needed to assess potential behavioral and physiological impacts at higher noise levels.

KEYWORDS

noise, energetics, gait, jet propulsion, finning

1 Introduction

There is a global investment in offshore wind (OSW) infrastructure as many countries increasingly prioritize renewable energies over fossil fuels (Gielen et al., 2019). The increased human presence in the ocean poses challenges to marine life since the pile driving noise emitted during OSW construction has been shown to cause physical damage (Halvorsen et al., 2012), sensory harm (Kastelein et al., 2016), and behavioral changes (Jones et al., 2020) to a myriad of marine taxa. Consequently, anthropogenic noise is recognized as a global pollutant of paramount concern (Halfwerk et al., 2011; Kunc et al., 2014; Duarte et al., 2021). Noise-induced behavioral changes can have direct fitness consequences, and the spatial extent is likely greater than that of noise-induced physical and physiological harm (Popper et al., 2022). However, movement responses are rarely quantified. Fine behavioral changes are difficult to measure in marine environments where animals are largely in accessible, leading to key knowledge gaps on the effects of noise on behaviors that can influence individual fitness.

Much of the existing research on noise-induced behavioral changes has focused upon large marine mammals, and to some extent fishes (Miller et al., 2000; Southall et al., 2007; Miller et al., 2012; Popper and Hawkins, 2019). There is scant data on marine invertebrates such as cephalopods. This is a surprising fact considering their central position in many ocean food webs (Clarke, 1996) and their high commercial value exceeding $1 billion USD per year worldwide (Hunsicker et al., 2010). Cephalopods have been shown to detect sounds within the same frequency range (<500 Hz) as pile driving noise, indicating a likely susceptibility to adverse effects of noise (Mooney et al., 2010; Mooney et al., 2020). Indeed, recent laboratory studies showed that solitary longfin squid (*Doryteuthis pealeii*), an important U.S. fishery taxon, exhibit alarm responses to pile driving playbacks (Jones et al., 2020; Jones et al., 2021). However these studies used solitary squid in tanks, which makes behavioral inferencing challenging since *D. pealeii* is an aggregating species and the acoustic field differed from field conditions (Birkett and Newton-Fisher, 2011; Jones et al., 2019). One field study examined caged squid (*Sepioteuthis australis*) behavioral responses to seismic air-gun surveys (Fewtrell and McCauley, 2012). The authors found that both the proportion of alarm responses (e.g., escape jetting) and swimming speed were positively correlated with received noise levels. Nonetheless, this preliminary study only assessed movement qualitatively, leading to important questions regarding the ecological consequences, energetics, and duration of the observed behavioral changes.

Most bioacoustic studies have not measured the duration of noise-induced behavioral changes (but see Miller et al., 2012) despite being a key consideration for policy makers (Finneran et al., 2017; Southall et al., 2021). Measuring the duration of noised-induced behavioral impacts is critical because it is inherently linked to impact severity and persistence of effect. For example, the energetic cost incurred from a transient increase in acceleration is less severe than a prolonged heightened acceleration state if an individual does not habituate or desensitize to a noise stimulus (Southall et al., 2007). The few studies measuring disturbance durations in aquatic animals have been restricted to large vertebrates capable of carrying motion sensor tags (Miller et al., 2012). For many marine species, quantifying individual movement is difficult, particularly over time scales comparable to pile driving operations; yet such data are needed to quantify behavioral changes and energetic costs. As a result, most studies on smaller and more abundant animals are conducted in tanks, providing key data but limiting the knowledge that can be applicable to actual noise exposures in field settings. New tools and methods are thus needed to accurately describe and quantify noise-induced behavioral changes, especially in more real-world conditions (Popper et al., 2022).

To date, there has been no field study quantifying the movement behavior of cephalopods, or any invertebrate, during real-time pile driving construction. Given that construction is imminent and considering the spatial overlap of cephalopod fisheries and planned OSW development (Figure 1), there is an urgent need to experimentally examine whether commercially-important cephalopods alter movement behaviors during pile-driving noise exposure, and if so, quantify how long those changes persist. In this context, our present aim was to quantitively examine the fine-scale swimming movements and kinematics of *D. pealeii* during field-based pile driving activities to assess potential ecological and energetic consequences of noise exposure. We utilized high-resolution movement sensors to measure individual-level swimming kinematics at sub-second to hourly temporal resolutions and at multiple spatial scales during the two main types of piling installation: continuous vibratory and impulsive impact hammering. Both installation methods are known to produce intense sounds, but the characteristics are vastly different (Amaral et al., 2020; Jézéquel et al., 2022). We then assessed the probability of squid changing their movement behavior associated with specific received noise levels, characterized the observed behavioral changes, and measured the durations of those alarm behaviors. These anthropogenically-induced alarm responses were then compared to natural swimming movements and gait disruptions observed throughout the course of quiet, control days to evaluate the potential biological and energetic implications of the noise-induced stress. To address these questions, we developed a new approach to quantify the movement of cephalopods that can be used to address similar questions for other species more broadly.

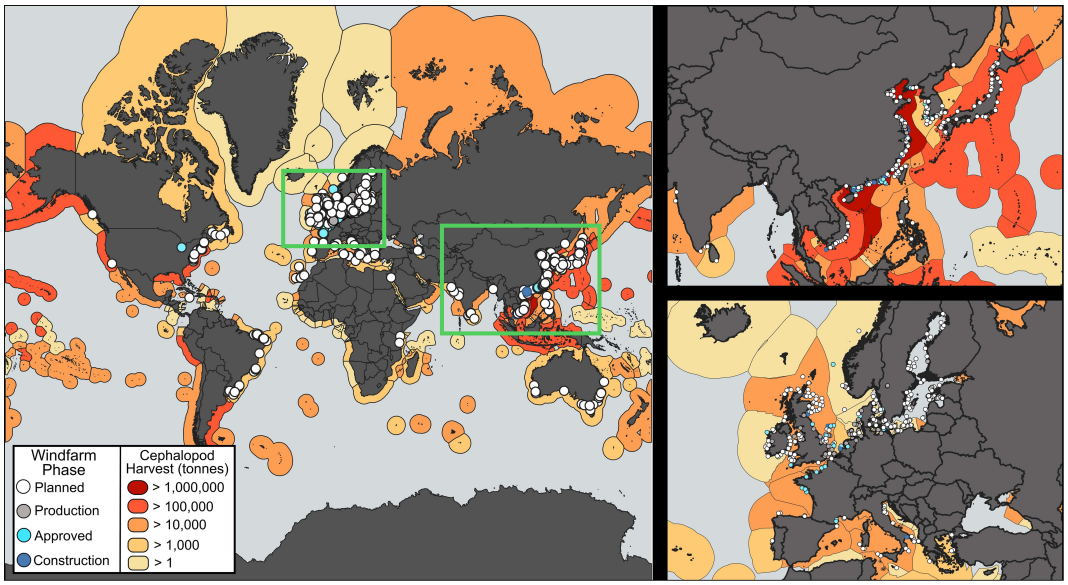

FIGURE 1
Future offshore windfarm construction largely overlaps with areas of high cephalopods harvest. The global map depicts individual OSW projects (dots) at four stages of development as well as the extend of cephalopod harvest within a country's ocean governance area (The Wind Power (www.thewindpower.net), Food and Agriculture Organization).

2 Methods

2.1 Study animals

Squid used in the present study were collected from Vineyard Sound, MA (41.22 N; 70.47 W). Animals were hand-selected and only animals without visible lesions and muscular damage were chosen for experimental use. Prior to the experiment, squid were held in multiple 1.2-m diameter cylindrical tanks constantly supplied with ambient, local seawater from the study area. Squid were fed mummichogs (*Fundulus heteroclitus*) and grass shrimps (*Palaemonetes* spp.) daily. Experimental squid were kept in holding tanks for no longer than three days before trials started, and new squid were used each experiment day. This study was carried out in accordance with the principles of the Basel Declaration and recommendations and approval of the Woods Hole Oceanographic Institution's (WHOI's) Institutional Animal Care and Use Committee scientific protocol to TAM.

2.2 Experiment procedure

Pile driving was conducted for 11 days in September 2021 off the WHOI's dock (Figures 2A, B). At the start of each pile driving day a cylindrical steel pile (length: 10 m, diameter: 0.3 m, wall thickness: 0.02 m) was positioned into the sediment using a vibratory hammer (VH, weight: 212 kg, H&M model 135) at 1150 blows per minute. Squid were then introduced into cages (see below for details) and given 15 minutes to acclimate. Exposures began as (1) a steel impact hammer (IH, weight: 1500 kg) was dropped at 1.2 m height at a rate of 8 -12 strikes per minute until the bottom edge of the steel pile was approximately 5 m into the substrate, taking (mean ± standard deviation) 14.9 ± 0.47 min. (2) The VH was then used to pull the pile out of the substrate and to reposition the pile in an adjacent location for another round of impact hammering. This process was repeated five times per experiment day, which lasted for three to four hours.

To assess potential dose-dependent responses, squid were monitored at two different distances from the pile (near site: within 8 m, far site: 50 m; received levels noted below). The exact distance from the noise source varied slightly because consecutive piles could not be driven in the exact same locations. Squid were placed in 1.5 m^3 cages constructed using a polyvinyl chloride frame covered with 1.5 cm knotless polyester mesh netting (Figures 2C, D). Each cage contained 4-7 squid of mixed sexes to represent wild aggregations (Shashar and Hanlon, 2013). Two underwater cameras (GoPro Hero 7 Black, San Mateo, CA) were placed in the cages for visual observations. Cages were lowered roughly 5 m and hovered 0.5 m above a sandy substrate. The largest squid (male) in each cage was affixed with a modified ITAG, a biologging tag designed for soft-bodied animals (Mooney et al., 2015; Fannjiang et al., 2019; Cones et al., 2022). The ITAG was used to measure fine-scale swimming kinematics during noise exposure and control

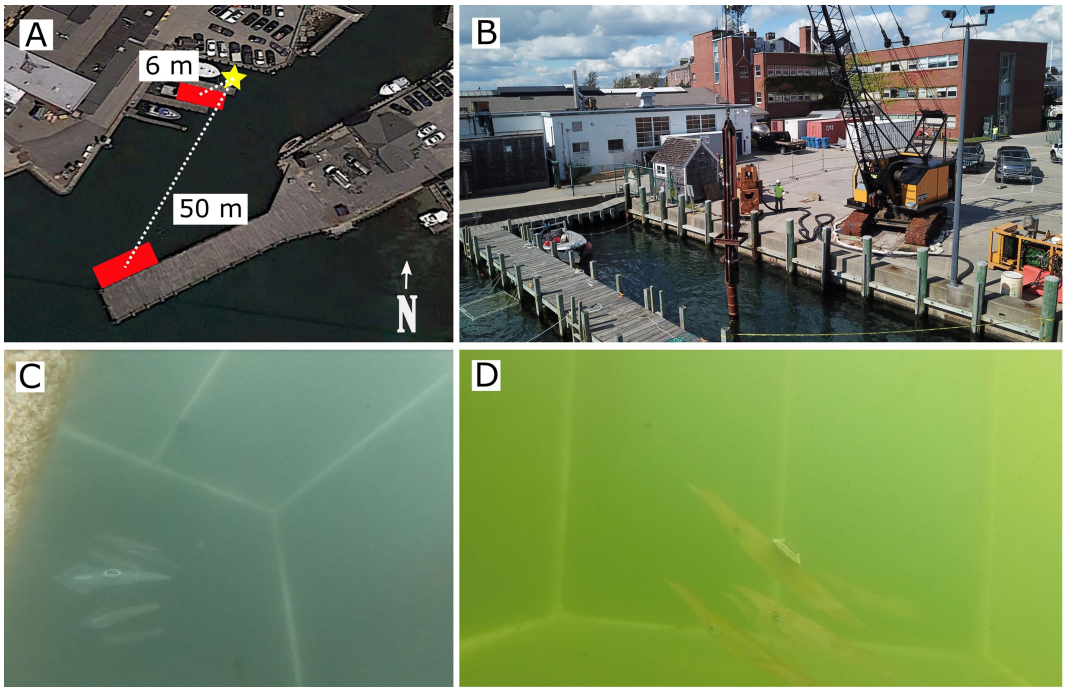

FIGURE 2
The experimental setup including a **(A)** map of the two sites: near (2-8 m) and far (50 m). The yellow star denotes the pile driving location, while the shaded red regions are the position of squid cages. The northern and western boundaries around the pile driving were solid sea walls. There were no physical barriers between the noise source and squid cages apart from a series of 0.3 m diameter piles supporting the dock slips. **(B)** Drone images during both impact pile driving. **(C, D)** Video footage from an experiment showing a focal tagged squid schooling with conspecifics.

periods (see Section 2.3). The analysis focused on the swimming behavior of the tagged squid. Hence, a typical squid group consisted of one large, tagged male (dorsal mantle length (DML): 25.2 ± 2.6 cm) associated with smaller untagged squid (DML: 16.3 ± 2.5 cm).

Control experiments (n=7) were conducted using the same methods, but without pile driving noise exposure. To compare metrics between the two experiment types, noise exposure time periods from experiment days were randomly assigned to control experiments.

2.3 Gait classification

ITAGs were used to measure squid movement dynamics. The sensor package was small (length: 7 cm, width: 3 cm, height: 1 cm) and was affixed using surgical sutures (Mooney et al., 2015; Flaspohler et al., 2019; Cones et al., 2022). Additionally, ITAGs were neutrally buoyant, hydrodynamic, and focal tagged squid exhibited normal swimming and schooling behaviors with other conspecifics. ITAGs contain an inertial measurement unit (IMU) which measures acceleration, magnetic field strength, and angular velocity. These high-resolution (100 Hz sampling rate) accelerometers allowed for the estimation of overall dynamic body acceleration (ODBA), a widely used metric to quantify behavior (Zhang et al., 2018) and estimate energetic cost (Wilson et al., 2006; Halsey et al., 2009). The ITAG IMU was used to measure two swimming gaits: jet propulsion and finning.

Jet propulsion is pulsatile and entails the intake of water into the mantle cavity and its expulsion through a flexible funnel (Bartol et al., 2001). Intense jet propulsion events are high acceleration movements employed in response to predators or during conspecific interactions, but is also the common response of squid to recorded pile driving noise (Wells and O'Dor, 1991; Hanlon et al., 2002; Jones et al., 2020). The jetting gait was quantified using similar methods described in detail in previous studies (Flaspohler et al., 2019; Cones et al., 2022). In brevity, a movement was deemed a jetting event if ODBA exceeded 0.3 gravities (g).

Finning is a more continuous movement generated by fin-mediated thrust from waves propagating down the length of the squid mantle-fin. In contrast to intense jet propulsion events, finning is frequently used during low-speed swimming and maneuvering (Stewart et al., 2010; Bartol et al., 2016). To measure finning rates, two small cylindrical magnets (diameter: 3 mm, height: 1 mm) were placed dorso-ventrally on one fin and remained in position without any additional measures. The position of the fin and magnet were coupled, and

movements distorted the ambient magnetic field measured by the ITAG magnetometer, resulting in fin position and magnetic field strength to be coupled. Concurrent video and tag data from a subset of six squid in preliminary lab control experiments revealed continuous fin-dominated swimming produced a sinusoidal curve with a frequency equivalent to fin rate (Supplementary Figure 1). First, a low-pass filter of 20 Hz was applied to the raw signal to smooth the high frequency noise. Then, a MATLAB (Mathworks, Natick, MA, USA) peak detector was used to enumerate crests in the signal which represented individual finning events. The technique was tested on 410 s of movement data from six squid. The algorithm had an average classification accuracy of 97.4%, and its worst segment performance was 95.8% correct detections.

The video data from the cages were used to corroborate and enumerate the number of intense jetting and startle alarm behaviors during noise exposure (defined in detail in Jones et al., 2020). For the impact hammer, only alarm behaviors coinciding with the impact hammer were considered. Alarm behaviors during agonistic encounters with conspecifics were not considered. Using kinematic data from the confirmed alarm behaviors, we created a custom MATLAB algorithm to identify similar movement patterns during the three noise treatment periods using the ITAG (control, vibratory hammer, impact hammer). If focal squid ODBA exceeded 0.3 g and had a concurrent two standard deviation change in finning rate, it was deemed a kinematic disturbance.

To assess if noise exposure impacted the overall swimming patterns, we applied the algorithm to all kinematic data (control and noise exposure sequences) to isolate all sequences, termed kinematic disturbances, during all noise treatments. For this analysis, noise exposure periods were treated as continuous, and all kinematic disturbances during impact and vibratory hammer periods were considered. This differs from the video analysis described above where only alarm behaviors coinciding with the hammer strike were considered.

Lastly, finning rates and ODBA were also used to measure the duration of a gait disruption. The disturbance duration was defined as the time required for the focal squid (1) to return within 25% of the mean finning rate for at least five consecutive finning events and (2) ODBA to decrease below 0.3 g. This method is analogous to Lowe (2002), which used tail-beat frequency as a metric to assess when captured sharks returned to baseline behavior after capture and handling.

2.4 Acoustic measurements

Given cephalopods sensitivity to low frequency (< 1 kHz) underwater particle motion (Mooney et al., 2010), the sound field was quantified in particle acceleration using a calibrated PCB triaxial accelerometer (model W356B11; sensitivity: x = 10.26 mV m s^{-2}, y = 10.38 mV m s^{-2}, z = 10.62 mV m s^{-2}) with a frequency sampling of 2 kHz. All acoustic measurements were taken during the behavioral experiments. The recording device was wired through a signal conditioner (Model 480B21, Piezotronics), which multiplied the recorded voltage by a factor of 10. The accelerometer signal was input to three analog filters (one per axis; Model FMB300B, Krohn-Hite), which each applied a bandpass filter between 0.06 and 2 kHz. Outputs of the filters were input to a data acquisition board (USB 6251, National Instruments), which was in turn connected to a laptop that ran a custom MATLAB script to record the audio files. Voltage values for each axis (x, y, and z) were calibrated to the sensitivity of the accelerometer and used to calculate the different following acoustic metrics. Recordings were taken at three distances from the pile (1, 8, and 50 m) during both IH and VH pile driving throughout the experimental period. For acoustic measurements, triaxial data were combined as the 3-D vector quantity.

For the IH, the pulse length (in ms) was measured as the time between 5% and 95% cumulative energy, and the rise time as the duration (in ms) from 5% of total energy to the peak acceleration of the signal (ISO standards 2017). The intensity was assessed by computing 0-peak accelerations (PAL_{zpk}; in dB re 1 μm s^{-2}). Next single strike sound exposure levels (SEL_{ss}; in dB re (1 μm s^{-2})^2 * s) were calculated by integrating PAL_{zpk} over the pulse length containing 90% of the signal energy, and cumulative sound exposure levels (SEL_{cum}; in dB re (1 μm s^{-2})^2 * s) were calculated using the following equation:

$$SEL_{cum} = SEL_{ss} + 10 * \log_{10}(N)$$

where N is the number of impulses.

Because VH signals were characterized as continuous (compared to transient IH signals), PAL was described in root mean square (PAL_{rms}; in dB re 1 μm s^{-2}) in the 90% energy window and the 0-1 kHz frequency range, as well as SEL_{ss}.

Finally, PAL_{rms} of the IH signals were calculated with identical methods as for VH signals. Based on PAL_{rms} datasets from both IH and VH, we estimated transmission losses (TL; in dB) by fitting nonlinear least-squared regressions using custom-made scripts in MATLAB (Ainslie, 2010). TL represents the loss of intensity due to the geometrical spreading of sounds in a physical medium (Ainslie, 2010), and was calculated as the slope of the logarithmic regression between PAL_{rms} and the distance from the noise source, which was expressed as:

$$TL = \alpha \times \log_{10}(r)$$

where r is the distance between the piling and the accelerometer (in m), and alpha is the geometrical TL term.

2.5 Statistical analyses

The non-parametric Mann-Whitney U test was used to test for differences in the number of alarm behaviors at the near versus

far site and between the IH versus VH. A two-sample t-test was used to test for differences in ODBA during alarm behaviors versus baseline schooling movements. Since our data fit normality assumptions, a one-way ANOVA was used to test for differences in finning rates during noise treatments and to test for differences in the frequency of kinematic disturbances during IH at the near site, far site, and control periods. Lastly, a two-sample Kolmogorov-Smirnov test was used to test if the duration of kinematic disturbances elicited during noise exposure and control periods had similar probability distributions.

3 Results

3.1 Acoustic field

A full summary of acoustic data is in Table 1. The IH and VH pile driving produced clear signals above background noise levels at both exposure sites, which allowed for isolation and analysis of all noise sequences (Figure 3A). Both rise time and pulse length increased with distance from the pile, with pulse length ranging from 190–990 ms and rise time increasing from 5.8 to 68 ms. PAL_{zpk} decreased from 122.96 dB re 1 μm s^{-2} at 1 m to 96.45 dB re 1 μm s^{-2} at 50 m. SEL_{ss} for the IH ranged from 81.30 at 1 m to 68.28 dB re (1 μm s^{-2})^2 * s at 50 m. In contrast, SEL_{ss} for the continuous VH signals were greater, ranging between 137.76, 134.62, and 126.96 dB re (1 μm s^{-2})^2 * s at 1, 8, and 50 m, respectively. SEL_{cum} for the IH was 102.04, 93.24, 88.32 dB re (1 μm s^{-2})^2 * s at 1, 8 and 50 m. Interestingly, TL values were similar for both IH and VH signals (α = 12.9 and 11.8, respectively) despite greater PAL_{rms} for the IH (Figure 3B), which was consistent with acoustic propagation in shallow waters.

3.2 Kinematic disturbances

Over 11 experiment days, we tagged 20 squid and each animal was considered an individual noise exposure experiment. In total, 1101 and 416 minutes of kinematic and video data were collected during IH and VH pile driving, respectively. Thirteen of the 20 experiments were located at the near site, while seven experiments were conducted at the far site. Additionally, we conducted seven control experiments (409 minutes of kinematic data) with identical methods but with no pile driving noise exposure. There were significantly more noise-induced alarm behaviors at the near site [compared to the far site (near site = 17 alarm behaviors, far site = 0 alarm behaviors, Mann-Whitney U test, z = 2.19, p = 0.0284)]. Alarm behaviors were high acceleration jet propulsion events coinciding with the impact hammer or at the onset of the vibratory hammer (Figure 4). Kinematic data from the ITAG revealed that alarm responses resulted in a significant increase in ODBA (two-sample t test, t = 2.11, p = 0.0438; Figure 5). At the near site, nine of the 13 focal squid exhibited one or multiple alarm behaviors in response to the impact and vibratory hammer. Five squid elicited more than one alarm behavior. Of the squid eliciting an alarm response at noise onset, there were more alarm behaviors in response to the IH (16 alarm behaviors) compared to the onset of VH (1 alarm behavior). Eighty-two percent of the alarm responses occurred during the first or second impact or vibratory hammer sequences within a given exposure day, and a separate 82% of the alarm responses occurred within the first three impact hammer strikes or at the onset of vibratory hammer. No focal squid at the far site reacted to either pile driving noise type.

3.3 Kinematic disturbance probability

Although alarm behaviors occurred in response to the IH, there was no significant change in the number of kinematic disturbances over the course of an experiment vs. control day. Indeed, focal squid at the near (0.037 ± 0.034 kinematic disturbance min^{-1}) and far (0.062 ± 0.048 kinematic disturbance min^{-1}) sites had statistically similar kinematic disturbance frequencies compared to the quiet control periods (0.058 ± 0.058 min^{-1}; One-way ANOVA, $F_{2,26}$ = 0.88, p = 0.43, Figure 6).

TABLE 1 Particle acceleration levels from the IH (black) and VH (red) at three different distances from the pile.

Distance from pile (m)	Pulse Length (ms)	Rise time (ms)	PAL_{zpk} (dB re 1 μm s^{-2})	PAL_{rms} (dB re 1 μm s^{-2})	SEL_{ss} (dB re (1 μm s^{-2})*s)	SEL_{cum} (dB re (1 μm s^{-2})*s)
1	190 ± 100	5.8 ± 9	122.96 ± 7.98	105.22 ± 1.7 95.21 ± 1.6	81.30 ± 9.1 137.76 ± 0.8	102.04 ± 9.8
8	270 ± 200	9.5 ± 20	112.32 ± 3.2	95.79 ± 2.4 82.88 ± 4.52	72.95 ± 4.0 134.62 ± 4.0	93.24 ± 2.6
50	990 ± 40	68 ± 30	96.45 ± 3.3	83.22 ± 1.9 75.26 ± 1.7	68.28 ± 2.6 126.93 ± 1.6	88.32 ± 1.6

Single strike sound exposure levels (SEL_{ss}) for the impact hammer were measured for individual hammer strikes, and a single strike for the VH was considered one pile driving sequence. Cumulative sound exposure levels (SELcum) at 1, 8, 50 meters for the impact hammer were calculated from, on average, 126, 118, 94 strikes respectively.

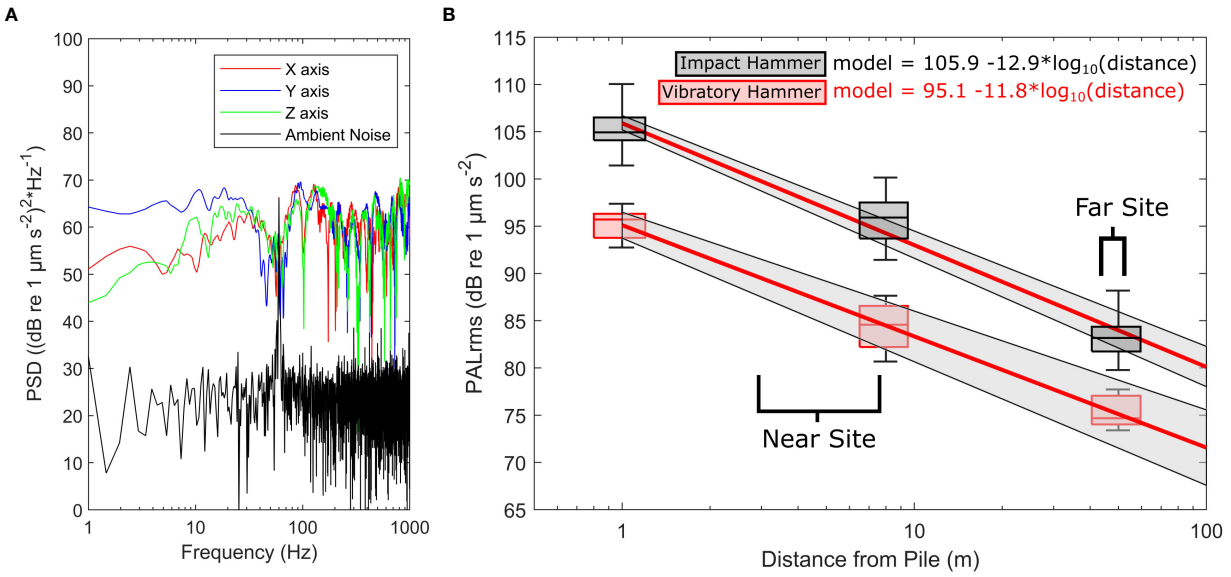

FIGURE 3
(A) PALrms propagation model labeled with the brackets denoting the distances of the experimental cages at the near and far sites. Particle acceleration was measured at multiple distances: 1, 8, and 50 m from the pile driving. The red line represents the empirically-based model fit, and the shaded region denotes the 95% confidence interval. **(B)** Power spectral density curves of the impact hammer and ambient noise measured at 1 m. The PSD curves were generated from a 1 min segment during both noise treatments, and the x (red), y (blue), and z (green) represent the three accelerometer axes during the impact hammer.

3.4 Duration of disturbances

Alarm behaviors during IH sequences persisted for 4.2 ± 4.7 s. This was significantly shorter than kinematic disturbances measured during 'quiet' control periods 6.1 ± 4.2 s (two-sample Kolmogorov-Smirnov test, $p < 0.001$, Figure 7A). For each noise-induced disturbance, focal squid accelerated rapidly (i.e., high ODBA), but ODBA for each disturbance returned to similar baseline levels within ca. 4 seconds (Figure 7B). However, for some individuals, the finning gait continued to deviate from baseline or individuals reacted to consecutive hammer strikes, resulting in longer recover times, with a maximum recovery time of 14.7 s.

Although finning behavior changed at short time scales during kinematic disturbances, average finning rates during IH periods were not significantly different at the near site (1.563 ± 0.13 fin s^{-1}), far site (1.624 ± 0.063 fin s^{-1}), and during silent control periods (1.587 ± 0.11 fin s^{-1}, One-way ANOVA, $F_{2,39} = 0.63$, $p = 0.54$, Figure 8A). Additionally, after combining all finning data across the two sites, there was no difference in average finning rates during noise exposure (IH: 1.584 ± 0.11 fin s^{-1}; VH: 1.583 ± 0.11 fin s^{-1}) and silent periods (1.587 ± 0.11 fin s^{-1}; One-way ANOVA, $F_{2,59} = 0.01$, $p = 0.99$, Figure 8B).

4 Discussion

We present the first study quantifying the fine-scale movement behaviors of a marine invertebrate in response to an actual field-based anthropogenic noise source. We used high-resolution movement sensors to quantitively measure changes in swimming kinematics and measure how long gait disruptions persisted. Our results demonstrate that while field-conducted pile driving noise elicited clear alarm responses at high received levels, these were short-term evasions that persisted for only 4 s on average. Further, these escape behaviors were found only at a site of relatively high received sound levels, although the measured noise levels corresponded to roughly 1 km from actual windfarm construction pile driving (Sigray et al., 2022). Interestingly, alarm behaviors were shorter in duration than similar high acceleration movements during natural, intraspecific agonistic encounters observed during quiet control periods indicating that the animals quickly returned to sensory vigilance. Additionally, when considering overall jetting and finning gait behaviors throughout an exposure or control day, there was no detectable impact of pile driving noise on swimming behavior. Although, the experimental cage may have constrained certain swimming behaviors, particularly horizontal dispersion from the sound source.

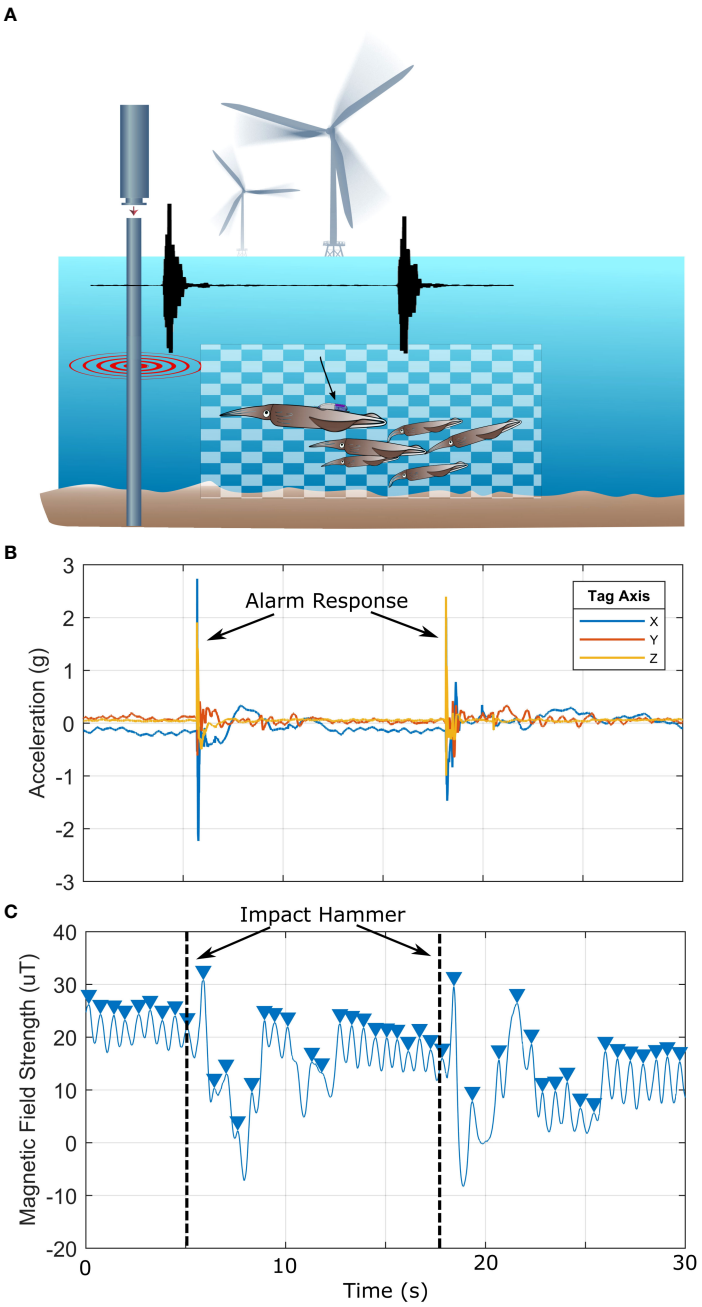

FIGURE 4
Squid elicit alarm behaviors in response to pile driving sound. **(A)** A schematic of the experimental setup with an overlaid example impact hammer signal. Black arrow highlights tagged large squid. **(B)** Focal tagged squid acceleration during a typical kinematic disturbance. Heightened acceleration occurs at the moment of the impact hammer strike. **(C)** Concurrent magnetic field strength data used to calculate finning rate. Magnetic field strength is a consistent sinusodial signal before impact hammer, but becomes irradic as the focal squid transitions to jet propulsion swimming.

This study used novel accelerometer-based particle acceleration measurements at multiple distances to create an acoustic propagation model and identify probabilities of movement behavior changes at specific received noise levels. Nine of 13 *D. pealeii* at the near site elicited at least one or more alarm movements in response to the IH between 122.96 and 112.32 PAL_{zpk} dB re 1 μm s^{-2}, which are noise levels greater than 880 m from a one OSW construction site (Sigray et al., 2022). We know of no other sites in which we there are comparable, published, particle acceleration data. This suggests that

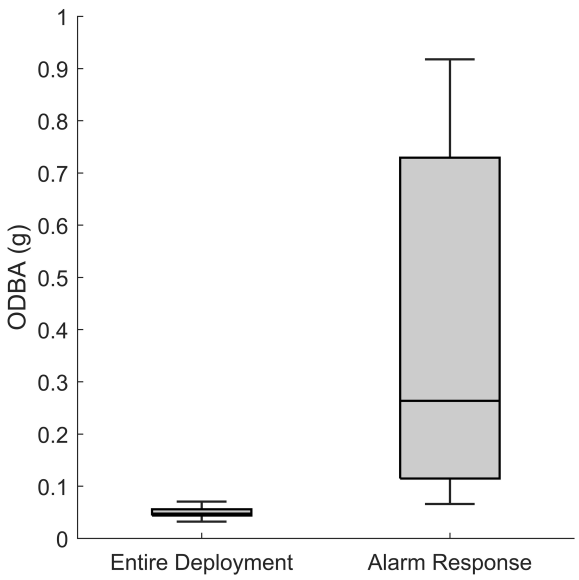

FIGURE 5
ODBA averaged over the entire experiment periods and (left) across all 17 alarm behaviors in response to pile driving noise (right).

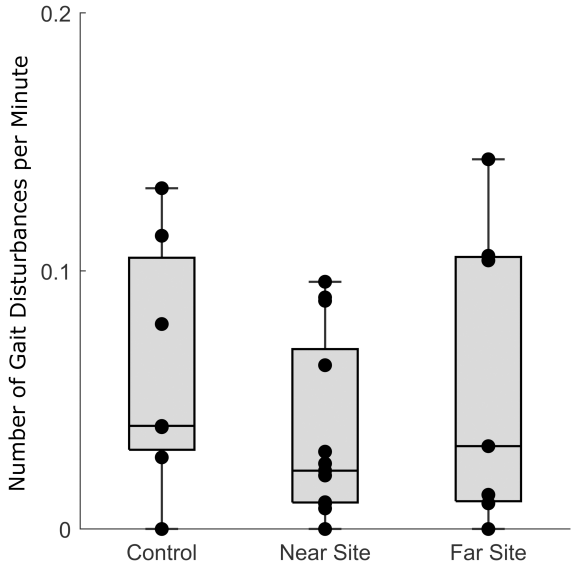

FIGURE 6
The number of daily gait disturbances calculated from kinematic algorithms trained by confirmed reactions. Although squid reacted to pile driving noise, it did not significantly increase the number of total gait disturbances over an experimental day.

behavioral disruption will likely occur at the kilometer scale and at a relatively substantial range, especially if we consider wind turbine pile spaces to be roughly 1 km apart and noise levels to stay consistent. More intense or persistent responses may occur within that 880 m range especially if larger pilings are used or if multiple platforms are constructed concurrently. Hence, the alarm responses described here may impact a significant majority of animals within the entire OSW development area, leading to potential regional impacts on squid populations. However, more information on noise-induced disruptions to group-level behaviors is needed to better assess impacts on populations.

Although there were clear alarm behaviors in response to pile driving noise, we found no significant difference in the

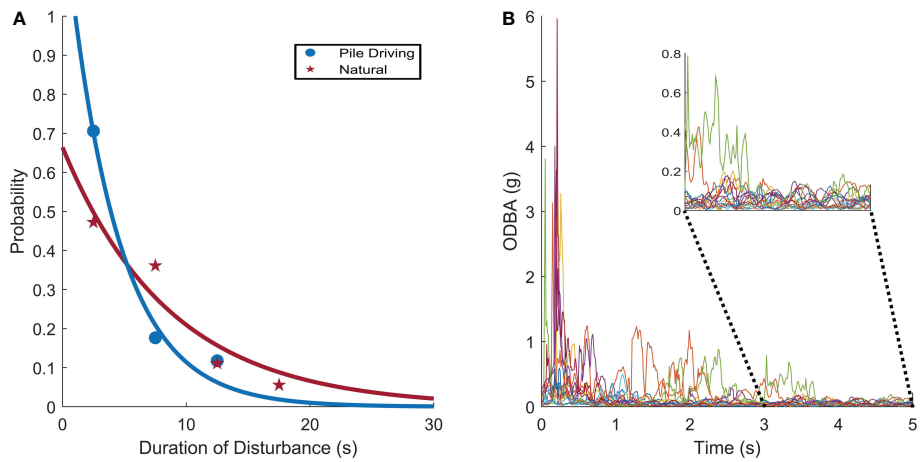

FIGURE 7
(A) Squid exhibited brief kinematic disturbances in response to pile driving noise, and these disturbances are similar in duration to natural kinematic changes during inter-individual interactions. The models compare the recovery time from both pile driving noise and naturally-induced kinematic changes. **(B)** ODBA during all 17 confirmed alarm responses to pile driving noise.

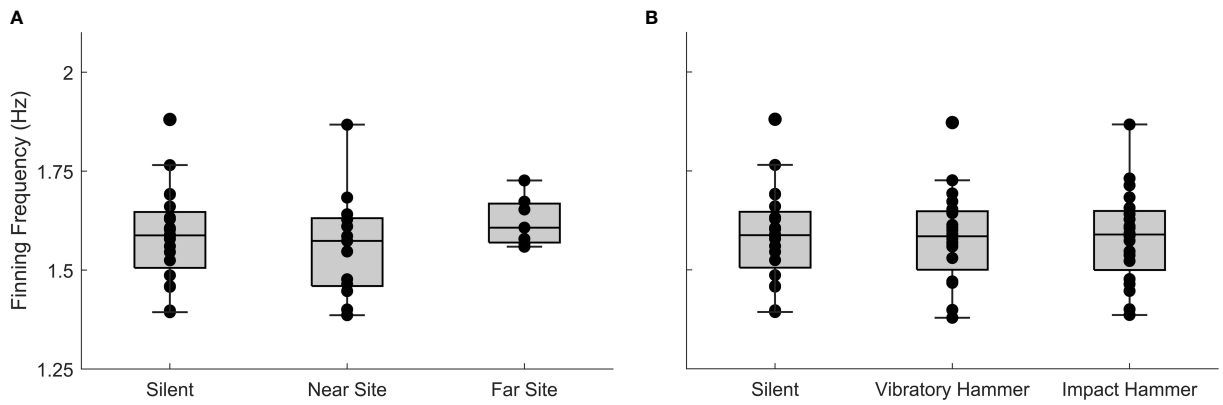

FIGURE 8
Focal squid finning rates averaged during over the impact pile driving periods, separated by **(A)** near and far site and the control quiet periods. **(B)** Finning rates for both near and far site separated by noise treatment.

number of kinematic disturbances measured from the ITAG between control and noise exposure periods (Figure 6). To be more representative of wild conditions, we used squid groups of mixed sexes in our experiments. *D. pealeii* are still reproductively active into September when our experiments took place (Stevenson, 1934), and squid are known to swim dynamically in breeding aggregations, and these movements were likely classified as kinematic disturbances in the present study (Shashar and Hanlon, 2013). This result provides more evidence that pile driving did not change long term swimming behaviors and it demonstrates the importance of considering the biology and group-level behaviors when quantifying noise-induced behavioral impacts. Future studies should avoid studying aggregating species in isolation because it may constrain individual behavior and limit interpretations.

Most alarm behaviors were associated with one or multiple rapid jet propulsion events; these jets resulted in elevated ODBA and a change in finning rate (Figure 4). An increase in ODBA and a transition to primarily jet propulsion indicates a higher energetic cost (Webber and O'Dor 1986, Halsey et al., 2009). Squid are thought to operate at or near their metabolic limit (O'Dor and Webber 1991), which suggests that an anthropogenically-induced high energy alarm behaviors may be detrimental to squid energy budgets. However, because the disruptions were transient and only elicited a maximum of three times per individual over 3-4 hours of pile driving, we suspect the impact to be non-substantial, especially considering squid frequently elicited similar dynamic kinematics during non-noise exposure periods. Additionally, free-ranging muscular squid naturally display high acceleration jet propulsion at rates, > 9 jets min^{-1} (Cones et al., 2022). Thus, the additional 0-3 jetting propulsion alarm responses over multiple hours of noise exposure are not likely detrimental to energetic expenditure.

No squid at the far site (with lower received levels) elicited alarm behaviors in response to either IH or VH pile driving noise despite noise levels occurring within *D. pealeii* sound detection abilities (Mooney et al., 2010). This result suggests there was either a dose-dependent response or there exists a minimum threshold that induces alarm behaviors, where animals detecting amplitudes 112-123 and 96 dB re 1 μm s^{-2} have a 69% and <1% probability of eliciting at least one alarm response, respectively. In fact, dose dependence behavioral responses were found in *S. australis* exposed to air gun noise (Fewtrell and McCauley, 2012). Squid elicited a higher proportion of alarm behaviors with increasing noise levels, implying the severity of noise impact on squid is related to the distance from the noise source.

Interestingly, 16 of the 17 alarm behaviors were observed during IH (7 alarm behaviors at the first hammer strike) pile driving, with only one instance of reaction to the onset of VH pile driving. This finding suggests that high amplitude and transient signals are more detrimental to squid swimming kinematics compared to low amplitude and continuous signals. Previous noise studies have largely focused upon IH noise impacts on marine life (Herbert-Read et al., 2017; Jones et al., 2020; van der Knaap et al., 2022), while fewer have directly compared noise impact with temporal variation (Neo et al., 2014; Shafiei Sabet et al., 2015). These studies also demonstrated that intermittent noises, rather than continuous, induced more severe behavioral changes including more alarm behaviors. Further research should seek impact severity comparisons between IH and VH techniques for a broader range of species. Considering some OSW farms have been successfully installed with only the VH, it could serve as an important mitigation technique in areas with suitable substrate type (OSPAR, 2014).

The duration of a behavioral disturbance is a key metric to address impacts to individual fitness, and it can inform models and evaluations of impacts by managers as they develop policy recommendations (Southall et al., 2007; Tyack et al., 2011;

Ranaweerage et al., 2015; Finneran et al., 2017). Observed *D. pealeii* alarm responses were transient and had similar movements as anti-predator behaviors observed in other squid species (Mather, 2010). By resuming baseline swimming within only a few seconds, squid may be selecting to maximize other sensory systems or detection needs, particularly audition, to enable vigilance for predators. In late summer, coastal Massachusetts waters and the habitat of this squid are turbid. Such conditions likely renders auditory cues more useful than vision for long-term sensory perception. Low acceleration swimming could serve to decrease chaotic flow around sensory hair cells, which aid in predator detection (Mooney et al., 2010; York and Bartol, 2014; Higham et al., 2015). Another explanation for the short-term alarm responses was that *D. pealeii* experienced temporary or permanent shifts in hearing thresholds as seen in other species (Smith et al., 2004; Mooney et al., 2009). If so, squid may lack perception of the noise stimulus, explaining the rapid decline in alarm behaviors throughout exposure. Future studies should aim to measure hearing thresholds before and after noise exposure to determine whether *D. pealeii* desensitized to pile driving noise or experienced physiological impairments.

There was no significant difference in finning rates over noise treatment periods, which is more evidence suggesting pile driving noise does not alter longer term natural swimming patterns. To our knowledge, these are the first data on squid finning rates in semi-wild conditions. Most research on squid locomotion, especially in the field, has focused upon jet propulsion despite finning being integral to squid energetics and ecology (Anderson and DeMont, 2005; Bartol et al., 2016; Cones et al., 2022). Fin-dominated movements increase propulsive swimming efficiency at certain speeds and allow for versatile maneuvers which are thought to aid in squids' ability to compete with fishes (Hoar et al., 1994; Bartol et al., 2016). Although we did not measure specific energetic costs throughout noise exposure, the finning detection method described here could be used in combination with other metrics (i.e., speed) in the future to estimate free-ranging squid energetics in response to real OSW constructions and more broadly (Anderson and DeMont, 2005; Bartol et al., 2008).

5 Conclusion

This work revealed that pile-driving noise induced clear but transient disruptions to squid swimming behavior. However, the scale of our experimental pile driving was much smaller than planned future pile driving associated with OSW development within the *D. pealeii* range in the U.S. eastern coast. The diameter of our steel pile was 0.3 m, while OSW turbines are using piles exceeding 8 m in diameter, perhaps approaching or exceeding 10 m diameter (Steelwind Nordenham, FHL Corporation). As a result, noise propagating from OSW constructions will likely be higher in amplitude and farther reaching, which would expand the volume of ocean where behavioral impacts may be elicited. It also indicates the alarm behaviors seen in our present study may be wide-spread or even more severe.

Consequently, this study represents a significant step toward understanding how an abundant and commercially important species will be impacted by current and planned offshore constructions. Our novel high-resolution movement and particle acceleration data allowed us to be the first study to document both the probability of behavioral change and its duration in multiple spatial scales and noise exposure contexts. Future studies should aim to assess if pile-driving causes horizontal displacement, which is of particular concern the management of commercial fisheries.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was carried out in accordance with the principles of the Basel Declaration and recommendations and approval of the Woods Hole Oceanographic Institution's (WHOI's) Institutional Animal Care and Use Committee scientific protocol to TM.

Author contributions

SC, YJ, and TAM designed research; SC, YJ, SF, and NA, performed research; SC and YJ analyzed the data; SC, YJ, SF, NA, and TAM wrote the paper. TAM acquired funding. All authors read and approved the last version of the manuscript.

Funding

This work was funded by the Bureau of Ocean Energy Management Cooperative Agreement #M20AC10009.

Acknowledgments

The authors thank the W.S. Schultz Co. for conducting the pile driving, especially Matt and Ben Karson. We thank Edward O'Brien and Kimberly Malkoski for facilitating SCUBA operations throughout the experiment. We also thank Rick Galat, Kerry Strom, Stephanie Madsen and other members of the WHOI Facilities team for coordinating dock space and vessel

traffic. Lastly, we also express gratitude to Roger Hanlon for his discussions on our results and data interpretations.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1070290/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Magnetic field strength as a method to measure fin rates. Fin and magnet position were linked, so propagating fin waves during swimming distorted the magnetic field at a frequency equivalent to the fin rate.

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 23 January 2023
DOI 10.3389/fmars.2023.1050148

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

RECEIVED 21 September 2022
ACCEPTED 09 January 2023
PUBLISHED 23 January 2023

CITATION
Sal Moyano MP, Ceraulo M, Luppi T, Gavio MA and Buscaino G (2023) Anthropogenic and biological sound effects on the maternal care behavior of a key crab species.
Front. Mar. Sci. 10:1050148.
doi: 10.3389/fmars.2023.1050148

Anthropogenic and biological sound effects on the maternal care behavior of a key crab species

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Introduction: Maternal care in marine decapods involves eggs caring in the brood compartment until the larvae hatch. This behavior mainly allows embryo mass oxygen supply, ensuring healthy embryonic development. The present study aimed to analyze the effect of different sound sources (anthropogenic and biologic) and their temporal patterns (low and high rate: 1 min of the sound stimulus + 5 min of silence and 1 min of the sound stimulus + 1 min of silence, respectively) on the maternal care of the key crab species, *Neohelice granulata*.

Methods: In the laboratory, three acoustic stimuli were played back: an artificial white noise (10 Hz – 20 kHz), and two sounds obtained from the crabs´ natural habitat, motorboat passages and biological signals from a crabs' predator fish. Three behavioral variables were quantified: still position, and two maternal care behaviors: abdominal flapping and chelae probing.

Results: Results demonstrated that the high rate anthropogenic stimuli, white noise and motorboat, affected all behavioral variables, increasing the still position and diminishing the maternal care behaviors. Otherwise, the predatory stimulus did not affect the still position although diminished the maternal care behaviors (high rate).

Discussion: The different behavioral response depending on the sound stimuli may indicate that crabs distinguish sound sources. The anthropogenic noise is suggested to cause distraction that is linked to the increased still position, while the predator stimulus would be associated with an alert behavior not affecting the locomotion behavior. The sound stimuli effect on the maternal care behavior revealed a negative effect that potentially could affect offspring survival. This is important considering the ecosystem engineering function of the studied key crab species. The reduction of the noise emission pattern rate is suggested as a mitigation action to diminish sound impact effects in the crab's natural habitat. The study contributes the first to assessing the effect of different sound sources on the maternal care behavior of a crustacean species.

KEYWORDS

maternal care, anthropogenic sounds, biologic sounds, negative effects, crustaceans

Introduction

Aquatic ecosystems are composed of a combination of sounds produced by animals (biophonies), physical agents (geophonies) and human activities (anthropophonies) which defines the soundscape (Pijanowski et al., 2011). These particular mixtures of sounds reflect the ecological pattern and processes of specific aquatic environments (Matsinos et al., 2008; Ceraulo et al., 2018). The complexity of the biophony component is directly related to the structure of the animal community (Kennedy et al., 2010).

Among biophonies, the sound production in crustaceans is well known. Species of more than 20 families of aquatic, semiterrestrial and terrestrial crustaceans are described to produce sound through substrate-borne vibrations and air/water-borne sound (Nakamachi et al., 2021). Specifically, in marine decapods, sound production is related to different communicative behaviors such as defensive (Clayton, 2005; Patek et al., 2009; Buscaino et al., 2011; Goh et al., 2019; Roberts, 2021), mating (Buscaino et al., 2015; Filiciotto et al., 2019; Flood et al., 2019), orientation (Stanley et al., 2012; Sal Moyano et al., 2021) and agonistic interactions (Boon et al., 2009; Goh et al., 2019; Taylor et al., 2019; Ceraulo et al., 2022). Despite the diverse decapod sound signals described and their associated behaviors, much less known is the effect of biologic sound signals (e.g. from predators) on the behavior. Very few studies were conducted on this topic, in fact, three studies in vertebrates (fishes: Luczkovich et al., 2000; Remage-Healey et al., 2006; whales: Miller et al., 2022) and only two in invertebrates that tested the effect of predatory sounds on the feeding and locomotion behavior of crabs (Hughes et al., 2014; Snitman et al., 2022). Sound is characterized by the pressure variation and the displacement of the particles of the medium in which the same sound is propagating (i.e. particle motion). Regarding the sensitivity of decapod crustaceans, diverse sensory mechanoreceptors such as statocysts, chordotonal organs and setae were described to be involved in the detection of substrate vibrations and sound particle motion (Popper et al., 2001). A variety of studies demonstrated that decapods appear more sensitive to low frequency acoustic stimuli resulting from the particle motion (see Roberts and Elliott, 2017, and references therein).

Among anthropophonies, the sound caused by different human sources (e.g. shipping, pile driving, seismic surveys) is considered a global pollutant adding noise to ecosystems and masking natural sounds (Clark et al., 2009; Ceraulo et al., 2018). Impacts on marine animals are known to depend on noise intensity and temporal patterns of exposure (Popper et al., 2014; Blom et al., 2019). The effect of anthropogenic sound on marine mammals and fishes is well studied (e.g. see reviews: Slabbekoorn et al., 2010; Erbe et al., 2016), while much fewer studies were conducted in invertebrates and, especially, in crustaceans. Those studies focused on crustaceans include anthropogenic noise effects on behavioral (predatory: Chan et al., 2010; Nousek-McGregor and Mei, 2016; predatory and foraging: Wale et al., 2013; locomotion and activity: Solan et al., 2016; Snitman et al., 2022) and physiological (Celi et al., 2015; Filiciotto et al., 2016) traits. However, no previous studies were conducted on crustaceans to test the effect of anthropogenic sound on behaviors that involve a direct impact on fitness, such as maternal care with important consequences in the offspring survival. In contrast, the effect of noise on parental care behavior was demonstrated in fishes (Picciulin et al., 2010; Nedelec et al., 2016; Nedelec et al., 2017; McCloskey et al., 2020; Nedelec et al., 2022).

Parental or maternal care includes all parental traits that enhance offspring fitness (Trumbo, 2012). Maternal care is widespread among many animal taxa. These care traits are associated with an evolutionary response to physically harsh environments, involving a selective advantage (Clutton-Brock, 1991). Parental care controls for physicochemical stress produced by, for example, abiotic factors such as temperature, anoxia and salinity (Clutton-Brock, 1991). In crustacean marine decapods, maternal behaviors involve the care of the eggs that females carry in their brood compartment until the hatching of eggs and larvae is released (Diesel, 1992). This behavior is related to the supply of oxygen to the embryo mass, exhibiting active brooding comportment directed towards their ventilation (Fernández and Brante, 2003). Oxygen limitation in the center of the embryo mass usually occurs given the large number of eggs (Strathmann and Strathmann, 1995). Moreover, oxygen availability varies throughout embryonic development in response to embryo oxygen demands: low oxygen consumption in early stages while high in late stages (Naylor and Taylor, 1999; Fernández et al., 2000). Decapod females can assess the oxygen consumption of the embryos and modify the brooding behavior according to the embryos' oxygen demands (Baeza & Fernández, 2002; Fernández and Brante, 2003). Since females provide the oxygen to the egg mass, their behavior is a critical factor during embryonic development, given that oxygen limitation was demonstrated to influence survival, growth rate and size of eggs and hatching larvae (Palumbi and Johnson, 1982; Strathmann and Strathmann, 1995; Baeza and Fernández, 2002).

In addition to ventilating and providing oxygen to the embryo mass, maternal behaviors allow cleaning and elimination of metabolites, avoiding microbial infections during egg development (Clutton-Brock, 1991). Besides, maternal care allows protection of eggs from predators or adverse abiotic conditions (i.e. temperature, salinity) (Strathmann, 1985; Thiel, 1999).

Decapod females show active maternal behaviors, being the abdominal flapping the most recognized behavior (Fernández and Brante, 2003; Silva et al., 2007). Other less frequent maternal care behaviors involve standing (raised body), chela and pereiopods probing (females introduced the chela/dactyls of the pereiopods into the embryo mass) (Baeza and Fernández, 2002). Abdominal flapping is currently related to increase the oxygen availability while chelae/pereiopod probing is associated with the assessment of oxygen conditions in the embryo mass (Baeza and Fernández, 2002; Fernández and Brante, 2003).

Neohelice granulata is a varunid semiterrestrial crab considered a key crab species in the intertidal zone of estuaries, salt marshes and mangroves of the South-western Atlantic Ocean, being distributed from San Jose Gulf, northern Patagonia, Argentina (42°82´S; 64°83´W), to Lagoa Araruama, Rio de Janeiro, Brazil (22°85´S; 42°85´W) (Spivak et al., 2019). The Mar Chiquita coastal lagoon is a wetland located in the Buenos Aires Province, Argentina (37°40′ S, 57°23′ W) declared as a Man and the Biosphere Reserve (MAB) by UNESCO, conforming to the UNESCO World Network of Biosphere Reserves. In this lagoon, *N. granulata* is a dominant species used as a model study given the great diversity of publications conducted on several topics of its physiology, ecology and behavior (see Luppi and Rodriguez, 2020; Rodriguez and Luppi, 2020). Moreover, this crab

is considered a key species and an ecosystem engineer because of its burrowing activity that regulates the estuarine ecosystem functioning (Gutiérrez et al., 2006).

The Mar Chiquita coastal lagoon soundscape was previously characterized, describing particular temporal and spatial patterns of anthropogenic (motorboat passages) and biologic (fish and crustaceans) sounds (Ceraulo et al., 2020). Particularly, a study revealed that motorboat passages affected the reproductive call rate of a fish species (Ceraulo et al., 2021). The sound production in *N. granulata* was recently reported, characterizing the specific signals and the associated reproductive behavior (Filiciotto et al., 2019; Sal Moyano et al., 2019). In addition, current studies demonstrated the effect of artificial and habitat anthropogenic and biological sounds on the physiological stress and locomotion behavior of this species (Filiciotto et al., 2018; Snitman et al., 2022). The maternal care behaviors in *N. granulata* were previously characterized (Silva et al., 2007). However, no earlier studies evaluated the effect of sound signals on the maternal care traits of this species.

In this context, the present study aimed to analyze the effect of different sound sources: anthropogenic (motorboat passages obtained from the crab habitat and an artificial white noise) and biologic from a crabs' predator (fish), considering two temporal patterns of emission (low and high rate), on the maternal care behaviors of *N. granulata*.

Material and methods

Origin and collection of experimental subjects

Ovigerous female crabs were collected manually from the field, the Mar Chiquita Coastal Lagoon. Following Silva et al. (2007), ovigerous females in a late stage of embryonic development were selected given that the frequency of maternal care is increased as egg hatching is closer. Similar-sized females ranging from 22.5 to 26.5 mm of carapace width were used due to the brooding behavior is associated with body size (Fernández et al., 2006). Females were transported to the laboratory and acclimated in natural seawater aquaria (30 × 35 × 25 cm, 26 L capacity, filled with 3 L), at a density of four crabs/aquarium, under a controlled photoperiod of 14:10 h, and continuous aeration. The ambient room temperature was 23.5 ± 2°C. Individuals were fed daily with rabbit pellet food and water was changed after feeding. Crabs were maintained for a maximum of one week in the laboratory and then replaced by fresh animals.

Experimental system

A circular experimental PVC tank (1.2 m diameter and 1.5 m depth) filled with seawater at a depth of 1.2 m was used. A subaquatic video camera (Barlus, UW-S2Z-CX10 model, connected to an NVR IP 16 channels, Hikvision, DS-7616NI-Q1 model) was placed on the top and center of the tank to allow visualization of the entire tank's bottom surface. An underwater loudspeaker (Model UW30, Lubell Labs Inc., USA, Rated Frequency Response between 100 Hz - 10 kHz) connected to a Power Amplifier (Model APXII-300, American Pro, 230V, 50 Hz, China) plugged into the stereo output of a laptop was located suspended 40 cm from the bottom and 10 cm from the tank lateral wall.

Acoustic stimuli selection

Three acoustic stimuli were used: white noise (bandwidth range of 10 Hz – 20 kHz), and two sound stimuli acquired from the natural habitat of the crab, Mar Chiquita Coastal Lagoon, obtained from a previous soundscape study of the lagoon (Ceraulo et al., 2020). The white noise stimulus was digitally created using the wgn Matlab-function "wgn". The natural habitat acoustic stimuli belonged to biologic sounds produced by the black drum fish (*Pogonias courbina*) and anthropogenic sounds emitted by motorboat passages. The fish *P. courbina* is a predatory species of *N. granulata* (Blasina et al., 2010) and emits choruses during the reproductive period (Ceraulo et al., 2020). To isolate the fish signals and avoid the co-presence of diverse sources of soundscape components, a specific 1000 Hz low-pass filter was applied to the fish selected files, while no filters were applied to motorboat passage signals.

For the selection of motorboat passages files, only passes with burst broadband noise (frequency below and above 700 Hz, C type-class, see Ceraulo et al., 2021) were used given that this type of noise was demonstrated to be the most intense and frequent in the lagoon (Ceraulo et al., 2021). From the dataset, 30-100 sec duration files were selected from different days and hours (N total = 45). Playlists were constructed by randomly choosing four different files. All playlists had a similar total duration. For fish stimulus, black drum choruses containing a high number of signals (more than 200 in the original dataset, Ceraulo et al., 2020) were selected from the dataset and one-min duration files from different days and hours were chosen (N total = 10). Each playlist consisted of only one file.

For each stimulus (motorboat, white noise and fish), two patterns of emission were considered: low and high rates. In the high rate pattern, playlists contained 1 min of the sound stimulus + 1 min of silence; while in the low rate one, playlists contained 1 min of the sound stimulus + 5 min of silence. For the stimuli obtained from the habitat (motorboat and fish), both conditions of temporal patterns were selected as proxies of the lagoon mouth soundscape during the warm season: high rate motorboat passes on weekend days while low rate during weekdays; and high rate fish choruses during peak hours (sunset: 19:00 to 21:00 h) while low rate fish choruses in the rest of the day given that these signals showed a strong daily circadian pattern.

Ten different playlists for each stimulus (motorboat and fish) and pattern of emission (low and high rate) were constructed. A control without sound was used. Thus, six treatments were conducted: (1) low rate motorboat, (2) high rate motorboat, (3) low rate white noise, (4) high rate white noise, (5) low rate fish, (6) high rate fish, and a control without sound.

Acoustic analysis

To test the experimental system, a calibrated hydrophone (model Reson TC4013, with a sensitivity response of -211 ± 3 dB re 1V/µPa between a wide frequency range of 1 Hz and 150 kHz) coupled with a

preamplifier (1-MHz bandwidth single-ended voltage and a high-pass filter set at 10 Hz, 20 dB gain, Avisoft Bioacoustics), connected to a digital acquisition card (Avisoft UltraSoundGate 116h) managed by the Avisoft Recorder USGH software (Avisoft Bioacoustics) was located in the center of the PVC tank at a depth of 20 cm from the bottom. The acoustic stimuli and tank background noise were acquired at the sampling frequency of 100 kHz with 16-bit resolution and analyzed by the Avisoft-SASLab Pro software (Avisoft Bioacoustics). Figure 1 shows the spectrogram and the sound pressure level ($L_{p,rms}$ dB re 1μPa) of an example of a motorboat, white noise and fish playlist. The power spectrum of the playlists with a high rate emission temporal pattern for the three stimuli is shown in Figure 2. The peak frequency for motorboat, white noise and fish stimuli were 3442 Hz (amplitude 130 dB re 1μPa), 6177 Hz (126 dB re 1μPa) and 195 Hz (121 dB re 1μPa), respectively.

Experimental protocol

A female was randomly taken from the maintenance aquaria and located in the center of the PVC tank using a net. After a 10 min habituation period in the experimental tank, the video recording started and the experiment began. The total experiment duration was 60 min, divided into two phases of 30 min each: the "before phase" without sound exposure, and the "test phase" with sound exposure when the different stimuli were played back. At the end of the experiment, both the transducer and video recording were turned off and the crab returned to different maintenance aquaria. Ten replicates for the control (no sound stimulus was emitted in any of the two phases) and per treatment (N = 6: low rate motorboat, high rate motorboat, low rate white noise, high rate white noise, low rate fish, high rate fish; without sound exposure in the before phase and under stimuli exposure in the test phase), were performed (N total = 70). For each replicate of the different treatments, a distinct playlist was randomly assigned and played back. Each female was used in only one trial to meet the assumption of experimental independence.

Behavioral observations

Following Silva et al. (2007), two maternal care behaviors were recognized: "abdominal flapping" (females moved the abdomen forwards and backwards beating rhythmically the egg mass) and "chela probing" (females used one or both chela to pierce the egg mass, sometimes taking and carrying particles to the mouth). The time duration (sec) of the two different maternal behaviors displayed by ovigerous females, flapping and probing, was considered. Both maternal behaviors were observed to occur while the female was

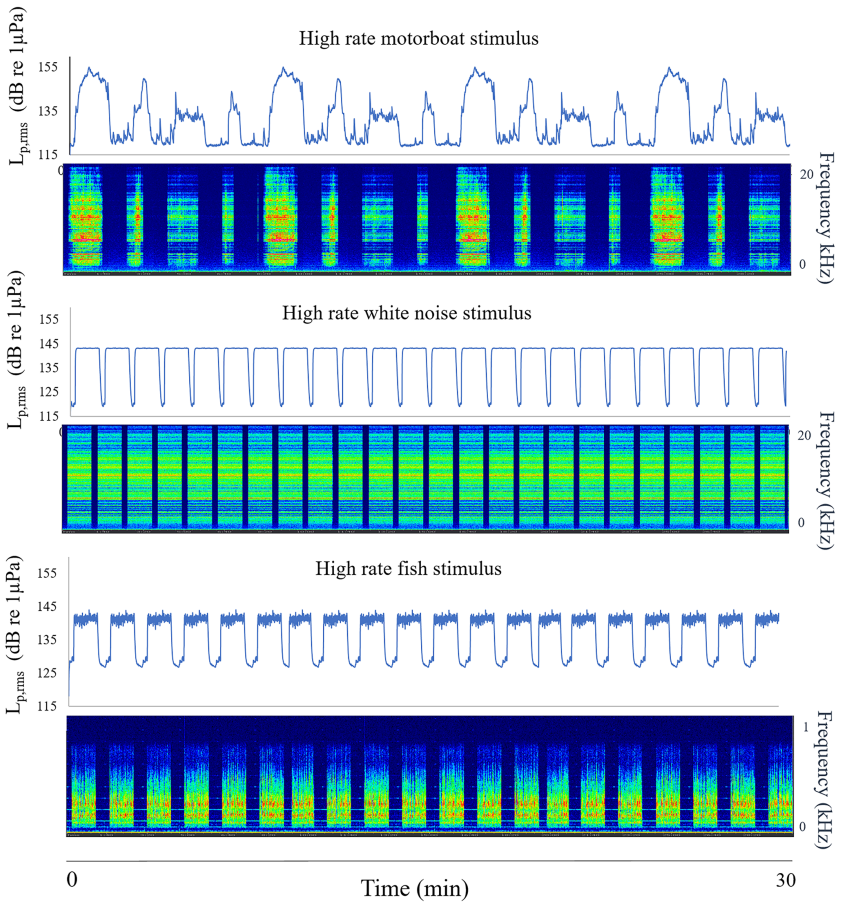

FIGURE 1
Spectrograms and sound pressure level ($L_{p,rms}$ dB re 1μPa) of 30 min duration of a playlist example of the different stimuli: motorboat, white noise and fish (8192 samples of FFT size, Hann window and signal superposition of 50%, Linear frequency scale).

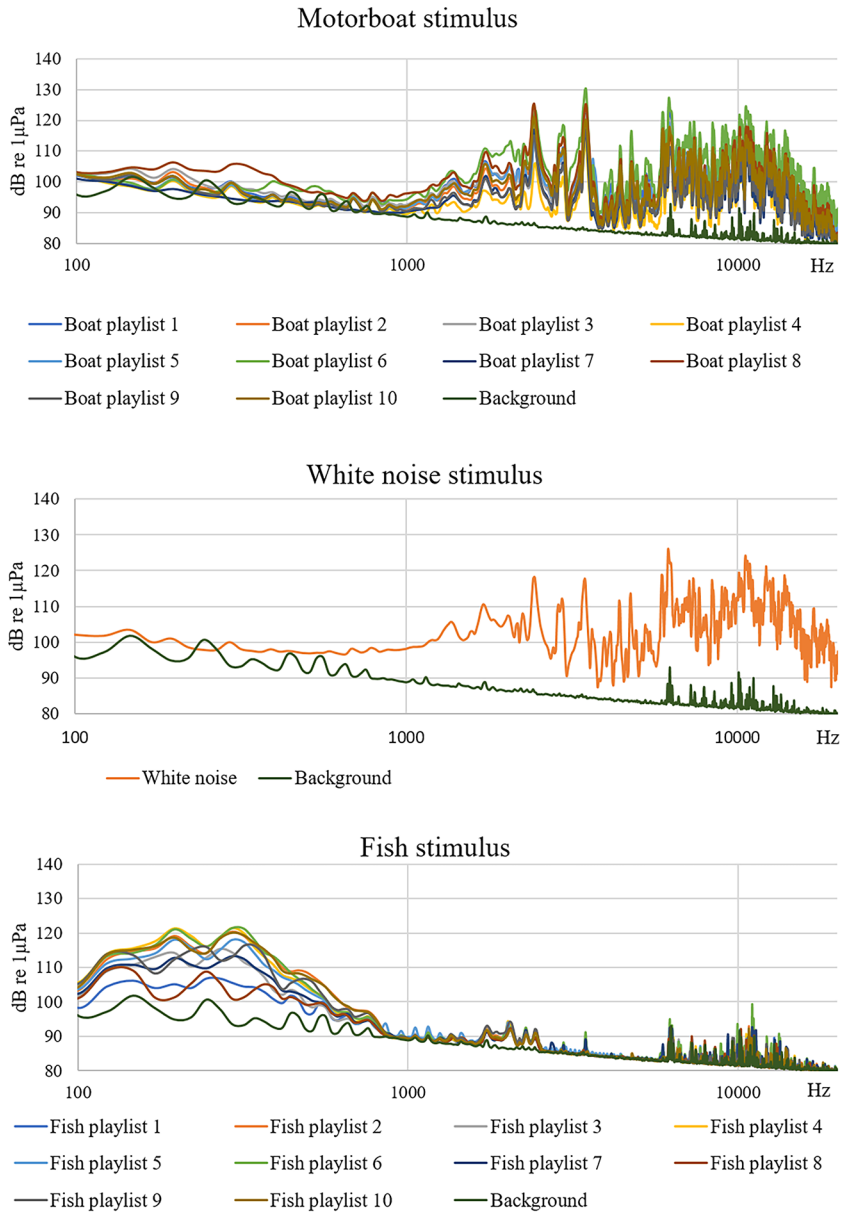

FIGURE 2
Power spectrum of all playlists with a high rate emission pattern of the motorboat, white noise and fish stimuli (FFT size 4096, hamming window, resolution 24.4 Hz).

walking or when she stopped locomotion. For the statistical analysis, the flapping was considered individually and, jointly with the probing and named generally "maternal care". The time in which females were observed still (without walking or moving the pereiopods nor the chelae) for 5 sec or longer was also quantified and considered as a "still" position. The duration in which the three behavioral variables were displayed (flapping, flapping + probing = maternal care, still position) was quantified in each phase (without sound exposure and with sound exposure) for each of the six treatments and the control.

Statistical analysis

Models with Poisson error distribution were fitted given that the nature of the data were counts (in seconds) of different behavioral variables. The residuals patterns and overdispersion were examined using the function testUniformity() and testDispersion() from the DHARMa package (Hartig, 2018). In all the cases, model validation of the residuals was applied to verify that underlying statistical assumptions were not violated. When overdispersion was detected, it was corrected incorporating an extra overdispersion parameter using a quasi-Poisson distribution (Ver Hoef and Boveng, 2007). Thus, to each behavioral variable quantified (still position, maternal care and flapping) in both before and test phases, a quasi-Poisson generalized linear model (GLM) with log link (See Zuur et al., 2009) was used to test the effects of the diverse stimuli (levels: low and high rate motorboat, low and high rate white noise, low and high rate fish). Finally, in the test phase, posthoc mean comparisons between the control and the low and high rate levels of each behavioral variable were conducted using an interaction means test in the "emmeans"

package (Lenth et al., 2018). All statistical analyses were performed in R 3.3.1 (R Core Team, 2016). A diagram of the experimental design used is shown in Figure 3.

Results

In the "before phase", no differences were found between the control and the different treatments (group of animals intended to test the diverse stimuli in the test phase) for any of the three behavioral variables quantified (still position: χ^2 = 8.52, *df* = 6, *P* = 0.2024; maternal care: χ^2 = 11.397, *df* = 6, *P* = 0.07686; flapping: χ^2 = 8.687, *df* = 6, *P* = 0.192).

When analyzing the "test phase", differences were encountered in the crab's response when exposed to the anthropogenic stimuli for all variables: the sound stimuli increased (both low and high rate motorboat, and high rate white noise) the still position while diminished the maternal care and flapping (high rate motorboat and white noise) compared to the control without sound stimuli (Table 1, Figure 4). In the case of the motorboat treatment, the effect on the still position was greater given that both patterns of emission boosted this behavioral variable. In the fish treatment, no differences were found in the still position (low and high rate), while the high rate pattern reduced the maternal care and flapping behaviors compared to the control (Table 1, Figure 4).

Consequently, differences among the behavioral response depending on the sound stimuli source were found: both anthropogenic stimuli increased the still position and diminished the frequency of the maternal behaviors; while fish predatory stimulus only reduced the maternal care behaviors in the high rate emission pattern but not the still position. Behavioral observations demonstrated that when the predatory stimulus started, the crab locomotion was interrupted for a few seconds (2-3) and immediately restored (75% of the cases considering the total number of replicates, low and high rate, N = 20); while when anthropogenic stimuli began, the crabs stopped walking and stayed for several seconds (>5) in still position (motorboat: 85%, white noise: 90%).

Discussion

Maternal care in decapods is directly related to the egg mass oxygen provision and healthy embryonic development having vital ecological consequences on the offspring's fitness. This study is the first to assess the effect of different artificial and habitat sound sources (anthropogenic and biologic) on the maternal care behavior of a crustacean species, the key crab *Neohelice granulata*. Results revealed a negative effect of sounds on maternal care traits. The high rate pattern of sound emission showed negative effects on the studied behaviors compared to the low rate one. Besides, the study demonstrates that this crab species is behaviorally responding differently according to the diverse sound stimuli, thus, it may be distinguishing sound sources. In this sense, only anthropogenic stimuli boosted the still position, suggesting a distraction effect, while the predatory stimulus may elicit an alert behavior without affecting the locomotion pattern. In addition, results are discussed in the context of the ecological importance of the crab species and the habitat.

The diverse sound stimuli emitted in the present study demonstrated that distinct sound sources elicited different behavioral responses: all stimuli, anthropogenic (motorboat passages, both high and low rate temporal patterns, and high rate white noise) and biological from a crab´s predator (high rate) reduced the maternal care behaviors, but only the first ones (white noise and motorboat passages) increased the still position of crabs. The similar response of crabs to white noise and motorboat stimuli (although the motorboat stimulus had a greater effect given that the low rate pattern also reduced the still position) could be due to the non-discrimination between them given that their peak frequency is higher than the known crustacean's sensitivity (see Roberts and Elliott, 2017). The

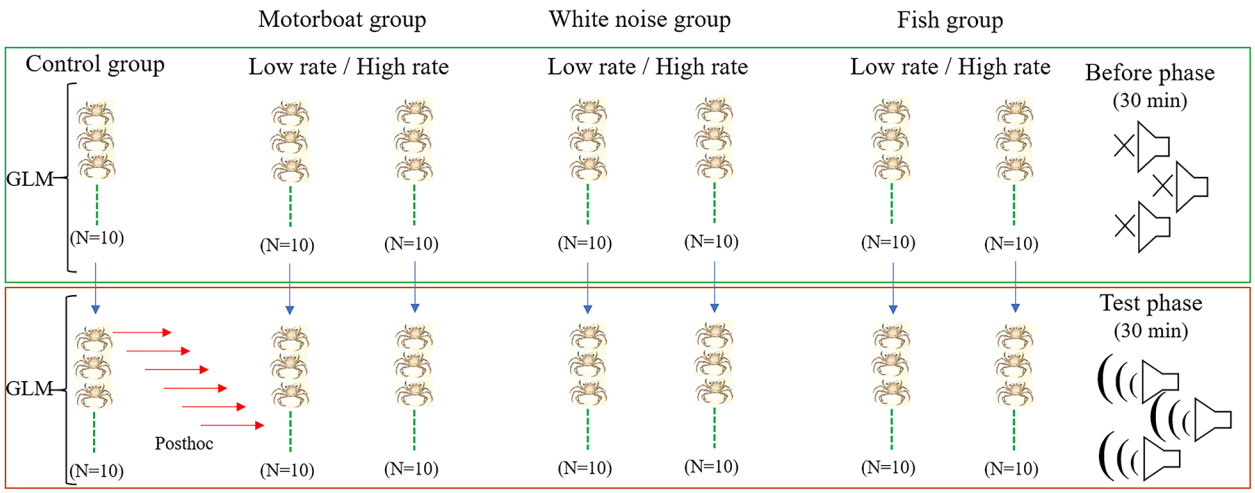

FIGURE 3
Diagram of the experimental design used showing both phases, before and test, with the control and six levels (low and high rate motorboat, low and high rate white noise, low and high rate fish) in each phase. For each phase, a GLM was performed. In the test phase, the six red arrows represent the posthoc comparisons between the control and levels. The same design was applied for the three behavioral variables (still position, maternal care (flapping + probing) and flapping).

TABLE 1 Results of the GLM of the "Test phase", showing the effects of the sound stimuli (motorboat, white noise, and fish) and both temporal emission patterns of sound (low and high rate) on the time duration (sec) of the different behavioral variables quantified (still position, maternal care (flapping + probing), and flapping).

TEST PHASE				
		χ^2	*df*	*P*
	Behavioral variable: Still position			
Factor	Time duration of behavioral variable	42.54	6	**< 0.001**
Posthoc Control vs	Low rate motorboat			**< 0.001**
	High rate motorboat			**< 0.01**
	Low rate white noise			0.06
	High rate white noise			**0.011**
	Low rate fish			0.84
	High rate fish			0.819
	Behavioral variable: Maternal care			
Factor	Time duration of behavioral variable	13.07	6	**0.041**
Posthoc Control vs	Low rate motorboat			0.106
	High rate motorboat			**0.019**
	Low rate white noise			0.186
	High rate white noise			**0.019**
	Low rate fish			0.686
	High rate fish			**0.015**
	Behavioral variable: Flapping			
Factor	Time duration of behavioral variable	15.76	6	**0.015**
Posthoc Control vs	Low rate motorboat			0.179
	High rate motorboat			**0.019**
	Low rate white noise			0.33
	High rate white noise			**0.002**
	Low rate fish			0.503
	High rate fish			**0.023**

Significant values are shown in bold.

reduced locomotion in *N. granulata*, when exposed to different anthropogenic sound sources, was previously described (Filiciotto et al., 2018; Snitman et al., 2022). Similarly, several studies conducted on decapods found reduced locomotion, a resting time increased or a response behavior (antipredator) diminished, in the presence of motorboat noise (Chan et al., 2010; Wale et al., 2013; Filiciotto et al., 2016; Nousek-McGregor and Mei, 2016; Solan et al., 2016). Considering the effect of biological sounds from predators, a study conducted in crabs demonstrated that reduced the feeding behavior (Hughes et al., 2014), in cetaceans diminished the foraging behavior (Miller et al., 2022), and in fishes affected the mating choruses and calling rates (Luczkovich et al., 2000; Remage-Healey et al., 2006). A study performed in *N. granulata*, showed reduced locomotion in the presence of predatory sounds from a fish and a crab (Snitman et al., 2022). In contrast, in the present study, the still position was not affected by predatory fish stimulus, although diminished the frequency of the maternal care behaviors displayed. The fact that distinct sound sources, anthropogenic and biologic, affected differently the still position (increased when exposed to anthropogenic stimuli and with no effect under fish stimulus exposure) may indicate that each stimulus promote a diverse behavioral response.

On one hand, anthropogenic stimuli may be associated with a distraction or confusion effect that would be linked with the increased still position (and reduced maternal care): immediately after hearing these stimuli, crabs were observed to stop locomotion for several seconds, retarding their return to the previous locomotion pattern. This retarded response or distracted behavior might imply an ecological disadvantage given crabs may be exposed to risks such as a predator attack. The distraction effect of ship noise disrupting the information gathering ability of animals was previously proposed to occur in hermit crabs (Yim-Hol Chan et al., 2010; Tidau and Briffa, 2019). On the other hand, predatory fish sounds might be related to elicit an alert behavior that could be explained by the observed

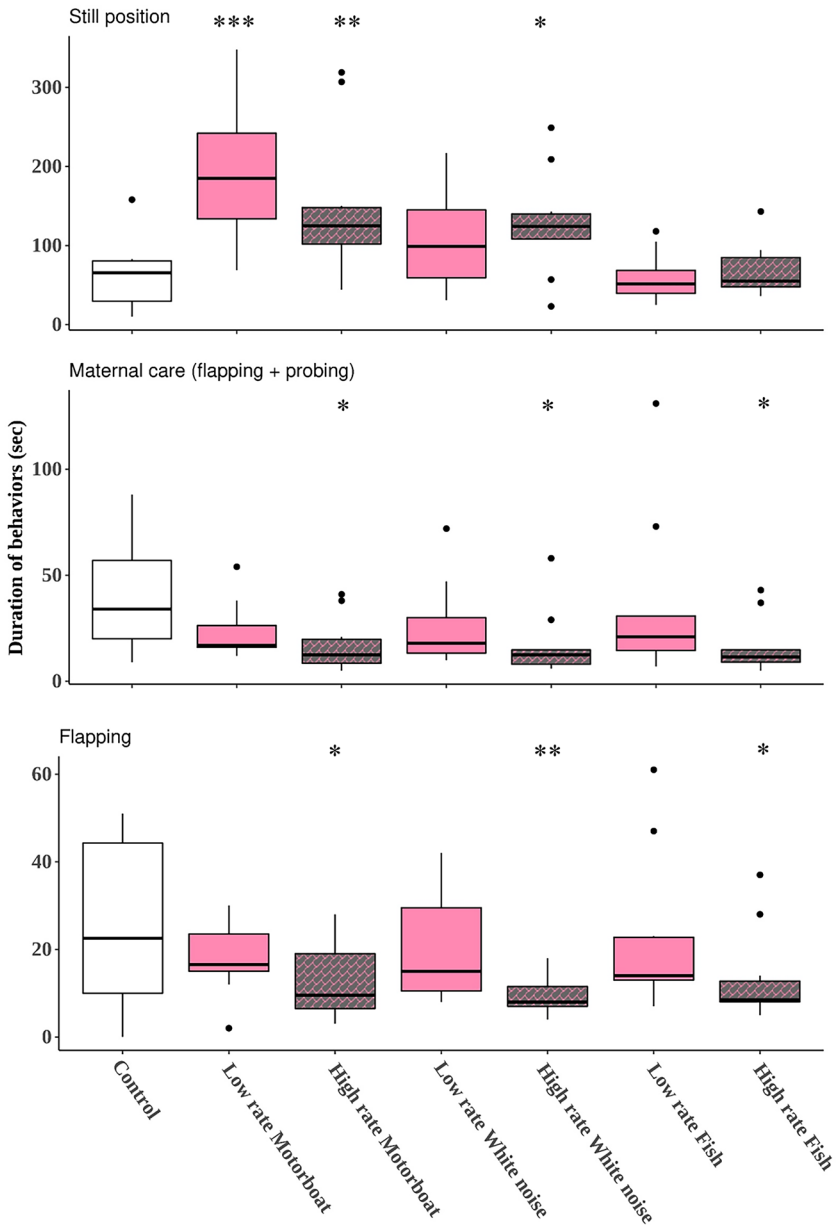

FIGURE 4
Results of the "test phase" showing the duration (sec) of the behavioral variables quantified: still position, maternal care (flapping + probing), and flapping, for each stimuli and patterns of emission (low rate motorboat, high rate motorboat, low rate white noise, high rate white noise, low rate fish, high rate fish) and the control without sound. High rate temporal emission patterns of the stimuli are represented with grey color, low rate temporal emission patterns of the stimuli with pink color and the control with white color. GLM, significant results: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. The asterisks represent the posthoc comparisons between the control and each treatment for the three behavioral variables.

behavioral response of stop walking for few seconds (2-3) when hearing the stimulus started and, immediately after, restoring the locomotion behavior. In its natural habitat, this species walks around the burrows showing a fast-running behavior to them in the presence of risk (del Valle Fathala and Maldonado, 2011). Thus, the potential alert behavior elicited immediately after hearing the predator sound would favor the fast response to return and hide in the burrow allowing survival against risk rather than causing a distraction (Snitman et al., 2022). Likewise, an alert behavior was previously described in the lobster *Palinurus elephas* in the presence of a predator (Buscaino et al., 2011).

The results above discussed that show different behavioral responses depending on the sound stimuli (considering their diverse band frequency ranges) are novel and interesting given that support the idea about crabs may be discerning diverse sound sources. Behavioral experiments demonstrating how animal react are useful to assess their hearing capability (Popper and Hawkins, 2021). In this sense, it is important to consider that the experiment was conducted in a tank, thus, conditions such as environmental variables were completely controlled which is important given the high variability of the natural habitat of the crab (coastal lagoon). However, it also may be highlighted that the sound properties of acoustic stimuli can get modified by the

surrounding environment (reflexions of the tank), thus, implying differences in tanks sound propagation compared to habitat sound propagation (Akamatsu et al., 2002). Besides, it was highly reported that crustaceans might only detect the particle motion component of the sound because the lacking of gas-filled organs inside the exoskeleton (Popper et al., 2001; but see Radford et al., 2022). A limitation of the present study was the lack of measurements of the particle motion sound component. To obtain results that are more representative of what occurs in nature and taking into account the fact that the description of the pressure variation alone is not exhaustive when studying the reactions to sound stimuli in crustaceans, future studies should be conducted in nature (or in bigger tanks) and with systems able to characterize also the particles motion.

Regarding the different temporal sequences of the sound stimuli emission pattern used in the present study, low and high rate, significant differences were found between patterns: the high rate ones showed an effect on the behavioral variables. In the Mar Chiquita coastal lagoon, both emission patterns are commonly represented by motorboat passages and fish choruses, mainly the high rate ones in the lagoon mouth, during the warmer months (spring and summer) (Ceraulo et al., 2020). Thus, the results found, demonstrate the importance of the potential effects of the high rate emission pattern of sound on this crabs species in its natural habitat. Similarly, a previous study conducted on a marine fish with parental care found that only a continuous noise (high rate temporal pattern) negatively affected nest inspection and spawning compared to the intermittent (low rate temporal pattern) treatment, thus, affecting reproductive success and offspring fitness (Blom et al., 2019). Also in a reef fish, it was demonstrated that motorboats affected parental behavior and offspring survival under a long-term exposure study (Nedelec et al., 2017).

Considering the effects of the diverse stimuli, all high rate patterns affected the maternal care quantified as probing + flapping, and the flapping behavior. Thus, flapping was the greatest behavior affected by the different stimuli. The flapping is the most frequent maternal care behavior displayed in this species (Silva et al., 2007), and the greatest related to oxygen provision to the embryo mass (Fernández et al., 2000). Consequently, this result may indicate a potential negative effect on the eggs oxygen supply. Mating behavior and maternal care, proxies for reproductive success, are behavioral traits conforming to important components of an individual's fitness given that reflect the survival capacity (Andersson, 1994). As well demonstrated in previous studies, brooding care in marine decapods is directly associated with the oxygen supply to the embryo mass (see Baeza and Fernández, 2002). Although we did not conduct a long-term study to evaluate costs on offspring, the negative effect of sound sources on the frequency of the maternal behaviors displayed may imply a reduction in oxygen supply, potentially affecting embryonic development, and consequently, offspring survival. Estuaries are changeable and vulnerable environments, characterized by high fluctuations in chemical and physical parameters, such as salinity, temperature and dissolved oxygen (Viaroli et al., 2007). The effects of salinity on embryonic development were previously reported in *N. granulata* through *in vitro* experiments (Bas and Spivak, 2000). The laboratory experiments performed in the present study contained oxygen-saturated seawater. However, given the variable conditions of the Mar Chiquita coastal lagoon estuary, e.g. dissolved oxygen (Luppi et al., 2013), the effect of sound on maternal behavior, and the potential consequent reduction in the oxygen supply, might be considered since it could add negative effects to the natural low dissolved oxygen concentrations of the habitat, impairing embryonic development. It was demonstrated that oxygen limitation retarded the development of inner embryos in gastropods (Cohen and Strathmann, 1996), and increased the risk of egg predation in a fish with parental care (Olsson et al., 2016). Specifically on aquatic invertebrates, the adverse impacts of hypoxia were widely assessed (reviewed by Galic et al., 2019). The physiological constraint of oxygen provision in marine invertebrates may have important ecological and evolutionary consequences at the population level (Baeza and Fernández, 2002). Future long-term studies conducted in *N. granulata* testing the effect of sound throughout the embryonic development and assessing direct traits associated to brood survival (e.g. number of hatched larvae, larvae weight and size, etc.) would help to better understand the effect of sound on offspring fitness and, consequently, the potential effects at a population level.

In this context, it is important to highlight that, although some studies evaluated the effects of anthropogenic sounds on invertebrates (e.g. Morley et al., 2018; Solé et al., 2018), very few have focused on the impacts of noise on marine ecosystem services considering how affects species that mediate ecosystem functioning (for an exception see Solan et al., 2016). The responses of marine invertebrates to anthropogenic noise are still little known, hindering the understanding of ecosystem impacts and the development of mitigation plans (Wale et al., 2019). In this sense, the present study contributes to a great extent to the knowledge of the anthropogenic sound effect on an ecosystem engineering key crab species in a coastal lagoon that provides important ecosystem services. Besides, the results demonstrating no negative effects on behavior of the low rate anthropogenic stimuli may suggest potential mitigation actions such as the reduction of the noise emission pattern rate. Thus, the present study provides important data to be used in the development of management plans and sustainable use in the Mar Chiquita coastal lagoon.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

MM, MC, TL and GB designed the experiment. MM conducted the experiment. MC and GB performed the acoustical analysis. MM wrote the draft of the manuscript. All authors reviewed and edited the manuscript. MM, MG, TL and GB provided resources for this study. All authors contributed to the article and approved the submitted version.

Funding

This project was funded by the Agencia Nacional de Promoción Científica y Tecnológica (PICT-2019-668, PICT-2017-373) and

Universidad Nacional de Mar del Plata (EXA 984/20). Financial support was also provided by the CAIMAR Joint Laboratory Italy-Argentina (Laboratori Congiunti Bilaterali Internazionali) of the Italian National Research Council (2017–2019) and the project BOSS: Study of bioacoustics and applications for the sustainable exploitation of marine resources (Projects of major importance in the Scientific and Technological Collaboration Executive Programs, funded by the Italian Ministry of Foreign Affairs and International Cooperation and the Argentinean Ministry of Science, Technology and Innovative Productivity).

Acknowledgments

The authors thank Jesús Nuñez for his help with the statistical analysis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 30 January 2023
DOI 10.3389/fmars.2023.1029003

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

RECEIVED 26 August 2022
ACCEPTED 05 January 2023
PUBLISHED 30 January 2023

CITATION
Song Z, Ou W, Su Y, Li H, Fan W, Sun S, Wang T, Xu X and Zhang Y (2023) Sounds of snapping shrimp (*Alpheidae*) as important input to the soundscape in the southeast China coastal sea. *Front. Mar. Sci.* 10:1029003. doi: 10.3389/fmars.2023.1029003

Sounds of snapping shrimp (*Alpheidae*) as important input to the soundscape in the southeast China coastal sea

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As a biological sound source widely distributed in temperate and tropical coastal waters, snapping shrimp produce strong pulses which can serve as honest signals to indicate habitat-specific soundscape. The past decade has witnessed the growing interest in investigating the acoustical activity of snapping shrimp across many shallow waters including the coastal line of the west Pacific Ocean. It was extended to the Southeast China coastal area where snapping sounds and the associated soundscape were recorded at four sites. Customized codes incorporating bandwidth and amplitude threshold operations were developed to detect snaps from the ambient noise to estimate snap rate and extract snaps individually. The subsequent analysis suggested that snaps recorded at different sites were unanimously stronger than background noise. Sound pressure level of the snaps ranged from 150 dB to 190 dB (re 1 µPa). The characteristics of snaps, including sound pressure level, duration, peak frequency, -3dB bandwidth from different sites are examined to evaluate the variability across the sites. Though snapping pulses had peak frequencies and the -3 dB bandwidth consistently below 10 kHz, snaps had considerable energy extending to the high frequency range over 200 kHz. The analysis of the acoustic data received for 7 consecutive days at one site indicated that the snap rate corresponded to tidal level periodicity. A high tide was accompanied with a local high snap rate regardless of light but this local snap rate peak was much higher at night. The mean rate fluctuated between 2000 and 4000 snaps per minute and more snaps were recorded after sunset suggesting that snapping shrimp living in the area snapped in response to light. These data may indicate that snaps are important communication means in light-limited condition and deepen our understanding on the correlation of snapping behavior and ecological environments.

KEYWORDS

snapping shrimp, west-Pacific Ocean, bioacoustics, animal behavior, coastal water

1 Introduction

The ocean is a natural reservoir for sounds originating from biological, geophysical, and anthropogenic processes (Wenz, 1962; Krause, 2008; Duarte et al., 2021). The increasing anthropogenic activities, including worldwide shipping, platform construction and wind farm operation are massive contributions to intensifying the ocean soundscape (McDonald et al., 2006; Slabbekoorn et al., 2010; Herbert-Read et al., 2017; Harding et al., 2019; Mooney et al., 2020). Marine animals, including invertebrates, have evolved to sense sounds and cue on environment acoustically to facilitate survival (Mann and Lobel, 1997; Hawkins and Amorim, 2000; Giorli et al., 2016; Van Oosterom et al., 2016; Erbe et al., 2017). For example, snapping shrimp are capable of producing sounds over 200 dB as an important input to the overall soundscape (Au, 1993; Au and Banks, 1998; Song et al., 2021). Biological activities from snapping shrimp along the coastal line have considerable effects on the habitat-specific soundscape (Everest et al., 1948; McClure and Wicksten, 1997; Fay, 2009; Monczak et al., 2017; Monczak et al., 2019).

Snapping sounds cover a wide range of frequency and present a highly diurnal dependence and seasonal variation, dominating over other sounds in shallow waters (Bohnenstiehl et al., 2016; Butler et al., 2017; Lillis and Mooney, 2018; Monczak et al., 2019; Mueller et al., 2020). Shrimp use its big claw to eject water, resulting in a cavitating bubble collapse and generation of a broadband pulse with energy extending to over 200 kHz (Versluis et al., 2000). Snapping shrimp has various dwelling sites including coral reefs, kelp, mangrove and oyster reefs. Sounds generated by snapping shrimps have induced numerous studies since World War II and snapping shrimp were previously thought to distribute in the tropical and subtropical zones. Many later studies have reported the sound activity of snapping shrimp in higher latitudes (Watanabe et al., 2002; Mathias et al., 2016; Bibikov and Makushevich, 2020; Lee et al., 2021).

Snaps can serve in various ways to meet the daily demands for shrimp in fighting for shelter protection, preying, rock-boring, excavation, and communicating (Nolan and Salmon, 1970; Schein, 1975; Schein, 1977; Schmitz and Herberholz, 1998). The size of the open major chela, and the resulting water jet and snap pulse are all signals produced during intraspecific encounters (Hughes, 2000). The snapping claw as a mechanosensory stimulus can be detected by setae on the major chela of the encountering competitors but both the physical size and water jet have a limited working distance (Herberholz and Schmitz, 1999). In comparison, the snapping pulse can propagate to a great distance and possibly used for group coordination (Toth and Duffy, 2005). The synchronizing snapping was reported in a previous study, raising a question on whether snapping shrimp can acoustically sense the snaps, which was examined recently in snapping shrimp (*Alpheus richardsoni*), suggesting this species is sensitive to sounds ranging from 80 to 1500 Hz (Dinh and Radford, 2021). Snaps are broadband signals with considerable energy below 1500 Hz, meaning that snaps may be used for acoustic signaling between conspecifics. The communication range will depend on the source level of the snaps.

The snapping either used for communication or as aggressive behaviors presented a diurnal pattern. Snaps were found to peak at dusk and dawn (Radford et al., 2008; Lammers et al., 2008; Lillis and Mooney, 2018), following a diurnal pattern to some extent. But they shift their show preferential snapping time from nighttime in the summer to daytime in the winter in the West Bay Oyster Reserve, Pamlico Sound North Carolina (Bohnenstiehl et al., 2016). A transition between dominant daytime at different seasons was observed and it may relate to light availability. These changes of snapping behavior with season were also reported in the Coastal Sea of Western Jeju, part of the western Pacific Ocean (Jeong and Paeng, 2022). The most frequent snapping events were found at night in late summer. But in late fall, snap rate was not the highest at night and dropped like the one during the day. A high tide was always accompanied with a higher snap rate by 13% than at low tide and the temporal variation of snap rate time was parallel to that of current speed during high/low tides with a time lag of about 1.25 h (Lee et al., 2021). These results paved us the way for further studies on what drives the temporal variations of snap rate during the day in different seasons and tidal levels. The biological characteristics of snapping shrimp may account for the snap rate variation to some extent.

Snaps produced from 42 snapping shrimp individuals of *Synalpheus paraneomeris* and 20 A. *angulosus* specimens were examined in acoustic measurements (Au and Banks, 1998; Song et al., 2021). They found that snaps have peak frequencies unanimously between 2 and 5 kHz. Peak-to-peak source levels varied from 183 to 189 dB (re 1 µPa) for *S. paraneomeris* and ranged from 164.9 to 187.7 dB (re 1 µPa) for *A. angulosus* (Au and Banks, 1998; Song et al., 2021). The laboratory measurements provide good controls to estimate the source level of snaps because the animals can be physically fixed 1 m away from the recording facility. In comparison, it is challenging to estimate the actual source level of snaps recorded from field. Snaps from the field and laboratory were compared for shrimp in the May River estuary, both showing a generally broadband property and the majority of energy was confined to below 10 kHz. Laboratory snaps had a much higher power spectral density than those of the field snaps, which may result from the attenuation due to the long travelling distance (Song et al., 2021). There might be other parameters that can lead to variability of snaps produced in the field, including anthropogenic noise (Spiga, 2022).

Among numerous studies on snapping shrimp sounds, only a few examine the acoustic characteristics of snaps in detail (Au and Banks, 1998; Song et al., 2021; Spiga, 2022) and in this paper, we added extra acoustic analysis on peak frequency, duration, -3dB bandwidth and sound pressure level of snaps recorded at four different sites in shallow water of southeast China coastal area. These sites located at two adjacent provinces and data were collected to probe into the temporal variation of the estimated snap rate and its correlation with tidal level. This study contributes to soundscape research concerning snapping shrimp in this region and provide information to probe into the sound habitat-specific underwater soundscape along the southeast China coastal line.

2 Materials and methods

2.1 Acoustic survey

Snaps were recorded at four different sites along the shallow waters of southeast China coastal area, with two sites at Fujian province and the rest two at Guangdong province (Figure 1).

A compact recording system, SoundTrap recorder (ST 300 HF, Ocean Instruments Ltd, New Zealand), with a linear frequency range of 20 Hz - 150 kHz was used in experiments at site 1, site 3 and site 4. Another sound recorder (ST 600 HF, linear frequency range: 20 Hz - 150 kHz) was deployed at site 2, which was physically fixed to a buoy. The ST 300HF and ST 600 HF are both compact underwater audio recording system containing a single channel, with a low self-noise level (less than 38 dB re 1 µPa above 2 kHz), a user programmable preamplifier and a 16-bit analog-to-digital converter. The sampling rate can reach as high as 576 kHz for ST 300 HF and 384 kHz for ST 600 HF. The preamplifier and ADC converter, batteries were embedded inside the main body and the tool functioned as one unit, with a 256 GB storage memory for ST 300 HF and Up to 2TB of data storage for ST 600 HF. The recorder was hung in the water column through either a steel bar with clips at the end or physically fixed to the buoy. The recording sites had a water depth of 12.0 m, 11.0 m, 12.0 m, and 4.0 m and the recorder was placed 2.0 m, 2.0 m, 3.0 m, and 1.5 m underwater at site 1, site 2, site 3, and site 4, with a sampling frequency of 576 kHz, 96 kHz, 192 kHz and 576 kHz, respectively.

2.2 Snap analysis

Shrimp snap is thought to have extremely typical characteristics of broadband energy and high amplitude (Au and Banks, 1998; Song et al., 2021). We referred to previous studies and used an envelope correlation algorithm combined with an amplitude threshold to detect snaps (Bohnenstiehl et al., 2016; Lillis and Mooney, 2018; Lee et al., 2021). Customized Matlab scripts were developed to extract individual snaps. Snap spectrogram was calculated using the short-time Fourier transform, using a 192-point fast Fourier transform (FFT) and a rectangular window of 1 ms. These settings provided a frequency resolution of 500 Hz.

After extraction, snaps from four recording sites were compared to address the potential variations. To begin, acoustic parameters including peak-to-peak sound pressure level (SPL), duration, peak frequency and -3dB bandwidth were calculated following previous studies (Madsen and Wahlberg, 2007; Au et al., 2016; Song et al., 2021). SPL was determined after calibration using the sensitivity of SoundTrap recorders. Duration was determined a time length covering 95% of the total pulse energy. Peak frequency was defined as the frequency point at which the spectral level had the highest value. The -3 dB bandwidth was the difference of two frequency points between which the spectral level was lower than the maximum level by 3 dB.

The snap parameters were tested to examine their normality using Shapiro-Wilk method. Either the ANOVA analysis or the Kruskal-Wallis analysis of variance (ANOVA) analysis was used to determine the differences in acoustic properties of the snaps recorded at different sites. All statistical analyses were tested at significance level of 0.05.

2.3 Examination on snap rate at site 2

The temporal pattern of the snap rate and soundscape concerning were examined using the data of the longest recording length (site 2) to probe into the soundscape and its temporal variation throughout the recording time. Considering the sampling frequency at site 2 was 96 kHz, we divided the recording bandwidth into 3 sub-bandwidths to quantify soundscape: a low sub-bandwidth from 0-1.5 kHz representing the fish sounds and boat noise; a middle sub-bandwidth from 1.5-10 kHz as an index of snapping shrimp sounds and a high sub-bandwidth from 10-48 kHz. The mean power spectral density was calculated for these bandwidths and the whole bandwidth from 0-48 kHz for every minute. The output was tracked with time for 7 consecutive days. The number of snaps per minute was calculated to estimate snap rate and the resulting temporal pattern. The sound

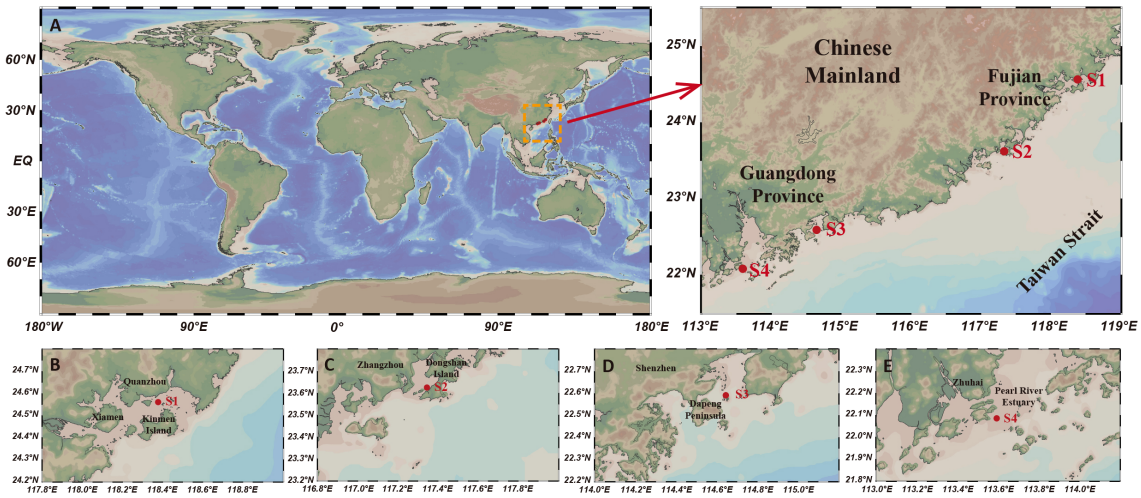

FIGURE 1
(A) An overall view of the location of the experimental sites S1, S2, S3 and S4 from both a global and regional context, with the four recording sites marked in red. **(B)** Recording site at Xiamen Bay (Xiamen, China), **(C)** Zhao'an Bay (Zhangzhou, China), **(D)** Daya Bay (Shenzhen, China) and **(E)** Pearl River estuary (Zhuhai, China). Figure was drawn using Ocean Data View (Schlitzer, R., Ocean Data View, https://odv.awi.de, 2021).

energy within bandwidth II was used to estimate the temporal change of snapping shrimp sounds because the peak frequency of the snap was confined to this bandwidth (Au and Banks, 1998; Schmitz, 2002; Song et al., 2021). The power spectral densities of bandwidth I, bandwidth II, bandwidth III and bandwidth IV can be compared to evaluate the influence of snapping shrimp sounds to the overall soundscape at different habitats.

3 Results

3.1 Acoustic properties of snaps across different sites

A representative shrimp snap train with continuous snaps showed that the thresholding method was effective to extract individual snaps from the long time series (Figures 2A, B). Snap (Figures 2C, D) presents a broadband property with frequency peaking at 3.8 kHz and it can be seen that energy was extended to over 100 kHz. The amplitude difference across 4 octaves beginning at peak frequency was less than 20 dB. The snap was composed of a precursor with relatively low amplitude and a short pulse characterized by its rapid onset and high amplitude. These features were similar to snaps reported in previous studies on snapping shrimp species, *A. heterochaelis*, *A. angulosus*, and *S. parneomeris* (Au and Banks, 1998; Versluis et al., 2000; Song et al., 2021).

The characteristic spectrum was similar among snaps recorded at different sites (Figure 3), all showing a broadband distribution. Snaps unanimously had higher energy than background noise. There were variabilities in snap and background noise amplitudes. Using power spectral density as reference (Figure 3), the greatest amplitude difference between snap and background noise was reflected in data from site 3, reaching 59 dB at peak frequency 2.3 kHz. Snaps from site 3 had the highest amplitude, followed by site 2 and site 4, and lowest amplitude snaps was recorded at site 1. The analysis of data from site 4 suggested the spectral lines of snaps and background noise were similar below 2 kHz. Background noise at site 4 decreased rapidly for frequencies greater than 2 kHz and was close to that of site 3 at frequencies higher than 10 kHz.

Duration of snaps from site 3 followed a normal distribution ($p=0.28$) and the rest parameters all followed a non-normal distribution ($p<0.001$), shown in Figure 4. Thus, the Kruskal-Wallis ANOVA was used to compare the data across different sites, showing a significant difference for duration ($p<0.001$), peak frequency ($p<0.001$), -3 dB bandwidth ($p<0.001$), and sound pressure level ($p<0.001$). Snaps recorded at site 3 had the highest sound pressure level, with a mean value of 186.2 ± 1.3 dB re 1μPa (n=750) and ranged from 183.8 to 190.1 dB re 1μPa. In comparison, the mean sound pressure level of snaps recorded at site 1 was 156.0 ± 4.4 dB re 1μPa (n=482), which was the lowest among these recording sites. These values were 172.5 ± 1.7 dB re 1μPa (n=629) and 165.5 ± 3.9 dB re 1μPa (n=111) for snaps from site 2 and site 4 respectively. The amplitude range of sound pressure level were 20.6, 8.9, 6.3 and 19.0 dB for site 1, site 2, site 3 and site 4 respectively. The mean durations were 312.8 ± 87.6, 476.1 ± 55.3, 452.9 ± 78.6, and 575.6 ± 206.5 μs accordingly. Snaps at all sites had a mean peak frequency consistently below 5 kHz and -3 dB bandwidth lower than 10 kHz, which may facilitate a long range propagation. More detailed comparisons of acoustic parameters are shown in Table 1.

3.2 Temporal pattern of snap rate

The temporal variation of the snap rate extracted from site 3 was shown for a series of 7 days and the dots represented the number of snaps per minute, which was smoothed out using a 120-point moving average filter and demonstrated in a black line (Figure 5). The snap rate presented a periodical pattern and ranged from 773 to 3875 snaps per minute, with a mean value of 2935.0, much higher than a previous study using a same thresholding method (Lee et al., 2021). The periodicity of the snap rate was reflected in the smoothed line, showing at least 2 distinct peaks within a day cycle, corresponding to the two high tides with a single day. The difference of snap rate between peak and valley can reach approximately 1000 within a day. The time of the peaks changed with the tidal level. On May 19, the first snap rate peaked at about 2:00 am, which emerged approximately an hour before the first high tide. On May 25, the first snap rate peak moved to around 5:00 am, which was behind the first high tide by almost 3 hours. These results demonstrated that snapping behavior was related to the tide. The first snap rate peak occurred as the tide was rising to reach high level (Peak 1 in Figure 5B). Peak 2 was associated with the high tidal level at night. Valley of snap rate consistently appeared during the daytime in current dataset, which occurred during the low tide periods. The number of the valleys changed during the recording period. At least two valleys were found in 5 of the 7 days during the daytime, including D19, D20, D21, D22, and D25, while only a single and significant valley was observed in D23 and D24. The valleys emerged between the two high tides and at least one valley was close to the low tide at daytime.

The snap rate of peak 1 which corresponds to the first high tide was unanimously than that of peak 2 (corresponding to the second-high tide) for each day during the recording period. The difference of the two snap rate during the high tide periods ranged from 17 to 420. The first snap rate peak (peak 1) occurred before the first high tide during the recordings while in comparison the times of valley and peak 2 were behind the low tide and second high tide in 5 of the 7 recording days (Table 2).

The snapping shrimp sounds were important contributions to the overall soundscape (Figure 6), accounting for a great proportion across the days, which can be seen in the power spectral density of bandwidth II between 1.5 and 10 kHz. This bandwidth was considered to estimate the soundscape input from snapping shrimp because the peak frequency was found in this range. For bandwidth I (0-1.5 kHz), the mean power spectral density was much lower during the night than daytime with peak-to-peak difference reaching approximately 10 dB, which was thought to be attributed to the commuting boat travels during daytime. The acoustic energy confined in frequency range from 10 to 48 kHz was the lowest (BW II). This proportion might be solely from snaps produced by shrimp as no other sound sources were found related to this part. Power spectral density across the whole recording band (up to 48 kHz) at site 3 fluctuated between 65.3 and 69.3 dB (BW IV).

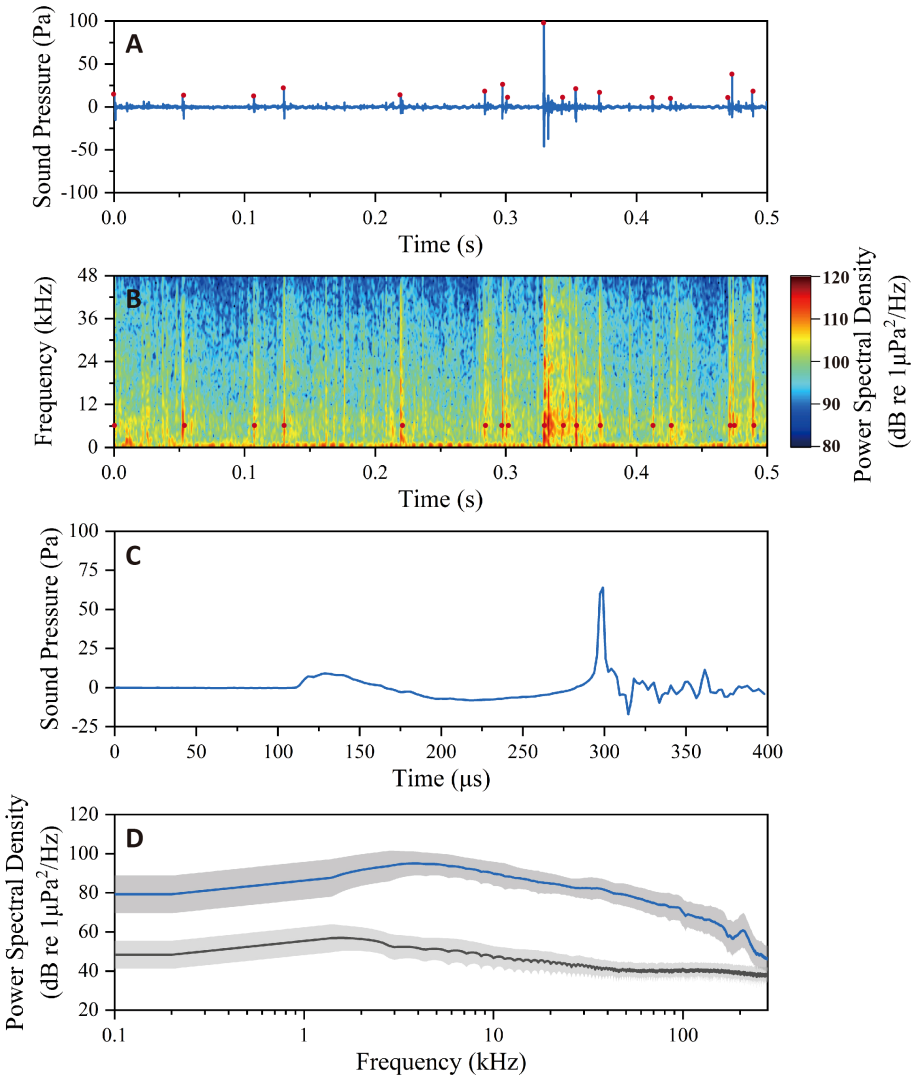

FIGURE 2
(A) A series of snaps in time domain and **(B)** time-frequency domain, with the red dots representing the detected snaps. **(C)** Waveform of a representative snap, showing a low amplitude precursor and a distinct high amplitude pulse. **(D)** Power spectral density of the snap (upper line) and its comparison to background noise (lower line). These data were recorded at site 1.

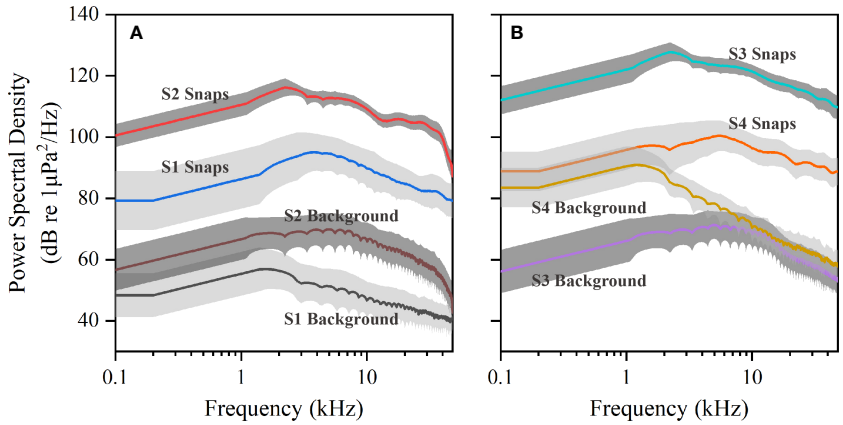

FIGURE 3
The comparison between the mean and SD (gray shading) frequency spectra of snaps and local background noise in decibels for **(A)** Site 1 and Site 2 and **(B)** Site 3 and Site 4.

4 Discussion and summary

Snapping shrimp, as a dominant source of ambient noise in shallow coastal waters, produce a strong pulse of a wide bandwidth. Several studies conducted on the acoustics of snapping shrimp have covered the topics on the temporal pattern of snap rate (Bohnenstiehl et al., 2016; Lee et al., 2021) and acoustic characteristics of snapping shrimp snaps (Au and Banks, 1998; Song et al., 2021; Spiga, 2022). The recordings in current paper provided additional data of the acoustics of shrimp snaps. However, the determinations on shrimp species and number of shrimp species were not yet achieved, limiting the comparison of snap characteristics to site-level instead of species-level. Significant differences were found in duration, peak frequency, -3 dB bandwidth and sound pressure level across the sites ($p<0.001$). The great ranges of sound pressure level of snaps recorded at different sites may be intrinsic to the animals. Song et al. (2021) found that snaps produced by shrimp can be significantly among different individuals for *A. heterochaelis* and *A. angulosus*. The individual variation may be the result of physiological processes, such as individual fitness. The number of species and individuals, as well as size of the shrimp can potentially influence the acoustic properties of the snaps in site-level. Though much remains to be done, the results present here show that shrimp produced snaps as loud as 190 dB and these sound pressure levels were underestimated values of respective source level if snaps had propagated a certain range before reaching the recording hydrophone. Peak frequencies were consistently below 10 kHz (Figure 4), similar to those of snaps recorded in laboratory conditions and field data in May River Estuary (Song et al., 2021). Among the papers on snapping shrimp acoustics, only one paper found the peak frequency of snaps can occasionally reach over 10 kHz and below 1 kHz, which was considered as response to the impulsive stimuli (Spiga, 2022).

Shrimp responded to light as well, reflected in the diurnal pattern of snap rate. Researchers found that snapping shrimp noise levels measured at nighttime were higher than those at daytime by 3–6 dB at Yacht Harbor in San Diego (Johnson et al., 1947; Everest et al., 1948), and by nearly 4 dB on Oahu, Hawaii (Lammers et al., 2008). A sharp increase in snapping abundance both at sunrise and sunset, raising the sound pressure level compared to daytime snaps, showing a potential relationship between light and snapping behaviors (Lammers et al., 2008). The number of snaps produced was correlated with season as well (Bohnenstiehl et al., 2016). Our current dataset had a limited recording length but based on the data available, we can interpret more snaps were recorded at night than during the daytime (Figures 5 and 6).

Besides, snap rate changed with tidal level. There was more than one snap rate peak across a single day and the peak location slightly shifted day by day, which may be attributed to the daily shift of tidal level. Snap rates at peak 1 and 2, which occurred at night, were consistently higher than those calculated for valley occurring during midday (Figure 5). Taking Day 23 as an example, the snap rates of peak 1 and peak 2 were 3561 and 3402, much higher than that of 2188 found in the valley. The snapping events came to its first crest at the earlier hours of the day before the emergence of the first high tide. The second snap rate peak was mostly happening after the second-high tide and slightly lower than that of the first snap rate peak. These two peaks may switch as the moon phase changes in the long term, which needs more data to address. The numbers of the valley and peak were changing across the seven days, which is probably influenced jointly

TABLE 1 Acoustic parameters of snaps recorded at different sites, compared to those reported in previous studies.

Site No.	Duration (μs)	Peak Frequency (kHz)	-3dB Bandwidth (kHz)	SPL (dB re 1μPa)
1	312.8 ± 87.6	4.29 ± 1.65	3.42 ± 1.46	156.0 ± 4.4
2	476.1 ± 55.3	2.54 ± 0.65	2.00 ± 0.89	172.5 ± 1.7
3	452.9 ± 78.6	2.53 ± 0.84	2.20 ± 1.31	186.2 ± 1.3
4	575.6 ± 206.5	4.16 ± 2.01	3.18 ± 1.76	165.5 ± 3.9
Kaneohe Bay (Au and Banks, 1998)	–	2 - 5	–	183 - 198
May River Estuary (Song et al., 2021)	–	4.10 ± 1.90	–	158.9 ± 4.0

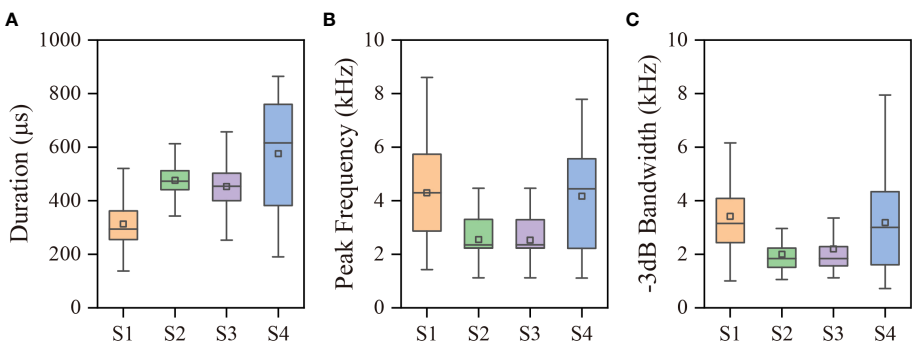

FIGURE 4
The comparisons of snap **(A)** Duration, **(B)** Peak frequency, and **(C)** -3dB bandwidth across the recording sites, where the box bottom and top denote the 25% and 75% percentile of the distribution, and the line extensions of the box represent the lower and upper edge values, respectively. The median and mean are represented by the line and square inside the boxes, respectively.

by tidal level and light. Lee et al. (2021) found that the significant correlation between snap rate and tidal level may be rooted in the change of current speed during tide fluctuation. Though the conclusions were based on data recorded in the water column at least 24 m deep and 100 km away from a local island, their results can still be representative for the snapping shrimp living in the benthic area. Jeong and Paeng (2022) used the 90 days' recording and find snap rate was higher at high tide and lower at low tide, showing a 13% variation. Using the long term monitoring and data analysis, they observed a complex pattern in snap rate, with a diurnal component dominating over the semi-diurnal component. The 7 consecutive days' recording in current paper was too short to drive any conclusion on tidal impact. Snap rate was the highest on D23, of which the high tide was not the greatest among the recording period. When comparing the two snap rate peaks (Peak 1 and Peak 2) corresponding to the two high tides within a single day, we found that the first snap rate peak (Peak 1) corresponding to the higher high tide was unanimously greater than that of the lower high tide (Table 2). These data altogether seem to show a positive relationship between snap rate and tidal level.

It remained to be investigated what drives the change of snap rate within a single day. There might be a possibility that shrimp produced more snaps to communicate when visual conditions are poor at night. Dinh and Radford (2021) found the snapping shrimp (*A. richardsoni*) was sensitive to low frequency sounds. There stands a possibility that snapping shrimp achieve communication through their snaps because there is a considerable proportion of energy spreading into the low frequency range (Figure 3). The snaps examined from four sites had significant energy below 1 kHz, overlapped with the tested audible frequency range of *A. richardsoni*. Supposing the snapping shrimp hear in the same way as *A. richardsoni* does (Dinh and Radford, 2021), we can hypothesize that shrimp can acoustically detect snaps if the sound pressure level exceeds hearing threshold. We turned to the audiogram of *A. richardsoni* and compared the amplitudes of hearing threshold and snaps. Shrimp present auditory responses to sounds up to 1500 Hz, at which the hearing threshold was approximately 125 dB (re 1 µPa). The lowest hearing threshold was approximately 90 dB (re 1 µPa) at 80 Hz. We calculated the spectrum of snaps using described in sound pressure level and found that snaps recorded at all sites unanimously have a higher mean sound pressure level than hearing threshold below 1 kHz. The hearing threshold increases to almost 125 dB at 1.5 kHz, surpassing the sound pressure level of snaps at site 1 and site 4. This indicates individuals of snapping shrimp at four sites

TABLE 2 The time of high and low tides during the 7-day recording period (D19-D25) at side 2, where the times of the snap rate peaks were tracked using the difference between times of the snap peak and high/low tides.

Date	1st high tide	Peak 1	Low tide	Valley	2nd high tide	Peak 2
19	3:00	-0.9 h (3368)	8:00	+1.0 h (2420)	15:00	+4.6 h (2948)
20	4:00	-1.2 h (3452)	9:00	+0.4 h (2426)	15:00	+4.7 h (3094)
21	5:00	-1.2 h (3481)	10:00	+0.2 h (2320)	16:00	+3.2 h (3276)
22	6:00	-1.0 h (3507)	11:00	+0.2 h (2315)	17:00	+2.9 h (3490)
23	7:00	-1.3 h (3561)	12:00	0 h (2188)	19:00	+1.8 h (3402)
24	8:00	-2.6 h (3443)	14:00	-0.7 h (2289)	20:00	-0.3 h (3237)
25	9:00	-3.9 h (3305)	15:00	+0.2 h (2128)	21:00	-1.6 h (3255)

The minus and plus signs represent time before and after tides respectively and the numbers in parentheses indicate the corresponding snap rate.

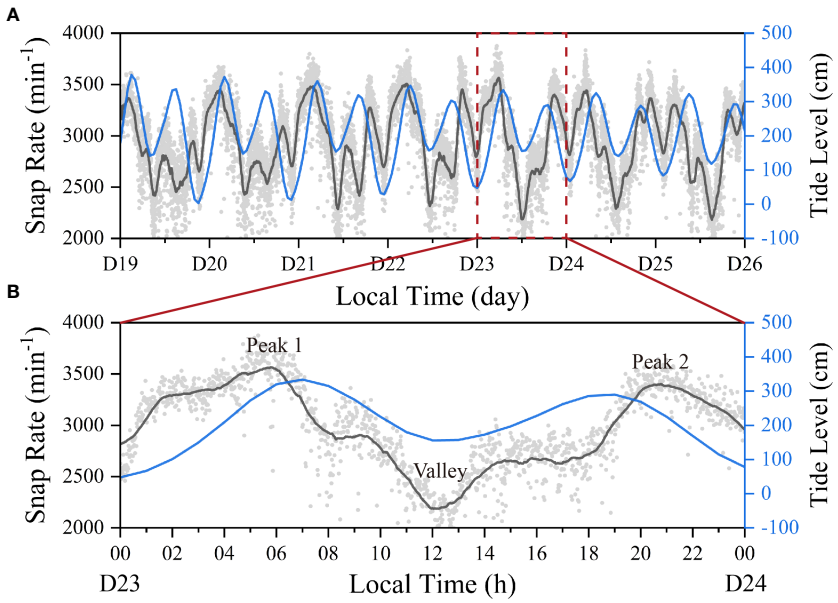

FIGURE 5
(A) Temporal variation of the snap rate extracted from Site 2 over a 7-day period (D 19–D 26) and the change of tidal level. **(B)** An enlarged layout of the snap rate within a single recording day.

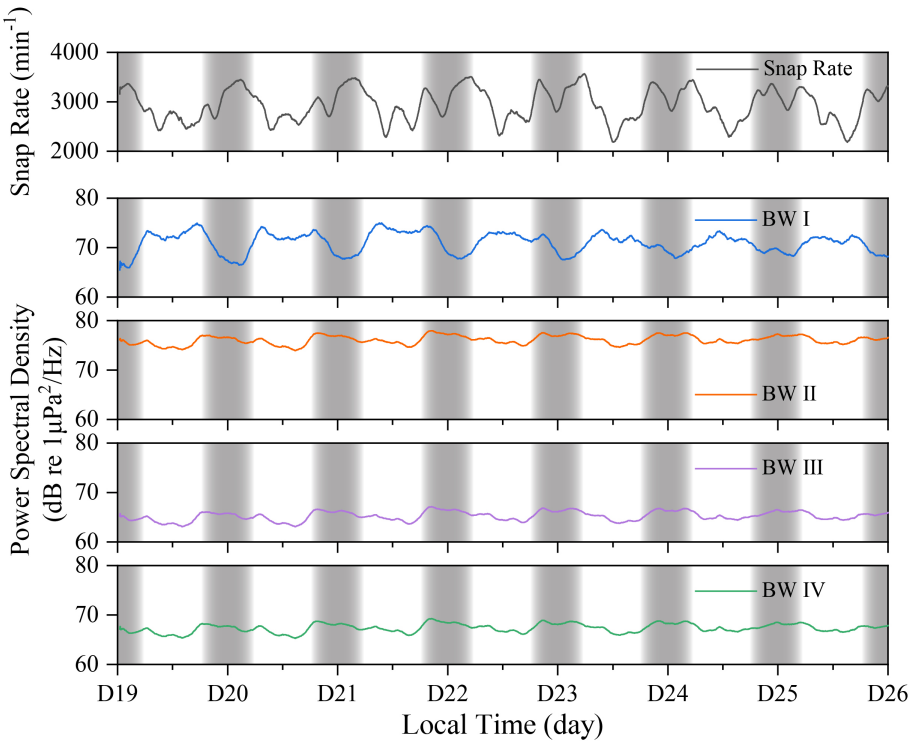

FIGURE 6
Relation between snap rate and the corresponding soundscape components at different frequency bandwidths, where BW I, BW II, BW III, and BW IV represent the mean power spectral densities (PSD) for frequency ranges of 0-1.5 kHz, 1.5-10 kHz, 10 kHz -48 kHz and 0-48 kHz. The PSD was calculated over a 2-minute window and their temporal variations were given as well. Gray bar areas define nighttime periods.

may be able to acoustically sense the snaps produced by conspecifics if the shrimp in the field have a same hearing threshold to *A. richardsoni*.

We followed a previous study and used snaps with peak amplitude exceeding four times the root-mean-square amplitude of the received signal to estimate snap rate (Lee et al., 2021). There was no doubt that more snaps can be detected using a smaller amplitude threshold but this would increase the probability of false detection. Snaps of smaller amplitude are probably from a greater distance. Using a same thresholding method, the snap rate in shallow water of southeast China coastal area (current paper) was much higher than that in the East China Sea (Lee et al., 2021). Lee et al. (2021) placed their recording hydrophones in the water column with depth between ~24 and ~80 m and the recording site was 100 km away from a nearby island. Our recordings were confined to the coastal region, close to local tidal zones where the shrimps inhabited, and this might increase the snap rate calculation due to the relatively lower propagation attenuation.

We conducted field experiments at four sites in southeast China coastal area to record the broadband pulses produced by snapping shrimp. Snaps dominated the overall underwater soundscape. Research was extended geographically into the Southeast China coastal area, providing data for the snapping shrimp in this region for the first time. The characteristics of snaps were significantly different across the recording sites. Snap rate examined in one site with 7 consecutive days of recording showed a diurnal pattern and snap rate had a correlation with the tidal level, indicating that the snap rate corresponded to the tidal level periodically. A high tide was accompanied with a local high snap rate regardless of light and this local snap rate peak was much higher at night. Data of a single site made it impossible to compare the snap rate across different sites. The long-term monitoring is important to probe into the monthly or seasonal snapping behavior and its coincidence with ocean environmental factors such as temperature, water depth and light performed in previous studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because Ethical review and approval were not required for this study because there is not an ethically questionable study. Experiments were conducted in the field where animals were in their natural conditions.

Author contributions

ZS, WO, YS, and YZ: primary writing. ZS and YZ: synthesis and overall coordination. ZS, WO, YS, HL, XX, and WF: experiment design and data collection. ZS, WO, and YS: acoustic data analysis.

WO, YS, XX, HL, and WF: ocean environment data analysis. ZS, WO, XX, WF, TW and YZ: paper revision and re-editing. All authors contributed to the article and approved the submitted version.

Funding

This work was financially supported by the Project supported by the National Natural Science Foundation of China (Grant Nos. 42106181; 12074323), the China National Postdoctoral Program for Innovative Talents (Grant No. BX2021168), the China Postdoctoral Science Foundation (Grant No. 2020M682086), the Outstanding Postdoctoral Scholarship and the PhD Fellowship of State Key Laboratory of Marine Environmental Science at Xiamen University, and the Science, Technology Major Project of Fujian Province (Grant Nos. 2021NZ033016), the Water Conservancy Science and Technology Innovation Project of Guangdong (2020-16), the Guangzhou Science and Technology Foundation (No. 202102020901) and the Financial Fund of the Ministry of Agriculture and Rural Affairs, P.R. China (No.NFZX2021).

Acknowledgments

The authors are grateful to Zhen Xiao from College of Ocean and Earth Sciences, Xiamen University, for his assistance in conducting this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Marine Science

TYPE Review
PUBLISHED 07 March 2023
DOI 10.3389/fmars.2023.1129057

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

RECEIVED 21 December 2022
ACCEPTED 23 January 2023
PUBLISHED 07 March 2023

CITATION
Solé M, Kaifu K, Mooney TA, Nedelec SL, Olivier F, Radford AN, Vazzana M, Wale MA, Semmens JM, Simpson SD, Buscaino G, Hawkins A, Aguilar de Soto N, Akamatsu T, Chauvaud L, Day RD, Fitzgibbon Q, McCauley RD and André M (2023) Marine invertebrates and noise.
Front. Mar. Sci. 10:1129057.
doi: 10.3389/fmars.2023.1129057

Marine invertebrates and noise

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Within the set of risk factors that compromise the conservation of marine biodiversity, one of the least understood concerns is the noise produced by human operations at sea and from land. Many aspects of how noise and other forms of energy may impact the natural balance of the oceans are still unstudied. Substantial attention has been devoted in the last decades to determine the sensitivity to noise of marine mammals—especially cetaceans and pinnipeds—and fish because they are known to possess hearing organs. Recent studies have revealed that a wide diversity of invertebrates are also sensitive to sounds, especially *via* sensory organs whose original function is to allow maintaining equilibrium in the water column and to sense gravity. Marine invertebrates not only represent the largest proportion of marine biomass and are indicators of ocean health but many species also have important socio-economic values. This review presents the current scientific knowledge on invertebrate bioacoustics (sound production, reception, sensitivity), as well as on how marine invertebrates are affected by anthropogenic noises. It also critically revisits the literature to identify gaps that will frame future research investigating the tolerance to noise of marine ecosystems.

KEYWORDS

marine invertebrates, marine noise pollution, sound production, sound detection, noise effects, statocyst, sound pressure, particle motion

1 Introduction

Marine invertebrates represent a hugely diverse taxa, playing a central role in food webs and ecosystem services, as well as constituting an important economical resource. Invertebrates make essential contributions to global biodiversity and provide major ecosystem functions (e.g., water filtering, habitat creation, organic matter processing, carbon transfer through food webs and nutrient recycling) (Collier et al., 2016). Many marine invertebrate species also have important intrinsic value to human society, including as food resources (shellfish protein), for health purposes (protection form algae eutrophication), as coastal protection from natural disasters and ocean acidification, through ornamental and recreational value, and in tourism.

Some agents of biodiversity decline in marine ecosystems (e.g., water pollution, overexploitation, habitat degradation, invasive species and climate change) have been analysed extensively (Collier et al., 2016). However, it is only relatively recently that noise and other forms of energy, like anthropogenic electromagnetic fields, have been considered critical stressors of the natural balance of the oceans. These pressure elements can have detrimental impacts on the survival and reproduction of individuals, with consequences for entire populations and species (van der Graaf et al., 2012; Hutchison et al., 2020; Popper et al., 2020). Recent findings have shown that marine invertebrates can be sensitive to anthropogenic noise and indicated that this sensitivity may have influence ocean biodiversity (André et al., 2011; Aguilar de Soto, 2016; Edmonds et al., 2016; Sordello et al. 2020), placing them as direct indicators of ocean health.

Ocean soundscapes are composed of a combination of biological, geological and anthropogenic sounds produced from a variety of sources (Pijanowski et al., 2011; Lindseth and Lobel, 2018; Duarte et al., 2021). As with other marine species, invertebrates have evolved around the extraction of information from soundscapes. Invertebrates are mainly sensitive to the particle motion of sound, rather than the sound pressure. As many of them live close to the seabed they are often affected by substrate vibration, which usually involves particle motion (Hawkins et al., 2021). Changing soundscapes due to a decrease of sound-producing animals and the introduction of man-made noises may thus alter vital invertebrate sensory abilities. Sources of marine underwater anthropogenic noise that generate vibration, include shipping (fishing boats, recreational motorboats, jet skis, trade vessels), oil and gas exploration and operation, the construction and operation of offshore wind farms and other renewable energy devices, dredging, construction of bridges and harbours, commercial and military sonar, and underwater explosions for construction or ordnance disposal. There are some natural sources of substrate vibration, including volcanos, earthquakes and breaking waves, animal movements/interactions and objects falling or rolling onto the seabed. Seabed substrates can propagate some seismic interface waves well, with particle motion existing in both the water and the sediment. Underwater sound sources can extend over large periods of time (continuous; e.g., shipping (Van der Graaf et al., 2008) and result in an increase in low-level background noise, or can be short and intense (tonal/impulsive; e.g., sonar, pile driving, air guns (Rako-Gospić and Picciulin, 2019). Impulsive sounds have a fast rise time reaching a maximum value followed by a fast decay. Impulsive sounds may be much higher in amplitude near the source than continuous sounds, but their energy decreases faster with distance (Hawkins and Popper, 2016). It is important to note that sound is not limited to just the water column but that the near-surface seabed can respond vigorously to in-water sound and the seabed transmits low-frequency energy well (Nedelec, 2021).

Impulsive sounds can be expressed in terms of their peak levels, but in some cases (e.g., seismic airguns) that is not sufficient for characterizing the energy. An alternative is the sound exposure level (SEL) – the time integral of the pressure squared for a single event – a measure reflecting the total acoustic energy received by an organism (Slabbekoorn et al., 2010). The metrics applied for continuous sounds are the root-mean-square sound pressure (RMS) and the peak sound pressure (Hawkins and Popper, 2016; Hawkins and Popper, 2017). In general it is accepted that the assessment of the sound sources and its potential impact on marine fauna needs to consider cumulative (repetition of a particular source) and aggregate (combined effects of different type of sources (Hawkins and Popper, 2016).

Sound can affect marine organisms depending on sound pressure level at the source, the pitch (frequency) and the distance between source and receiver (Richardson et al., 1995). Table 1 provides a summary of the typical characteristics of different common anthropogenic sound sources in the marine environment.

Given the increasing introduction of anthropogenic noise to the oceans, it has become essential to design tools to monitor and regulate the effects of sounds on marine fauna. Anthropogenic noise is recognized as a major component of environmental change in the 21st Century and a pollutant of international concern, featuring prominently on international directives and agendas. Although additional scientific and technical progress is still required to support the further development of criteria related to acoustic impact on marine environment (including in relation to impacts of introduction of energy on marine life, relevant noise and frequency levels), two indicators were published for Descriptor 11 (Noise/energy) of the Marine Strategy Framework Directive (MSFD EU, 2008) in the EC Decision 2010/477/EU on criteria and methodological standards on GES of marine waters (Dekeling et al., 2014):

Indicator 11.1 Distribution in time and place of loud, low and mid frequency impulsive sounds.

- Proportion of days and their distribution within a calendar year, over areas of a determined surface as well as their spatial distribution, in which anthropogenic sound sources exceed level that are likely to entail significant impact on marine animals, measured as Sound Exposure Level (in dB re 1µPa2.s) or as peak sound pressure level (in dB re 1µPa-peak) at one meter, measured over the frequency band 10 Hz to 10 kHz.

Indicator11.2 Continuous low frequency sound.

- Trends in the ambient noise level within the 1/3 octave bands 63 and 125 Hz (centre frequency) (re 1µPa^2; average noise level in these octave bands over a year) measured by a statistical representative sets of observation stations and/or with the use of models if appropriate.

TABLE 1 Acoustic properties of some anthropogenic noises.

Sound	Source level (dB re 1 µPa-m) *	Bandwidth (Hz)	Major amplitude (Hz)	Duration (ms)	Directionality	Sound type
TNT (1-100 lbs)	**272–287 Peak**	**2–1000**	**6–21**	**~ 1–10**	**Omnidirectional**	**Tonal/ impulsive**
Pile driving	**228 Peak/ 243–257 P-to-P**	**20–>20 000**	**100–500**	**50**	**Omnidirectional**	**Tonal/ impulsive**
Offshore industrial activities						
Dredging	**168–186 rms**	**30–>20 000**	**100 - 500**	**Continuous**	**Omnidirectional**	**Continuous**
Drilling	**145–190 rms****	**10–10 000**	**< 100**	**Continuous**	**Omnidirectional**	**Continuous**
Wind turbine	**142 rms**	**16–20 000**	**30 - 200**	**Continuous**	**Omnidirectional**	
Shipping						
Small boats and ships	**160 –180 rms**	**20–>10 000**	**>1 000**	**Continuous**	**Omnidirectional**	**Continuous**
Large vessels	**180–190 rms**	**6–>30 000**	**>200**	**Continuous**	**Omnidirectional**	**Continuous**
Sonar						
Military sonar low- frequency	**215 Peak**	**100 –500**	**_**	**600–1 000**	**Horizontally focused**	**Tonal/ impulsive**
Military sonar mid-frequency	**223–235 Peak**	**2800–8200**	**3 500**	**500–2 000**	**Horizontally focused**	**Tonal/ impulsive**
Echosounders	**235 Peak**	**Variable**	**Variable 1500 – 36 000**	**5–10 ms**	**Vertically focused**	**Tonal/ impulsive**
Seismic surveys						
Airgun array	**260–262 P-to-P**	**10–100 000**	**10–120**	**30–60**	**Vertically focused***	**Tonal/ impulsive**
Other activities						
Acoustic deterrent/harassment Devices	**132–200 Peak**	**5 000–30 000**	**5 000–30 000**	**Variable 15–500 ms**	**Omnidirectional**	**Tonal/ impulsive**
Tidal and wave energy devices***	**165–175 rms*****	**10–50 000**	**_**	**Continuous**	**Omnidirectional**	**Continuous**

* Nominal source, ** Higher source levels from drill ships use of bow thrusters, *** Projection based on literature data with levels back-calculated at 1 m (Modified from Götz, 2009).

In this review, we provide a synthesis of the peer-reviewed literature published from the late 1960s to 2022 reporting marine invertebrate bioacoustics (detection and production of sound) and responses to anthropogenic noise in different life stages, in populations and ecosystems. This work documents prominent trends in research topics and methods, the kinds of noise sources that have been studied, the measurements used to characterise them, and the gaps and perspectives in research coverage that merit attention in future research. We outline the necessity/utility of existing scientific information concerning anthropogenic noise effects on marine invertebrates for predicting potential consequences of noise exposure. We also scale up to influences on ecological and evolutionary processes, and consider how this information is important for biodiversity conservation and the implementation of meaningful mitigation measures.

2 Marine invertebrate bioacoustics

Sound travels about five times faster in water (ca. 1500 m/s) than in air (ca. 340 m/s) because the density of water is greater, and also attenuates less over the same distance. This characteristic allows long-distance communication in water, but also implies a long-distance impact of noise on aquatic animals (Slabbekoorn et al., 2010). Particle motion is an important component of sounds travelling through the water and it is detected by invertebrates (Popper & Hawkins, 2019). Sound is an important sensory modality for marine organisms, especially because other senses (vision, smell or taste) may be limited due to information loss in marine ecosystems (Popper and Hawkins, 2019). The scientific knowledge of the biological significance of sound perception and production in marine invertebrates is scarce. Animals produce acoustic signals for

communication about, for instance, predators, prey, territorial defence, social and sexual behaviour, and identity. They have evolved to detect sounds both as part of communication and to make use of acoustic cues in the environment, aiding in, for instance, settlement and habitat choice. In this section, we summarize the current knowledge regarding marine invertebrate bioacoustics including analysis methods, receptor organs, sound detection and production.

2.1 Measurements: Imaging, electrophysiology, respirometry, biochemistry

The different techniques used to study invertebrate bioacoustics are summarized and described below.

2.1.1 Imaging techniques

Scientific and diagnostic imaging allow visual representations of invertebrate sensory structures, organs or tissues for various purposes such as the study of normal anatomy and function, or the diagnosis of the effects of sound on these structures. Imaging techniques include Electron Microscopy and 3D imaging techniques (Figure 1).

Electron microscopes have a higher resolution than light microscopes and are capable of a higher magnification (up to 2 million times) (Rudenberg and Rudenberg, 2010), allowing the visualization of structures that would not normally be visible by optical microscopy. There are two major types of electron microscopes used in invertebrate bioacoustics: Transmission Electron Microscopes and Scanning Electron Microscopes. Scanning Electron Microscopy produces images of a sample by scanning it with a focused beam of electrons that interact with atoms in the sample, providing information about its surface topography and composition (Butterfield et al., n.d.) and achieving resolution better than 1 nanometre (Suzuki, 2002). In invertebrates, this technique allows description of the surface of sensory epithelium and effects of noise upon it (Figures 1A–E) (Solé et al., 2013a; Solé et al., 2013b; Day et al., 2016; Solé et al., 2016; Solé et al., 2018; Day et al., 2019). In Transmission Electron Microscopy, a beam of electrons is passed through an ultrathin specimen and an image is formed from the interaction of the electrons transmitted through it. This technique is used in the description of invertebrate ultrastructural sensory epithelia, allowing the inner cellular organelles to be visualised and analysis of the effects of sound on them. (Figure 1F) (Solé et al., 2013b)

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique that allows creation of a 3D image of a body's internal organs using powerful magnetic fields and radio waves. This technique has been used to construct models of the morphological structure of invertebrate sensory systems (Ziegler et al., 2018). Computer tomography (CT) relies on differences in X-ray attenuation of biological tissues to do a 3D reconstruction of them. Major molluscan organs have been visualized using CT techniques (Ziegler et al., 2018).

2.1.2 Electrophysiology

Auditory evoked potential recordings have been used in a variety of invertebrate taxa as a measurement of sound sensitivity (Figure 2A). The evoked potential technique for hearing was popularized by Hong Yan's work on fishes before to spreading it among invertebrates (Yan, 2002). This method involves measuring responses from neurons associated with sound detection and the resulting conduction of responses toward a brain or central set of ganglia (Hall, 2007). Recording may be thus from nearby sensory organs, such as the statocyst, or if sound detection comes from more peripheral hair cells or organs, it may occur nearby the brain/central ganglia area (Jezequel et al., 2021). While evoked potential methods have been widely applied to measure hearing abilities in many aquatic vertebrates e.g., (Supin et al., 2001; Kastak et al., 2005; Nachtigall et al., 2007; Mooney et al., 2012; Piniak et al., 2016; Jones et al., 2021), it has

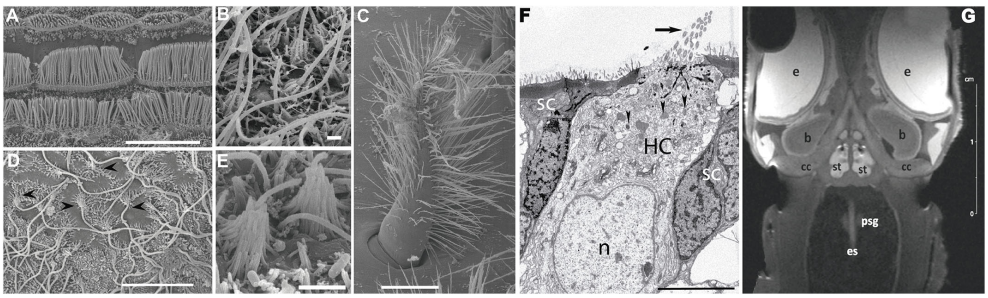

FIGURE 1
Imaging Techniques. **(A–E)**: Scanning Electron Microscopy. **(F)**: Transmission Electron Microscopy. **(G)**: Magnetic Resonance Imaging. **(A–F)**: Different types of sensory epithelia (hair cells) depending on the marine invertebrate group (**A, F**: Cephalopods. **(B, E)**: Cnidarians. **C**: Crustaceans. **D**: Gastropods). **(A)**: View of three rows of hair cells (bundle of kinocilia) in statocyst *crista* epithelium of *Sepia officinalis*. **(B)**: Statocyst sensory epithelium of the jellyfish *Cotylorhiza tuberculata*. Hair cells carry an only nonmotile kinocilia surrounded by a short crown of stereocilia (Solé et al., 2016). **(C)**: A seate (bearing hairs) of the medial group sensory epithelia in the hermit crab *Dardanus calidus* statocyst. Setae are typical hair cell on crustaceans. **(D)**: Apple snail (*Pomacea maculata)* inner statocyst sensory epithelia. Arrowheads point to the hair cells exhibiting their lonely kinocilia surrounded by a crown of stereocilia. Between them microvilli of the supporting cells is visible (Solé et al., 2021a). **(E)**: Statocyst sensory epithelia of the sea anemone *Calliactis parasitica*. Similarlly to other groups of cnidarians **(B)** their hair cells present a solitary kinocilia surrounded by a crown of stereocilia. **(F)**: Apex of a *S. officinalis* hair cell (HC) in between two supporting cells (SC). The HC shows kinocilia (arrow), nucleus (n) and cytoplasmic mitochondria (arrowheads) (André et al., 2011). **(G)**: Coronal view -anterior section- of squid (*Loligo vulgaris*) head (B: Brain, cc: cranial cartilage, e: eye, es: oesophagus, m: mouth, psg: posteror salivary gland, st: statocyst. (Solé et al., 2013b). Scale bar: **(G)** = 2 cm. **(C)** = 25 µm. **(A)** = 10 µm. **(D, F)** = 5 µm. **(E)** = 2 µm. **(B)** = 1 µm.

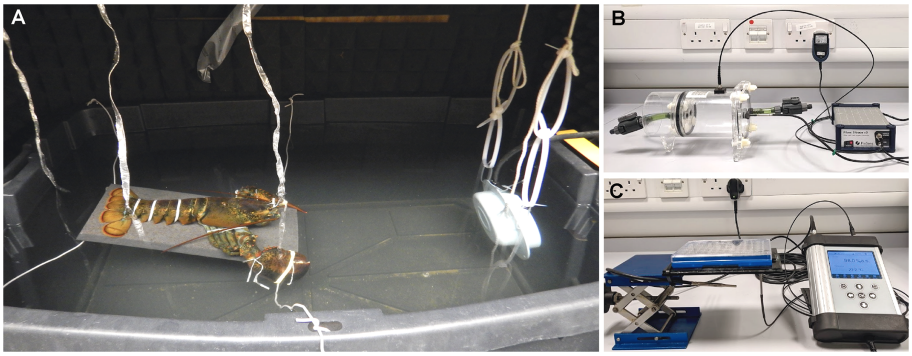

FIGURE 2
(A) Electrophysiology. **(B, C)**: Respirometry. **(A)**: Evoked potential hearing test of an American lobster (*Homarus americanus*) **(B)**: Respiration set-up for adult invertebrates; calibrated volume sealed respiration chamber connected to a fibox 3 trace v3 fibre-optic trace oxygen meter (Presens – Precision Sensing, Regensburg, Germany) *via* fibre-optic cable to a PSt3 oxygen sensor spot (detection limit: 0.03% oxygen, 15ppb). **(C)**: Plate set-up used for larvae and gametes; 64 well plate with PSt7 oxygen sensor spots (detection limit: 0.03% oxygen, 15ppb) attached to a fibox 4 trace hand held oxygen meter (Presens – Precision Sensing, Regensburg, Germany). Both (**B, C** use non-destructive oxygen measurements, measuring luminescence decay time by stimulating an immobilised luminophore with monochromic light.

only been sparingly applied to invertebrates, including squid (Mooney et al., 2010), prawns (Lovell et al., 2005), snapping shrimp (Dinh and Radford, 2021), lobsters (Jezequel et al., 2021) and other crustaceans (Hughes et al., 2014; Radford et al., 2016). Some of its advantages include that it can be applied to a variety of taxa, including wild caught animals, and it can be non-invasive. Although often times it is a more invasive method involving sedation, needle electrodes and surgery to access nerve structures. Evoked potential methods are generally cost-effective and permit to reach a relatively high animal sample size of (i.e. > 10), that is higher than psychophysical methods, and whole audiograms can be measured quickly (tens of minutes to a few hrs).

2.1.3 Respirometry

There are a number of techniques used to assess the effects of a stimulus on the metabolic rate of an organism. One such method, respirometry, provides an indirect calorimetric approach to the measurement of metabolic heat changes through monitoring and measurement of variations in oxygen uptake (Figures 2B, C). For marine invertebrates, changes in respiration rate are observed indirectly through changes in the dissolved oxygen of the surrounding water. Animals are encapsulated in a sealed, water-filled chamber and dissolved oxygen is measured either at the start and end points of the exposure using an oxygen probe, or continuously throughout the exposure using an oxygen sensor. During long exposures, intermittent flow respirometry may be used (Steffensen et al., 1984; Steffensen, 1989) when periodic flushing of the respirometry chamber is performed to maintain sufficient oxygen saturation. In both static and intermittent-flow respirometry, oxygen consumption is calculated accounting for bacterial respiration, water volume, exposure time and environmental conditions, and calibrated against the animal's mass to allow comparability between individuals and across species. Respirometry has been used to investigate the effects of anthropogenic noise on decapods (Regnault and Lagardere, 1983; Wale et al., 2013b; Ruiz-Ruiz et al., 2020), bivalves (Shi et al., 2019; Wale et al., 2019) and cephalopods (Woodcock et al., 2014).

2.1.4 Cellular–biochemical–molecular aspects

Several techniques for the assessment of invertebrate stress are based on cellular, biochemical and molecular aspects. It is possible to determine the physiological state of an animal using stress analysis after sound exposure. Stress bioindicators can be measured in invertebrate haemolymph. Total haemocyte count (THC), heat shock protein 27 (Hsp27) expression in haemocyte lysate, total protein concentration (PT) and phenoloxidase activity (PO) in cell-free haemolymph, were considered potential biomarkers of stress (Filiciotto et al., 2014; Celi et al., 2015).

In aquatic invertebrates, the homeostasis of total haemocyte density and composition may be considered an important well-being predictive parameter. Decreases of total haemocyte count (THC) under stressful conditions, usually carried out with cell counter chambers, have been reported for several aquatic crustacean species (Le Moullac et al., 1998; Sánchez et al., 2001; Mercier et al., 2006), suggesting the possibility of immune depletion as well as an increased risk of infection (Filiciotto et al., 2014; Celi et al., 2015). Although the variation in differential haemocyte count in the presence of different stressors is not well understood, it has been used as a stress indicator in crustaceans (Jussila et al., 1997; Johansson et al., 2000; Filiciotto et al., 2014) (Figure 3). The measurement of this parameter is easily feasible under the microscope after on slide cell fixation and stain.

Another parameter useful to evaluate the disturbance of the homeostatic balance of animals is the measurement of glucose haemolymphatic. Hyperglycemia is a primary response typical of many aquatic animals to different stressors (Lorenzon, 2005; Fazio et al., 2013; Faggio, 2014). Glucose haemolymphatic, which can be measured in haemolymph using commercial kits, increases in marine invertebrates under exposure to acoustic stimulu (Filiciotto et al., 2014; Vazzana et al., 2016). In the haemolymph, it is possible to measure the total protein concentration. This parameter is non-destructive, easy, cheap and measurable through fluorimetric methods. It can be used as a "warning" of poor environmental conditions such as noise (Filiciotto et al., 2014; Vazzana et al.,

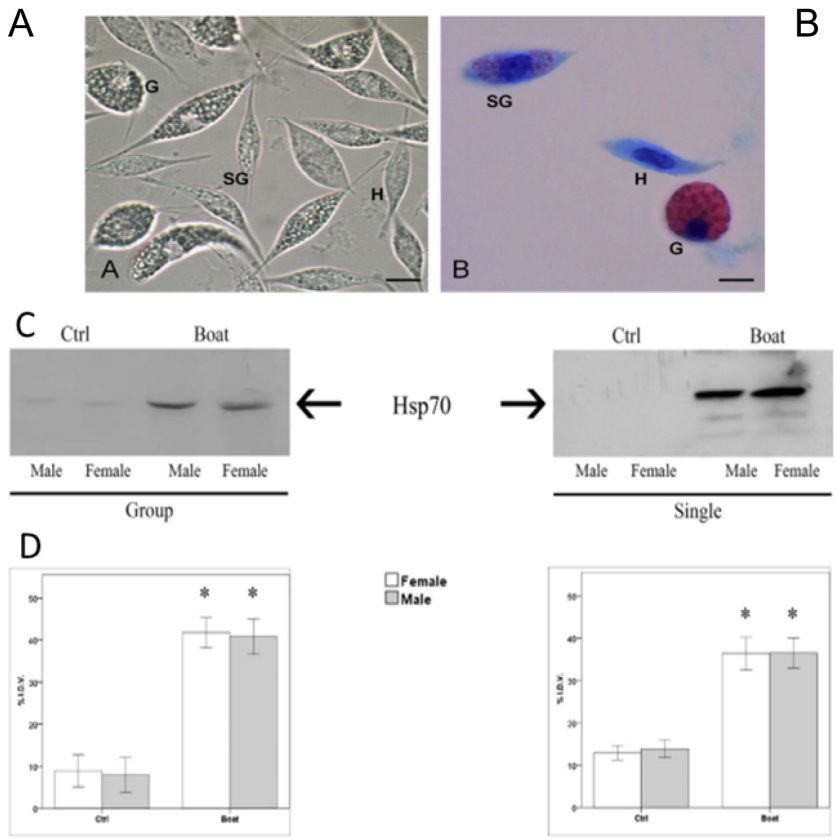

FIGURE 3
Light Microscopy. Haemocytes of the spiny lobster *Palinurus elephas* **(A)** no staining and **(B)** stained with May–Grünwald–Giemsa. H: hyalinocytes; SG: semigranulocytes; G: granulocytes. Scale bars: **(A, B)** = 8 µm. Effect of the acoustic stimuli on the expression levels of the protein Hsp70 in *P. elephas*; **(C)** Representative western blot of Hsp70 levels in single and grouped animals. **(D)** Integrated density value (% IDV) of the Hsp70 protein bands. Data are the means ± standard error (N = 18 control and N = 18 test specimens). Asterisks represent significant differences between CTRL and BOAT condition (*= $p < 0.01$). (Filiciotto et al., 2014).

2016). A further indicator of the negative effect of altered conditions on invertebrates is a change in enzyme activities. There are still few studies on the variations of enzymes in stressed invertebrates, but some have shown a modulation of peroxidase, alkaline phosphatase and esterase activity measured through rapid colorimetric methods (Vazzana et al., 2016; Vazzana et al., 2020a; Vazzana et al., 2020b) after acoustic stimulus. Among bioindicators of stressful conditions in crustaceans is also included expression of heat shock proteins (Snyder and Mulder, 2001; Liberge and Barthelemy, 2007). Some authors showed, through the use of western blot analysis and Real-Time PCR (RT-PCR), that, in marine invertebrates exposed to acoustic stimuli, occurs a protein and gene overexpression of the Hsp70 (Filiciotto et al., 2014; 2016; Vazzana et al., 2016; 2020a). The latter aspect is useful to understand better the variations of the complex cellular–biochemical–molecular network of organism in stress condition.

2.1.5 Measurement of underwater sound

In a sound wave, particles of the medium (e.g., water) oscillate around a point of origin ('particle motion') causing local compressions and expansions ('sound pressure') that transfer the sound energy to neighbouring particles (ISO 18405:2017; Gray et al., 2016). Thus, all sound involves both pressure and particle motion fluctuations. The number of oscillations per second is the frequency in Hertz (Hz). Sound pressure fluctuations are omnidirectional and are measured as force per unit area in Pascals (Pa), typically using piezoelectric hydrophones, which have been readily available for many years (ISO 18405:2017, Robinson et al., 2014). Sound particle vibrations are directional and are described by displacement (m), velocity (ms^{-1}) or acceleration (ms^{-2}); three metrics that have a frequency-dependent relationship to one another (Nedelec et al., 2016, ISO 18405:2017). The directional information is described by angles relative to references such as magnetic north and gravity. Particle acceleration can be measured using capacitive, piezoresistive or piezoelectric accelerometers, while particle velocity can be measured using geophones, all of which are proof-mass instruments (a proof mass is a known quantity of mass used in a measuring instrument as a reference for the measurement of an unknown quantity) that are becoming more readily available (Nedelec, 2021). Particle acceleration can also be measured using a pressure gradient between hydrophone pairs (Chapuis et al., 2019). Finally, in simplified acoustic conditions (deep water and far from the source relative to wavelength), particle velocity magnitude but not direction can be estimated from pressure measured by a single hydrophone (Nedelec, 2021). Underwater sound is often reported in decibel units (dB), which are represented on a logarithmic scale relative to 1 µPa for pressure, 1 pm for displacement, 1 nm s^{-1} for velocity and 1 um s^{-2} for acceleration (ISO 18405:2017).

The statolith organs of many invertebrates measure the relative motion of the body of the animal to the dense statocyst, which moves

with a lag due to its greater mass and inertia, creating a biological analogue of a proof-mass instrument (Packard et al., 1990; Kaifu et al., 2011). Therefore, measuring the whole-body vibration of animals is of interest because it links acoustic stimulus and sound detection. Piezoresistive accelerometers that measure acoustic vibrations of solid objects they are fixed to exist, however their scale relative to the bodies of aquatic invertebrates means that the accelerometers themselves would alter the vibration of the whole body. Recently, the availability of non-contact laser Doppler vibrometer techniques, that have already been applied to research on hearing in several amphibian, reptile and crustacean species (Hetherington and Lindquist, 1999; Hetherington, 2001), has opened the possibility of measuring whole-body vibration of aquatic animals. Whole-body vibrations of cephalopods and scallops that were exposed to air borne sound (<360 Hz) were successfully measured using a laser Doppler vibrometer, confirming the hypothesis that particle motion can vibrate the whole body of invertebrates (André et al., 2016). However, to report the particle motion levels measured by an instrument, it is necessary to calibrate the instrument for its coupling to the medium in which the sound is to be measured. The coupling of animal bodies to the water column remains poorly understood, thus measuring whole-body motion gives us a limited understanding of responses to particle motion levels in the water. Further advancement of measurement techniques on whole-body vibration of aquatic animals elicited by propagating acoustic waves will improve understanding of particle motion reception in invertebrates. This will involve calibrating the animals themselves as well as any accelerometers that are attached to them.

2.2 Detection of sound: Vibration, reception and sensitivity

2.2.1 Physical aspects: Acoustic pressure vs particle motion

The motion of the 'particles' that make the medium (e.g., air, water, or solid substrate) is an intrinsic aspect of sound. Sound pressure can be described by its magnitude and its temporal and frequency characteristics, but at a single point, sound pressure does not contain directional information. Particle motion can be described by its magnitude, temporal and frequency characteristics, but additionally it always contains directional information because of its inherent 'back and forth' action (Hawkins and Popper, 2017). Many aquatic invertebrates sense and use particle motion, including to detect the direction of the source, (André et al., 2016; Nedelec et al., 2016). Particle motion and sound pressure are proportional in 'plane wave' conditions (far from the source and from any boundaries that may cause reflections relative to the wavelength). Close to the source in the 'near field', particle motion is higher than would be expected from equivalent pressure in plane wave conditions in the 'far field' due to interactions between the wavelength, frequency and distance from the source. This interaction, which causes additional particle motion near to the source decreases with inverse proportion to the distance from the source until it can be treated as negligible after approximately one wavelength. A good rule of thumb is therefore that the boundary of the near field region with additional particle motion is one wavelength from the source. Therefore, particle motion is present wherever there is sound and a good rule of thumb is that the boundary of the near field region with additional particle motion is one wavelength from the source. Sensory hair cells in the sensory systems (see below) are stimulated by mechanisms that respond to particle motion and convert these motions to electrical signals that stimulate the nervous system. Because aquatic invertebrates lack gas-filled cavities, it seems that they mostly perceive the particle motion of the sound. But recent experiments put this statement in question: particle motion may not be the sole component implied in sound lesions in invertebrates (Solé et al., 2017).

2.2.2 Receptor systems

2.2.2.1 Cilia-based mechanosensory systems

Mechanoreceptors are sensory cells (hair cells) detecting mechanical forces that usually bear specialized cilia (Figure 1). These mechanosensory cells are the starting point of mechanotranduction processes in which the hair cells express transmembrane channels that convert force into cellular signal. Hearing, proprioception or gravity mechanisms are based in these mechanosensory cells (Bezares-Calderón et al., 2020). These receptor systems can be found on the body surface of animals or enclosed in fluid-filled cavities. Hair cells possess unique features including the presence of cilia (microtubule with a basal body which contains organelles) that can be motile or not and, a tuft of stereovilli (actin-filled microvilli). Unlike vertebrates that are characterized by the presence of a single cilia with a 9 + 2 axoneme and a group of stereovilli, invertebrates generally have kinocilia (with an internal structure of 9 x 2 + 2 microtubules in the axoneme) in their hair-cell-based receptor systems. The number of kinocilia per cell varies according to the group of invertebrates (e.g., cnidarians: monociliary cells with a concentric or eccentric bundle of stereovilli; cephalopods: multiciliary cells with microvilli; crustaceans: monociliary cells without microvilli; Figure 1). Some mechanosensory systems present accessory structures (statolith, statoconia, cupula) above the hair cells which stimulate the underlying sensory epithelia. The kinocilia are mechanically directly or indirectly (via a cupula) coupled with the surrounding fluid. An external stimulus causes the movement of an accessory structure or fluid which leads to the mechanical deflection of the cilia, and stimulates the sensory cells. These hair cells may appear in the form of primary (specialized neurons with an axon leaving the cell) or different types of secondary sensory cells (without an axon) that make afferent synaptic contacts with first-order afferent neurons. Hair cells and neurons receive numerous efferent endings (Budelmann, 1989) and are responsible from the information transmission to the nervous system. Depending on the direction of deflation of the kinocilia, the amount of neurotransmitter release will be different, causing an excitation or inhibition response and serving to regulate a wide range of behaviours.

Invertebrates can detect underwater sound (i.e., of mechanical disturbance of water) through three types of sensory systems: the body superficial receptor systems, the internal statocyst receptor system and the chordotonal organs (Budelmann, 1992b) (Figure 4).

2.2.2.2 Superficial receptor systems

Epidermal detector systems for vibration and other local water movements known as "hydrodynamic receptor systems" are found all over the external body surface and are analogous structures to fish and amphibian lateral lines (Budelmann, 1992b) (Figure 5). Their

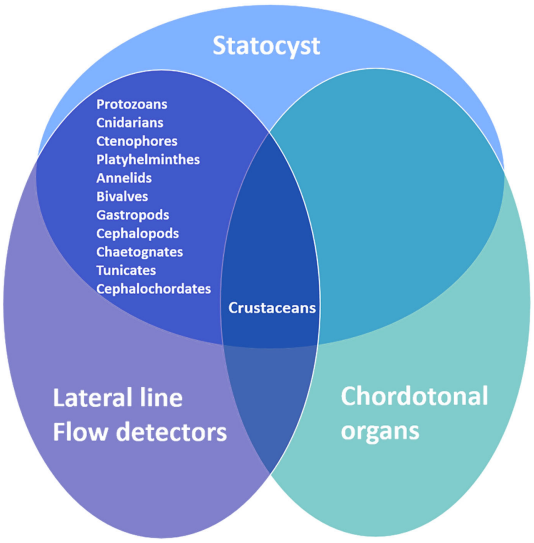

FIGURE 4
Marine Invertebrate sound sensory systems.

receptor cells are epidermal sensory cells carrying kinocilia that can be mechanically deflected by local movements that occur relative to the animal's body surface. In some cases, the cilia are embedded in an accessory cupula structure (Budelmann, 1989) (Figure 5).

Some species of **protozoans** respond to vibrations and water disturbances (Kolle-Kralik and Ruff, 1967). Unicellular organisms commonly respond to mechanical stimuli impinging upon them. Motor responses in ciliated cells result from alterations in motility of the cilia. The resulting behaviour is cellular contraction or alteration in locomotion (Budelmann, 1992b).

Cnidarians are sensitive to low-frequency water oscillations. Horridge (Horridge, 1966) showed sensitivity to low-frequency oscillations by the hydromedusa *Eutonia*. The sea anemone *Sagartia* reacts to water currents (Frings, 1967). The sensory structures are monociliary hair cells with a concentric bundle of stereovilli (Budelmann, 1989). Cnidarian's polyp and medusa stages

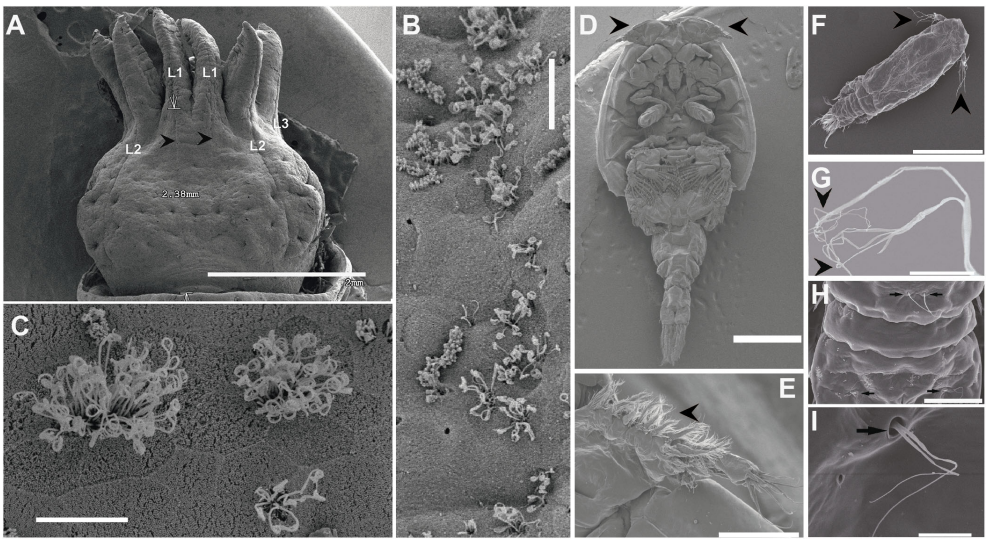

FIGURE 5
Scanning Electron Microscopy. (**A–C**: Cephalopod. **D–I** Crustacean). **(A)**: Epidermal lines (lateral line analogue) on the head of *Sepia officinalis* larva. Lateral lines on three arms and above the eye (L1–L3) that run in anterior/posterior direction are visible. White arrows show the length of the lateral line L1 (black arrowheads). **(B)**: Epidermal line L1. **(C)**: Detail from **(B)**. Hair cells' kinocilia of L1. **(D)**: Ventral view of an adult whole body of sea lice *(Lepeophtheirus salmonis)* showing the first antenna (arrowheads) responsible from the sound perception. **(E)**: First antenna of an adult of *L. salmonis*. **(F)**: Dorsal view of a *L. salmonis* copepodid showing the first antenna (arrowheads). **(G)**: Detail from the first antenna setae showing their irregular branching tips. **(H)**: Dorsal view of the *L. salmonis* copepodid abdomen showing some paired setae (arrows). **(I)**: Detail from H showing the structure of a birrame setae (arrow). (**A–C**: Solé et al., 2022; **D–I**: (Solé et al., 2021b). Scale bar **(A, D)** = 2 **mm**. **(F)** = 300 µm. **(E)** = 100 µm. **(H)** = 30 µm. **(B, C, G)** = 10 µm. **(I)** = 5 µm.

to detect vibrations in water associated with prey movement. Hydrozoan and Cubozoan polyps show mechanoreceptors bearing specialized cilia located in their tentacles (Golz and Thurm, 1993; Golz and Thurm, 1994; Bouillon et al., 2006; Tardent and Schmid, 1972) which inform the animals about surrounding environment changes. Albert (Albert, 2011) described light, touch, gravity, chemicals, sound pressure waves, direction, vibration and hydrostatic pressure receptors in medusa. Behavioural observations in *Aurelia labiata* under turbulent water evidenced its sensitivity to sound pressure waves and vibration mediated by sensory ciliary hairs (Albert, 2007).

Ctenophores possess sensory organs able to detect vibrations in water associated to prey movement (Tamm, 2014). The comb jelly *Leucothea* and the sea walnut *Pleurobrachia* are sensitive to water oscillations. The receptor cells are monociliary hair cells with a specialized basal body (Budelmann, 1992b).

Platyhelminthes have many sensory cells that sense local water movements. In flatworms, each cell has a single kinocilium surrounded by either a collar of eight separate stereovilli or a collar with eight columnar ridges, closely filled with microfilaments (Budelmann, 1989).

The receptor organs for water movements and vibrations on **annelids** are the "segmental sensilla" which are disk-like-sensory buds containing three types of ciliated epidermal cells distributed all over the body surface, tentacular cirri and palps (Budelmann, 1989). When low-frequency vibrations stimulate their tentacles, tube worms withdraw into their tubes (Laverack, 1968).

Among **Mollusks, Cephalopods** also have superficial receptor systems sensitive to local water movements. These receptors are analogous in structure and function to the amphibian and fish lateral lines. Late embryonic stages and hatchlings of cephalopods have epidermal lines (Villanueva and Norman, 2008), consisting of ciliated primary sensory hair cells that carry cilia (Hanlon and Budelmann, 1987) and non-ciliated accessory cells, running in anterior-posterior direction and located on the arms, head, anterior part of dorsal mantle and funnel (Figures 5A–C). Cuttlefish present eight, and squids ten, "epidermal lines" of ciliated sensory cells (Budelmann, 1992b; Solé et al., 2018) which are sensitive to local water oscillations (0.5–400 Hz) and are able to perceive hydrodynamic pressure. In addition to the epidermal lines in the head and arms, on cephalopods, there are others ciliated cells with shorter cilia that occur on the body surface, also involved in the detection of water movements (Budelmann, 1992b; Preuss and Budelmann, 1995).

In **gastropods**, several types of receptor endings were identified in the skin of the tentacles, lips, dorsal surface of the head and mouth region of the pond snails *Lymnaea stagnalis* and *Vivipara viviparus* (Zaitseva and Bocharova, 1981). The **bivalve** abdominal sense organ (ASO) of scallop *Patinopecten yessoensis* is highly sensitive to water-born vibrations (Zhadan and Semen'kov, 1984; Zhadan et al., 2004). It is the largest of the mechanosensory organs studied, containing about 4 million sensory cells (Haszprunar, 1983; 1985).

Chaetognathes are predators of marine plankton. They wait motionless until the water oscillation produced by a prey or another source of vibration arrives (Budelmann, 1992b; Feigenbaum, 2011). Chaetognates exhibit "ciliary fences" on the body surface, consisting of stiff kinocilia polarized in the same direction. All fences together are able to detect the direction of water movements (Horridge and Boulton, 1967; Budelmann, 1992b).

The sessile ascidians (**Tunicates**) are sensitive to water movements through cupular organs present in the exhalent siphon of the animal (Bone, and Ryan, 1978; Mackie and Singla, 2004). The cupular organ exhibit primary sensory cells embedded in a gelatinous cupula, structure considered an analogue of neuromasts in vertebrates. In ascidians, mechanoreceptors of the oral area are involved in monitoring the incoming water flow. In the coronal organ of the oral siphon, the sensory cells present different morphologies depending on the species (*Enterogona* order show multiciliate cells, *Pleurogona* present one or two cilia accompanied by stereovilli). The coronal organ presents a line of secondary sensory cells with a hair bundle also comprising graded stereovilli. These hair cells resemble vertebrate hair cells for morphology, embryonic origin and arrangement, and this organ is considered homologous to the vertebrate octavo-lateralis system (Burighel et al., 2011). *Molgula socialis* presents a coronal organ with a few associated rows of sensory cells running the whole length of the oral velum and the tentacles (Caicci et al., 2007). *Oikopleura* exhibit another organ sensitive to water oscillations, the Langerhans receptor (with monociliary cells that lack a cupula) on either side of the trunk (Bone and Ryan, 1979).

Two types of ciliated sensory cells sensitive to water movements are shown in the lancelet Branchiostoma (*Amphioxus*) (**Cephalochordates**) (Bone and Best, 1978). On the buccal cirri, the hair cells carry a normal kinocilium. On the velar tentacles, the sensitive cells bears a shorter and thicker modified cilium (Burighel et al., 2011).

Crustaceans exhibit superficial receptor systems sensitive to water disturbances over the body surface. The receptors systems can present a single cuticular hair ("sensillum") or a group of hairs. The structure of the hair(s) consists of one to four sensory cells with a flexible basal joint. When the water oscillations bend the hairs the sensory cells are mechanically stimulated (Budelmann, 1992a). Decapod crustaceans, especially lobsters and crayfish, present cuticular cells on their carapace and over the body surface, on the two large and small antennae and on the telson (Budelmann, 1992a; Jezequel et al., 2021). In addition to sensory sensilla distributed around the body surface, some planktonic crustaceans present sensory sensilla responsible for the water disturbance and sound perception on the antenna (Solé et al., 2021b) (Figures 5D–I).

2.2.2.3 Statocyst receptor systems

Invertebrate statocysts can be defined as internal receptor systems, analogous to the vertebrate inner ear (otolith organ), that act as equilibrium receptor systems, although most are thought to be gravity receptor systems only (Anken and Rahmann, 2002). In addition, statocysts of cephalopods and decapod crustacea include angular acceleration detector systems (Budelmann, 1988; Budelmann, 1992a). In these groups, the statocyst as linear accelerometers can also detect acoustic particle motion (since the whole animal vibrates together with the water column) and are involved in underwater hearing (Budelmann 1992a; Budelmann 1992b).

Statocysts present different range of complexity from the simplest gravity receptor systems to the more complex organs of cephalopods which show receptor systems for linear and angular accelerations (Budelmann, 1992b). However, all these different systems have only

two basic structural elements: a mass, the statolith or statoconia, the position of which varies as a function of the forces applied; and sensory elements (hair cells that carry kinocilia in contact with the mass) that are mechanically affected by the position of the mass (Figure 6). Changes in orientation cause the movement of the statolith into the statocyst and thereby the stimulation of different groups of hair cells. In some cases, the heavy mass is surrounded by, or included in, the sensory cell lacking kinocilia (Budelmann, 1992b).

In **cnidarians**, statocysts can be external or internal pendulum-like projections bearing internally the mass (Budelmann, 1988; Solé et al., 2016). The position of the pendulum is monitored by one or several hair cells. Scyphozoan medusae shows marginal sense organs bearing statocysts (Werner, 1993). Numerous small crystals collected in sac-like statocyst are located at the distal ends of their rhopalia (sensory organs associated with pulsing, swimming, orientation and gravireception) (Passano, 1982) (Figure 6E). Statocysts lacking hair cells occur in cnidarian polyp *Corymorpha* (Campbell, 1972), in the nemertine worm *Ototyphlonemertes* (Brüggernann and Ehlers, 1981), and in some flatworms (Ferrero, 1973). The process of stimulus detection in the statocyst is mediated by the differential contact of the statolith and the surrounding sensory cell(s), or alternatively by membrane distortions (Budelmann, 1988).

Ctenophores have only a single statocyst containing a single large statolith in the aboral organ (apical organ). The frequencies of the eight locomotory comb rows are controlled by four compound motile mechanoresponsive cilia (balancers), which support the statolith, and consequently regulate the position of the animal respect to gravity perception (Budelmann, 1992b; Tamm, 2014).

Lacking on the sessile adults, the **ascidian tunicate** *Ciona* present a unique statocyst in their its larvae, consisting in a single cell carrying a large pendulum-like projection without cilia (Budelmann, 1992b).

Bivalve, scaphopod mollusks and most **gastropods** exhibit the "typical" invertebrate statocyst. (Figure 6D) (Cragg and Nott, 1977; Budelmann, 1992b) that is shown from the pediveliger stage (Cragg and Nott, 1977). It is a sphere filled with endolymph which walls are lined by between 10 and 3,000 hair cells, each bearing kinocilia and contains either a single statolith or a mass of statoconia (Budelmann, 1988).

With the exception of the **Nautiloids**, which present a simplest statocyst that resemble gastropod and bivalve molluscs equilibrium organs, all **cephalopods** have a couple of statocysts generally located within the cephalic cartilage. The cephalopod statocysts are sophisticated balloon-shape bodies filled with endolymph that contain the sensory hair cells which lie on the inside wall of the inner sac and are grouped into two main areas of sensory epithelium (macula and crista). In **octopods**, the statocyst is a sphere-like sac. It contains a single gravity receptor system, the macula plate with a compact attached statolith. The angular acceleration receptor system is a ridge of cells that runs along the inside of the statocyst sac, divided into nine crista segments. Either a large or a small cupula is attached to each segment (Budelmann, 1988). In **decapods**, such as cuttlefish and squid, the statocysts are even more complex (Figures 6A–C). Its

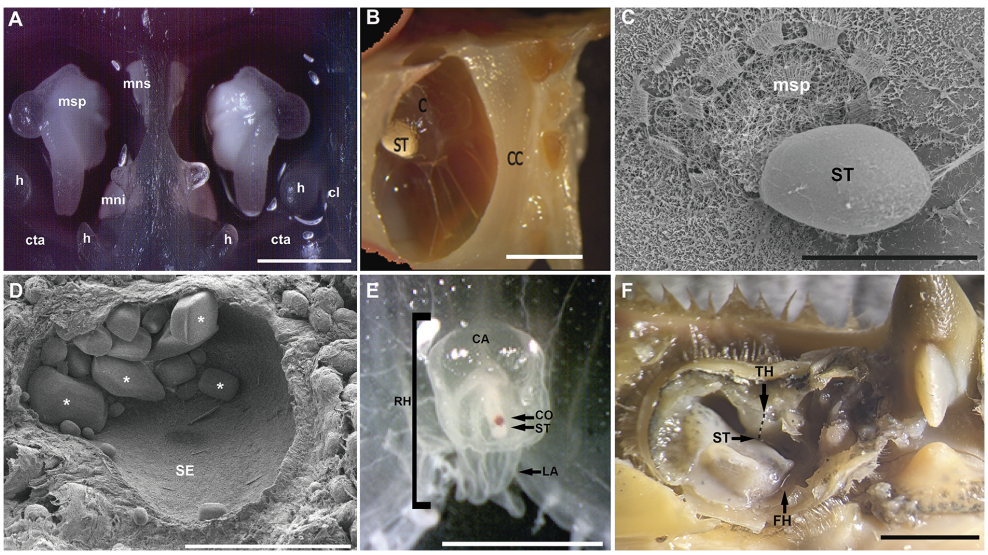

FIGURE 6
Invertebrate marine statocyst (**A–C**: Cephalopods. **D**: Gastropods. **E**: Cnidarians. **F**: Crustaceans). **(A,B, E, F)**: Photomicrograps. **(C, D):** SEM. **(A)**: *epia officinalis* statocyst cavities opened transversally (Anterior view). Each cavity shows the three macula-statolith systems (msp, mns, mni) and two of the crista-cupula systems (cta, cl)(Solé et al., 2017). **(B)**: Lateral view of the interior of a *Octopus vulgaris* statocyst. The spherical inner sac is suspended in the cephalic cartilage cavity by fibrous strands. The statolith is attached to the macula. The crista lies on the inside wall of the sac-like structure (André et al., 2011). **(C)**: *Illex coindetii* hatchling inner statocyst morphology. The transversally opened statocyst cavity shows the statolith attached to the macula statica princeps. Note the hair cell kinociliary groups arranged in nearly concentric rings around a center (Solé et al., 2018). **(D)**: Inner cavity of apple snail (*Pomacea maculate)* statocyst covered by sensory epithelium. Some aragonite crystals are visible (asterisk) (Solé et al., 2021a). **(E)**: Anterior view of the jellyfish *Aurelia aurita* rhopalium bell margin. There is a mass of sensory cells with a single layer of pigment cells (pigment-cup ocellus) on the oral side near the statocyst (Solé et al., 2016). **(F)**: Transversally opened statocyst cavity of a blue crab (*Callinectes sapidus*). Arrows point to the location of the different ciliary areas (ST, TH, FH). TH hair cells run following a line distribution as it is shown in the image (Solé et al., 2023) (ca, rhopalar canal; C, Crista; CC, Cephalic cartilage; cl, crista longitudinalis; co, pigment-cup ocellus; cta, crista transversalis anterior; FH, Free-hook hairs; h, hamuli lobe; LA, lappet; mni, macula neglecta inferior; mns, macula neglecta superior; msp, macula statica princeps; RH, rhopalium; SE, Sensory epithelium; ST, statolith; TH, Thread hairs). Scale bars: **(A, B)** = 2 mm. **(F)** = 0,5 mm. **(E)** = 400 µm. **(D)** = 200 µm. **(C)** = 20 µm.

angular acceleration receptor system is subdivided into only four segments. Its gravity receptor system is subdivided into three systems. Each system has a unique pattern of morphological and physiological polarization of its hair cells, depending on the position of the basal foot structure and the internal tubuli content of its kinocilia (Budelmann, 1979). One of these three systems is covered by a large calcareous statolith, whereas the others are covered by statoconial layers. In cephalopods statocysts, the sensory hair cell organization is highly complex and receive a high degree of efferent innervation (Colmers, 1981).

Crustaceans are sensitive to low frequency acoustic stimuli (Salmon and Horch, 1972; Goodall et al., 1990; Roberts et al., 2016). Mechanical disturbances of water/sediment (associated to sound waves) are detected by a pair of statocysts (Figure 6F), chordotonal organs linked to joints of antenna or legs (Figure 7) and internal and external sensilla (Figure 5) (Popper et al., 2001; Breithaupt, 2002). The statocyst in **crustaceans** shows a similar basic structure among all species and can be located on the basal segment of the antennule (in decapods) and the uropod or telson of the tail (mysids and isopods). The statocyst presents cuticular sensory hairs polarized in one particular direction due to its asymmetric basal joint. They have an overlying statolith mechanically connected to the cuticular hair which stimulates three sensory hair cells. Depending on the species the cuticular hairs per statocyst is variable but in general they are arranged in two to four rows and are polarized towards the centre (Budelmann, 1992a; Rose and Stokes, 1981).

2.2.2.4 Chordotonal organs

Chordotonal organs which are associated with flexible articulations of the appendages, are common among **crustaceans** (Bush and Laverack, 1982; Cooper, 2008; Atkins et al., 2021) (Figure 7). The oscillations of the water column stimulate the chordotonal sensory cells sited in the appendages. The hermit crab *Petroehirus* exhibit chordotonal organs with sensory cells in the basal segment of the antennal flagellum. The rock and the spiny lobster present a similar organs in the large and small antenna and, the crayfish *Astaeus* in intersegmental joints of the first and second antenna (Laverack, 1964; Rossi-Durand and Vedel, 1982). The chordotonal organ is a proprioceptive organ that monitors joint movement, direction of movement and static position and in some cases could be related with sound perception (Figure 7). Fiddler and ghost crabs present specialized Barth's myochordotonal organs (Bart's MCO) located on each walking leg; these resembles a distinct, thin-walled "window" in the exoskeleton. The males of these species produce acoustic signals detected by their females. Thanks to Barth's myochordotonal organs, ghost crabs are sensitive to both substrate-borne and airborne sounds and, fiddler crabs responds to substrate-born vibrations.

2.2.3 Acoustic sensitivity in molluscs and crustaceans

Using a broad definition – the reception of vibratory stimuli of any kind and nature, provided that the sound source is not in direct contact with the animal's body (Budelmann, 1992b) – hearing is widespread among invertebrates. Although the research on invertebrate acoustic sensitivity is scarce, some studies on bivalves, cephalopods and crustaceans have determined some important aspects about the invertebrate threshold sensitivities.

Early studies on sound detection by **bivalves** reported induced burrowing behaviour in clam species (Mosher, 1972; Ellers, 1995). Recent work has quantified sensitivity of marine bivalves to substrate-borne vibration (Zhadan, 2005; Kastelein, 2008; Roberts et al., 2015). By exposure to vibration under controlled conditions using valve closure as the behavioural indicator of reception and response

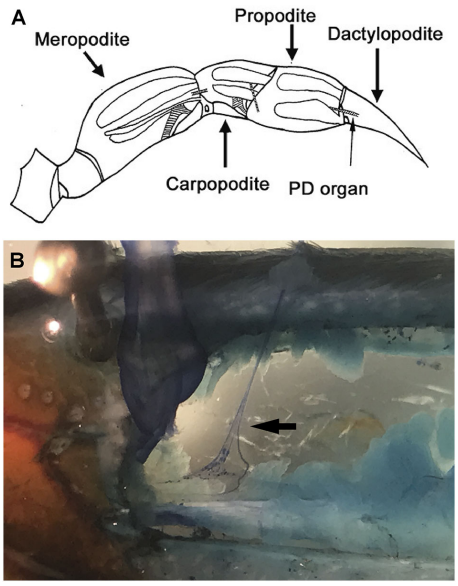

FIGURE 7
Crab chordotonal organ. **(A)**: Drawing of the first walking leg of a crab showing the anatomical location of chordotonal organs (hatched regions). PD organ spans the most distal joint in the limb between the propodite and dactylopodite. **(B)**: Innervation of chordotonal organs. Image of a dissected first walking leg of a blue crab (*Callinectes sapidus*). PD nerve dissected away from the main leg nerve (arrow). The individual neurons stained with methylene blue are visible. (PD: Propodite-dactylopodite chordotonal organ) (Image courtesy of Dr. Robin L. Cooper**).**

(Roberts et al., 2015), the thresholds were shown to be within the range of vibrations measured in the vicinity of anthropogenic operations such as pile-driving and blasting. Using pure-tone exposures and an accelerometer fixed to the shell to detect valve closure, Japanese oysters (*Crassostrea gigas*) were shown to have maximum sensitivity from 10 to 200 Hz (Charifi et al., 2017). The **bivalve abdominal sense organ (ASO)** is highly sensitive to water-born vibration in the range 20–1500 Hz (Zhadan and Semen'kov, 1984; Zhadan et al., 2004).

While there is uncertainty regarding the biological importance of particle motion sensitivity versus acoustic pressure, recent behavioural (including changes in ventilation rhythm) and electrophysiological studies confirmed **cepaholopd** sensitivity to frequencies under 400 Hz (*Sepia officinalis*, (Packard et al., 1990); *Sepioteuthis lessoniana*, (Hu et al., 2009); *Octopus vulgaris* (Packard et al., 1990; Kaifu et al., 2007; Kaifu et al., 2008; Hu et al., 2009; Kaifu et al., 2011), *Loligo vulgaris*, (Packard et al., 1990), *Loligo pealeii*, (Mooney et al., 2010). Whole body vibrations due to particle motion were detected in cuttlefish *Sepia officinalis* (André et al., 2016) through an experimental set-up based on laser Doppler vibrometer techniques (frequencies 60, 120 and 320 Hz). This work confirmed the hypothesis that particle motion can encompass the whole body of cephalopods and cause it to move with a similar phase and amplitude. Mantle movement (lengthened ventilation or jetting) has been used as an indicator of the sound perception to understand the perceptionmechanism (Kaifu et al., 2007; 2008 Packard et al., 1990) or to understand the biological significance of their acoustical perception (Wilson et al., 2007; Samson et al., 2014; Mooney et al., 2016; Jones et al., 2021). In most cases, unconditioned animals were used to observe their baseline behavior. Mantle muscle movements were recorded using an electromyograph (Kaifu et al., 2007; Kaifu et al., 2008) or measurement of the changes of mantle muscle thickness based on impedance between two electrodes inside and outside the mantle (Packard et al., 1990). Cephalopod behavioural responses were then categorized to response type (e.g., inking, jetting, startle, colour change, fin movement, no response).

Among **crustaceans**, Lovell and colleagues studied the mechanism of the reception of sound and hearing abilities of the prawn *Palaemon serratus* using a combination of anatomical techniques, electron microscopy and electrophysiology (Lovell et al., 2005). They concluded that *P. serratus* is sensitive to sounds with frequencies ranging between 100 and 3000 Hz. The same authors (Lovell et al., 2006) demonstrated that all *P. serratus* individuals were able to hear sound with a frequency of 500 Hz, regardless of their size. Although data are not available on frequency-specific hearing/particle motion detection capability, preliminary experiments demonstrated *Nephrops norvegicus* postural responses to water vibrations (Goodall et al., 1990). The hermit crab (*Pagurus berhnardus*) showed antenna/maxilliped movement and forward locomotion in response to particle motion (Roberts et al., 2016). Auditory evoked potential (AEP) analyses of *Panopeus* sp. crabs evidenced their sensitivity to particle motion (Hughes et al., 2014). This response range overlaps with peak frequencies associated with airgun, pile-driving, sonar activities and biologically sources of underwater noise (Jeffs et al., 2003; Radford et al., 2007). Marine crustaceans present sensory hairs covering their bodies, which, when stimulated by water or substrate-borne vibrations associated with changes in acceleration hydrodynamic flow or sound, help animals sense nearby biological movements (Tautz and Sandeman, 1980; Radford et al., 2016). The American lobster *Homarus americanus* shows sensory hairs sensitives to low frequency (Derby, 1982) and ontogenic variations in AEP response up to 5 kHz (Pye and Watson, 2004; Jezequel et al., 2021). **Crustacean chordotonal organs** are stimulated by vibrations. One specialised organ, present on fiddler and ghost crabs, Barth's myochordotonal organ (Barth's MCO), is sensitive to frequencies above 300 Hz. All walking legs contain the sensory organ and if an individual loses a walking leg, it would still be able to detect vibrations through its other walking legs (Derby, 1982). Pelagic crab larva with capacity to detect specific underwater sounds/vibrations are able to use sound as an orientation cue to settle (Montgomery et al., 2006; Stanley et al., 2010; Stanley et al., 2012) (Jeffs et al., 2003; Radford et al., 2007).

Relevant studies on marine invertebrate acoustic sensitivity are detailed in Table 2.

2.3 Production of sound

Marine invertebrates can produce and use sounds to reveal their presence and for a broad variety of behaviours. They can generate the sound unintentionally during moving or feeding (Radford et al., 2008; Di Iorio et al., 2012) or deliberately for communication (Salmon, 1984; Popper et al., 2001; Chitre et al., 2012) (e.g. reproduction (Lucrezi and Schlacher, 2014) or defence (Patek, 2001; Buscaino et al., 2011). The capacity to produce sounds is known in only three groups of marine invertebrates: bivalves, echinoderms and crustaceans.

Many mussels (**bivalves**) produce snapping sound by stretching and breaking byssal threads, which the animals use to attach themselves to hard substrates. In addition, mussels can produce sound with the valve movements (Ubirajara Gonçalves et al., 2020).When expelling water and faeces from their central inner cavity, scallops "cough" by the contraction of the two valves of their shell. In this process, scallops produce a sharp "crack" followed by a long puffing noise as the two valves close (Di Iorio et al., 2012).

Among **Echinodermata**, there are some examples of sound producers. The long-spined sea urchin (*Diadema antillarum*) produces, during movement, crackling sounds by stridulation of its stiff spines and with a special feeding structure, the Aristotle's lantern. This animal uses the five teeth of the lantern to scrape kelp or invertebrates from the substrate. In addition, sea urchin have a calcified test that act as a resonator. The sound originated by the feeding noises of sea urchins, which frequencies are in the range of 800 to 2800 Hz, are amplified by the ovoid calcareous skeleton of urchins acting as a Helmholtz resonator (Radford et al., 2008). There is noise associated with *Kina (*a sea urchin from New Zeland*)* caused by feeding apparatus and spines and by the fluid inside the Aristotle's lantern that produces sound by resonance. Sounds associated with grazing *Kina* urchins contribute to the surrounding soundscape, increasing ambient sounds level 20– 30 dB during the sunrise/sunset periods (Radford et al., 2010).

Crustaceans are the only marine invertebrates in which communication *via* acoustic signals is well known (Aicher and Tautz, 1990; Budelmann, 1992a; Schmitz, 2002; Buscaino et al., 2011; Staaterman et al., 2011; Edmonds et al., 2016). In marine crustacea, the production of sound has been described only in two

TABLE 2 Relevant studies on marine invertebrate acoustic sensitivity.

Species	Common name	Acoustic Perception	Method	Study
Bivalves				
Donax variabilis	coquina	Sounds below 4096 Hz	Burrowing behaviour responses to sound	(Ellers, 1995)
Macoma balthica	Baltic clam		Digging movements after vibratory stimulation	(Mosher, 1972)
Mytilus edulis	blue mussel	Vibration stimulus (Sinusoidal excitation -tonal signals (5–410 Hz). Thresholds 0.06–0.55 m/s^2 (RMS)	Behavioural changes (valve closure)	(Roberts et al., 2015)
Crassostrea gigas	Japanese oyster	10–200 Hz pure tones	Valve closure (accelerometer oyster shell)	(Charifi et al., 2017)
Mizuhopecten yessoensis	Japanese scallop	30–1000 Hz	Behavioural (shell oscillations) directional sensitivity of ASO to water-borne vibrations.	(Zhadan, 2005)
Chlamys swifti	swifti scallop	30–1000 Hz	Behavioural (shell oscillations) directional sensitivity of ASO to water-borne vibrations.	(Zhadan, 2005)
Patinopecten yessoensis	Ezo giant scallop	ASO Fibres I: 20–1000 Hz (max 250–300 Hz) ASO Fibres II: 20–340 Hz	Electrophysiological study ASO	(Zhadan and Semen'kov, 1984)
Cephalopods				
Sepia officinalis	European common cuttlefish	Particle motion (acceleration) <4x 10^{-3} m/s^2	Behavioural changes in breathing and jetting activity	(Packard et al., 1990
Sepia officinalis	European common cuttlefish	Fit the frequency dependence of particle motion sensitivity model	Physical model of the sensory system	(Kaifu et al., 2011)
Sepia officinalis	European common cuttlefish	PM encompass the whole body of cephalopods and cause it to move with same phase and amplitude	Experimental set based on laser Doppler vibrometer techniques	(André et al., 2016)
Sepioteuthis lessoniana	oval squid	400–1500 Hz	Auditory brainstem response (ABR) approach	(Hu et al., 2009)
Octopus vulgaris	common octopus	400–1000 Hz	Auditory brainstem response (ABR) approach	(Hu et al., 2009)
Octopus vulgaris	common octopus	Fit the frequency dependence of particle motion sensitivity model	Physical model of the sensory system	(Kaifu et al., 2011)
Octopus vulgaris	common octopus	Particle motion (acceleration) <4x 10^{-3} m/s^2	Behavioural changes in breathing and jetting activity	(Packard et al., 1990)
Amphioctopus fangsiao/ Octopus ocellatus[1]	*webfoot octopus*	50–150 Hz	Behavioural changes (respiratory activities)	(Kaifu et al., 2007)
Amphioctopus fangsiao/ Octopus ocellatus[1]	*webfoot octopus*	141 Hz particle motion at particle accelerations below 1.3 × 10 -3 m/s^2	Behavioural changes (respiratory activities)	(Kaifu et al., 2008)
Amphioctopus fangsiao/ Octopus ocellatus[1]	*webfoot octopus*	Fit the frequency dependence of particle motion sensitivity model	Physical model of the sensory system	(Kaifu et al., 2011)
Loligo vulgaris	European squid	Particle motion (acceleration) <4x 10^{-3} m/s^2	Behavioural changes in breathing and jetting activity	(Packard et al., 1990)

(Continued)

TABLE 2 Continued

Species	Common name	Acoustic Perception	Method	Study
Loligo pealeii	longfin squid	30–500 Hz (lowest thresholds between 100–200 Hz)	Auditory evoked potentials (AEPs) with electrodes placed near the statocysts	(Mooney et al., 2010)
Crustaceans				
Palaemon serratus	*common* prawn	100–3000 Hz	Anatomical techniques, electron microscopy and electrophysiology	(Lovell et al., 2005) (Lovell et al., 2006)
Neprhops norvegicus	*Norway lobster*	20–180 Hz	Behaviour responses to water vibrations	(Goodall et al., 1990)
Pagur Panopeus sp.*us berhnardus*	hermit crab	[5–400 Hz at particle velocities of 0.03–0.044 m/s^2 (RMS)]	Behavioural responses to particle motion	(Roberts et al., 2016)
Panopeus sp.	mud crabs	predatory fish sounds (or vibrations) 90–200 Hz, (vibrations <0.01 m/s^2)	Electrophysiological, auditory evoked potential (AEP)	(Hughes et al., 2014)
Cherax destructor	Australian freshwater crayfish	150–300 Hz	Electrophysiological recordings (Sensory hairs located on the claws)	(Tautz & Sandeman, 1980)
Ovalipes catharus	paddle crabs	100–200 Hz	Medical imaging technology, microCT, and auditory evoked potentials (AEP)	(Radford et al., 2016)
Homarus americanus	American lobster	20–300 Hz	Electrophysiological recordings (Sensory hairs, cuticular sensilla)	(Derby, 1982)
Uca sp. *Ocypode* sp.	fiddler crab ghost crab	≥300 Hz	Barth's myochordotonal organs (Barth's MCO)	(Popper et al., 2001)
Alpheus richardsoni	snapping shrimp	≥1500 Hz. (more sensitive: 80–100 Hz)	Electrophysiological, auditory evoked potential (AEP) in response to only particle motion and to both particle motion and sound pressure.	(Dinh & Radford, 2021)

([1]*Octopus ocellatus* has been accounted as a junior synonym of *Amphioctopus fangsiao* (Norman and Hochberg, 2005).

groups – barnacles (*Cirripeda*) and decapods (*Decapoda*) – but the detection of sound is widespread. In barnacles, the sound is produced incidentally when the chitinous appendages scrape on its shells during feeding (Fish, 1967). This movement produces rhythmic crackling (Budelmann, 1992a). In decapods, stridulatory movements during which several body parts are scratched against each other produce creaky sounds on spiny lobster, crayfish, shrimps and crabs (Budelmann, 1992a). These sounds may serve to scare off potential predators (Takemura, 1971; Patek, 2002). Patek showed the slip-stick mechanism (similar to bowing a violin) in the spiny lobsters (Patek, 2001). This was the first description of this mechanism in the animal kingdom, which is similar to the system underlying pectoral spine stridulation in blue catfish (Mohajer et al., 2015).

There is scarce knowledge about which sounds are incidentally produced or used for intra/extra-species communication. Snapping shrimp produce explosive clicks (Au and Banks, 1998; Versluis et al., 2000; Kim et al., 2009). These clicks have a fundamental role in the territorial behaviour of the shrimp and are used to stun prey or interspecific opponents (Au and Banks, 1998). Crustaceans produce acoustic signals that span a wide range of frequencies (Edmonds et al., 2016). Stomatopod mantis shrimp (*Hemisquilla californiensis*) and American lobsters *(Homarus americanus)* produce low-frequency rumblings. European spiny lobsters *(Palinurus elephas)* emit ultrasonic signals (Patek and Caldwell, 2006; Staaterman et al., 2011). *P. elephas* use a stridulating organ (plectrum) and rigid file to produce audible rasps associated with anti-predator responses (Buscaino et al., 2011). Jézérel experimentally investigated the propagation features of the sounds from various sizes of European spiny lobsters (*Palinurus elephas*) in natural conditions (Jézéquel et al., 2020a). The sound propagation and its attenuation with the distance on European spiny lobsters varied significantly with the body size. California spiny lobsters (*Palinurus interruptus*) produce pulsatile rasps using frictional structures located at the base of each antenna when interacting with potential predators (Patek et al., 2009). American lobsters produce carapace vibrations (Henninger and Watson, 2005), by simultaneously contracting the antagonistic remotor and promotor muscles located at the base of the second antenna. These sounds may serve in addition as a territorial or

courtship role (Stocker, 2002). Red swamp crayfish (*Procambarus clarkii*) produce sound signals related to a territorial role (Buscaino et al., 2012). The sound-producing and acoustic behaviour of 11 large crustacean species of North East Atlantic such as moving, feeding, mandible rubbing, swimming, species-specific behaviour were analysed (Coquereau et al., 2016a; Coquereau et al., 2016b). The male of European lobsters (*Homarus gammarus*) use buzzingsounds for intraspecific communication during agonistic interactions (Jézéquel et al., 2018; 2020b).

Relevant studies on sound production are detailed in Table 3.

3 Effects of anthropogenic noise in marine invertebrates

Acoustic impact generally refers to activities of anthropogenic origin that generate sounds with frequencies that overlap those of the auditory range of marine organisms (Richardson et al., 1995). The underwater sounds that can affect marine biota can be differentiated between acute and chronic effects. Acute effects are those that cause immediate hearing damage or body injuries due to intense sound sources. Chronic effects are produced by prolonged exposure to moderate pressure level sounds. In addition, sounds can be differentiated between intentional (produced by seismic surveys, navy sonar, etc.) and unintentional (associated to pile-driving, shipping, harbour construction, etc.) sources whose potential effects range from behaviour changes, immediate hearing damage, body injuries or physiological trauma due to intense sound sources, to habitat degradation or expulsion from preferred habitats for prolonged periods. Much of the damage comes from the vibration of the invertebrate body created by the particle motion travelling through the water or the substrate (André et al., 2016). These impacts can affect individuals, populations or even entire ecosystems to unpredictable levels.

Relevant studies on invertebrate effects of noise are detailed in Tables 4–7.

3.1 Early life stages

There are few scientific studies which have directly investigated the effects of low-frequency sound on larvae and other early life stages of invertebrates. Acoustic impacts can be expressed throughout the life cycle of marine invertebrates, 2/3 of whose species have a bentho-planktonic life cycle (Thorson, 1964), i.e., they have a pelagic larval stage of variable duration. This section focuses on the larval, paralarval and juvenile stages, which can exhibit developmental impact (body malformations, higher hatchlings mortality, lower hatch rate and immature hatchlings and slower growth rate) after sound exposure.

Anthropogenic sound exposure resulted in delayed hatching and development of crustaceans eggs, and impaired embryonic development or significantly increase larvae abnormality and mortality rates in crustaceans, bivalve and gastropod (Christian et al., 2003; Courtenay et al., 2009; Stanley et al., 2010; Aguilar et al., 2013; Nedelec et al., 2014). Nedelec et al. (2014) showed negative effects on sea hare *Stylocheilus striatus* larvae of exposure to boat noise, whilst Aguilar de Soto, 2013 found a negative impact of exposure to high levels of seismic air gun noise on *Pecten novaezelandiae* larvae.

Two more general studies focused on the impacts of anthropogenic noise on zooplankton or some of its permanent components (copepods, krill) as invertebrate larvae are temporarily found there (meroplankton). Fields et al. conducted an *in situ* experiment on seismic air gun impacts on *Calanus* spp. showing low mortality (Fields et al., 2019). McCauley et al. through an *in situ* sampling strategy estimated major impacts on zooplankton (copepods, cladocera in particular; mass mortality for krill larvae) after seismic surveys (McCauley et al., 2017). Although the results of these two works could seem contradictory, the opposite results can be explained by the size of the plankton species. McCauley et al. (2017) showed that seismic mostly affected small copepod species, while *Calanus finmarchicus*, the species assessed by Fields et al. (2019) is a very large species. This reinforces the idea that the effects on one species is not applicable on taxonomically near species.

A recent study suggests a critical period of increased sensitivity to acoustic trauma in three species of cephalopod hatchlings *(Sepia officinalis, Loligo vulgaris* and *Illex coindetii*) after sound exposure (Solé et al., 2018). This is the first analysis of noise damaged sensory epithelia in the statocyst and lateral line system on cephalopod hatchlings.

For decades, barnacles have been a study model of choice for research in larval ecology, particularly because of their major role in the 'fouling' of ship hulls. More than three decades ago, Branscomb and Rittschof (1984) demonstrated that the primary settlement of young cypris stages of *Amphibalanus amphitrite* fails when exposed to low-frequency noise (Branscomb & Rittschof, 1984). Testing the impact of continuous ultrasound on their larvae collected from plankton there were delays in metamorphosis, which highly reduces primary settlement of cypris larvae (Guo et al., 2012; Choi et al., 2013). This last study further reveals that the other classical components of sessile epibiosis (polychaetes, bryozoans, ascidians and algae) are not affected by these low-frequency, low intensity ultrasound. Mussel larvae could use low-frequency sounds to select the natural habitat of mussel adults in a high-energy coastal area as suggested after exposure of *Mytilus edulis* to boat sounds (Jolivet et al., 2016).

Many other benthic invertebrates have a free-swimming larval stage and use biotic sounds for orientation, habitat selection and settlement (Jeffs et al., 2003; Montgomery et al., 2006; Lillis et al., 2013). Anthropogenic can lead to developmental delays during the metamorphosis and settlement stages after tidal and wind turbines sound exposure (Pine et al., 2016). In this study, the times to metamorphosis of megalope larvae of the crabs *Austrohelice crassa* and *Hemigrapsus crenulatus* decreased in ambient sound recorded in a natural estuarine environment and tidal and wind turbine sounds treatments. This reduction classically corresponds to a positive effect in larval ecology but the authors also suggest that spectral composition rather than sound level is more relevant to explain the observed results.

Whiteleg shrimp *Litopenaeus vannamei* exposed to aquaculture production system soundscapes (sound recordings of a commercial recirculating aquaculture system, RAS) showed no effects on early stages of this species probably due to a rapid habituation or higher

10.3389/fmars.2023.1129057

TABLE 3 Relevant studies on sound production on marine invertebrates.

Species	Common Name	Sound Type	Sound Origin	Study
Bivalbes				
Perna perna	brown mussel	Impulsive activities: 4–6 kHz band with a max SPL between 43 to 105 dB re 1µPa	Valve movements	(Ubirajara Gonçalves et al., 2020)
Pecten maximus	great scallop	Coughing sounds: 20–27 kHz	Valve movements	(Di Iorio et al., 2012)
Echinoderms				
Diadema antillarum	long-spined sea urchin	Crackling sounds	Stridulation of its stiff spines and Aristotle's lantern (calcified test act as a resonator)	(Radford et al., 2008)
Evechinus chloroticus	Kina	Grazing sounds (800 Hz–28 kHz)	Feeding apparatus and spines Fluid inside the Aristotle's lantern (produces sound by resonance)	(Radford et al., 2010)
Crustaceans				
Cirripeda	*barnacle*	1–3 ms pulses peak amplitude 70 dB (measured at 50 cm of distance)	Chitionous appendages scrape on its shell during feeding	(Fish, 1967)
Linuparus trigonus	spear lobster (spiny lobster)	2 type series of pulses: *A* type; slow repetition rate (10–80 times/sec) - weak at the low frequency range below 3 kHz; *B* type sound, powerful at low frequency. Repetition rate very high	Creaky sounds by rubbing the protuberance of the antennal coxa against the white tubercle in front of its optic stalk	(Takemura, 1971)
Palunirus argus *Palinurus elephas*	spiny lobsters	Stick-and-slip' sounds	Rubbing the base of each antenna against the antennular plate	(Patek, 2002)
Synalpheus paraneomeris	snapping shrimp	Explosive clicks, source levels between~175–220 dB re 1 µPa (peak–peak) @ 1 m; frequency spectrum 2-200 kHz with (peak energy at 2 kHz))	Forceful closing of the chela (in addition to a strong jet of water)	(Au and Banks, 1998) (Kim et al., 2009) (Versluis et al., 2000)
Hemisquilla californiensis	mantis shrimp	Low frequency rumblings (20–60 Hz)	Vibrating their posterior mandibular remoter muscles	(Edmonds et al., 2016)
Palinurus elephas	European spiny lobster	Ultrasonic signals (20–55 kHz)	Stridulating organ (plectrum) and rigid file	(Patek & Caldwell, 2006) (Staaterman et al., 2011)
Palinurus elephas	European spiny lobster	Audible rasps in the 2–75 kHz range (15 kHz peak frequency)	Stridulating organ (plectrum) and rigid file	(Buscaino et al., 2011)
Panulirus interruptus	California spiny lobster	Pulsatile rasps (150.4+/-2.0 dB re 1 microPa) at distances from 0.9 to 1.4 m.	Frictional structures located at the base of each antenna	(Patek, 2002)
Homarus americanus	American lobster	Mean frequency of 183.1·Hz (range 87–261·Hz), range in duration from 68 to 1720·ms (mean 277.1·ms) and lead to waterborne acoustic signals	Produce carapace vibrations, by simultaneously contracting the antagonistic remotor and promotor muscles located at the base of the second antenna	(Henninger & Watson, 2005)
Procambarus clarkii	red swamp crayfish	Sound signals [multi-pulsed, 0.4 ms duration, 128 dB re 1 µPa (zero-peak), mean bandwidth 20 kHz]		(Buscaino et al., 2012)
Cancer pagurus *Carcinus maenas* *Necora puber* *Pachygrapsus marmoratus*	11 large crustacean species of NE Atlantic	Single pulse and pulse train signals distributed across a peak frequency of 3 to 45 kHz with received levels	34 sounds were associated with behaviours such as moving, feeding, mandible rubbing, swimming, species-specific	(Coquereau et al., 2016b)

(Continued)

TABLE 3 Continued

Species	Common Name	Sound Type	Sound Origin	Study
Galathea squamifera *Lophozozymus incisus*		between 93 and 142 dB re 1 µPa (peak to peak)	behaviour and other unidentified behaviours	
Alpheus heterochaelis *Alpheus angulosus* *Alpheus* sp.	Snapping shrimp	Snaps	collapse of a cavitation bubble upon the rapid closure of their specialized snapping claw	(Lillis et al., 2017) (Lillis & Mooney, 2018)
Homarus gammarus	European lobster	"Rattles"	*Rattles when feeding*	(Jézéquel et al., 2018)
Homarus gammarus	European lobster	Buzzing sounds	When stressed vibrated its carapace, producing a low-frequency sound similar to 'buzzing' sound of the American lobster	(Jézéquel et al., 2020b)
Palinurus elephas	European spiny lobster	SL, at one meter from the animals, varied with size (largest SLup to 167 dB re 1 µPa2)		(Jézéquel et al., 2020a)

hearing thresholds of hatchery-produced individuals, (Slater et al., 2020).

3.2 Adults

Animals under exposure to low-frequency sounds may suffer physical damage such as changes in the hearing threshold or barotraumatic ruptures. Morphological or histological analysis allows detection of physical trauma (internal injuries, sensory cell damage of statocysts, epidermal sensory cells and neurons) that can lead to death. This trauma can affect structures involved in sound perception. Invertebrates can behaviourally respond to sound (increased aggressiveness, alarm responses, predator defence, orientation, habitat selection which could have consequences for reproduction and survival). Stress bioindicators such as hormones, immune responses, heat shock proteins, cardiac physiology and overall degraded body condition are the main physiological responses. Metabolic rate, which is the most direct indicator of stress, can be measured from respiration, oxygen consumption or feeding rate. In some cases, irreversible DNA damages has been reported.

3.2.1 Physical effects

In **bivalves**, field studies of airgun exposure found no evidence of increased mortality in adult scallops and clams (La Bella et al., 1996; Parry et al., 2002; Harrington et al., 2010). In another field study, a dose-dependent increase scallop mortality was found four months after exposure to an airgun (Day et al., 2016). In addition, scallops exhibited abnormal reflexes that may indicate damage to mechanosensory organs (Day et al., 2017). The opposite results of these works could be explained by the time of monitoring. Harrington et al. (2010) only monitored scallops for two months, whereas Day et al. (2016) showed that significantly higher mortality rates only occurred towards the end of the 4-month period. Parry and Gason (2006) also stated that to detect mortality in such studies, very significant mortality level would be needed.

Low-frequency noise exposure causes anatomical damage in **cephalopods**. After an increase in the frequency of strandings in North Spain (Guerra et al., 2004), recent findings showed that exposure to artificial noise had a direct consequence on the functionality and physiology of cephalopod statocysts, which are the sensory organs responsible for equilibrium and movements in the water column (André et al., 2011; Solé et al., 2013a; Solé et al., 2013b; Solé et al., 2017). Exposure to noise was challenging the life of exposed individuals in laboratory and offshore conditions (feeding and mating cancellation and irregular swimming). Lesions present on the exposed animals were consistent with a manifestation of a massive acoustic trauma observed in vertebrate species.

Cnidarians and **ctenophores**, both in the polyp and the medusa stage, possess sensory organs located in their tentacles, able to detect vibration in water associated to prey movement and changes in their surrounding environment. A study described morphological effects (severe damages to the statocyst sensory epithelia) after noise exposure on two species of Mediterranean Scyphozoan medusa, *Cotylorhiza tuberculata* and *Rhizostoma pulmo* (Solé et al., 2016).

Among **crustaceans**, blue crabs (*Callinectes sapidus*) suffer mortality as a result of underwater explosions (Moriyasu et al., 2004). Although no lethal effects of underwater noise have been described for *C. pagurus, Homarus gammarus* or *Nephrops norvegicus*, sub-lethal effects of continuous, low-frequency anthropogenic noise have been reported among the Decapoda (Edmonds et al., 2016).

Although no significant effects were detected in snow crabs after exposure (Christian et al., 2003), airgun exposure caused ultrastructural statocyst damages in rock lobsters up to a year later (Day et al., 2016). In a recent study, lobsters showed impaired righting and significant damage to the sensory hairs of the statocyst after exposure equivalent to a full-scale commercial assay passing within 100–500 m (Day et al., 2019). Reflex impairment and statocyst damage persisted over the course of the experiment – up to 365 days post-exposure – and did not improve following moulting.

3.2.2 Behavioural effects

Behavioural responses, not necessarily associated with startle responses, has been observed in **bivalves** (e.g., valve closure and

TABLE 4 Relevant studies on noise impact on bivalves.

Bivalves						
Species	Common name	Stage	Sound effects	Sound source	Received Levels	Reference
Pecten fumatus	Southern Australian scallop	Larva	**Impaired development** Significant under development Body malformations (D-veliger larva)	Seismic pulses playback	SEL pulse 165 dB re 1 Y/ μPa^2	(Aguilar et al., 2013)
Pecten fumatus	Southern Australian scallop	Larva	**High Mortality** Behaviour and reflex responses disruption Permanent Immunosuppression	Seismic airgun	Max SEL_{cum} 198 dB re 1 µPa	(Day et al., 2017)
Perna canaliculus	New Zealand green-lipped mussel	Larva	**Behaviour** Faster settlement with decreased size of the settlers	Ship noise	126 and 100 dB re 1µParms	(Wilkens et al., 2012)
Mytilus edulis	blue mussel	Adult	**Physiology (stress)/Behaviour** Increased clearance rates/valve movement	Pile driving playback	SELss 153,47 dB re 1µPa	(Spiga et al., 2016)
Mytilus edulis	blue mussel	Adult	**Physiology (stress)** Higher breaks in the DNA Lower algal clearance rates, higher oxygen-consumption rates	Ship noise playbacks		(Wale et al., 2019)
Mytilus edulis	blue mussel	Adult	**Physiology (stress)** Changes in biochemical and immunological parameters in digestive gland	Playback	high frequency acoustic treatment (100–200 kHz)	(Vazzana et al., 2020a)
Mytilus edulis	blue mussel	Larva	Larva settlement increase	Low frequency vessel noises	127 ± 3 dB re 1 µ Pa between 100 and 1,000 Hz	(Jolivet et al., 2016)
Mytilus edulis	blue mussel	Adult	**Behaviour** Reduction responsiveness over sequential exposures Mostly respond to the onset of a pulse train.	single pulses and pulse trains (laboratory conditions)	150 and 300 Hz tones	(Hubert et al., 2021)
Mytilus galloprovincialis	Mediterranean mussel	Adult	**Physiology (stress)/Behaviour** No changes in behaviour Changes in plasma and tissue biochemical parameters (glucose, total proteins, total haemocyte number (THC), heat shock protein 70 (Hsp70) expression, and Acetylcholinesterase (AChE) activity)	Low frequency	linear chirp 0.1-5 kHz SPL 150 dB re 1µPa rms	(Vazzana et al., 2016)
Mytilus galloprovincialis	Mediterranean mussel	Adult	**Physiology (stress)** Changes in biochemical and immunological parameters in digestive gland	Linear chirp Playback	SPL 145-160 dB 1µPa rms high frequency acoustic treatment (100–200 kHz)	(Vazzana et al., 2020a)
Magallana gigas	Pacific oyster	Adult	**Physiology** Lower growth rate (2.6 time slower) **Behaviour** Decreased valve activity (lower metal contamination/ decreased grow)	Cargo ship noise (with trace metal contamination, Cd)	150 dBrms re 1µPa	(Charifi et al., 2018)
Ruditapes philippinarum	Manila clam	Adult	**Behaviour** Reduced maximum depth of sediment particle redistribution Reduced valve activity Effects on benthic ecosystem **Physiology** Tissue biochemistry effects due to perturbations in the delivery of oxygen to tissues	Continuous Broadband Noise (CBN) and Impulsive Broadband Noise (IBN) (similar offshore shipping and construction)	SEL 135-150 dB re 1 µPa	(Solan et al., 2016)
Sinonovacula solanconstricta	razor clam	Adult	**Behaviour** Avoidance response: deeper digging **Physiology (stress)** Changes in metabolic activity (O:N ratios) Altered expression of metabolic genes Affected activity of Ca2+/Mg2+-ATPase	White noise and sine wave	80 dB re 1 µPa (induced gens expression) 100 dB re 1 µPa (repressed gens expression)	(Peng et al., 2016)
Cardium edule	common cockle	Adult	**Behaviour** Cockles retracted their siphons and closed the shells	Seismic operations		(Kastelein, 2008)

(Continued)

TABLE 4 Continued

Bivalves						
Species	Common name	Stage	Sound effects	Sound source	Received Levels	Reference
Paphia aurea	golden carpet shell	Adult	**Physiology (stress)** Hydrocortisone, glucose and lactate Ievel increase	Seismic operations	210 dB re to 1µPa	(La Bella et al., 1996)
Crassostrea virginica	Eastern oyster	Larva	**Behaviour** Higher levels of oyster settlement in larval cultures	Acoustic signatures ambient reef sound	1.5–20 kHz	(Lillis et al., 2013)
Crassostrea gigas	Pacific oyster	Larva	**Behaviour** No response to sound on unfed larvae Increased swimming activity fed larvae	Natural and anthropogenic sound (laboratory conditions)		(Stocks et al., 2012)
Mytilus coruscus	Korean mussel	Adult	**Physiology** reduced byssal threads secretion mechanical performances (strength, extensibility, breaking stress, toughness and failure location) wakened	Ambient underwater condition	~50 dB re 1 µPa	(Zhao et al., 2021)
			Physiology reduced byssal threads secretion mechanical performances (strength, extensibility, breaking stress, toughness and failure location) wakened	Playbacks of pile-driving	~70 or ~100 dB re 1 µPa	
Placopecten magellanicus	giant scallop	Adult/ juveniles	**Behaviour** repeated valve closures (stronger effects for juveniles)	Pile driving sounds in field experiments	single strike levels: VH (near site = 136.60 ± 4.98 dB re (1 µm·s^{-2})^{2}s, far site = 116.20 ± 4.03 dB re (1 µm·s^{-2})^{2}s) IH (near site = 94.39 ± 1.34 dB re (1 µm·s^{-2})^{2}s, far site = 72.48 ± 2.51 dB re(1 µm·s^{-2})^2.s	(Jézéquel et al., 2022)
Limecola balthica	Baltic macoma Baltic clam	Adult	**Behaviour** Potential anti-burrowing stress response	"noise eggs"	low-frequency multi-tone ~ 100 Hz – 200 Hz	(Wang et al., 2022)
Pecten maximus	King scallop	Larva	**Mortality** <4% mortality rates without any noise influence **Physiology/Growth** Interactive impact on postlarval growth between trophic environment and noise level /spectra No change in fatty acid profiles	Pile Driving playback Drilling playback	*Pile driving (increasing levels P1, P2, P3)* SPLpp 147.6 (P1) up to 187.6 dB (P3)re 1 mPa s SEL24h 186.9 (P1) up to 215.8 dB (P3) re 1 mPa s *Drilling (increasing levels D1, D2, D3)* SPLrms 107.0 (D1) up to 175.4 dB (D3) re 1 mPa s SEL24h 153.4 (D1) up to 221.7 dB (D3) re 1 mPa s	(Olivier et al. 2023)

recessing reflex behaviour). These responses were used to establish thresholds of sound detection (Roberts et al., 2015). In addition to classic behavioural patterns (i.e., persistent alterations in recessing reflex behaviour), a novel flinching behaviour (a rapid retraction of the velum and then returned to position) was observed on commercial scallops (*Pecten fumatus*) after exposure to a seismic survey. This behaviour was observed before the acoustic wave reached the animal, suggesting that it was a response to the faster traveling ground roll wave (Day et al., 2016). Changes in scallop behaviour and reflex responses disruption were observed at least 120 days after seismic survey exposure (Day et al., 2017).

Among **cephalopods**, behavioural startling responses (jetting and inking) were observed in squids during seismic surveys (Fewtrell & McCauley, 2012) and in response to noise in laboratory conditionss (Samson et al., 2014). Squid show fewer alarm responses with subsequent exposure to noise from seismic surveys (Fewtrell & McCauley, 2012). This process of habituation has been observed in different species of cephalopods (McCauley et al., 2000; Samson et al., 2014; Mooney et al., 2016). While other studies also reported behavioural response to acoustic stimuli in a context of anti-predator defence (Hanlon and Budelmann, 1987; Kaifu et al., 2007); the capture of *Todarodes pacificus* reportedly increased in the presence of underwater sound (Maniwa, 1976). Feeding and foraging behaviour has been shown to be altered in response to different noise stimuli in cephalopods (Jones et al., 2021).

Decapod **crustaceans** exposed to seismic sound exhibited alarm behaviour (startle responses) when they were very near from the sound source (Goodall et al., 1990; Christian et al., 2003). *Carcinus*

TABLE 5 Relevant studies on noise impact on cephalopods.

Cephalopods						
Species	Common name	Stage	Sound effects	Sound source	Levels	Reference
Loligo vulgaris	European squid	Adult	**Damage to sensory systems** substantial, permanent, cellular damage to the statocysts and neurons	Sinusoidal wave sweeps	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(André et al., 2011) (Solé et al., 2013a)
Loligo vulgaris	European or common squid	Larva	**Damage to sensory systems** cellular damage to the statocysts and lateral line system	Sinusoidal wave sweeps	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(Solé et al., 2018)
Illex coindetii	Southern shortfin squid	Adult	**Damage to sensory systems** substantial, permanent, cellular damage to the statocysts and neurons	Sinusoidal wave sweeps	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(André et al., 2011) (Solé et al., 2013a)
Illex coindetii	southern shortfin squid	Larva	**Damage to sensory systems** cellular damage to the statocysts and lateral line system	Sinusoidal wave sweeps	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(Solé et al., 2018)
Sepioteuthis australis	southern reef squid	Adult	**Stress** Alarm responses Aggression jetting	Seismic airgun	168-173 dB re 1 µPa	(Fewtrell & McCauley, 2012)
Architeuthis dux	giant squid	Adult	**Mortality** **Damage to sensory systems** Nine strandings Extensive damage to internal muscle fibres, and organs including statocysts	Seismic airgun		(Guerra et al., 2004)
Sepia officinalis	common Mediterranean cuttlefish	Adult	**Damage to sensory systems** Substantial, permanent, cellular damage to the statocysts and neurons	Sinusoidal wave sweep	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(André et al., 2011) (Solé et al., 2013b)
Sepia officinalis	common Mediterranean cuttlefish	Adult	**Damage to sensory systems** Injuries to the statocysts the severity of the injuries was greater, the closer the distance to the sound source	Sinusoidal wave sweep	139-142 dB re 1 µPa2 at 1/3 octave bands centred at 315 Hz and 400 Hz (off-shore experiments)	(Solé et al., 2017)
Sepia officinalis	common Mediterranean cuttlefish	Larva	**Damage to sensory systems** Cellular damage to the statocysts and lateral line system	Sinusoidal wave sweep	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(Solé et al., 2018)
Sepia officinalis	common Mediterranean cuttlefish	Adult	**Physiology** Changes on the statocyst endolymph proteomic composition	Sinusoidal wave sweep	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(Solé et al., 2019)
Sepia officinalis	common Mediterranean cuttlefish	Adult	**Behaviour** Escape responses (inking, jetting) Body patterning changes and fin movements Sound habituation	Pure-tone pips	Pure-tone pips from 80 to 300 Hz (> 140 dB re. 1 µPa rms and 0.01 m s–2) and (Solé et al., 2022)Part. accel. of 0–17.1 m s–2 80 and 300 Hz 200Hz	(Samson et al., 2014)
Sepia officinalis	common Mediterranean cuttlefish	Adult/ Larva/ Eggs	**Damage to sensory systems** cellular damage to the statocysts and lateral line system (adult and larva) Decreased larva survival rate Decreased hatching success	Pile-driving playback Drilling playback	Max. 170 dB re 1 µPa2 Max: 167 dB re 1 µPa2,	(Solé et al., 2022)
Octopus vulgaris	common octopus	Adult	**Damage to sensory systems** Substantial, permanent, cellular damage to the statocysts and neurons	Sinusoidal wave sweeps	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(André et al., 2011) (Solé et al.2013a)

TABLE 6 Relevant studies on noise impact on crustaceans.

Crustaceans						
Species	Common name	Stage	Sound effects	Sound source	Levels	Reference
Daphnia magna	water flea	Adult	**Behaviour** No effects on swimming speed or depth	Ambient noise (continuous regular and irregular intermittent)	122 dB re 1 µ Pa	(Sabet et al., 2015)
Palaemon serratus	common prawn	Adult	**Behaviour** Change in locomotor patterns **Physiology (stress)** Change in haemolymph and brain total protein content, DNA fragmentation Change in brain protein (HSP 27, HSP 70) level expression	Boat noise (Laboratory experiments)	Power spectrum peaks up to 140 dB re 1µPa rms in the frequency band 0.1-3 kHz	(Filiciotto et al., 2016)
Litopenaeus schmitti *Farfantepenaeus subtilis* *Xyphopenaeus kroyeri*	southern white shrimp southern brown shrimp Atlantic Seabob	Larva/Adult	**Catch rate** No significant deleterious impact	Seismic survey	635 cu. 196 dB peak re 1 µ Pa	(Andriguetto-Filho et al., 2005)
Crangon crangon	southern brown shrimp	Adult	**Physiology (stress)** Significant growth and reproduction rates reduction Increased **Mortality** rate	High ambient sound-level in tanks	30 dB (25 to 400 Hz)	(Lagardère, 1982)
Crangon crangon	southern brown shrimp	Adult	**Behaviour** Increased cannibalism Increased food intake **Physiology (stress)** Increased ammonia excretion Increased O_2 consumption	High ambient sound-level in tanks	105 dB re 1 µPa	(Regnault & Lagardere, 1983)
Balanus amphirite	barnacle	Larva	**Impaired development** Larva metamorphosis and settling reduction	Low frequency sound (30Hz)		(Branscomb & Rittschof, 1984)
Jasus edwardsii	southern rock lobster	Larva	No effects on larva hatching and morphology	Airgun	>185 dB re 1 µPa^2.s	(Day et al., 2016)
Jasus edwardsii	southern rock lobster	Adult	**Physiology (stress)** Suppressed total haemocyte count 120 days post-exposure, but biochemical haematological homeostasis resilient to seismic signals after 365days Chronic impairment of nutritional condition	Air-gun seismic signals/ controlled field experiments	(2000-40000 cu.in.) 185 dB re 1 µPa^2.s at 20 m range	(Fitzgibbon et al., 2017)
Nephrops norvegicus	Norway lobster	Adult	**Physiology** Tissue biochemistry effects due to perturbations in the delivery of oxygen to tissues **Behaviour** Reduced maximum depth of sediment particle redistribution reduced burying and bioirrigation	Continuous Broadband Noise (CBN) and Impulsive Broadband Noise (IBN)	135-150 dB re 1 µPa	(Solan et al., 2016)
Nephrops norvegicus	Norway lobster	Adult	No effects on catch or size	Air-gun seismic operations	210 dB re to µPa/m.	(La Bella et al., 1996)
Homarus americanus	American lobster	Adult	**Behaviour** Increase in food intake **Physiology** Change in serum biochemistry **Mortality** No effect on delayed mortality No effects on catch	Airgun sounds	227 dB re 1 µPa (peak–peak) @ 1 m] at 144-169 dB re 1 µPa^2/Hz average peak energy density 187 re 1 µPa^2/Hz	(Payne et al., 2008)

(Continued)

TABLE 6 Continued

Crustaceans						
Species	Common name	Stage	Sound effects	Sound source	Levels	Reference
			No damage to equilibrium sensory systems **Physiology** Sub-lethal physical changes in serum biochemistry and hepatopancreatic cells **Behaviour** changes in feeding level	Airgun exposure on aquarium	[202 dB re 1 µPa] at 144-169 dB re 1 μPa^2/Hz	
			No effects	Vessel noise	< 1kHz	
			Physiology Increase haemolymph glucose	Mid-frequency sonar	1-s 1.67 kHz /2.5 to 4.0 kHz 1-s	
Palinurus elephas	European spiny lobster	Adult	**Physiology (stress)** Total haemocyte count (THC), henoloxidase (PO) activity in cell-free haemolymph activity decreased significantly, total protein and Hsp27 expression increased significantly	Ships noise (tank experiments)	Power spectrum peaks up to 120 dB below 10 kHz	(Celi et al., 2015)
Palinurus elephas	European spiny lobster	Adult	**Behaviour** Increased locomotion **Physiology** Increased levels of haemolymph stress bio indicators (glucose, total protein, heat-shock proteins (HP 70), and total haemocyte count)	Ship noise (tank experiment)	Power spectrum peaks up to 120 dB below 10 kHz	(Filiciotto et al., 2014)
Carcinus maenas	shore crab	Adult	**Physiology (stress)** Size-dependent response as oxygen consumption (higher metabolic rate and potentially greater stress) **Behaviour** Effects on feeding Behaviour (remaining immobile). Slower to retreat to shelter. Faster righting reflex	Ship noise playback	148–155 dB re 1 µPa Rms	(Wale et al., 2013a) (Wale et al., 2013b)
Carcinus maenas	shore crab	Adult	Reduced food aggregation in crabs and released competition for shrimp	Playback of a broadband artificial sound	129.5 to 142.0 dB re 1 µPa depending on the location	(Hubert et al., 2018)
Coenobita clypeatus	Caribbean hermit crab	Adult	**Behaviour** Delayed response to predator risk	Boat motor playback	98.1 dB SPL re 1 µPa at 1 m range	(Chan et al., 2010)
Pagurus bernhardus	common hermit crab	Adult	**Behaviour** Faster shell selection (critical for reproduction and survival)	Anthropogenic noise/ playback experiments	165 dB re 1 µPa	(Walsh et al., 2017)
Cancer magister	dungeness crab	Larva	**Mortality** For immediate and long-term survival and time to molting, the field experiment revealed no statistically significant effects	Air guns (controlled field experiments)	Mean sound pressure 231 dB re 1 µPa cumulative energy density up to 251 J/M^2	(Pearson et al., 1994)
Chionoecetes opilio	snow crab	Adult	**Catch rates** No change in catch (limited statistical power)	Airgun seismic array	Max 155–163 dB re 1 µPa at 1m	(Morris et al., 2018)
Jasus edwardsii	rock lobster	Adult	**Behaviour** Impaired righting reflex **Damage to sensory systems** Damaged statocyst	Airgun seismic array	109–125 dB re 1 µPa	(Day et al., 2019)
Callinectes sapidus	blue crab	Adult	**Mortality** **No effects**	Underwater explosions Vessel noise	< 1kHz	(Moriyasu et al., 2004)
Callinectes sapidus	blue crab	Adult	**Behaviour** Changes competitive behaviour	Mid-frequency sonar	1-s 1.67 kHz /2.5 to 4.0 kHz 1-s	(Hudson et al., 2022)

(Continued)

TABLE 6 Continued

Crustaceans						
Species	Common name	Stage	Sound effects	Sound source	Levels	Reference
			Physiology Increase haemolymph glucose			
Chionoecetes opilio	snow crab	Adult	**Physiology** No significant acute effects upon adult snow crabs (haemolymph, hepatopancreas, heart, and statocysts)	Seismic airgun	[broadband received levels 197–220 dB re 1 µPa (zero-peak)]	(Christian et al., 2003)
		Larva	Slower developmental rates and higher mortality or abnormality rates in larvae of crabs	Seismic airgun	[224–227 dB re 1 µPa (zero-peak) @ 1 m]. peak sound levels of 216 dB re 1 µPa every 10 s for 33 min	
Chionoecetes opilio	snow crab	Adult	**Physiology** Bruised hepatopancreas and ovaries on adult crabs resultant larvae of exposed eggs were smaller than controls	Seismic survey		(Christian et al., 2004)
Austrohelice crassa	tunnelling mud crab	Larva	**Physiology** Delayed due to interference with natural sound associated with mudflats which has been shown to mediate crab metamorphosis	Wind and tidal	125–245 dB re 1 µPa, up to 10 kHz	(Stanley et al., 2012)
Hemigrapsus crenulatus	hairy-handed crab or papaka huruhuru	Larva	**Physiology** Delayed due to interference with natural sound associated with mudflats which has been shown to mediate crab metamorphosis	Wind and tidal	125–245 dB re 1 µPa, up to 10 kHz	(Pine et al., 2012) (Pine et al., 2016)
Hemigrapsus sexdentatus *Cyclograpsus lavaux* *Macrophthalmus hirtipes* *Grapsidae*	hairy-handed crab smooth shore crab stalk-eyed mud crab	Larva	Reductions between 34–60% metamorphosis time	Exposure to underwater reef noise		(Stanley et al., 2010)
Amphibalanus amphitrite	Acorn barnacle	Larva	**Behaviour** Fails on primary settlement **Physiology** Delays in metamorphosis up to nearly 2 weeks	Exposure to low frequency noise	30 Hz but no specified level	(Branscomb & Rittschof, 1984)
Amphibalanus amphitrite	Acorn barnacle	Larva	**Behaviour** significantly reduced cyprid settlement	Exposure to ultrasound (antifouling treatment)	(ultrasound - continuous sound at 23 kHz) - discontinuous sound: 5 min at 20-25 kHz/20 min pause).	(Guo et al., 2012)
Amphibalanus Amphitrite Elminius sp.	Acorn barnacle	Larva	**Behaviour** significantly reduced fixation rates above 260 Hz	Exposure to low frequency sounds (fouling study)	70-445Hz	(Choi et al., 2013)
Carcinus maenas	Shore crab	Adult	**Behaviour** increase in activity and antennae beats (males higher activity than females) **Physiology** No effects on oxygen consumption	Geophones supported on a softly sprung frame to induce a seabed vibration	20 Hz	(Aimon et al., 2021)
Lepeophtheirus salmonis	Sea lice	Adult Larva (copepodids, chalimus and pre-adults)	**Damage to sensory systems** Damaged sensory setae of the first antenna Damaged cells involved in frontal filament production Damaged nervous system	Continuous acoustic signals (SEL at a level that induces sufficient lesions in the sensory organs to disrupt vital functions)	**Laboratory experiments**: Discrete frequencies 100Hz - 1kHz **Field experiments**: continuous exposure to individual 350 Hz and 500 Hz signals) during, respectively, a	(Solé et al., 2021b)

(Continued)

TABLE 6 Continued

Crustaceans						
Species	Common name	Stage	Sound effects	Sound source	Levels	Reference
					cumulative cycle of 2 h and 1 h, played back every 4 h	
Homarus gammarus	European lobster	Adult (young-of-year)	**Behaviour** Increased exploring time and decreased hiding time	"noise eggs"	low-frequency multi-tone ~ 100 Hz	(Leiva et al., 2021)
Nephrops norvegicus	Norway lobster	Larva/ Juvenile	**Mortality** Larval mortality, antagonistic to cadmium toxicity. **Physiology** Delays in larval development **Behaviour** differences in swimming behaviour juvenile stage.	combination of pile driving playbacks and cadmium combined synergistically at concentrations >9.62 µg [Cd] L^{-1}	170 dBpk-pk re 1 µPa	(Stenton et al., 2022)
Corophium volutator		Adult	**Behaviour** lower bioturbation rates and shallower luminophore burial depths	"noise eggs"	low-frequency multi-tone ~ 100 Hz – 200 Hz	(Wang et al., 2022)
Callinectes sapidus	Blue crab	Adult	**Behaviour** No impact on olfactory-mediated foraging No cross-modal effects	Natural sounds of predators and soundscape	Atlantic croaker (*Micropogonias undulates)* and red drum (*Sciaenops ocellatus)* and marine background sounds	(Solé et al., 2023)
			Behaviour No impact on olfactory-mediated foraging No cross-modal effects **Physiology** Righting reflex Damaged sensory statocyst epithelia No damaged antennule or eye sensory epithelia	Sinusoidal wave sweep	171 dB of 1 μPa^2 ; max 180 dB of 1 μPa^2	

maenas subjected to boat noise were more likely to suspend their search for food, although their ability to find food was not affected (Wale et al., 2013a). Crabs subjected to boat noise took longer to find refuge than when subjected to ambient noise (Wale et al., 2013a). Increased respiration, decreasing escape responses and reduction on foraging activity in the presence of sound from its predatory species suggests that crustaceans use sound as a sensory cue for the presence of fish (Regnault and Lagardere, 1983; Hughes et al., 2014). *Nephrops norvegicus* showed a reduced activity, bury less deeply and flush their burrows less regularly under impulsive anthropogenic noise (Solan et al., 2016). Anthropogenic noise can modify foraging interactions, reducing food aggregation in crabs (*C. maenas*) and thereby release competition for shrimps (*C. crangon*) (Hubert et al., 2018).

Variables related to locomotion such as distance travelled, linear and angular velocity, or single events such alarm responses, intraspecific aggressive encounters and sheltering behaviour were found in crustacean species exposed to underwater noise (Celi et al., 2013; Filiciotto et al., 2014; De Vincenzi et al., 2015). Lobsters and common prawn exposed to boat noises modified their locomotor activities (distance moved, velocity, proximity with conspecific) when exposed to ship noise (Filiciotto et al., 2014; Filiciotto et al., 2016). Roberts et al. showed modification on the hermit crab (*Pagurus bernardus*) antennae movement under sound exposure (Roberts et al., 2016). Righting reflex (time to right itself) of the rock lobster (*Jasus edwardsii*) was delayed after exposure to airguns (Day et al., 2016). Shrimp *Procrambarus clarkii* showed decreased agonistic behaviour under frequencies between 100 and 25,000 Hz (Celi et al., 2013).

Behavioural effects on movement of snow crabs (*Chionoecetes opilio*) after 2D seismic noise exposure, analysed by positioning telemetry, were similar to natural vibrations, and smaller than the responses of crabs to handling, temperature and time of day (Morris et al., 2020a). Habituation to vibrations in crabs has been shown and crabs maintained in captivity for short periods of time presented greatest sensitivity to particle motion (Roberts et al., 2016).

Hermit crabs (*Pagurus bernhardus* show interaction of ship noise exposure with predator presence reaction, shell size and the mean duration *to* accept or reject the optimal empty shell (Tidau and Briffa, 2019b). Ship noise, but not loud natural ambient noise, causes adverse effects on the shore crabs (*C.maenas*) capacity to change the carapace colour to improve camouflage and predator escape responses (Carter et al., 2019). Bioturbation may affect intra and inter-specific behaviour on lobster (*Nephrops n*orvegicus) and after exposure to continuous and impulsive low-frequency noise (Solan et al., 2016).

3.2.3 Physiological effects

A few studies conducted on marine **bivalves** exposed to sound have highlighted its effects on physiological and molecular

TABLE 7 Relevant studies on noise impact on Gastropods, Bryozoa, Echinoderms, Cnidarians, Tunicates and zooplankton.

Other taxa							
Species	Taxa	Common name	Stage	Sound effects	Sound source	Levels	Reference
Stylocheilus striatus	Gastropod	sea hare	Larva	**Impaired development** Reduced embryos development Increased larva mortality	Boat noise playback (field experiment)		(Nedelec et al., 2014)
Bolinus brandaris	Gastropod	purple dye murex	Adult	**Behaviour** Reduction of Motility No mortality	Air-gun seismic operations	210 dB re 1 µPa/m.	(La Bella et al., 1996)
Bembicium nanum	Gastropod	striped-mouth conniwink	Larva	**Behaviour** Increased swimming activity	Natural and anthropogenic sound (laboratory conditions)		(Stocks et al., 2012)
Pomacea maculata	Gastropod	apple snail	Adult	**Damage to sensory systems** Cellular damage to the statocysts	Sinusoidal wave sweep	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(Solé et al., 2021a)
Ciona intestinalis	Tunicate	sea squirt	Larva	**Physiology** Increase rate of settlement, metamorphosis and survival	Vessel generator noise (biofouling study)	127.5-140.6 dB re 1 µ Pa	(McDonald et al., 2014)
Zooplankton (copepods, Cladocera, krill)	Multiple taxa		Larva/ Adult	**Mortality** Increase in dead zooplankton All immature krill (shrimp-like zooplankton) killed	Airgun	156 dB re 1 µ Pa2 s−1 sound exposure levels and 183 dB re 1 µ Pa peak-to-peak	(McCauley et al., 2017)
Zooplankton (*Calanus* sp.)	Multiple taxa		Larva/ Adult	**Mortality** Increase in dead zooplankton	Airgun	1363 kPa, yielding SEL 221 dB re 1 mPa2 s, and 25 kPa yielding SEL 183 dB re 1 mPa2 s	(Fields et al., 2019)
Bugula neritina	Bryozoan	brown bryozoan	Larva	**Behaviour** Decrease swim activities	Boat noise (laboratory conditions)		(Stocks et al., 2012)
Amphiura filiformis	Echinoderm	brittle star	Adult	**Physiology** Tissue biochemistry effects due to perturbations in the delivery of oxygen to tissues **Behaviour** Reduced maximum depth of sediment particle redistribution	Continuous Broadband Noise (CBN) and Impulsive Broadband Noise (IBN)	135-150 dB re 1 µPa	(Solan et al., 2016)
Heliocidaris erythrogramma	Echinoderm	Australian sea urchin		**Behaviour** No differences on swimming behaviour	Natural and anthropogenic sound (laboratory conditions)		(Stocks et al., 2012)
Arbacia lixula	Echinoderm	Black sea urchin	Adult	**Physiology** Changes in enzyme activity, expression of the HSP70 gene and protein	Laboratory condition, linear chirp 100-200 kHz	145-160 dB re 1 µPa rms	(Vazzana et al., 2020b)
Cotylorhiza tuberculate	Cnidarian	fried egg jellyfish	Adult	**Damage to sensory systems** Extruded or missing hair cells Bent, flaccid or missing kinocilia	Sinusoidal wave sweeps	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(Solé et al., 2016)
Rhizostoma pulmo	Cnidarian	barrel jellyfish	Adult	**Damage to sensory systems** Extruded or missing hair cells Bent, flaccid or missing kinocilia	Sinusoidal wave sweeps	157 dB re 1 µPa (peak levels up to 175 dB re 1 µPa	(Solé et al., 2016)
Styela plicata	Ascidian	pleated sea squirt	Adult	**Behaviour** increased the frequency and longevity of siphon closure events	3 separate stimuli: boat motor, song recording, water current to simulate turbulence.		(White et al., 2021)
Arenicola marina	Polychaete	lugworm sandworm	Adult	**Behaviour** Increased shallower particle burial dephts	"noise eggs"	low-frequency multi-tone ~ 100 Hz – 200 Hz	(Wang et al., 2022)

mechanisms. Increased sound intensity result in an alteration in metabolism related genes (Peng et al., 2016) or increases in the levels of biochemical stress parameters measured in their plasma and tissues (La Bella et al., 1996; Vazzana et al., 2016; Vazzana et al., 2020a). The long-term capability of scallops to maintain homeostasis was reduced after airgun exposure (Day et al., 2016).

Among **cephalopods**, analysis of statocyst endolymph of the Mediterranean common cuttlefish (*Sepia officinalis*) showed changes in the protein content immediately and 24 h after sound exposure (Solé et al., 2019). The affected proteins were mostly related to stress and cytoskeletal structure. Hemocyanin isoforms, tubulin alpha chain and intermediate filament protein were down-regulated after exposure.

Among **crustaceans** sub-lethal physiological changes (serum biochemistry and hepatopancreatic cells) were observed in American lobsters (*H. americanus*) after one month of sound exposure (Payne et al., 2007). Permanent high-level exposure to sound caused a significant reduction in the rate of growth and reproduction, an increase in the level of aggressiveness (cannibalism) and the mortality rate, and a reduction in feed intake of shrimp *Crangon crangon* (Lagardère, 1982; Regnault and Lagardere, 1983). Reduced growth and reproductive rates are known tertiary effects of stress response (Barton, 2002). Some crustaceans show alterations on respiration (increase on metabolic rate) in high ambient noise conditions (Regnault and Lagardere, 1983; Wale et al., 2013b). European spiny lobsters are affected by noise in both cellular and biochemical parameters. Filiciotto et al. (Filiciotto et al., 2016) found in laboratory experiments that the common prawn *Palaemon serratus* exhibits stress responses to playback of boat noise. In particular, noise exposure produced alterations in total protein concentrations in the haemolymph and brain, in DNA integrity, in the expression protein levels of HSP 27 and 70 in brain tissues.

Respiratory responses to noise exposure are often species-specific with some animals, such as the shore crab *Carcinus maenas* (Wale et al., 2013b), displaying an increased oxygen consumption in response to noise exposure, whilst others, such as the blue mussel *Mytilus edulis* (Wale et al., 2019) and the blood clam *Tegillarca granosa* (Shi et al., 2019), showing decreased respiration during noise exposure. Among the echinoderms, brittle stars (*Amphiura filiformis*) showed signs of physiological stress after low-frequency noise exposure (Solan et al., 2016) and in the sea urchin *Arbacia lixula* significant change was found in enzyme activity and in gene and protein expression of the HSP70 (Vazzana et al., 2020b).

3.3 Effects on populations and ecosystems

Noise exposure could have an enormous impact on the regional population structure of a species because of the induced emigration, unbalanced prey–predator relation, and the effects on larva development that leads to a reduced recruitment (Peng et al., 2015). Physical, behavioural and physiological effects may result in a reduction of the population within a given area that leads to a decline in the fisheries catch. Some studies analysed the effects of seismic noise exposure on regional catch rates (snow crabs in Canada (Christian et al., 2004) and rock lobsters and scallops in Australia (Parry and Gason, 2006; Harrington et al., 2010). A recent study found no negative effects on catch rates of snow crab (*Chionoecetes opilio*) after 3D seismic noise exposure (Morris et al., 2020b). No statistical significance was found on catch rate of different marine invertebrate groups after seismic exposure (cephalopods (La Bella et al., 1996), bivalves (Parry et al., 2002; Harrington et al., 2010), gastropods (La Bella et al., 1996; Christian et al., 2003; Parry and Gason, 2006; Boudreu et al., 2009), and stomatopods (La Bella et al., 1996).

Acoustic noise pollution can disrupt the antagonistic behaviour, the communication, the social grouping and associations (including their dominance hierarchies and mating systems) and consequently their capacity to act collectively or mate normally by altering the medium through which signals are transmitted or directly altering physiology (Fisher et al., 2021).Changes in mating behaviour and grouping behaviour are shown in crustaceans (Ruiz-Ruiz et al., 2020; Tidau and Briffa, 2019a) demonstrating noise-induced changes in social interaction. Population level could be compromise due to changes in predator avoidance behaviours, if sound exposure induces behavioural changes in prey (i.e. recessing reflex, or decreasing the time of shell selection (Walsh et al., 2017) and consequently, the predation rates increase (Chan et al., 2010). Avoidance behaviours have a greater impact than startling responses on populations that migrate from the areas where seismic surveys are conducted. More research is needed to determine if marine invertebrates avoid other types of noise or can modify their sound characteristics (e.g. amplitude, frequency, and signal timing) in the presence of noise as in some terrestrial invertebrate species, which have shown the physical ability to adjust the frequencies of their courtship signals to avoid anthropogenic masking (Cator et al., 2009) limiting the effects on their population.

4 Gaps and perspectives: The responses to noise

This review provides the current information concerning marine invertebrate bioacoustics and effects of anthropogenic noise. This effort can assist scientists, natural resource managers, industries and policy-makers to predict potential consequences of noise exposure on marine ecosystems and may allow implementing mitigation measures and define a successful strategy for a complete marine noise risk management. On the basis of this review, we identified gaps in our current knowledge on the potential effects that noise exposure may trigger in marine invertebrates:

(1) The biological mechanisms of sound detection and production lack of descriptive data for most species.

(2) Some marine invertebrate groups are very poorly investigated (i.e., annelids and echinoderms). Expanding taxonomic sampling will provide tools to identify species that are especially vulnerable to noise, including those that play an important role in local ecosystems. Priority should be devoted to biological productivity, vulnerability and sensitivity to noise exposure in addition to legal protection aspects and commercially importance of target species.

(3) The physical and physiological variables related to stress, energy metabolism and hormones responses need to be

improved (including proteomic and metabolomics methods), especially how these changes may influence individual and population health.

(4) Sound impacts in populations, communities and ecosystems involves referring to sensory systems and auditory capabilities, social structure, life history, ecological role, and evolutionary adaptation. Gathering more information will help predicting noise responses of understudied species or species that could be presumably unaffected by noise because they survive in noisy habitats or possess lower hearing sensitivity to noise sources.

(5) There is a need to undertake and compare large-scale/long-term field and laboratory studies. Very few research studies have explored the effects of noise at large scales or over long periods of time (e.g. seasonal, yearly) due to the logistical and experimental challenges that they represent. Large-scale studies can provide interesting outputs on cumulative effects of noise exposure related to population persistence, ecological integrity, and evolutionary processes. In addition, it is necessary to increase the number of opportunities to investigate the effects of exposure to a gradient noise in contrast to the traditional research that compares quiet/noisy treatments. This would allow to determine the levels of noise at which a response is initiated and the changes in response when increasing noise levels. In laboratory studies, it is necessary to work in an acoustic environment that would be as close as possible as the invertebrate's natural environment, particularly to what concerns particle motion effects.

(6) Given the short life cycle of most invertebrates, adaptation and habituation to long-term noise exposure or a potential recovery from chronic noise exposure effects are not likely to occur but this has not been investigated.

(7) Current literature references mostly lack of detailed metrics to interpret results. A standardised protocol in future publications should always include duration, frequency range, weighting filters applied, reference pressure used, source and received levels, distance and duration of recordings, including data on the magnitude and direction of particle motion respect to the source.

(8) When performing field studies, particularly under Controlled Exposure Experiments, a previous characterisation of the local soundscapes should be provided to extract the contribution of noise exposure to potential effects.

(9) Changes in environmental factors do not usually occur independently from other stressors. Different changes can operate simultaneously and have antagonistic or synergistic effects (in addition to noise introduction, artificial light, habitat fragmentation, global warming, acidification, etc.). The interactions between these different stressors (multistressors) must be considered when describing noise effects.

(10) Dose-response data is necessary to provide regulators and decision-makers with proper information.

5 Conclusions

(1) We reported on the current scientific knowledge on marine invertebrate bioacoustics (detection and production of sound) and their responses (physical, physiological and behavioural effects) to anthropogenic noise at different life stages, population and ecosystem levels. Although the impact of noise pollution in marine invertebrates is understudied, an exhaustive and systematic revision of literature provided evidence that anthropogenic noise is detrimental not only to these species but also to the natural ecosystems they inhabit.

(2) Considering that the effects of noise can be elicited from cellular to ecosystems level, the understanding of noise impact requires an interdisciplinary expertise to embrace a holistic vision of the problem.

(3) Further research must include a detailed protocol that would ideally provide not only accurate acoustic metrics and methods, but also long-term experiments, cumulative effects, gradients of noise exposure, potential recovery from chronic noise in a variety of taxonomic groups and noise sources.

(4) Multiple stressors effects have to be considered when assessing potential impacts of noise exposure.

(5) This review represents a valuable reference to provides guidance to natural resource managers when evaluating anthropogenic noise effects and developing future operations at temporal and spatial scales that are relevant to oceanic ecosystems.

Author contributions

MS and MA wrote a first version of the manuscript that was completed and significantly improved with the expert input of all co-authors. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to thank Prof. Robin Cooper (Department of Biology. University of Kentucky), for kindly provide the images of Figure 5 and Dr. Youenn Jézéquel (Woods Hole Oceanographic Institution) for the 2A picture.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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10.3389/fmars.2023.1129057

Glossary

GLOSSARY OF TERMS 1

Marine noise pollution: Noise produced by human activities which can potentially damage marine organisms by interfering with or masking biological relevant signals, causing physiological stress, physical damage on sensory systems or behavioural reactions

Vibration: Mechanical oscillation able to propagate in an elastic medium (air, water, etc.).

Impulsive sound: Sound of short duration and wide frequency bandwidth reaching a rapid maximum value followed by a fast decay. (e. g. explosions, military sonar, pile driving, airgun arrays, cetacean echolocation signals).

Continuous sound: Sound of a narrow frequency range that extends over long periods of time (e.g. dredging, drilling, wind turbines, tidal and wave energy devices, ships, etc.).

Sound Pressure: component of the underwater sound waves consisting on the pressure fluctuations of the local hydrostatic pressure in the medium (ISO/DIS, 2016) .

Particle motion: component of the underwater sound waves consisting on the back-and-forth motion of particles in the medium (ISO/DIS, 2016)

GLOSSARY OF TERMS 2

Statocyst: Invertebrates internal sensory receptor that act as an equilibrium and sound/vibration perceptor system.

Hydrodynamic receptor systems: Invertebrate epidermal sensory systems located all over external body surface that are used to detect movement and vibration.

Lateral line system: sensory organ (analogous to fish lateral line) used to detect movement and vibration in some invertebrate larvae. Usually they are ciliated cell lines running over the head and arms.

Chordotonal organs: proprioceptive organs associated with flexible articulations on the crustacean appendages that monitor joint movement, direction of movement, static position and sound perception.

Stridulation: Mechanism of sound-production where the vibrations are produced by rubbing two rigid structures against each other.

Dose–response: Relationship between the sound exposure level and the magnitude of the response.

Physical effects: damage produced after noise exposure consisting in barotraumatic ruptures, massive internal injuries, statocyst sensory cell ultrastructural damages, epidermal sensory cells and neurons that can lead to death.

Behavioural effects: changes produced in the species normal behaviour after noise exposure related to reproduction and survival, increased aggressiveness, alarm responses or predator defence.

Physiological effects: changes in physiological parameters after noise exposure. Stress bioindicators such as hormones, immune responses, heat shock proteins, cardiac physiology and metabolic rate are main physiological responses to noise exposure.

Cortisol (stress hormone): corticosteroid hormone or glucocorticoid involved in response to stress after sound exposure.

Masking: Situation where a biological signal occurs at the same time as noise, leading to an increase of the threshold for detection by the receiver.

Mitigation: Procedure to reduce harmful effects, in this case from exposure to underwater sound.

frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 16 March 2023
DOI 10.3389/fmars.2023.1104526

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

RECEIVED 21 November 2022
ACCEPTED 01 March 2023
PUBLISHED 16 March 2023

CITATION
Ledoux T, Clements JC, Comeau LA, Cervello G, Tremblay R, Olivier F, Chauvaud L, Bernier RY and Lamarre SG (2023) Effects of anthropogenic sounds on the behavior and physiology of the Eastern oyster (*Crassostrea virginica*). *Front. Mar. Sci.* 10:1104526. doi: 10.3389/fmars.2023.1104526

Effects of anthropogenic sounds on the behavior and physiology of the Eastern oyster (*Crassostrea virginica*)

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Introduction: Noise pollution is a major stressor in the marine environment; however, responses of economically and ecologically important invertebrates, such as oysters, are largely unknown.

Methods: Under laboratory conditions, we measured acute behavioral and physiological responses of eastern oysters (*Crassostrea virginica)* to sound treatments mimicking human activity in the environment.

Results: Oysters immediately reduced their valve gape under simulated pile driving sound, but not drilling or boating sound. Pile-driving sound also reduced adductor muscle glycogen, but not triglyceride. None of the sound treatments affected longer-term (12 hours) valve activity levels after the administration of sounds. Interestingly, neither acute nor longer-term valve gaping responses were correlated with glycogen content on the individual level, suggesting that the observed behavioral responses to sound were not mechanistically driven by energetic physiology.

Discussion: Our results suggest that *C. virginica* responds to some, but not all, anthropogenic sounds. Future studies assessing downstream effects on growth, reproduction, and survival in the wild are needed to better understand the effects of anthropogenic sounds on oyster populations and the biological communities they support.

KEYWORDS

animal behavior, coastal ecosystem, energetic physiology, environmental stressors, global change biology, noise pollution

1 Introduction

In recent years, the ecological consequences of sound caused by human activity in the marine environment have become a topic of contemporary interest (Williams et al., 2015; Popper and Hawkins, 2016; Wale et al., 2019; Duarte et al., 2021). With increasing nautical activities in coastal areas (e.g., pile driving, cargo shipping, drilling, recreational activities), marine organisms are increasingly exposed to anthropogenic noise pollution. Anthropogenic noise is expected to have wide ranging effects on marine organisms, including both lethal and sub-lethal impacts (Tyack, 2008; Johansson, 2011; Popper and Hawkins, 2016). At present, studies regarding the impact of noise on marine organisms have focused largely on fish and mammals (Peng et al., 2015). Yet, despite representing >90% of marine organisms, there is a lack of information regarding the effects of sound on invertebrates (Nedelec et al., 2014). Further studies are thus urgently required to better understand the impact of noise pollution on these marine organisms (Solé et al., 2023). Among invertebrates, bivalves are some of the most commercially and ecologically valuable. In 2018 bivalve aquaculture yielded a global production of 17.7 million metric tons, more than doubling the production of marine and coastal finfish aquaculture (FAO, 2020). Bivalves are increasingly recognized not only for their substantial ecological value, but for their economic importance as well (Clements and Comeau, 2019a; Van der Schatte Olivier et al., 2020). While shellfish aquaculture and fisheries are important economic activities for current and expanding coastal communities, these activities expose bivalves to various sounds (e.g., boat engines, mechanical sorting), the impact of which is still poorly understood. Although bivalves can tolerate a wide range of environmental stressors (Pourmozaffar et al., 2019), little is known of their susceptibility to anthropogenic noise pollution (Firestone and Jarvis, 2007; Bittencourt et al., 2014; Peng et al., 2015; Williams et al., 2015; Jolivet et al., 2016; Bonnel et al., 2022). One indicator of stress in bivalves is valve gaping behavior (Clements and Comeau, 2019b). A wide valve opening in bivalves can be indicative of an unstressed animal (Tran et al., 2011; Tran et al., 2016), while partial or complete valve closure can be considered as a protective response when threatened or stressed (Charifi et al., 2017; Charifi et al., 2018). Behaviorally, some cockles (*Cardium edule*) are known to close their valves in response to vibrations (Kastelein, 2008). Valve closures in response to sound are also reported for mussels (*Mytilus edulis*) (Roberts et al., 2015). Pacific oysters, *Magallana gigas*, were reported to engage in transient valve closures in response to sound in a frequency-dependent manner, responding to sound frequencies of 10 to <1000 Hz, with maximum responses occurring between 10 to 200 Hz (Charifi et al., 2017). In a recent field experiment, Doyle et al. (2020) reported that giant clams, *Tridacna maxima*, responded behaviorally to sound by increasing the frequency of mantle retractions, and may become "distracted" (i.e., alter predator avoidance behaviors) by sound in areas of water flow. Stress responses, however, usually involve adjustments to all levels of animal organization, and physiological and molecular impacts may therefore accompany behavioral responses to stressors. For example, stress can impact energetic physiology and reduce the amount of energy available for growth and reproduction (Calow, 1985). With respect to sound, Peng et al. (2016) reported that marginal effects of sound on digging behavior in Asian razor clams (*Sinonovacula constricta*) were accompanied by increased O:N ratios (oxygen consumed versus nitrogen excreted), although metabolic and excretion rates were unaffected. Likewise, Charifi et al. (2018) reported that Pacific oysters exhibited reduced valve gaping and gill function in response to noise pollution, which positively resulted in less metal accumulation, but negatively drove reductions in feeding and growth. Reductions in physiological energetic parameters such as glycogen or lipids (i.e., triglycerides) can also be used as indicators of stress in bivalves (Widdows, 1985). Overall, however, few studies have assessed the effects of sound on bivalves, and those consolidating behavior and physiology are lacking. Notably, studies have yet to consolidate valve gaping behavior and physiological energetics in the context of noise pollution. As part of the National Ecosystem Stressors Program, the Department of Fisheries and Oceans Canada (DFO) recently designated acoustic disturbance as a priority stressor of national importance. Likewise, stressor effects on ecological and economically valuable bivalves are of significant interest and importance under DFO's mandate to protect Canada's aquatic ecosystems from negative impacts. The eastern oyster (*C. virginica*) is a valuable commercial species with a wide geographic distribution occupying an important place in the marine ecosystem (Lacoste et al., 2016). In Atlantic Canada, *C. virginica* supports the local economy through commercial fisheries and aquaculture activities and is of significance for the ecosystem services it provides (Clements and Comeau, 2019a). Given the paucity of information regarding the effect of sound on bivalves, coupled with the importance of noise pollution and bivalves to DFO's mandate, the goal of this study was to experimentally determine whether anthropogenic noise could affect the valve gaping behavior and energetic physiology of adult Eastern oysters (*Crassostrea virginica*) under a laboratory setting. Based on previous observations of bivalve molluscs in response to noise pollution (e.g., Kastelein, 2008; Roberts et al., 2015; Charifi et al., 2017), we hypothesized that exposure to sound would affect the behavior of the Eastern oyster by reducing the opening of the valves and that exposure to sound would result in lower concentration of energy reserves in the form of glycogen and triglycerides – physiological responses that have yet to be documented in response to noise pollution in bivalves (to the best of our knowledge).

2 Methods

2.1 Animal collection and laboratory acclimatization

In October 2019, 72 adult oysters (*C. virginica*; mean ± SD shell length: 59.2 ± 3.9 mm) were collected from an oyster aquaculture site in Lamèque Bay, New Brunswick, Canada (64° 40' 6" W, 47° 47'14.7" N). The oysters were then transported to the Institute of Marine Sciences in Rimouski (ISMER) where they were each connected to a non-invasive valvometry system (DC-204R, Tokyo Sokki Kenkyujo Co., Japan) described in Nagai et al. (2006) (see *2.4 Behavioral measurements* section below for details). The oysters were then placed in a recirculating seawater system (Multi-stressor

units, Aquabiotech System) and were acclimatized to laboratory conditions for seven days prior to behavioral experiments. The seawater temperature was initially kept the same as the field conditions from which the oysters came (≈12 °C) and was gradually increased to 18 °C over the seven-day acclimatization period, where they remained for approximately two days; temperature during the experimental period was held constant at 18 °C. Salinity was held constant at approximately 27 PSU during both the acclimatization and experimental periods. During acclimatization, oysters were fed 1% of their dry mass daily with a tri-species microalgal mixture (1:1:1 ratio of *Isochrysis galbana, Pavlova lutheri*, and *Chaetoceros gracilis*).

2.2 Sound emission system

Alongside a control treatment (i.e., no sound), three sound treatments were selected for the experiment: 1) Fishing boat; 2) Drilling; and 3) Pile driving (Figure 1). Sound treatments were recorded in each individual experimental unit with a Loggerhead LS1 underwater acoustic recorder (sampling frequency of 44.1 kHz) equipped with a HTI-96-MIN hydrophone (sensibility -170 dB re 1VµPa-1). Sound levels were adjusted to match field realistic situations. The boat sound used was the same and at similar levels, as described in Jolivet et al. (2016) and displayed a mussel culture boat of 11 meters long equipped with a diesel motor (300 hp). Drilling and pile driving sounds were recorded during the offshore wind farm installation in the bay of Saint-Brieuc (France) with a calibrated hydrophone (High Tech, Inc., HTI-99-HF: sensitivity −169.7 dB re 1 V/µ Pa; frequency range 2 Hz to 125 kHz). Spectral composition and source sound level were determined using the MATLAB (The MathWorks, Inc.) software to select a 30 s sequence that was repeated during emission. We specifically sought to test the three sounds without interference from other natural sounds in the aquatic environment (and any interpolation of our results to natural systems should therefore be made with caution). Sounds were administered in the *Larvosonic* system (Figure 1) previously developed for studying the impacts of anthropogenic noises on the early stages of benthic marine invertebrates. Olivier et al. (2023) provide a comprehensive description of the *Larvosonic* system and its acoustic characteristics. In summary, integrated acoustic panels (diffuser and bass trap components) effectively dampen the reflection of the whole frequency bandwidth, and as already detailed in Olivier et al. (2023), when the source level increases by N dB, both Pressure Energy and Kinetic Energy increase by N dB even if impedance (ratio between KE/PE) i) evolves nonlinearly as a function of the source–receiver distance and ii) for a given source–receiver configuration, the impedance evolves nonlinearly as a function of frequency. All oysters were placed at the bottom of each cylinder so that the impedance ratio is similar for a fixed frequency for the four external cylinders but slightly varies from the internal ones (lower speaker distance). As described in Figure 1, our study design incorporated one *Larvosonic* system per sound condition, which each system consisting of a large tank

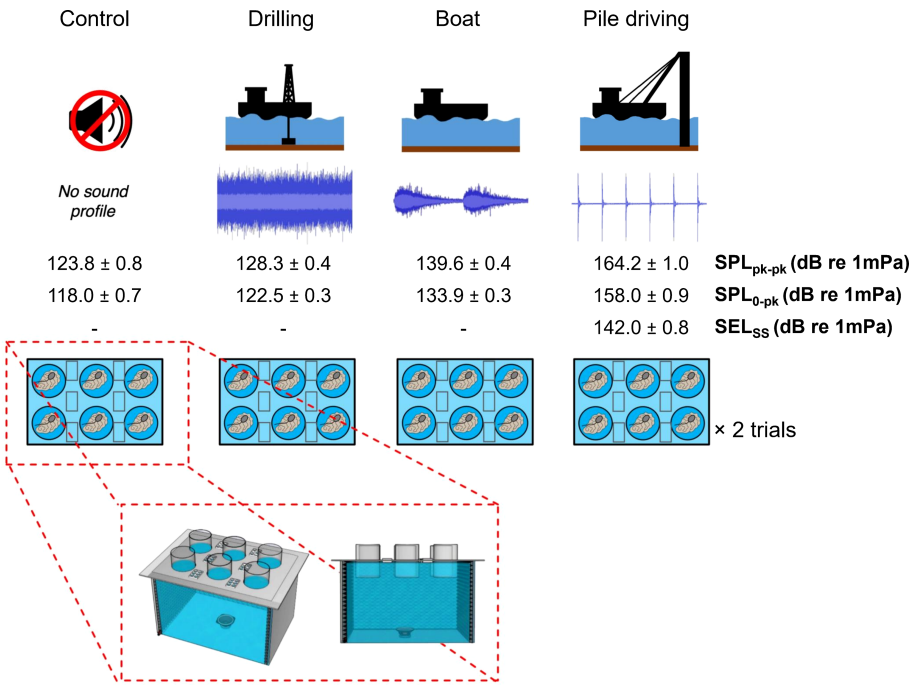

FIGURE 1
Experimental design used to test for effects of anthropogenic sounds on behavioral and physiological responses in Eastern oyster, *C. virginica*, including sound treatment conditions. The bottom panel (in dashed red box) is a 3D representation of the experimental tanks developed at the Institut des Sciences de la Mer à Rimouski – ISMER (*Larvosonic* system; Olivier et al., 2023). Note that the experiment was repeated two times and that some oysters were removed prior to analysis due to technical and/or logistical issues (see *2.3 Experimental design* section for details). Ambient SPL peak to peak in the room (Local) was 114.50 ± 0.10 dB re 1µPa.

(120 cm length × 68 cm width × 68 cm depth) filled with freshwater and supporting 6 semi-submerged experimental cylinder units that constitute 6 replicates. An underwater loudspeaker (Clark Synthesis AQ339, Diluvio, 8Ohms/20-17 000Hz) was positioned in the center of each tank to diffuse the sound. Speakers were connected to a Denon amplifier (DN-300Z/16–bit/20-20 000Hz/44.1KHz), then to a matrix mixer with a signal processor (Yamaha 26x8 MTX3, Buena Park, CA, USA). In each tank corresponding to a specific sound, comparative $SPL0_{0\text{-}pk}$ measurements were obtained between each experimental unit, as less than~ 6dB were measured between central and external cylinders (see Table 1 in Olivier et al., 2023 for additional information). There was a weak contamination of the control sound treatment (no added sound) by sound emissions of other tanks estimated to + 9 dB re 1 µPa (compared to the sound level of the experimental room without any sound emission).

2.3 Experimental design

Each tank contained six semi-submerged cylinder units (n = 1 oyster cylinder^{-1}, 6 oysters tank^{-1}), each filled with eight liters of filtered seawater (10 and 1 µm filters). Once the oysters were placed at the bottom of each individual cylinder, they acclimatized for nine hours after which they were continuously exposed to their respective sound treatment for 12 hours; oysters were not fed during this time. Valve gaping behavior was continuously measured throughout the acclimatization and experimental periods, and individual tissue samples (adductor muscle and digestive gland) were collected at the end of the sound exposure period. The experiment was repeated twice (n = 12 oysters treatment $^{-1}$ total) over a period of three days. Technical issues (i.e., malfunctioning sensors) with some of the valvometry systems resulted in the loss of data for some individuals, resulting in final sample sizes of 7, 8, 6, and 10 oysters for the Boat, Drilling, Pile Driving, and Control treatments, respectively.

2.4 Behavioral measurements

Each individual oyster was connected to a non-invasive valvometry system (DC-204R, Tokyo Sokki Kenkyujo Co., Japan) described in Nagai et al. (2006). A Hall element sensor (HW-300a) was attached to the external ventral margin of one valve with UV resin (Solarez, Wahoo International, Vista, CA, USA) and a small magnet was attached to the external ventral margin of the opposite valve. Functionally, the Hall sensor measures the magnetic flux (flux density) between it and the magnet, which is proportional to the distance between the sensor and the magnet. This flux density was then translated to a microvoltage (µV) *via* Dynamic Strain Recorders (DC 204R) and recorded on a SD card. For the purposes of this experiment, data were recorded at a frequency of one measurement per second. Upon completion of each experiment, the linear relationship between µV and valve opening (i.e., the µV value at a range of known mm distances between the ventral margins of the two valves) was derived for each individual oyster to calculate the valve opening for each point µV measurement. We then computed the relative change (%) in the oyster valve opening in response to each sound using the following equation:

$$Relative\ \ change\ \ (\%) = -\frac{VO_b - VO_a}{VO_b} \times 100$$

where VO_b and VO_a represent the mean valve opening (in mm) 5 mins before (VO_b) and 5 mins after (VO_a) the application of sound. Herein, a negative number indicates a valve closure (avoidance) in response to sound, while a positive value indicates an opening in response to sound. Alongside the relative change (%) in oyster valve opening, we also computed the longer-term valve activity levels by adding up the total distance moved (in mm) for each oyster after the administration of sounds. Herein, the absolute (+ sign) distances of each measured valve opening, and closure (in mm) were summed for each individual oyster to compute the "total distance moved" over the 12 hours observation period following the administration of the sound treatment.

2.5 Physiological measurements

To document physiological energetics and relate them back to any observable behavioral effects, glycogen and triglyceride concentrations were measured in each oyster. Glycogen concentration was determined in the adductor muscle using a slight modification of the method described in Keppler and Decker (1974). Briefly, 30 mg of adductor muscle was homogenized in 5 volumes of 6% perchloric acid using a sonicator (Q55 Sonicator). The homogenate was then neutralized with 1.5 volumes of 2M $KHCO_3$. Then, 50 µl of the slurry was transferred to a clean tube and the glycogen was then hydrolyzed by adding 100 µl of amyloglucosidase (56 U ml^{-1}) in a 0.4 M sodium acetate buffer (pH 4.8). Following a 120-minute incubation at 40°CC, the hydrolysis was stopped by adding 50 µl of 6% PCA and the acid was neutralized by adding 50 µl of 2M $KHCO_3$. The sample was then centrifuged at 2000 g for 5 mins and the supernatant was kept. Each sample was also processed without hydrolysis by adding PCA before amyloglucosidase to determine and remove the concentration of free glucose from that of hydrolyzed glycogen. The glucose concentration was then measured using a coupled enzyme test as described by Williams et al. (2019). The glycogen content is reported as µmoles glycosyl units · g of tissue^{-1}. Triglyceride concentration was determined in the digestive gland. Triglycerides were extracted according to Bligh and Dyer (1959) with slight modifications. Approximately 45 mg of digestive gland tissue was homogenized in 1 ml of methanol using a sonicator followed by the addition of 2 ml of chloroform. The solution was incubated at room temperature for 120 mins and mixed by inversion every 15 minutes. Then, 0.6 ml of distilled water was added to the mixture to generate phase separation. Following a centrifugation at 1000 g for 5 mins, the organic phase was removed and transferred to a new tube and the chloroform was completely evaporated under a fume hood. The extracted lipids were resuspended in 200 µl of ethanol and the triglyceride concentration was measured using a commercial kit according to the supplier's instructions (InfinityTM Triglycerides Liquid Stable Reagent, Thermo Scientific Inc.). The triglyceride concentration is reported as µmoles · g of tissue^{-1}.

2.6 Statistical analysis

To test for sound effects on behavioral (relative change in valve opening and valve activity levels) and physiological (glycogen and triglyceride content) responses, we built mixed linear effects (LME) models and used ANOVA to test for the effect of treatment on each response variable (significance level of $p \leq 0.05$). Models included sound treatment as a fixed categorical factor with four levels (boat, drilling, pile driving, and control) and experiment as a categorical random variable. Linear regression was used to determine whether physiological energetics (glycogen and triglyceride content) were related to changes in both valve gaping responses to sound. Assumptions of homoscedasticity and normality of the residuals were verified using Levene's tests and Shapiro-Wilk tests, respectively. A logarithm transformation was applied to the glycogen content variable. Statistical analysis was performed with R software (RStudio version 3.6.2; R Core Team, 2020). Linear mixed models were built using the lmer() function from the *lmerTest* package (Kuznetsova et al., 2017), and the Anova() function from the *car* package (Fox and Weisberg, 2019; with Type 3 sum of squares) was used to obtain fixed effect significance. Where significant overall effects of sound were detected, Tukey HSD *post hoc* comparisons were used to determine pairwise group differences using the glht() function in the *multcomp* package (Hothorn et al., 2008).

3 Results

3.1 Valve gaping activity

Examples of typical individual valve gaping responses in each sound treatment are depicted in Figure 2. Linear mixed effects results indicated a significant overall effect of sound treatment on the relative change in valve opening (LME ANOVA: $X^2_3 = 10.47$, $p = 0.015$). Tukey HSD pairwise comparisons revealed that valve opening was significantly decreased in oysters from the Pile Driving treatment as compared to the Control ($p = 0.0086$), while valve gaping was unaffected by Boat ($p = 0.8541$) and Drilling ($p = 0.8503$) sounds (Figure 3A). In contrast to the relative change in valve opening, valve activity levels (total distance moved (in mm) after the administration of sounds) were not affected by any of the sound treatments (LME ANOVA: $X^2_3 = 4.22$, $p = 0.2385$; Figure 3B).

3.2 Energetic reserves

Oyster glycogen reserves were significantly affected by sound (LME ANOVA: $X^2_3 = 11.4$, $p = 0.0098$). Tukey HSD pairwise comparisons indicated that oyster glycogen concentrations were significantly lower in the Pile Driving treatment as compared to the Control ($p = 0.0062$), while the other two sound treatments were statistically similar to the Control (Figure 4A). In contrast to glycogen, triglyceride content was unaffected by any of the sound treatments (LME ANOVA: $X^2_3 = 0.44$, $p = 0.9308$; Figure 4B). Although sound treatments appeared to affect valve opening and glycogen in similar ways, linear regression revealed no relationship between individual changes in valve opening and individual glycogen content ($F_{1,29} = 2.34$, $p = 0.137$, $R^2 = 0.07$). Likewise, triglyceride content was not related to relative change in valve gaping (Linear regression: $F_{1,29} = 0.25$, $p = 0.621$, $R^2 = 0.009$). Linear regression also revealed no relationship between individual valve activity levels and individual glycogen content ($F_{1,29} = 0.000012$, $p = 0.997$, $R^2 = -0.03$), nor triglyceride content ($F_{1,29} = 0.56$, $p = 0.462$, $R^2 = -0.02$).

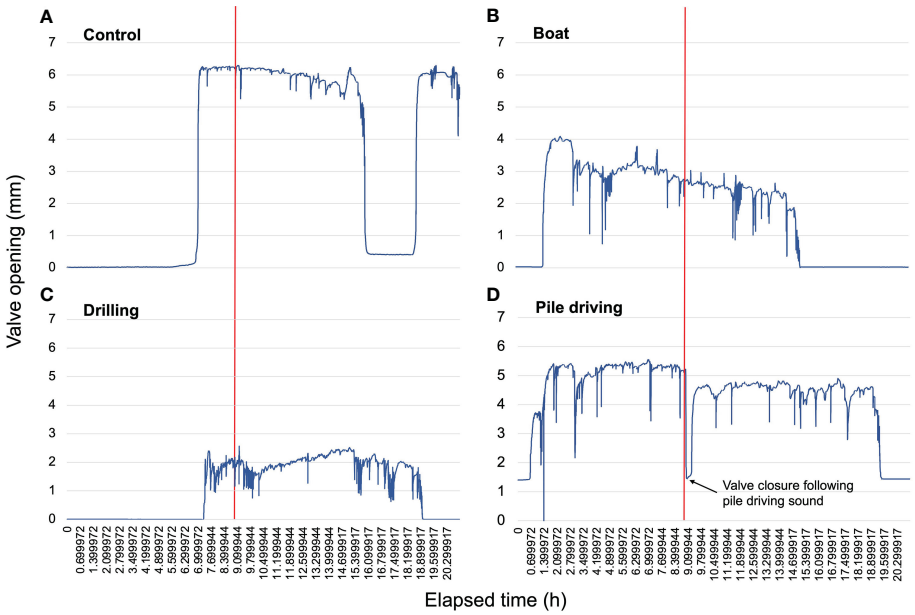

FIGURE 2
Recordings of valve gaping activity of a single representative oyster responding to the **(A)** Control, **(B)** Boat, **(C)** Drilling, and **(D)** Pile driving sound treatments over a period of 21 hours. The vertical red line indicates the start of sound exposure. Note the immediate and sustained decrease in valve gaping after sound exposure in panel **(D)**.

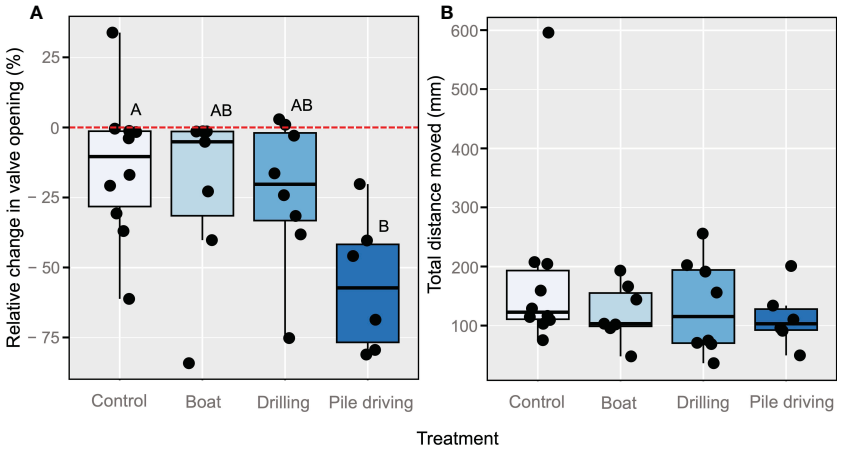

FIGURE 3
(A) Relative change (%) in oyster valve opening between five minutes before and after the start of sound treatments. Letters above the bars represent pairwise differences according to Tukey HSD tests. **(B)** Valve activity levels (total distance moved, in mm) by oysters after the administration of sound treatments. Each point represents an individual oyster.

4 Discussion

To our knowledge, this is the first study to link behavioral and physiological responses to anthropogenic noise in bivalves. Our results suggest that certain sounds such as pile driving can affect oyster behavior and physiology by reducing valve gaping amplitudes and glycogen concentration in the adductor muscle, while other sounds such as boating and drilling into the sea floor appear to have a negligible effect. These results indicate that bivalve behavior and physiology may be sensitive to some, but not all, anthropogenic noises when applied acutely. Based on these experimental observations, the noise created by prolonged periods such as pile driving sound near oyster beds has the potential to exert population and community level impacts where oysters are present in high abundance; however, more direct research is necessary. In this experiment, pile driving sounds were characterized by short pulses of high-level soundwaves, while the boating and drilling sounds were characterized by more sustained levels of lower-level sound. Bivalves thus appear to be sensitive to anthropogenic sounds commonly occurring in coastal regions. Varied responses to the different treatments could be attributed to both the frequency and amplitude of certain sounds. Indeed, Charifi et al. (2017) reported that the valve gaping responses of Pacific oysters, *Magallana gigas*, to sound were frequency dependent. Such sound-specific responses have been observed in other studies as well. For example, behavioral responses of coral reef fishes to boat noise depend on engine type, which is likely a result of different types of sounds produced by different types of engines (McCormick et al., 2018; McCormick et al., 2019). The magnitude of behavioral responses in squid (*Sepioteuthis australis)* are also reported to increase incrementally as sound levels from air guns increased (Fewtrell and McCauley, 2012). In the context of other studies, our results ultimately suggest

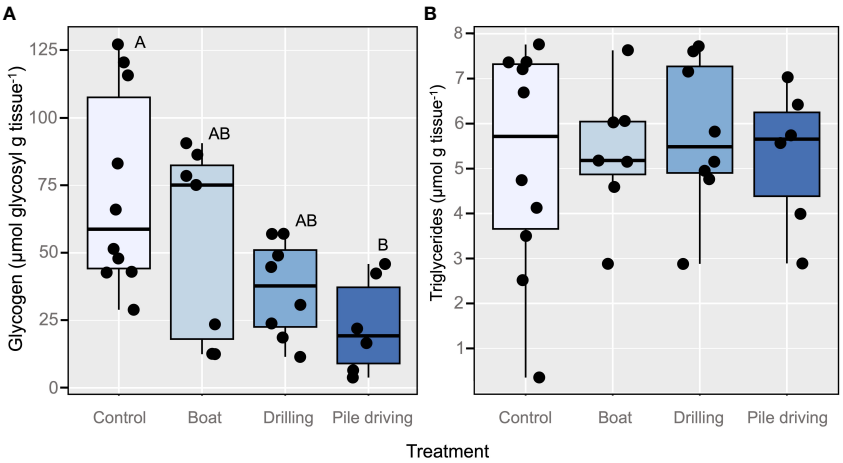

FIGURE 4
Concentration of glycogen in the adductor muscle **(A)** and triglyceride in the digestive gland **(B)** of oysters from each of the four sound treatments. Each point represents an individual oyster. The letters above the bars indicate the results of the multiple comparison test (Tukey) for the significant effect of sound treatments on the amount of glycogen.

that responses of marine organisms to sound are likely complex, appearing both species and sound specific. Given the paucity of information on the responses of bivalves and other invertebrates to anthropogenic noise, additional research is strongly warranted.

One indicator of stress in bivalves is valve gaping behavior (Clements and Comeau, 2019b). Valve gaping has been used to monitor bivalve stress in experiments involving chemical and nutritional stressors (Di Fiori et al., 2012; Cordeiro et al., 2017), environmental fluctuations (Palais et al., 2011; Dowd and Somero, 2013), and other stressors such as oxygen and salinity (Tang and Riisgård, 2016; Woodin et al., 2020). In many circumstances, bivalves tend to completely or partially close their valves to avoid stressful conditions. For example, bivalves tend to partially close their valves in response to the threat of predation, perhaps to 'hide' from predators (Smee and Weissburg, 2006; Carroll and Clements, 2019; Clements et al., 2020; Clements et al., 2021). Likewise, oysters tend to close in response to stressful low oxygen conditions (Porter and Breitburg, 2016; Coffin et al., 2021). Shell closure and the restriction of filtration are behavioral responses by which oysters can also limit soft tissue exposure to noxious or stressful agents (Hegaret et al., 2007). In our experiments, we observed that oysters in simulated pile driving noise exhibited rapid valve closures (almost completely in some circumstances; Figure 2D) in the first seconds-minutes following exposure to noise, followed by a gradual reopening of valves. While drastically understudied, valve closure responses to sound in bivalves have also been reported in blue mussels (Roberts et al., 2015) and Pacific oysters (Charifi et al., 2017). As such, pile driving sounds (or at least sounds with similar characteristics to our pile driving treatment) appear to represent an acute anthropogenic stressor for eastern oysters. Given this species' remarkable latitudinal distribution range (4,000 km) along North America's coastline (Carriker and Gaffney, 1996), and the requirement of pile driving for bridge and wharf construction, it is possible that numerous oyster populations have been impacted over time. In contrast, however, there were no significant differences in valve activity levels during the 12 hours following sound exposure, suggesting no long-term behavioral impacts.

Alongside behavioral responses to sound intensities mimicking pile driving, we also observed significant reductions in glycogen content in oysters exposed to simulated pile driving sound. Glycogen content in bivalves is known to decrease in the presence of various other stressors as well. For example, Encomio and Chu (2000) reported that glycogen content was reduced in the adductor muscle of *C. virginica* with increased exposure to polycholobiphenyl (PCBs). Similarly, acute exposures to heavy metals such as $HgCl_2$ and $CdCl_2$ can reduce glycogen content in freshwater bivalves, *Lamellidens marginalis* (Sonawane and Sonawane, 2018). Increases in water temperature are also widely reported to affect glycogen content in bivalves (Andrade et al., 2018; Clements et al., 2018; Weber et al., 2020). Such reductions in glycogen content likely reflect the need for energy utilization to avoid stressful conditions. The reduction in glycogen may be associated with a " flight" response (McCarty, 2016) where oysters mobilized glucose molecules *via* glycogenolysis (Wright et al., 2008), perhaps in attempt to avoid physical stress caused by pile driving sound (Hegaret et al., 2007; Sampaio and Freire, 2016). While it may thus be tempting to associate the observed reductions in glycogen content with changes in valve gaping, we did not observe any correlation between individual glycogen content and either of our valve gaping responses (acute valve closures nor longer-tern valve activity levels). This lack of correlation is not totally unexpected given that bivalve adductor muscles are comprised of both smooth and striated muscle fibers, allowing for rapid and prolonged valve closures (either full or partial) without expending additional energy (i.e., "catch contractions"; Galler et al., 2010). It thus seems that the utilization of glycogen supplied energy to some other process involved in stress avoidance that we, unfortunately, did not measure. As such, a mechanistic understanding of sound-related changes in glycogen content awaits further research.

Although we observed significant reductions in glycogen content in response to pile driving sound, triglyceride content remained unaffected. This lack of effect on triglycerides is probably related to the acute nature of our experiments. For example, Vinagre et al. (2012), showed that it takes exposure to a stressor for more than 15 days in order to observe effects on triglyceride stores. In our study, the period of exposure to anthropogenic noise was twelve hours. Likewise, Plaistow et al. (2001) reported that physiological stresses of shorter duration will deplete glycogen reserves while prolonged stresses will draw on triglyceride reserves. While the lack of effect on triglycerides is not surprising, experiments with longer exposure times are needed to understand the chronic impacts of anthropogenic noise on coastal bivalves. Interestingly, the degree of valve opening following sound was only a fraction of the pre-sound valve opening, and this reduced valve gaping was evident for many minutes following exposure to simulated pile driving noise (e.g., Figure 2D). Coupled with the significant reduction in glycogen content, these results suggest that anthropogenic noise associated with pile driving sound may have broad-reaching effects on coastal bivalves. In oysters, stress can reduce the energy available for growth and reproduction. For example, Bøhle (1972) reported reduced filtration activity under stressful salinity conditions, which has been linked to reduced valve gape amplitudes under low salinity (Casas et al., 2018; but see Dodd et al., 2018 for contrasting results whereby stress does not reduce filtration). As such, behavioral and physiological responses to the pile driving sound herein have the potential to impact oyster growth rates and thus have implications for bivalve fisheries and aquaculture production. Bivalves also provide important ecosystem services such as water filtration, which could be affected in areas where pile driving, or exposure to various sounds are prevalent. Indeed, noise-driven changes in valve gaping behavior and gill function have been linked to depressed feeding and growth in Pacific oysters, *Magallana* (*Crassostrea*) *gigas*, during 14-day exposures (Charifi et al., 2018). However, it is important to note here that such effects remain speculative, as our experiment measured acute (<12 hours) responses to sound, and that of Charifi et al. (2018) was also short-term (14 days). Indeed, recent evidence suggests that bivalves may be able to adapt to repeated exposures to stress (Clements et al., 2021) and it is certainly possible that oysters are able to habituate to repeated sound for longer exposures, particularly under natural conditions. Indeed, we observed no effects of any sound treatment on activity levels 12 hours following the sound, suggesting that sound impacts on bivalve behavior may be restricted to acute

responses. Furthermore, as previously mentioned, exposure to anthropogenic sounds in our experiments was administered in the absence of natural coastal soundscapes, further complicating direct inferences to natural systems. Recent laboratory and field studies revealed that habitat-related sound can affect settlement in oyster larvae (Lillis et al., 2013). Field experiments testing effects of prolonged sound intensities similar to our pile driving treatment on oyster behavior, physiology, feeding, growth, and survival are ultimately needed to determine if the anthropogenic noises tested herein can have population- and community-level effects on oyster-associated systems. As with any experiment, our study is subject to limitations. Of particular note is that our sample size is low (6–10 individuals per sound treatment), and our results should be interpreted with some caution. To overcome this limitation, future studies should include more individuals. Nonetheless, the trends in the data are relevant and align with the results of other studies on anthropogenic noise and bivalves (Roberts et al., 2015; Vazzana et al., 2016; Charifi et al., 2017). Additionally, we are unable to determine if it is the intensity of sound, the frequency of sound, or both that resulted in valve gaping behavior changes with our data. Other studies have documented that blue mussels (Roberts et al., 2015) and Pacific oysters (Charifi et al., 2017) are sensitive to a wide range of sound frequencies. To the best of our knowledge, studies testing the effects of anthropogenic noise on bivalves have not mechanistically determined which attribute of sound (e.g., intensity vs. frequency) results in animal responses. Indeed, it is possible that different attributes of sound may drive responses of different biological traits. For example, it may be that the intensity of sound affects gaping behavior while the frequency or duration of sound affects physiology. While this mechanistic understanding is not possible from our data, future studies would thus benefit from teasing out which sound attributes drive bivalve responses to sound. On top of that, we only used one sound recording per sound treatment. In order to generalize these recordings to various noises with similar intensities and frequencies, it would require using multiple recordings from multiple sources for each treatment (e.g., multiple recordings from multiple boats). Future studies should try to use multiple recordings from multiple sources of the same sound treatment.

Over the past decade, noise pollution has been a topic of contemporary importance in the marine environment, including coastal areas habited by bivalves. Coupled with previous studies, the behavioral and physiological responses to sounds detected in this study suggest that Eastern oysters (*C. virginica*) may be sensitive to some, but not all, sounds created acutely by anthropogenic activity in coastal systems. As oysters play important economic and ecological roles in nearshore coastal communities, more studies regarding the effects of various noises on oysters in their natural environment are warranted. Studies including chronic effects on ecologically and economically critical traits such as growth, reproduction, and survival are needed to better understand the potential effects of anthropogenic noise on bivalve fisheries and aquaculture production, as well as the ecosystem services that bivalves provide.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval were not required for the study on animals (bivalve molluscs) in accordance with the legislation and requirements of the Canadian Council of Animal Care.

Author contributions

TL conceptualized, designed, and setup the experiments, analyzed data, and wrote and revised the manuscript. JC assisted with analyzing data and writing/revising the manuscript. LAC provided financial and supervisory support, assisted with conceptualizing and designing the experiment, experimental setup, data analysis, and revising the manuscript. GC realized and analyzed implementation of sound profiles. RT provided in kind support, assisted with conceptualizing and designing the experiment, set up and conducted the experiment, and revised the manuscript. FO and LC assisted with the design and implementation of sound profiles, and revised the manuscript. RB provided financial and supervisory support, and revised the manuscript. SL provided financial and supervisory support, assisted with conceptualizing and designing the experiment, assisted in data collection, and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Ecosystem Stressors Program's Operational Funds (Fisheries and Oceans Canada, Gulf Region) and a NSERC Discovery grant to S.G.L. (RGPIN-2019-05751).

Acknowledgments

We want to thank Michelle Maillet and Erica Watson at Fisheries and Oceans Canada, along with Viviane Baldwin, Loïck Ducros, and Chloé Melanson in Dr. Simon Lamarre's lab at Université de Moncton, for assistance with laboratory procedures. Thanks also to the team at ISMER for technical assistance with animal husbandry and sample processing. We thank Delphine Mathias (SOMME consulting company, Brest, France) for conducting the acoustic analysis. A special thanks to Dr. Rémi Sonier and Dr. Luke Poirier at Fisheries and Oceans Canada, for constructive feedback on earlier versions of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1104526/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 20 April 2023
DOI 10.3389/fmars.2023.1134792

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

RECEIVED 30 December 2022
ACCEPTED 24 March 2023
PUBLISHED 20 April 2023

CITATION
Kühn S, King F and Heubel K (2023) Decreased feeding rates of the copepod *Acartia tonsa* when exposed to playback harbor traffic noise.
Front. Mar. Sci. 10:1134792.
doi: 10.3389/fmars.2023.1134792

Decreased feeding rates of the copepod *Acartia tonsa* when exposed to playback harbor traffic noise

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Introduction: Copepods present the largest and most diverse group of zooplankton and their feeding behavior can affect top-down and bottom-up processes. Thus, how efficient feeding is executed determines the abundance of copepods' prey and their predators and, with that, carbon transfer and storage in ecosystems. The rise of anthropogenic underwater noise from shipping, oil exploration and exploitation, wind farm construction and operation, and more, is increasingly changing the marine acoustic environment. This acoustic pollution can have detrimental effects on biological life. Studies on this topic increasingly indicate that anthropogenic underwater noise adversely affects primary producers, marine mammals, fish, and invertebrates. However, little data exist on the effects of anthropogenic underwater noise on the feeding behavior of zooplankton.

Methods: Here, we investigated the ingestion and clearance rates of the copepod *Acartia tonsa* on a motile phytoplankton as a function of prey density under ambient aquarium sound conditions and, when exposed to playback, harbor traffic noise.

Results: We measured significantly decreased ingestion rates and clearance rates of *A. tonsa* when exposed to harbor noise compared to ambient conditions. The negative impact of noise on the ingestion rates was found at all given phytoplankton cell densities between 1k to 10k cells ml^{-1}. Clearance rates were fitted to the Rogers random predator equation which revealed significantly decreased capture rates on phytoplankton under the exposure of harbor noise while handling times remained the same in both sound treatments.

Discussion: Our results call for follow-up studies to focus on noise driven community-effects in field experiments to confirm laboratory results and to predict the outcome of a changing world with multiple stressors. Further, the underlying mechanism on how noise affects the feeding behavior of copepods is still unknown. Noise may distract copepods or mask hydromechanical cues of the prey. Noise may also adversely affect copepod physiology or morphology that would lead to changes in the feeding behavior. All potential mechanisms need to be investigated rigorously in future experiments.

KEYWORDS

underwater noise effects, continuous underwater noise, zooplankton, copepods, ingestion rates, *clearance rates*, predator–prey, functional response

Introduction

Research on the feeding ecology of key species is essential to predict human impacts on natural dynamics linked to trophic energy transfer within and between ecosystems. This information is crucial for the integration of strategies that lead to and protect a good environmental status of the marine environment (Directive 2008/56/EC). Crustacean zooplankton, especially copepods, are of exceptional importance due to their linkage between primary production and higher trophic levels. Hence, the magnitude of grazing and predation has direct effects on the community structure of phytoplankton and other planktonic animals as well as (in-) directly on bottom-up carbon transfer (Turner, 2015; Lynam et al., 2017; Steinberg and Landry, 2017). Human activities have led to climate warming and ocean acidification in combination with various pollutants that are continuously added to the oceans (Doney et al., 2012). Those environmental stressors have the potential to affect copepod species abundances and impede top-down and bottom-up planktonic food web structures (Garzke et al., 2016; Cole et al., 2019; Moreno et al., 2022).

In copepod feeding ecology, one pollutant has so far been overlooked even though it has become a major topic in science and politics (Directive 2008/56/EC; Duarte et al., 2021). The increase of anthropogenic underwater noise in marine ecosystems through construction work, energy exploration and exploitation, and ship traffic (Duarte et al., 2021; Jalkanen et al., 2022) is motivating studies to unravel the impacts of this acoustic pollution across sensory modality-based processes in a variety of marine animals (Halfwerk and Slabbekoorn, 2015). The reason for the growing attention is that noise-related effects may have the potential to change the composition of communities and, in turn, compromising essential ecosystem functions through masking and altering morphology, physiology, and behavioral processes in various taxa from primary producers, to small invertebrates to large marine mammals (Erbe et al., 2019; Murchy et al., 2020; Duarte et al., 2021; Solé et al., 2021a).

Shipping, as the main source of continuous underwater noise in the North Sea, can lead to an increase in noise levels of more than 30 dB above the natural ambient sound, especially in coastal areas (Farcas et al., 2020; Kinneging and Tougaard, 2021). Crustaceans, including copepods, produce sound (Tolstoganova, 2002; Jézéquel et al., 2018; Jézéquel et al., 2019; Kühn et al., 2022) and detect and react to hydromechanical disturbances perceived through sensory hair structures (Fields, 2014; Lenz and Hartline, 2014). This mechanoreception is a crucial sensory mechanism for copepod inter- and intraspecific interactions i.e. in feeding, mating, and predator avoidance (Yen and Strickler, 1996; Fields, 2014). In order for a copepod to perceive a fluid signal, these sensory structures, setae located on antennules, must be "moved" (10 nm bend; see Yen et al., 1992) remotely *via* vibrations and other fluid disturbances inducing "suspicious" fluid velocities and velocity gradients (Yen et al., 1992; Kiørboe et al., 1999) or, potentially, through strong pressure changes (Yen and Okubo, 2002). Some copepods are highly sensitive to vibration frequencies, from 40 Hz to 1 kHz (Yen et al., 1992), that fall in the frequency range of continuous underwater noise (10 Hz to > 10 kHz; Duarte et al., 2021). There is, to our best knowledge, however, no study that investigates the stimulus sound and how its different compounds are perceived by copepods.

Nevertheless, there is an increasing number of studies on the effects of noise on crustaceans: In benthic species, continuous underwater noise altered feeding, predator-avoidance, camouflage (Wale et al., 2013; Carter et al., 2020; Leiva et al., 2021), mating, and metabolic rates (Ruiz-Ruiz et al., 2020). Studies focused on crustacean meroplankton, showing no and negative effects of continuous noise on parameters related to swimming, development, and settlement (e.g. Pine et al., 2012; Sal Moyano et al., 2021). Previous studies on the effects of continuous anthropogenic underwater noise on marine crustacean holoplankton found significant physiological and morphological impacts (Solé et al., 2016; Tremblay et al., 2019; Solé et al., 2021b) from which only two investigated marine copepods (Tremblay et al., 2019; Solé et al., 2021b; see also review Vereide and Kühn, 2023). Further investigations of the effects of anthropogenic underwater noise on copepod behavior are therefore needed.

In the present study we experimentally tested the hypothesis that shipping noise alters the feeding response of the pelagic copepod, *Acartia tonsa*, on phytoplankton compared to ambient sound conditions. To do so, we investigated the effect of noise at different prey densities. In general, it is known that copepod feeding behavior depends on prey cell density (Frost, 1972), and the effect of environmental stressors varies between different prey densities (see van Dinh et al., 2019 for copepods and Fulfer and Menden-Deuer, 2021 for dinoflagellates). With common functional response equations (Holling, 1959; Rogers, 1972; Abrams, 2022), it is possible to investigate the effect of prey density and noise on the capture rates and handling times in copepods. The capture rate describes the rate at which the consumer encounters and detects prey items per unit of prey density. The handling time is the time spent to process the prey item (Holling, 1959; Rogers, 1972). These models predict that with increasing prey density, the probability of encountering and detecting a prey item is increasing, leading to a decrease in searching and detection time, while handling, feeding, and digestion remain the same (Holling, 1959). Little is known about the effect of underwater noise on copepod foraging efficiency and whether this would be density-dependent. Noise pollution may affect prey encounter, detection, and handling. We predict the effects of underwater noise on copepod feeding may be more pronounced at higher prey densities because of the increased number of prey encounters (Holling, 1959) on which noise can have an effect. In the present study, copepods were fed with green algae and exposed to playbacks of shipping noise from a harbor traffic underwater recording while a control group was incubated under ambient aquarium sound conditions. We quantified the effect of prey cell density on the ingestion and clearance rates in both groups at the end of the experiment. These results will be of relevance for discussions on the inclusion of noise in predictions on future zooplankton-based food web dynamics in a world with multiple stressors (Pirotta et al., 2022).

10.3389/fmars.2023.1134792

Methods

Study location

Feeding experiments were performed in the laboratory in September 2021 at the Research and Technology Centre West Coast (FTZ) in Büsum, Kiel University, Germany. Experimental copepods were caught in an artificially built lagoon in Büsum (54°08'01.78"N 8° 50'32.11"O) with access to the Wadden Sea but without tidal influences.

Experimental organisms

The pelagic copepod *Acartia tonsa* (0.5–1.5 mm length) can be found year-round in coastal and estuarine environments at high biomasses (Brylinski, 1981). *Acartia* sp. use mechanoreception for feeding (DeMott and Watson, 1991; Gonçalves and Kiørboe, 2015) and are sensitive to low-frequency vibrations (≤ 1000 Hz; Yen et al., 1992), which makes it, in addition to its trophic role in ecosystems, the optimal model species for the present noise study. As prey, we used small (< 20 µm) highly motile phytoplankton instead of micro-zooplankton, for instance, ciliates, to exclude potential effects of noise on the escape behavior of small prey animals. The chosen phytoplankton prey, *Tetraselmis chuii*, is a genus of green algae within the order Chlorodendrales, characterized by a flagellated cell body. Species of this genus are found in both marine and freshwater ecosystems around the world including the German Wadden Sea and are widely used in copepod feeding experiments (Thor et al., 2002; Scholz and Liebezeit, 2012). *T. chuii* were cultivated and provided by BlueBioTech GmbH, Büsum.

Collecting copepods

A. tonsa were caught with four light traps (see Kühn et al., 2022) deployed for 1–2 h during dark hours (21:00–23:00). The light traps were positioned at 20–50 m distance to the shore towards the center of the lagoon and positioned on the bottom (1.4 m depth). At this location, there is no direct input of underwater noise through boat traffic and pilot sampling showed a permanent high catching success of *A. tonsa* individuals. Animals caught in the traps were carefully poured into two cooling boxes where they were maintained (<2 h) until selected in the laboratory for experimental acclimatization. A new population sample of *A. tonsa* was caught for every experimental day. Additionally, the ambient sound of the lagoon was recorded with a SoundTrap-HF [sampling rate: 96 kHz; calibration Information; End-to-End: 176.3 dB (High); RTI Level @ 1kHz 135.4 dB re 1 µPa; Ocean Instruments, Auckland, New Zealand].

Feeding experiment

The caught copepods were brought to the laboratory and anesthetized with 15 g L^{-1} (sea water) magnesium chloride (adapted from Isinibilir et al., 2020). The animals stopped moving after 1–10 min (high individual variations, pers. obs. SK and FK) and were sorted into groups of roughly same-sized, copepodite, and adult *A. tonsa* stages, irrespective of sex, under a stereomicroscope (Stemi 508, Carl Zeiss Microscopy GmbH). Nauplii stages were excluded from the experiments. The number of *A. tonsa* in experimental grazing plastic vials (110 ml Kautex wide neck PET containers) varied slightly with prey density (Table 1) and availability (number of copepods caught in light traps also depended on weather conditions and fluctuations in local natural density). In addition, the number of copepods as grazers was determined to ensure a reduction of optimally 40–50% in phytoplankton cell concentration under ambient sound conditions conservatively enabling the detection of measurable effects, if any, that could go both directions, i.e. decreased or increased feeding rates. Seven to 19 A. *tonsa* individuals per experimental unit were put together (Table 1), in Eppendorf tubes filled with purified (filtered, UV-treated, and ozonized) North Sea water (provided by IMTE, Büsum). The copepods were checked after 30 min and only those that displayed normal swimming behavior were selected for the experimental runs. This was tested by looking for an increased jumping behavior when triggered with white light while being in the Eppendorf tubes to reduce handling stress. Dead or non-normal swimming copepods (< 5%, pers. obs.) were sorted out. Copepods selected for the experiment were then put in Eppendorf tubes without lids (13 ml) closed with a mesh net (5 µm) and acclimatized for 12 h in the experimental aquarium in the dark without food. The aquarium (100 * 50 * 30 cm) was filled with 130 L purified North Sea water (provided by IMTE) that was filtered continuously. Water temperature, oxygen, and salinity were kept constant at 18 ± 0.2 °C, 9.5 mg L^{-1} ± 0.1, and 36 mS cm^{-1} ± 0.1, respectively. An overview of sample preparation, experimental design, and work flow is depicted in Figure 1. The experiments took place in a shed located around 100 m away from the main building of the FTZ to exclude low-frequency building sound during the experiments. The experimental room was additionally equipped with sound absorption material (molded pulp egg-texture cartons) on the walls. Further, similar to a setup used by Amorim et al. (2013), we reduced the influence of ground vibrations by placing the aquarium onto a box (120 * 80 * 16 cm) that was filled with a combination of fine and coarse sand up to the top. A marble slate (120 * 80 * 3 cm) was placed onto that box with 10 equally

TABLE 1 Number of copepods in *grazer* vials per experimental phytoplankton cell density.

Cells ml^{-1}	Mean number of copepods in vial and ranges
1K	12.4 ± 0.3 (11–13)
3K	12.5 ± 0.4 (9–14)
5K	11 ± 0.7 (7–14)
8K	17 ± 0.6 (13–18)
10K	14.6 ± 0.9 (10–19)

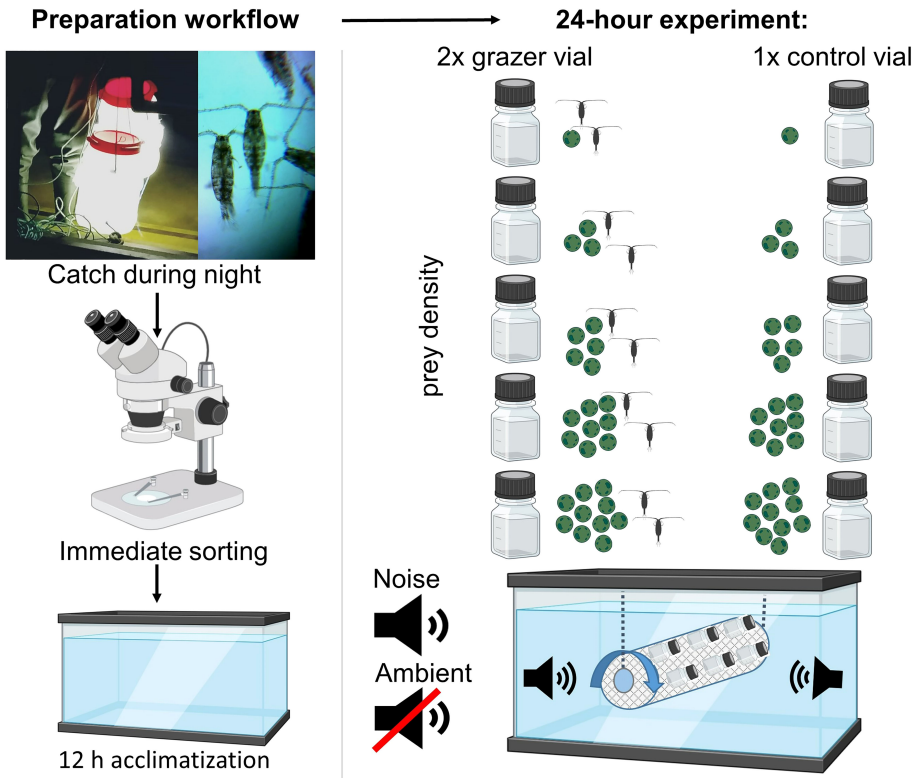

FIGURE 1
Overview Experimental Setup. Left column: preparation workflow. Capture of copepods *Acartia tonsa* using light traps at night. Immediate sorting under the stereoscope, and 12 h acclimatization in the dark. Right column: experimental design. Preparation of five different phytoplankton cell concentrations (1000–10000 cells ml^{-1}) using *Tetraselmis chuii* as prey and filled in experimental vials: *control* vials and *grazer* vials. Groups of copepods were then added to the *grazer* vials after Almeda et al. (2018). Both *control* and *grazer* vials with all different cell concentrations were then mounted to an underwater phytoplankton wheel and incubated for 24 hours exposed to either ambient sound or harbor noise playbacks. Partly created with BioRender.com.

distributed circular rubber studs (6 cm in diameter and 3 cm in height) on which the aquarium was placed. The whole setup was standing on a wagon with rubber wheels.

After acclimatization, we obtained the functional response curves by quantifying the ingestion and clearance rates through vial incubations. For this, *T. chuii* was filtered through a 50 µm plankton mesh net to remove cell aggregates. The experimental prey densities (1000, 3000, 5000, 8000, and 10000 cells ml^{-1}) were prepared by diluting the start prey cell density with purified seawater and amending it with 40 µl 110 ml^{-1} growth medium (provided by BlueBioTech GmbH) to avoid variations in phytoplankton growth between vials. For each phytoplankton cell concentration (prey density level) prepared, we also set aside and preserved vials for later confirmation of estimated *initial* cell concentrations while preparing the different prey density levels by adding 40 µl of Lugol solution (15 g KI + 500 ml dest H_2O + 10g I_2) (Almeda et al., 2018). Information on variations in the initial cell concentrations for the different densities can be found in Table S1 in the Supplementary Material. Grazer vials, each equipped with a group of copepods, were either exposed to ambient aquarium sound or to playback of harbor traffic noise. The days with runs alternated between ambient and noise treatments to ensure experimental copepods would have been exposed to similar environmental Wadden Sea conditions prior to being tested. Further, all five levels of different prey densities were tested simultaneously within a run. On each experimental day, one baseline *control* vial per prey density level was also included in the run to check for baseline phytoplankton mortality or growth in the absence of grazers during incubation. One to two experimental *grazer* vials on each experimental day were included in the incubation run to obtain the feeding rates of the copepods for all respective different prey density levels either exposed to ambient aquarium sound or to playback harbor traffic noise (Figure 1, Table 2). We consider each vial community as an experimental unit. Copepods are known to feed with abnormally high rates in the beginning of a feeding experiment because of starvation or handling stress (Mullin, 1963 and emphasized in Frost, 1972). Therefore, each run of baseline *control* vials and experimental *grazer* vials was incubated for 24 h. Additionally, we conducted the experiments in the dark to ensure constant nocturnal feeding conditions throughout the incubation (see Stearns, 1986). In all experimental *grazer* vials, copepods were allowed to graze down the suspensions without phytoplankton prey cell replacement.

We built a slowly rotating underwater plankton wheel for continuous phytoplankton mixing in the experimental vials. Pictures of the plankton wheel can be found in Figure S1 in the Supplementary Material. All experimental *control* and *grazer* vials were prepared with a window of 3.5 cm diameter fitted with a 5-µm

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TABLE 2 Overview experimental *grazer* vials per day and sound treatment.

Total Days	Noise	Ambient
8	4	4
Cell density		
1000	4 vials/ 2 days	4 vials/ 2 days
3000	7 vials/ 3 days	4 vials/ 2 days
5000	4 vials/ 2 days	6 vials/ 3 days
8000	4 vials/ 2 days	4 vials/ 2 days
10000	6 vials/ 3 days	8 vials/ 4 days

mesh net to ensure exchange with the surrounding aquarium water. The plankton wheel was made of a stainless-steel circular wire frame (35 cm length, 8.5 cm width, and 21 cm width with added vials) onto which a matrix of three by five stainless-steel baskets were mounted. This was done for easy mounting and removing of experimental vials onto and from the plankton wheel. To each of the five columns, one prey density level had been assigned while within each column, the baskets in rows (three rows per column) were randomly assigned to *control* and experimental *grazer* vials within respective prey densities. The wheel was placed in the middle of the aquarium's water column (4.5 cm of water below and above the vials) and was rotated by a synchronous AC 12 V Motor (CHANCS Motor, TYC-50; 1 rpm) connected to the plankton wheel *via* gear wheels and closed timing belts. An overview of the experimental setup and workflow is summarized in Figure 1 and Table 2.

Sound exposure

The described workflow for the feeding experiments was conducted under ambient aquarium sound conditions and under the exposure of playback harbor traffic noise (Figure 1). Harbor traffic noise was recorded at the Büsum port (54°07'20.63"N 8°51'32.67"E) on June 8, 2021. The underwater sound recordings were done with an AS-1 hydrophone (Nauta Scientific, Milano; (cross-calibrated at the FTZ) sensitivity: −211 dB re 1V/µPa) that were connected with a P48 hydrophone preamplifier (26 dB gain) to a Zoom H5 Handy recorder (gain=3). The whole setup was calibrated at 1 kHz (sensitivity of −188 dB re 1V/µPa). The harbor noise recording reflects a typical composition of harbor traffic with consecutive passings of a small shrimp trawler, a foot passenger ferry, a former larger trawler now used by an operator for guided nature trips, and a sailboat, with a total duration of 20 ' 11". The first boat passed after 1'36" for 38 s. The second boat passed at 8'40" (49 s). The third one passed from 14'34" to minute 15'14" (40 s) of the recording and Boat 4 passed at 17'42" (60 s) (Figure 2A). All boats on the recording (sampling rate: 48 kHz) were passing at a distance of 10–15 m from the hydrophone. The hydrophone was positioned ~10 cm off the harbor wall at 1 m depth. The original recording was played back in the experimental aquarium with two UW30 underwater speakers (Electro-Voice; frequency response 0.1–10 kHz) connected to a self-designed mobile waterproof audio-suitcase amplifier (MAK2x30/4, Bela P. Event-Studiotechnik) that was connected to a laptop. The original sound file was played *via* Audacity (Version 2.3.0). For comparison of actual harbor noise and its representation in the laboratory, we played back the harbor traffic noise file using the section capturing the first boat passing and recorded it in an experimental vial mounted onto the plankton wheel at a fixed position with a SoundTrap-HF (calibration Information see above; sampling rate: 96 kHz). Finally, the distance of the plankton wheel to the two UW30 speakers and the gain that was added to the original recording for the exposure were both decided upon the playback sound's similarity towards the original harbor recording. We analyzed the sound recordings in SpectraPlus-SC (V 5.3.0. 12A, Pioneer Hill Software LLC, Sequim, WA, USA) for frequency and power spectral density (PSD) characteristics visualized in spectra and spectrograms [FFT size: 16384 (for sampling rates of 48 kHz) and 32768 (for sampling rates of 96 kHz); Hanning window, 0.5 overlap]. For comparison, the root mean square (rms) power levels were calculated in SpectraPlus-SC from the PSD spectral data of the different sound recordings (Figure 2B). Based on analysis of original recordings and recordings of playbacks in the laboratory, we decided on the following sound exposure setup. One UW30 underwater speaker was placed on both sides along the plankton wheel with a distance of 15 cm to the experimental vials maintaining the noise exposure during the plankton wheel rotation on the right and on the left side. This recording of harbor noise was then played back in an infinite loop from the start to the end of each experimental exposure day *via* Audacity (Version 2.3.0).

The spectrogram of the measured original harbor noise recording (Figure 2A) and the spectra of the measured exposures in the aquarium versus harbor noise and ambient sound measured in the field (Figure 2B) are presented. To compare original recordings with its representation as experimental stimuli, Figure 2B shows the spectrum of the harbor noise at the time of the first boat passing (38 s) from the original harbor recordings (173 dB re 1 $\mu Pa^2\ Hz^{-1}$) in comparison to the recordings of the playbacks made in the vial in the aquarium (174 dB re 1 $\mu Pa^2\ Hz^{-1}$), the ambient aquarium sound treatment (155 dB re 1 $\mu Pa^2\ Hz^{-1}$), and the ambient sound in the artificial lagoon (144 dB re 1 $\mu Pa^2\ Hz^{-1}$). Note that ambient lagoon sound levels as recorded at the place of copepod origin are lower than ambient sound conditions in the laboratory that included potential noise from the water filter and the plankton wheel motor noise (Figure 2B). For boat noise in the original and in the playback recording, most energy was found between 100 and 2000 Hz. We are fully aware of the fact that the playback is not reflecting true harbor noise with shipping traffic found in the field. In an aquarium setup, the experimental sound exposures will divert from the real frequency-sound level distribution due to aquarium wall reflection, aquarium vibration, and the size of the aquarium that does not allow full soundwave cycles for low frequencies. Further limitations of the acoustic setup are pointed out in the discussion.

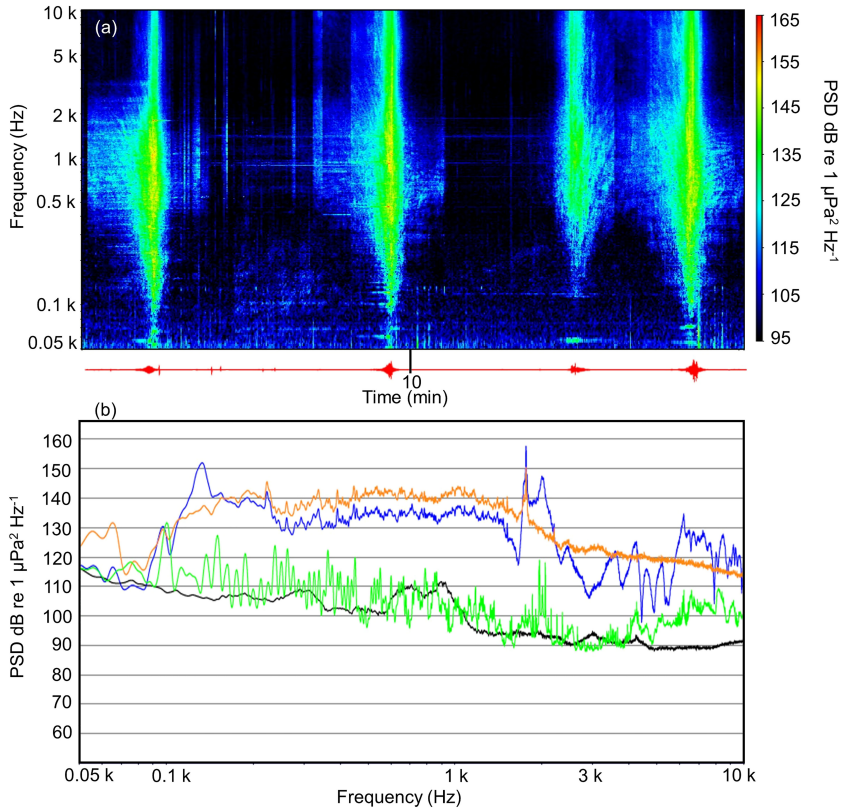

FIGURE 2
Sound exposure. **(A)** Spectrogram from the original harbor traffic noise recording. The left y-axis shows the frequency in Hertz. The right y-axis presents the color code for the power spectral density (dB re 1 µ Pa^2 Hz^{-1}). The x-axis presents the recording as time series in minutes (red line). **(B)** Spectrum of the first boat (see **(A)** 1'36" to 2'14") passing the hydrophone in the original recording (orange line) and the exposure sound measured in the aquarium in the experimental beaker (blue line). The green line shows the ambient aquarium sound spectrum. The black line represents the ambient field sound from the copepod catching site. All samples had a length of 38 s. The figures are presented in a Hanning window (50% overlap), frequency resolution is 3 Hz, visualized in SpectraPlus-SC (V 5.3.0. 12A).

After the incubation

The feeding incubation was ended after 24 hours (except one case that ended after 28 h) by gently taking out each experimental vial from the aquarium plankton wheel. Each *grazer* vial was then visually checked for swimming activity. Here, individuals were noted as "not active" or "active". A copepod was assigned and noted "not active" when there was no movement or only sinking after a few seconds when triggered with white light. Lugol solution 40 µl (15 g KI + 500 ml dest H_2O + 10 g I_2) was then added to the baseline *control* and *grazer* vial to preserve copepods and phytoplankton before taking out the next one. The plankton wheel was set on hold, and for the noise treatment, the playback was stopped only after all vials were taken out, preserved, and stored in a cooling box. In the laboratory, we counted the *initial*, baseline *control* and *grazer* prey cell concentration using a Fuchs-Rosenthal counting chamber (area: 16 mm^2, depth: 0.2 mm, volume: 3.2 µl) under microscopes (100x; Zeiss Axioscope and Leitz Aristoplan). After counting, copepods were removed from the vial and their prosome length (µm) was measured under a microscope using a calibrated Moticam X3 Plus laboratory camera (Software Motic Images Plus Version 3.0.19.108b) attached to a microscope (50 x; Leitz Aristoplan). Sizes were only noted for intact (not damaged) animals. Clearance rates (volume swept clear from prey cells in ml copepod^{-1} h^{-1}) and ingestion rates (ingested prey cells h^{-1} copepod^{-1}) were calculated after Frost (1972).

Data and statistical analysis

Descriptive statistics are presented as mean ± standard error if not stated otherwise. The cell concentrations between 1K and 10K cells ml^{-1} were decided on being treated as a categorical fixed factor with five levels of prey cell concentrations (PC). However, choosing a regression model with cell concentrations as a continuous variable led qualitatively to the same results.

Statistics were performed in R version 3.4.4 (R Core Team, 2022). Figures for data presentation are based on the package ggplot2 (Wickham, 2016).

Mixed-effect model for ingestion rates

A linear mixed effect model (function *lmer* in package *lme4*; Bates et al., 2015) was fitted on the obtained data based on results per experimental vial community. We decided on using a mixed

model with varying intercept per *day* to account for potential variation in the copepods that were caught daily from a certain population in the field. Hence, we included the ingestion rate of *A. tonsa* as the response variable and included the fixed effects of underwater noise treatment (A.N., noise *vs.* ambient), prey cell concentration (PC, five levels), mean body size per vial (MS, centered), number of copepods per vial (D, integer), and the number of animals "not active" after the incubation (S, integer). In addition to these fixed effects and continuous variables, we included *day* as a random factor to account for differences in general conditions among different experimental runs. We stepwise selected the optimal model with all reasonable combinations of the fixed variables (see Zuur et al., 2009) based on the lowest Akaike's information criterion (AIC) with the fewest parameters (ΔAIC < 2; Burnham and Anderson, 2002). Note that *p*-values were computed using Kenward-Roger standard errors and *df* (package: jtools, Long, 2022).

Clearance rates feeding response curves

Clearance rates were fitted to the Rogers random predator model as it accounts for prey depletion over time (Rogers, 1972). For this, we used the *gnls* function in R (package *nlme*; Pinheiro et al., 2022), in order to get the coefficient estimates for the ambient sound and harbor traffic noise treatment, and integrated the following equation:

$$Ne = N0(1 - e^{a(Ne*h-T)})$$

Ne is the number of prey items eaten, *N0* is the initial number of prey, *a* is the capture rate, and *h* and *T* are handling time and the incubation time, respectively. In order to receive the estimates for the clearance rates' coefficients *a* and *h*, this equation was divided by the initial number of prey (*N0*) (similar to Hollings disk equation calculations in Schultz and Kiørboe, 2009). Note that the number of prey items eaten (*Ne*) is found on both sides of this equation which is solved by applying the Lambert W function (Bolker, 2008).

Results

In total, 51 experimental runs – 26 ambient and 25 noise treatment vial communities – were analyzed (Table 2). A total of 688 copepods were either exposed to boat noise playbacks (343 copepods) or to ambient aquarium sound (345 copepods). The mean length of all measurable (see methods) *A. tonsa* was 647 µm ± 2 µm (n = 602) and width was 204 µm ± 3 µm (n = 235). For within-vial length – width size distributions see Table S2 in the Supplementary Material.

The prey algae *Tetraselmis chuii* ranged in length from 8 µm to 16 µm (normal distributed; mean = 12 µm ± 0.2; *n* = 80) and in width from 7 µm to 12 µm (normal distributed; mean = 9 µm ± 0.1; *n* = 80). In the control vials, which maintained prey algae without copepods, there was no significant difference in growth or mortality between incubation in ambient and noise treatments (Welch's *t*-test, *n* = 26, *df* = 23.97, *t* = −0.49, *p* = 0.631).

Feeding rates

The experimental results on the copepods' ingestion rates (y-axis) at increasing prey densities from 1000 to 10000 cells ml^{-1} (x-axis) when exposed to ambient aquarium sound and playback harbor traffic noise are shown in Figure 3. Mean ingestion rates decreased by 48%, 24%, 64%, 48% and 29% at prey densities of 1000, 3000, 5000, 8000, and 10000 cells ml^{-1}, respectively, when exposed to playback harbor traffic noise compared to ambient sound conditions. In both sound treatments, ingestion rates increased with increasing prey density (Figure 3, Table 3). The optimal AIC-based model structure that describes the ingestion rates pattern is shown in Table 4 in bold ($n_{\text{each model}}$ = 49, *df* = 8, AIC = 681, *delta* = 0.36). It was found that a combination, not an interaction, of the fixed variables *sound treatment* (A.N.) and *density of prey cells ml^{-1}* (PC) described the obtained data from the feeding experiments best. Mean copepod lengths per vial (MS), the density of copepods per vial (D), and the number of animals "not active" in the end of the experiments (S) were not included in the

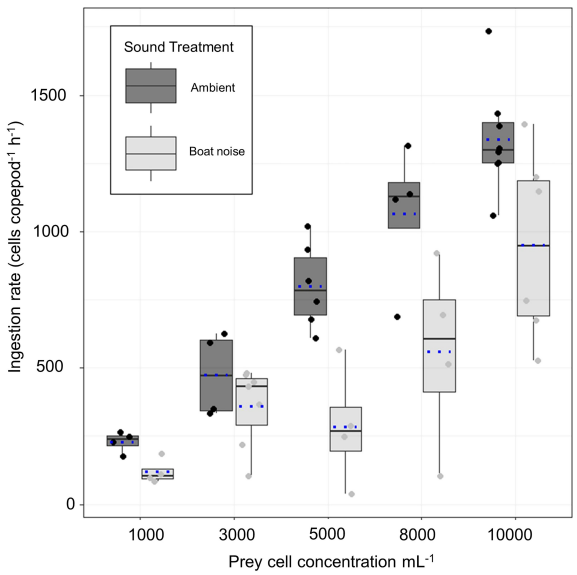

FIGURE 3
Ingestion rates under ambient aquarium sound and harbor noise conditions. The x-axis shows the initial phytoplankton prey cell concentrations from the categories 1000 to 10000 cells ml^{-1}. The y-axis presents the ingestion rates that are defined as the number of phytoplankton cells consumed per individual copepod per hour (cells copepod^{-1} h^{-1}) calculated after Frost (1972). The boxplots are drawn from the first to the third quartile with a black horizontal line denoting the median and a blue dashed line denoting the respective mean. The whiskers of the plots reaching to the lowest and highest values that is within 1.5 interquartile range. The jittered dots are presenting all values in the data set. Box plots shaded in grey and corresponding dots represent ingestion rates obtained under ambient sound conditions, while light grey colored boxes show ingestion rates when exposed to underwater harbor noise.

TABLE 3 Overview descriptive statistics of the calculated ingestion rates (cells copepod^{-1} h^{-1}) after Frost (1972).

Cells ml^{-1}	Treatment	n Vials	Mean ± se	Median
1000	A	4	230 ± 19	238
	N	4	119 ± 23	104
3000	A	4	475 ± 78	470
	N	7	361 ± 55	433
5000	A	6	800 ± 64	781
	N	4	285 ± 109	268
8000	A	4	1065 ± 134	1128
	N	4	558 ± 173	605
10000	A	8	1339 ± 69	1298
	N	6	949 ± 141	948

A, ambient sound; N, Noise treatment.

optimal model. The optimal model (R^2 marginal = 0.76 and R^2 conditional = 0.77) shows that increasing prey densities (PC) from 1000, over 3000, 5000, 8000, to 10000 cells ml^{-1}, significantly increased copepod ingestion rates (Table 5, Figure 3). Specifically looking into the sound treatments, the model estimated a significant decrease by 330 ingested cells hour^{-1} copepod^{-1} when exposed to harbor noise compared to ambient aquarium sound conditions (LMM, n = 51, df = 3.92, t = −4.26, p < 0.010).

Figure 4 shows the clearance rates with increasing prey density. For the ambient sound conditions, the estimated capture rate a was 0.013, which significantly decreased by 0.008 under the exposure of harbor traffic noise (GNLS; n = 51, df = 47, t = −3.7, p < 0.001). There was no significant difference in handling time h between ambient (estimate 0.005) and harbor noise conditions (GNLS; n = 51, df = 47, t = −0.12, p = 0.9).

TABLE 4 Model selection AIC ranking table.

Rank	Model	*df*	logLik	AIC	delta	Weight
1	~ A.N.*PC	12	−328.324	680.6	0.00	0.337
2	**~ A.N.+PC**	**8**	**−332.505**	**681.0**	**0.36**	**0.281**
3	~ A.N.*PC+MS	13	−328.248	682.5	1.85	0.134
4	~ A.N.+PC+MS	9	−332.455	682.9	2.26	0.109
5	~ A.N.*PC+MS+D	14	−328.197	684.4	3.75	0.052
6	~ A.N.+PC+MS+D	10	−332.440	684.9	4.23	0.041
7	~ A.N.*PC*MS	22	−320.733	685.5	4.82	0.030
8	~ A.N.*PC*MS+D	23	−320.714	687.4	6.78	0.011
9	~ A.N*PC+MS+S	20	−325.048	690.1	9.45	0.003
10	~ A.N.*PC+MS+D+S	21	−325.039	692.1	11.43	0.001
11	~ A.N.+PC+MS+S	16	−330.070	692.1	11.49	0.001
12	~ A.N.*PC*MS+S	29	−317.819	693.6	12.99	0.001
13	~ A.N.+PC+MS+D+S	17	−330.039	694.1	13.43	0.000
14	*~ A.N.*PC*MS+D+S*	*30*	*−317.771*	*695.5*	*14.90*	*0.000*

Number of observations per model = 49 (out of 51, due to no mean copepod size values for two vials, see Table S2 in the Supplementary Material). Displayed are all considered models with different variable combinations that may describe the observed pattern in ingestion rates. Here, the response variable is ingestion rate. Independent variables and their abbreviations are: Treatment (ambient and noise) = A.N.; Prey cells ml^{-1} = PC; Mean Copepod Size Vial^{-1} = MS; Density (the number of copepods in each grazer vial) = D; "not active" (Number of copepods that did not display normal jumping behavior when triggered with white light directly after the feeding incubation) = S. * denotes additive and interaction term in statistical model. Columns: *df* = degrees of freedom; logLik = log-likelihood (goodness of fit of the model); AIC = Akaike information criterion (prediction error of a model); delta = change in values from model with lowest AIC. Weight = prediction power of each model. A random intercept was included for day in all models to account for variation among daily runs. The selected best model is highlighted in bold (rank = 2). The best model is the one with the fewest df of all model that have a delta < 2. The null model is marked in italics.

TABLE 5 Final optimal model summary of estimated noise treatment and prey density effects on copepod ingestion rates.

	Est.	*t*-Value	*df*	*p*-Value*
(Intercept)	338.33	3.62	19.60	< 0.0001
Harbor noise	−336.20	−4.26	3.92	< 0.01
3000 cells	270.23	2.6	41.21	< 0.01
5000 cells	393.26	3.62	44.86	< 0.0001
8000 cells	637.03	5.77	40.21	< 0.0001
10000 cells	977.68	9.65	44.65	< 0.0001

Number of observations = 51. R^2 marginal = 0.76 and R^2 conditional = 0.77. *p*-Values calculated using Kenward-Roger standard errors and df (package: jtools, function, summ). * denotes statistical significance ($p < 0.05$).

Discussion

We present novel data on the impact of anthropogenic underwater noise on the feeding rates of the crustacean zooplankton *Acartia tonsa* when fed with small phytoplankton. Overall, we found a common pattern for ingestion rates as a function of prey density (Frost, 1972; Almeda et al., 2018) in both sound treatments. However, with a mean decrease of 40%, the animals in added playback harbor noise conditions failed to ingest every second to third prey they would have ingested under ambient sound conditions. In terms of inference, one may rather want to better compare a playback harbor traffic playback treatment versus a treatment playing back ambient lagoon sound. However, despite taking all measures to limit disturbing noise in the setup, in a controlled laboratory setup, operating background noise running the plankton wheel would be higher than any realistically playback of ambient lagoon sound as a silent treatment (Figure 2B). Therefore, we consider our choice of comparing a treatment with added harbor traffic noise playback to ambient aquarium sound conditions showing a similar effect as if there was an underlying playback of silent lagoon recordings added to the ambient sound control. We predicted prey density-dependent effects of noise would be more pronounced and easier to detect at high prey densities. Although we did not find this interaction between prey density and noise exposure, we found an increased dispersion in the data at higher prey density (cf. Table 3, Figure 3). This might be due to variations and plasticity in individual behavioral responses (Liu et al., 2018; Holm et al., 2019) or due to small differences in sensory structures (Yen et al., 1992; Fields, 2014). van Dinh et al. (2019) found a combined effect of prey density and pyrene, especially at high prey densities. They hypothesized that this might be due to the narcotic effect of pyrene that affects the handling time of the copepod *Calanus finmarchicus*. They calculated lower searching rates and longer handling times, which explains the increased effect at high prey density (van Dinh et al., 2019). In contrast, we calculated a lower capture rate in the noise treatment, while handling time remained the same between the two sound treatments. It therefore appeared that the capture rate of copepods in particular was affected by noise in our study.

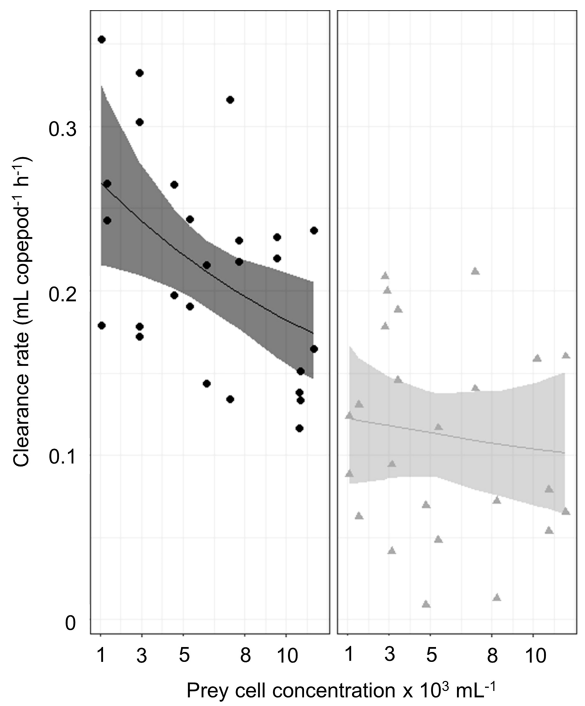

FIGURE 4
Clearance rates under ambient sound and harbor noise conditions fitted to the Rogers random predator equation. The x-axis shows the initial phytoplankton prey cell concentrations (continuous variable) from min 1042 to max 11563 cells ml^{-1}. The y-axis presents the clearance rate that is defined as the volume of water from which cells are removed by feeding copepods (ml $copepod^{-1}$ h^{-1}) calculated after Frost (1972). The left panel shows the clearance rate under ambient aquarium sound conditions and the right panel the clearance rate under playback harbor noise exposure. The jittered dots are presenting all values in the data. The 97.5% confidence intervals were calculated using a bootstrap method in R version 3.4.4 (R Core Team, 2022).

Mechanisms of how underwater noise could affect capture rates

One of the mechanisms at hand would be masking or distraction. It is suggested that noise can mask essential natural sound cues for invertebrate settlement (as found in other meroplanktonic species Pine et al., 2012). Copepods detect prey through visual, chemical and mechanical signals (Fields, 2014), and the magnitude of importance for each detection mechanism is

feeding mode- and species-specific (see Jakobsen et al., 2005; Fields, 2014), although mechanoreception may be the most common means of perception in different feeding modes (DeMott and Watson, 1991; Gonçalves and Kiørboe, 2015). *Acartia tonsa* is able to switch between feeding modes and prey types depending on the prey size (Jonsson and Tiselius, 1990), prey motility (Kiørboe et al., 1996), prey density (Kiørboe et al., 1996), and external disturbances such as turbulences (Saiz and Kiorboe, 1995; Kiørboe et al., 1996; Strickler and Costello, 1996). In ambush-feeding mode, copepods wait motionless in the water column until they perceive a hydromechanical signal generated by a potential prey, then reorientate themselves towards the prey, and attack it by directional jumps (Tiselius and Jonsson, 1990; Saiz and Kiorboe, 1995). On the other hand, feeding on small non-motile prey involves the generation of a feeding current with feeding appendages and thoracopods (Tiselius and Jonsson, 1990; Gonçalves and Kiørboe, 2015). Note that, in our current study, we cannot differentiate which feeding mode had been used. Saiz and Kiorboe (1995) studied the effect of turbulent water on the two feeding modes of *A. tonsa* and found only decreased clearance rates when exposed to turbulences exceeding natural turbulence levels in the field probably due to impaired prey perception (ambush feeding) and eroded feeding currents (suspension feeding). Even though turbulence and sound are not directly comparable, high levels of underwater noise could impair similar mechanisms as turbulences.

The detection of the potential prey, in general, depends on the strength of its velocity difference to the ambient to elicit a behavioral response of the copepod. In the copepods *Labidocera madurae* and *Acartia fossae* a velocity strength of only 20 μm s^{-1} in the vibration frequency range from 40 to 1000 Hz is sufficient to trigger antennal neuroreceptors to fire (Yen et al., 1992) and a study on the escape response of *A. tonsa* has shown a threshold signal strength or velocity difference for deformation at 150 μm s^{-1} and accelerations as low as 130 μm s^{-2} in the near field of a siphon flow (Kiørboe et al., 1999). Due to the high sensitivity of copepods to fluid disturbances, the harbor noise exposure used in the present study may have been above detection thresholds, which could have led to masking or distraction, but further measurements of particle motion velocities in an aquarium setup are needed to test this.

Our results are inconsistent with a feeding experiment by Tremblay et al. (2019) in which *A. tonsa* was exposed to a noise egg, a waterproof device that produces low-frequency sound (de Jong et al., 2017). Nevertheless, they found a physiological response correlated with oxidative stress (Tremblay et al., 2019). Hydromechanical disturbances from different prey types are sensed by mechanoreceptive setae on the first antenna of calanoid copepods (Yen et al., 1992; Gonçalves and Kiørboe, 2015), such as *Acartia* sp. Solé et al. (2021b) performed an ultrastructural analysis of the setae on the first antenna of the ectoparasitic copepod species *Lepeophtheirus salmonis*, which uses mechanoreception similar to calanoids, but for host detection. They found that the setae had fused when *L. salmonis* was exposed to noise for 4 h. Maximum setae fusion occurred when exposed to a combination of 350 Hz and 500 Hz sound (cf. Figure 2). The mechanism was hypothesized to be related to oxidative stress, possibly followed by acoustic trauma. Similar mechanisms may also occur in *A. tonsa* upon exposure to harbor noise, where setae fusion may lead to impaired prey perception. However, the noise frequency ranges that affect *A. tonsa* most may be different from those of *L. salmonis*.

Whether the impact of noise on the ingestion rates of copepods feeding on phytoplankton is due to masking the hydromechanical signals of potential prey or distraction or related to physiological or morphological changes remains open. The magnitude and direction of responses in zooplankton when exposed to underwater noise is further most probably species- and stage-specific and depends on the sound source level and experimental design (Vereide and Kühn, 2023; Tremblay et al., 2019; Solé et al., 2021b). For future studies, we suggest a combination of empirical and modeling approaches investigating how noise impacts feeding in different copepod species, sexes, and stages.

Acoustic setup design

We were limited to measure only the sound pressure part of the harbor traffic exposure even though particle motion is known being detected by invertebrates rather than sound pressure (Nedelec et al., 2016). Nedelec et al. (2015) and Simpson et al. (2016) measured 20–40 dB (μm s^{-2})^2 Hz^{-1} higher particle acceleration in laboratory tank experiments compared to *in situ* recordings while sound pressure levels measured in these tanks were similar to the *in situ* recordings. We therefore may underestimate the true exposure in terms of particle motion. Further we would like to address that copepods most probably perceive velocity rather than acceleration (Kiørboe et al., 1999) which should be considered when reporting particle motion in sound-related future work on copepods. Playback of harbor traffic noise is partly distorted in small tanks from the original recordings (see Akamatsu et al., 2002; Jones et al., 2019) as seen in Figure 2B. However, at higher frequencies (>1000 Hz), such distortions may be less biologically relevant due to copepod sensitivity to lower frequencies (Yen et al., 1992; Solé et al., 2021b). Further, the continuous rotation of the plankton wheel during incubation imposes some variation onto the vials housing the experimental copepod communities per day, potentially leading to random noise exposures instead of the regular exposure from the looped playback. Differences in noise regularity, from e.g. ship traffic versus operational wind turbine noise, may lead to different behavioral outcomes (Nedelec et al., 2015).

Anthropogenic underwater noise effects on trophic cascades from a zooplankton perspective

We consider our results to be realistic for near-field shipping noise levels, for example in ports or along shipping lanes, servicing e.g. offshore wind farms or the oil industry and construction work.

Ingestion rates are known to be linearly correlated to egg production in *A. tonsa* and other calanoid copepods species (Kiørboe et al., 1985). Further, copepod growth is limited by food quantity (Anderson et al., 2021). Food quantity, in this case phytoplankton density, is known to vary throughout the year and being site- and species-specific. In general, phytoplankton densities ranging from low ($\times 10^3$ l^{-1}) to high abundances ($\times 10^6$ l^{-1}) especially during spring blooms (Lefebvre et al., 2011; Alprol et al., 2021). Our results on ingestion rates at different prey densities are therefore representative for natural phytoplankton abundances. Reduced feeding due to anthropogenic underwater noise at all phytoplankton prey densities, as presented in this study, may thus lead to decreased egg production, limited growth and development, and, in turn would lead to lower abundances of certain copepod species. Thus, the decrease of certain copepod species affects both the interactions to lower levels, e.g. phytoplankton and smaller zooplankton, as well as to higher trophic levels and thus may alter the fate of organic carbon's transfer through the food chain (Steinberg and Landry, 2017) and its storage (Turner, 2015).

Our study did not consider ontogenetic and developmental aspects and sex differences. It is known that older copepod developmental stages are more mechanoreception-sensitive compared to younger developmental stages (Fields and Yen, 1997; Kiørboe et al., 1999) and hence adult animals may be more vulnerable to underwater noise. The effects of continuous noise on different developmental stages in copepods should be further investigated like done in a previous study on the effects of impulsive underwater noise on *A. tonsa* (Vereide et al., 2023). Further, differences in feeding efficiencies between female and male copepods are known but this difference is mainly explained by body size (van Someren Gréve et al., 2017), which was included in the statistical analysis of our study our study. Note that if noise affects the detection and response to hydrodynamic signals from prey, it could also alter the perception of fluid signals from potential mates and predators (Fields, 2014), thus affecting community dynamics.

At this point we cannot extrapolate from the feeding response of a single species on a single prey species when exposed to a single stressor to whole community-level dynamics. Our results, however, underline the need to further investigate the consequences of anthropogenic underwater noise on zooplankton.

In conclusion, we found that elevated noise levels similar to those measured the North Sea (Farcas et al., 2020; Kinneging and Tougaard, 2021) impair copepod feeding. However, noise exposure should be further investigated in the field to disentangle the potential effect of real-life shipping noise from playback noise in small tanks.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SK conceptualized the project. SK, FK, and KH designed the research. SK and FK conducted the experiments. SK analyzed the data. SK wrote the initial draft of the manuscript. SK, FK, and KH wrote the paper. KH supervised and acquired funding and resources. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the Federal State Funding at Kiel University and the JPI Oceans Noise project ORCHESTRA (03F0932B). We acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds.

Acknowledgments

We would like to thank the staff at the Research and Technology Centre West Coast as well as BlueBioTec, especially Dr. Daniela Martensen-Staginnus, and IMTE (all Büsum, Germany) for supporting the present study. Special thanks to Dawid Jasecki for his support constructing the underwater plankton wheel.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1134792/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 25 April 2023
DOI 10.3389/fmars.2023.1058414

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Marine Biology, a section of the journal Frontiers in Marine Science

RECEIVED 30 September 2022
ACCEPTED 10 March 2023
PUBLISHED 25 April 2023

CITATION
McCloskey KP, Radford AN, Rose A, Casiraghi G, Lubbock N, Weschke E, Titus BM, Exton DA and Simpson SD (2023) SCUBA noise alters community structure and cooperation at Pederson's cleaner shrimp cleaning stations. *Front. Mar. Sci.* 10:1058414. doi: 10.3389/fmars.2023.1058414

SCUBA noise alters community structure and cooperation at Pederson's cleaner shrimp cleaning stations

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Recreational SCUBA diving is widespread and increasing on coral reefs worldwide. Standard open-circuit SCUBA equipment is inherently noisy and, by seeking out areas of high biodiversity, divers inadvertently expose reef communities to an intrusive source of anthropogenic noise. Currently, little is known about SCUBA noise as an acoustic stressor, and there is a general lack of empirical evidence on community-level impacts of anthropogenic noise on coral reefs. Here, we conducted a playback experiment on Caribbean reefs to investigate impacts of SCUBA noise on fish communities and interspecific cooperation at ecologically important cleaning stations of the Pederson's cleaner shrimp *Ancylomenes pedersoni*. When exposed to SCUBA-noise playback, the total occurrence of fishes at the cleaning stations decreased by 7%, and the community and cleaning clientele compositions were significantly altered, with 27% and 25% of monitored species being affected, respectively. Compared with ambient-sound playback, SCUBA-noise playback resulted in clients having to wait 29% longer for cleaning initiation and receiving 43% less cleaning; however, cheating, signalling, posing and time spent cleaning were not affected by SCUBA-noise playback. Our study is the first to demonstrate experimentally that SCUBA noise can have at least some negative impacts on reef organisms, confirming it as an ecologically relevant pollutant. Moreover, by establishing acoustic disturbance as a likely mechanism for known impacts of diver presence on reef animals, we also identify a potential avenue for mitigation in these valuable ecosystems.

KEYWORDS

anthropogenic noise, SCUBA, community-level impacts, cleaning mutualism, coral reefs, marine invertebrates, reef fishes, interspecific behaviour

1 Introduction

SCUBA diving is a multibillion-dollar industry and is one of the largest and fastest growing recreational sports globally, with over 28 million certified divers and one million new divers being certified annually (Lück, 2016; PADI, 2021). Because divers seek out areas of high biodiversity, and many reef organisms are small and visibility rarely exceeds 30 m, divers often move close to habitat and site-attached animals, meaning that this popular pastime can have negative impacts on coral reefs (Davenport and Davenport, 2006). SCUBA divers can cause physical damage to reef habitat (Hawkins and Roberts, 1993; Zakai and Chadwick-Furman, 2002; Giglio et al., 2020), but the mere presence of divers can also elicit stress and behavioural changes in marine mammals, fishes and invertebrates, thus affecting aquatic communities and disrupting ecosystem services (Curtin and Garrod, 2008; Lindfield et al., 2014; Titus et al., 2015a; Giglio et al., 2022). However, the mechanisms underpinning these detrimental diver-presence effects have not been established. Given that standard open-circuit SCUBA equipment is inherently noisy (Lobel, 2005; Radford et al., 2005), acoustic disturbance is a plausible but untested reason for organismal responses to diver presence.

Anthropogenic noise from a wide range of sources (e.g., pile-driving, sonar, shipping, motorboats) pervades almost all aquatic ecosystems (Duarte et al., 2021), with increasing evidence demonstrating a suite of negative impacts across many taxa (see reviews: Shannon et al., 2016; Cox et al., 2018; Kunc and Schmidt, 2019; Duarte et al., 2021). However, most of the research to date focuses on how underwater noise affects individual animals; there has been little investigation of how noise effects scale up to interspecific interactions and community compositions (Kunc and Schmidt, 2019). For example, only one aquatic study that we know of has considered community-level demographics (Nedelec et al., 2017), and only a small handful of studies have demonstrated that noise can alter interspecific relationships among fishes, such as predator–prey interactions (Simpson et al., 2016; Ferrari et al., 2018) and cooperative mutualisms (Nedelec et al., 2017).

While there is a paucity of investigations into community-level responses to noise in aquatic ecosystems, terrestrial anthropogenic noise (e.g., traffic noise near roads) has been shown to have a range of effects on avian communities, including to abundance, species richness and community structure (Francis et al., 2009; Slabbekoorn and Halfwerk, 2009; Herrera-Montes and Aide, 2011; Cooke et al., 2020). That body of work includes experimental application of traffic noise (a 'phantom road') to a roadless landscape, identifying noise as the principal mechanism for the negative impacts of roads on avian populations and communities (McClure et al., 2013, Ware et al., 2015; McClure et al., 2017). Whilst early studies suggested overall population reductions in response to road traffic (Reijnen and Foppen, 1994; Reijnen et al., 1995), more recent investigations show that community-level changes can be more complex, as species can respond differently to noise (Cooke et al., 2020; Senzaki et al., 2020). Applying this foundational knowledge (i.e., acoustic stressors driving community-level responses) to aquatic ecosystems, noise might underpin previously documented impacts of diver presence on coral reefs. For example, the presence of divers has been shown to affect coral reef fishes (Benevides et al., 2019; Branconi et al., 2019; Giglio et al., 2022) and fish communities, including species-specific changes to diversity and abundance (Lindfield et al., 2014; Andradi-Brown et al., 2017); in these studies, SCUBA noise was suggested as a potential contributing factor but was not evaluated experimentally in isolation.

To investigate impacts of SCUBA noise on coral reefs, we focused on ecologically important cleaning stations, considering potential changes to the local community composition and disruption to cooperative interactions between cleaners and clients. Mutualistic services play an integral part in the complex web of interactions that help maintain ecosystem health and function (Grutter et al., 2003; Clague et al., 2011; Waldie et al., 2011). On coral reefs, cleaning symbioses are iconic interspecific mutualisms between cleaners, such as gobies, wrasse and shrimp, and a diverse range of client fishes (Grutter, 1999; Becker and Grutter, 2004; Vaughan et al., 2017). These complex and highly developed associations positively impact the health of individual fishes and influence community-wide diversity (McCammon et al., 2010; Clague et al., 2011; Waldie et al., 2011). Furthermore, cleaner species are thought to modify movement patterns, habitat choice, activity and local abundance of reef fishes (Grutter et al., 2003), and may also play a role in determining the distribution of territorial fishes (Whiteman et al., 2002). Typically, a cleaner species will occupy discrete microhabitats that serve as cleaning stations and are visited by clients. During cleaning interactions, client fish will pose motionless, making them vulnerable to predation while cleaners inspect, remove and ingest ectoparasites and dead tissue. Conversely, cleaner species often service clients that would otherwise be natural predators. Because cleaning imposes costs and potential risk to participants (Cheney and Côté, 2001; Chapuis and Bshary, 2009), involves multiple species that are likely to differ in their sensitivity to stressors (Vaughan et al., 2017), and is important for ecosystem function (Losey, 1972), cleaning symbioses are ideal interactions for testing hypotheses about how anthropogenic stressors, such as noise, can have impacts beyond those on individual species.

Here, we experimentally assessed the effects of SCUBA noise on the local community structure and cooperative interactions at the cleaning stations of a well-studied cleaner shrimp species, the Pederson's cleaner shrimp *Ancylomenes pedersoni* (Titus et al., 2015a, Titus et al., 2015b; Titus et al., 2019). Pederson's cleaner shrimp are obligate cleaners (i.e., species that clean throughout juvenile and/or adult life), with their cleaning stations visited by over 20 reef fish families (Huebner and Chadwick, 2012a; Titus et al., 2015b; Gilpin and Chadwick, 2017; Huebner et al., 2019). These established locations facilitate observation of important interspecific mutualistic behaviours, allowing experimental exposure of cleaners and clients to different acoustic treatments to test for a mechanism underpinning previously documented impacts of diver presence on coral reef organisms. We conducted a playback experiment at *A. pedersoni* cleaning stations to evaluate the impacts of SCUBA noise on: 1) community structure near the stations, 2) clientele composition, and 3) interspecific behaviour during cleaning interactions.

2 Methods

2.1 Experimental overview

We conducted a playback experiment at 40 *Ancylomenes pedersoni* cleaning stations on Coral View Reef (N 16° 05' 17.87" W 86° 54' 38.56") on the Bay Island of Utila, Honduras, which is located at the southern end of the Mesoamerican Barrier Reef. Coral View Reef is a fringing reef on the southern coast of the island that slopes from ca. 3 to 30 m depth and is a typical contemporary Caribbean reef in terms of oligotrophic nutrient conditions, coral cover, fish abundance and reef community structure (Titus et al., 2019). This site has been visited regularly by snorkelers and SCUBA divers for more than 20 years (Titus et al., 2015a). On Caribbean reefs, Pederson's cleaner shrimps inhabit corkscrew sea anemones *Bartholomea annulata* to form mutualistic and ecologically important cleaning stations. Reef fish use sea anemones as visual cues to locate cleaning stations and engage in cleaning interactions with resident shrimp (Huebner and Chadwick, 2012b; Gilpin and Chadwick, 2017). Similarly, for research purposes, seeking *B. annulata* facilitates the finding and observing of cleaning stations. We located and monitored 40 *B. annulata* cleaning stations occupied by *A. pedersoni* at depths of 4–18 m across a continuous stretch of reef (ca. 600 m^2 study area), and with a minimum distance of 5 m between experimental stations. We monitored cleaning activity and numbers of shrimp at all cleaning stations throughout the field season using a rotation of static cameras every 5–7 days.

At each cleaning station, we administered two acoustic treatments: playback of local reef soundscapes (ambient sound) and playback of local reef soundscapes with added noise from SCUBA (SCUBA noise), presented in a counterbalanced, repeated-measures design. By comparing responses to playback of local reef soundscapes with those to the soundscapes with SCUBA noise, we isolated SCUBA noise as the experimental stressor without any visual presence of divers, and also controlled for any influences of the acoustic playback itself and/or electromagnetic interference from the loudspeakers. To avoid disturbance by observers, and to allow analysis of replicate trials while blind to the treatment, we used video cameras to record the local fish community and cleaning activity during each deployment.

2.2 Playback tracks and sound analysis

We made field acoustic recordings using a digital recorder (H6-BLACK field recorder, sampling rate 48 kHz; Zoom Corporation, Tokyo, Japan). This was connected to an omnidirectional hydrophone (HTI-96-MIN with inbuilt preamplifier, High Tech Inc., Gulfport MS; manufacturer calibrated sensitivity -164.3 dB re 1 V μPa^{-1}; frequency range 0.2–30 kHz) to measure sound pressure, and to a triaxial accelerometer (M20-040: sensitivity following a curve over the frequency range 0–3 kHz; calibrated by manufacturers; Geospectrum Technologies, Dartmouth, Canada) to measure particle acceleration. We took recordings in sea states between 0 and 2 on the Beaufort Scale in the absence of rain, with recording equipment suspended approximately 1 m above the seabed using a submerged stand.

We made three 5-min daytime recordings each of ambient coral reef soundscapes and of open-circuit SCUBA noise at coral reefs. In each SCUBA-noise recording, a pair of divers approached the recorder, remained stationary approximately 1 m from the recorder for 4 min, and then swam away from the recorder to simulate a recreational visit to inspect/observe/photograph a cleaning station. We used the original field recordings to create experimental playback tracks for each 45-min trial using *Audacity 2.2.1* (http://audacity.sourceforge.net/). We constructed three replicate tracks per treatment and used those in rotation to avoid pseudoreplication on single exemplars for each treatment. Each replicate used a different recording of ambient sound or SCUBA noise and was played on a loop. For the SCUBA-noise treatment, this resulted in six SCUBA disturbances per trial, at randomised intervals of 4 ± 1 min (mean ± SD). We re-recorded, analysed and compared playback tracks to original recordings.

We analysed recordings using *PAMGuide* (sound pressure; Merchant et al., 2015) and *paPAM* (particle acceleration; Nedelec et al., 2016a) in *MATLAB R2017b* across a frequency range of 0–2 kHz, which covers the likely auditory range of coral reef fishes (Wright et al., 2011; Ladich and Fay, 2013) and decapods (Popper et al., 2001; Roberts and Elliot, 2017). We calculated spectrograms, power spectral densities (PSD), root-mean-square levels (SPL_{rms} and SAL_{rms}) and cumulative sound exposure levels (SEL_{cum} and AEL_{cum}) in both the sound-pressure and particle-acceleration domains. Calculations were made over batch-processed 30-s subsamples of the recordings (n = 3 per recording type) for each of the four recording types (original ambient-sound recording, ambient-sound playback, original SCUBA-noise recording and SCUBA-noise playback; Figures 1, 2, and Table 1).

2.3 Experimental procedure

For experimental playbacks, we used recreational SCUBA to reach experimental stations and place equipment. Underwater loudspeakers (University Sound UW-30; max output level 156 dB re 1 µPa at 1 m, frequency response 0.1–10 kHz; Lubell Labs), kept in position using custom-made stands (PVC piping with loudspeaker attached using elastic bungee cord), were placed ca. 0.5 m away from and facing focal cleaning stations. Each loudspeaker was powered by an amplifier (M033N, 18 W, frequency response 0.40–20 kHz; Kemo Electronic GmbH), an MP3 player (SanDisk Clip Jam) and a battery (12V 12Ah sealed lead-acid) housed at the surface in a waterproof barrel. For each trial, we also placed a GoPro Hero 5 camera at 1 m from the focal cleaning station (Supplementary Figure S1). We administered both acoustic treatments (ambient-sound and SCUBA-noise playback) to a cleaning station on the same day, and two stations were treated simultaneously (with random allocation of one station to each treatment order). Trials were completed between 0800 and 1300 h, with previous research showing that cleaning interactions

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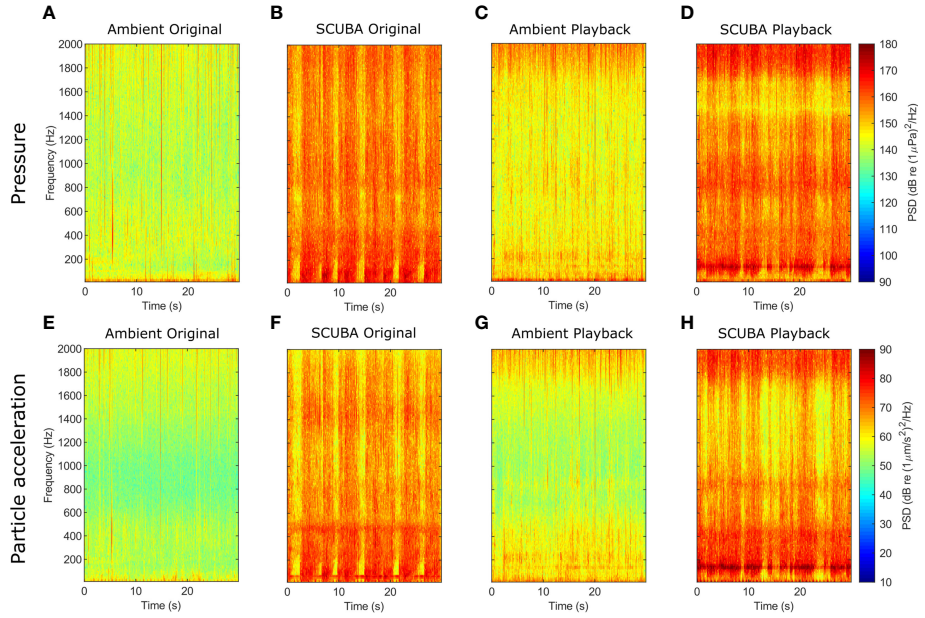

FIGURE 1
Spectrograms of sound-pressure **(A–D)** and triaxial particle-acceleration levels **(E–H)** for original ambient-sound **(A, E)** and SCUBA-noise **(B, F)** recordings, and playback tracks for ambient-sound **(C, G)** and SCUBA-noise **(D, H)** treatments. For these comparisons, 30-s subsamples of one SCUBA-diving pair and one ambient recording were analysed (fft window length = 4048, Hamming filter, 75% overlap, 0–2 kHz).

at *A. pedersoni* stations on this same study reef do not change predictably throughout the day (Titus et al., 2015b).

Our study was designed to evaluate noise as an underpinning mechanism behind previously identified impacts of SCUBA diver presence on interspecific interactions at *A. pedersoni* cleaning stations, using the same study system at the same location (Titus et al., 2015a). While we used SCUBA to access the cleaning stations, the first 10 min of a trial consisted of silent playback to allow the local fish and resident *A. pedersoni* to resume normal behaviour following disturbance from placing equipment (Titus et al., 2015a; Nedelec et al., 2016b; Nanninga et al., 2017); this was double the acclimation period from the previous work on the study system, to ensure a return to pre-disturbance behaviour (Titus et al., 2015a). There followed the administration of each treatment (SCUBA noise and ambient sound) over two 45-min segments separated by a 10-min gap of silent playback between the first and second treatment. We video-recorded both treatments at 40 cleaning stations over 20 non-consecutive days.

We cropped all videos collected in the field using *ffmpeg 4.13* (ffmpeg.org). For each treatment, we cut 45-min segments and

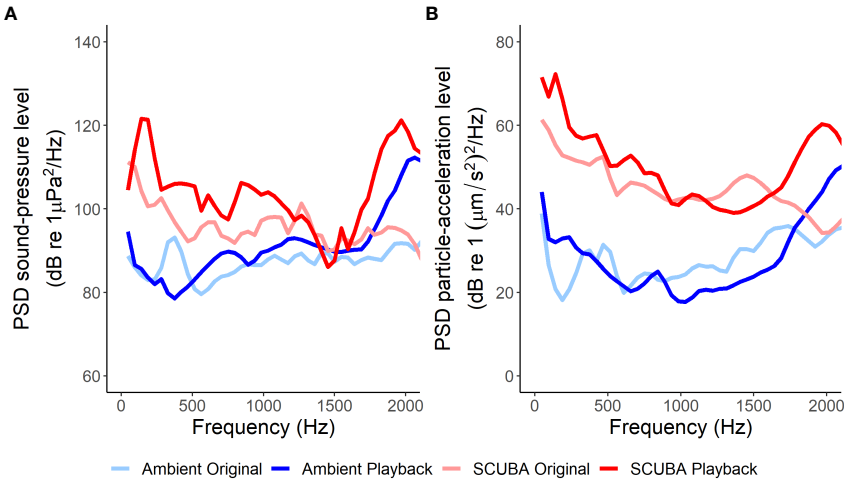

FIGURE 2
Mean power spectral densities (PSD) of the original ambient-sound recordings, ambient-sound playback tracks, original SCUBA-noise recordings and SCUBA-noise playback tracks in terms of sound pressure **(A)** and particle acceleration **(B)**, giving an average sound profile for each treatment (fft length = 1024, Hamming evaluation window, 50% window overlap, 0–2 kHz).

TABLE 1 Root-mean-square and cumulative sound-exposure levels in both sound pressure (SPL_{rms} and SEL_{cum}) and triaxial particle acceleration (SAL_{rms} and AEL_{cum}) for each recording type.

Recording	SPL_{rms} (dB re 1 µPa)	SEL_{cum} (dB re 1 μPa^2 s)	SAL_{rms} (dB re (1µm/s^2)	AEL_{cum} (dB re (1µm/s^2)^2 s)
Ambient-sound original	100.1	119.7	94.8	110.5
Ambient-sound playback	112.4	131.9	98.5	113.8
SCUBA-noise original	115.4	135.0	105.7	118.5
SCUBA-noise playback	129.0	148.6	117.0	129.4

saved them with coded file names. Videos were watched with no sound so that the observer (K.P.M.) was blind to the acoustic condition. We scored community assessments and individual behaviours from the videos using the behavioural observation software *BORIS 7.6.1* (Friard and Gamba, 2016).

2.4 Community-wide assessment and analysis

To test for impacts of SCUBA noise on the local community at *A. pedersoni* cleaning stations, we collected data on the frequency of fishes passing directly over the cleaning station and identified individual fish to species level during each trial. Analyses of the local fish communities were carried out for 39 of the possible 40 cleaning stations; one station was removed due to unintended interference by passing SCUBA divers. Similarly, we identified to species level all fish cleaned by *A. pedersoni* (hereafter clientele), and limited assessment of clientele composition to stations where at least one clean was observed (n = 22 stations). To analyse local community and clientele composition, we removed species with extremely low occurrences (< 1 % of total individuals; nine species from surrounding community: *Acanthurus chirurgugs, Acanthurus coeruleus, Chaetodon capistratus, Chaetodon striatus, Emblemariopsis diaphana, Haemulon flavolineatum, Lutjanus jocu, Serranus tigrinus*, and *Stegastes viride*; six species from clientele composition: *E. diaphana, H. flavolineatum, Hypoplectrus unicolor, L. jocu, Scarus taeniopterus*, and *Stegastes leucostictus*). We performed multivariate analyses in *R v4.0.0* (R Core Team, 2020) using the *Vegan 2.5-7* package (Oksanen et al., 2020), and conducted univariate analyses using generalised linear mixed models (GLMMs) fitted with AICc selection using the *lme4 1.1-26* package (Bates et al., 2015). Levels of significance were determined for fixed effect terms via comparisons to null models without the term of interest (i.e., sound treatment). Test assumptions were checked by visualising and evaluating model residuals for normality, homogeneity of variance, collinearity and influence of outliers with Cook's distance.

We measured total fish occurrence, recorded as the total number of fish for each species observed in the videos. This video-based method precludes a complete assessment of abundance, because it is possible that the same fish can re-enter the frame of view and any fish out of frame cannot be counted, but it avoids disturbance caused by observers in the water. We used species ID and measures of occurrence to calculate species composition for each station, and assessed these using GLMMs with a Poisson distribution; we included acoustic treatment and station as fixed and random factors, respectively. We compared species assemblages between ambient-sound and SCUBA-noise playback using unrestricted one-way nested PERMANOVA (maximum permutations = 9999), with acoustic treatment as a fixed factor and cleaning station as a random factor. Variation in fish species assemblages between ambient-sound and SCUBA-noise treatments was visualised using non-metric multidimensional scaling (nMDS) based on a Bray-Curtis similarity matrix. Lastly, we assessed species-level variation between the two treatments in separate GLMMs with either Poisson or negative binomial distributions (dependent on model fit). Our analyses were conducted across 15 species for local community analysis and eight species for clientele analysis, after the removal of those with < 1 % occurrence, using False Discovery Rate (FDR) to correct for multiple test comparisons.

2.5 Cleaning behaviour assessment and analysis

To investigate the impact of SCUBA noise on interspecific interactions at cleaning stations, we collected data on several cooperative behaviours of *A. pedersoni* and their clients: time that the shrimp was visible within the camera view (i.e., 'in-frame'), 'antenna whipping' by the shrimp (hereafter signalling; Caves et al., 2018), fish 'poses' at the cleaning station (Titus et al., 2017; Caves et al., 2018), time to initiate a cleaning interaction (hereafter delay; Nedelec et al., 2017), cleaning rate and time, and cheating rates (Titus et al., 2019; Table 2). First, we determined whether acoustic treatment (SCUBA-noise or ambient-sound playback) affected the likelihood that each of signalling, posing and cleaning occurred, using separate McNemar's tests for paired binomial data from all 39 stations. For sites where cameras recorded at least one cleaning interaction in either treatment (n = 22 stations; 113 cleaning interactions in total), we then determined whether acoustic treatment affected the rate (for counts) or activity-budget proportion (for durations) of each cleaning-related behaviour; paired t-tests or Wilcoxon signed-ranks tests were used, depending on whether the data met the assumptions for parametric testing. In some cases, behavioural measures are dependent on the occurrence of another behaviour and therefore only cleaning stations where the latter behaviour occurred were included in analyses. For example, cheating and cleaning delays are functions of cleaning interactions, and therefore analyses require that both treatments experienced at least one cleaning interaction (n = 8). These considerations were made to ensure statistical robustness,

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TABLE 2 Ethogram for the recorded interspecific behaviours by *Ancylomenes pedersoni* and client fishes.

Behaviour	Description	Variables
In-frame	Shrimp visible within the view of the camera	Duration
Signalling	Shrimp vigorously waves or 'whips' antennae	Count
Poses	Client fish arrives within a body length of the station and remains motionless for a brief period; often accompanied by a flaring of the opercula and/or fins	Count, duration
Clean	Shrimp makes physical contact and begins to clean the client fish	Count, duration
Cheating	Client fish 'jerks' or 'twitches' during a clean	Count
Delay	Time between the client fish arriving and remaining motionless until the first shrimp makes visible contact	Duration

emphasise biological context and relevance, and maintain confidence and conservativeness in the resulting conclusions.

We analysed all data using *R v4.0.0* (R Core Team, 2020). Statistical significance was assumed where $p < 0.05$. We also derived effect sizes for significant results using the *rstatix 0.6.0* package: Cohen's *d* for t-tests and Wilcoxon's effect size *r* for Wilcoxon tests (Kassambara, 2020).

3 Results

3.1 Local fish community

Fishes passed over the cleaning stations at a mean ± SE rate of 0.73 ± 0.09 events per min during the 45-min trials. For the local fish community, there was no significant difference in species richness between acoustic treatments (GLMM: $X^2_1 = 0.24$, $p = 0.62$, treatment = −0.05 ± 0.11, intercept = 1.51 ± 0.08, station ID = 0 ± 0). However, there was a 7% lower total occurrence of fishes during SCUBA-noise playback compared to the ambient-sound control ($X^2_1 = 4.23$, $p = 0.04$, treatment = 0.08 ± 0.04, intercept = 3.29 ± 0.12, station ID = 0.48 ± 0.70; Figure 3A), and the species composition of the local fish communities was significantly different between treatments (PERMANOVA: Pseudo-F = 0.77, df = 1, $p = 0.03$, 9999 permutations; Figure 3B). Three species were present significantly less during SCUBA-noise playback compared to ambient-sound playback (Figure 4): 22% fewer bicolour damselfish *Stegastes partitus* (GLMM: $X^2_1 = 7.34$, FDR-adjusted $p = 0.025$), 61% fewer cocoa damselfish Stegastes varibilis ($X^2_1 = 19.01$, FDR-adjusted $p < 0.001$) and 80% fewer bluehead wrasse Thalassoma bifasciatum ($X^2_1 = 30.14$, FDR-adjusted $p < 0.001$). Conversely, one species was present significantly more during SCUBA-noise playback: 259% more beaugregory *Stegastes leucostictus* ($X^2_1 = 26.34$, FDR-adjusted $p < 0.001$; Figure 4). None of the other species were found to differ significantly between the acoustic treatments (Supplementary Table S1).

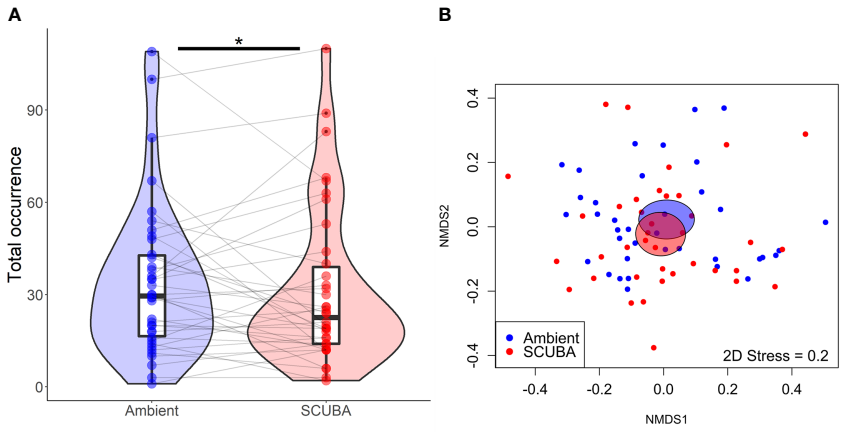

FIGURE 3
Community-level differences in total fish occurrence between the two acoustic treatments (playback of ambient sound or SCUBA noise). **(A)** Total fish occurrence. Boxes show median and interquartile range; violin plots show the kernel probability density of the data at different values; coloured points show treatment responses; grey lines join paired data from the same cleaning stations. *$p < 0.05$. N = 39 cleaning stations. **(B)** Nonmetric multidimensional scaling (nMDS) ordination showing variation in fish community. Individual dots show replicates at cleaning stations (n = 39); shaded ellipses represent the standard error of the weighted average for each treatment.

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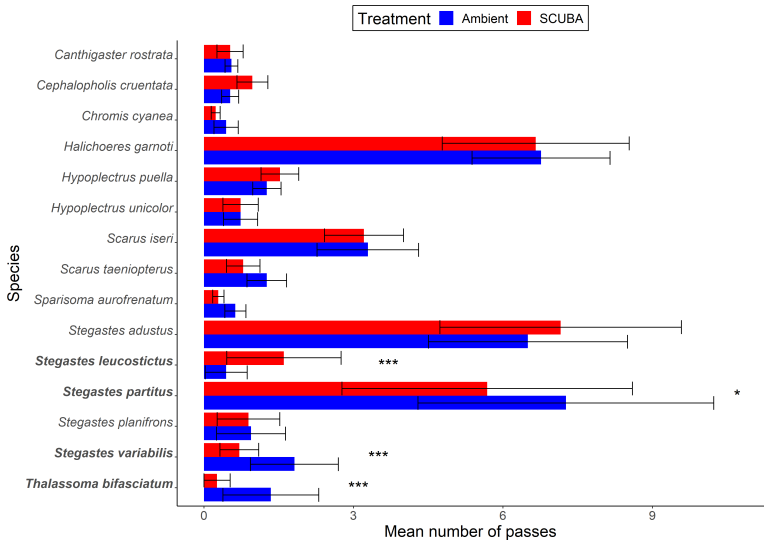

FIGURE 4
Occurrence of fish species in the surrounding community during the acoustic treatments (playback of ambient sound or SCUBA noise). Shown are mean ± SE number of passes by fishes for those species above the occurrence threshold (> 1%). *p < 0.05, ***p < 0001. N = 39 cleaning stations.

3.2 Clientele community

There was no significant difference between acoustic treatments in the overall composition of clientele across all eight species at cleaning stations (PERMANOVA: Pseudo-F = 0.16, df = 1, p = 0.90, 9999 permutations). However, when considering individual species, two were present significantly less during SCUBA-noise playback compared to the ambient-sound control (Figure 5): 89% fewer Caribbean sharp-nose puffer *Canthigaster rostrata* (GLMM: X^2_1 = 7.36, FDR-adjusted p = 0.034) and 71% fewer dusky damselfish *Stegastes adustus* (X^2_1 = 6.92, FDR-adjusted p = 0.034). None of the other species were found to differ significantly between the acoustic treatments (Supplementary Table S2).

3.3 Cleaning behaviour

There were no significant differences between the two acoustic treatments (SCUBA-noise and ambient-sound playback) in the likelihood that any of the three cleaning-related behaviours occurred: signalling by *Ancylomenes pedersoni* (McNemar's test: X^2_1 = 1.13, p = 0.29, n = 39 pairs), poses by client fishes (X^2_1 = 0,

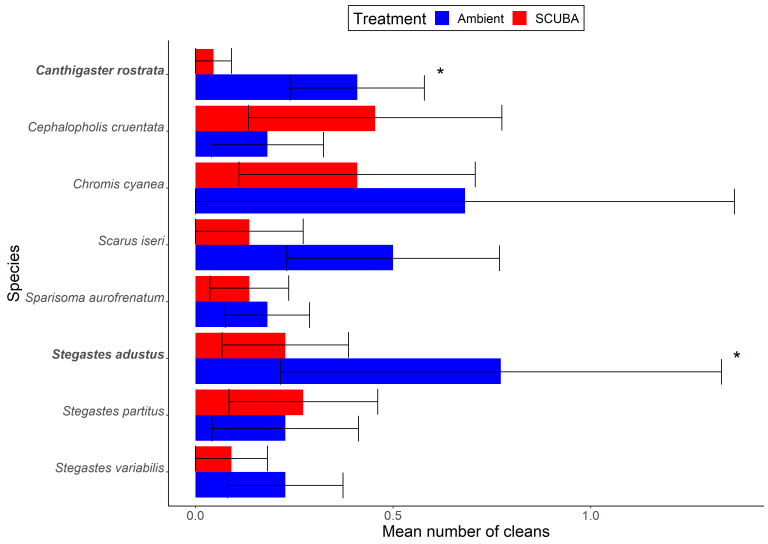

FIGURE 5
Client occurrences, identified to species level, during the two acoustic treatments (playback of ambient sound or SCUBA noise). Shown are mean ± SE number of cleans of fishes for species above the occurrence threshold (> 1%). *p < 0.05. N = 22 cleaning stations.

p =1, n = 39 pairs) and cleaning interactions between *A. pedersoni* and clients (X^2_1 = 1.79, p = 0.18, n = 39 pairs) .

There was no significant difference between the two acoustic treatments in the total time that *A. pedersoni* spent in-frame at the focal cleaning stations (mean ± SE : 43 ± 1 min; Wilcoxon test: $V_{22} = 128$, $p = 0.98$). There was also no significant treatment difference in the signalling rate by *A. pedersoni* (23:9 ± 0:5 events per hour for time spent within view; V_{22} = 161; p = 0:28), nor any significant difference between the two treatments in posing behaviour by client fishes posing behaviour by client fishes (posing rate: 4.9 ± 0.1 events per hour, V_{22} = 110, p = 0.56; total posing time: 60 ± 3.8 s, V_{22} = 119; p = 0.82).

Acoustic treatment did significantly affect the delay to initiate cleaning when a client fish arrived at the station (mean ± SE : 1.9 ± 0.5 s; Wilcoxon test : $V_8 = 0$, $p = 0.008$, $d = 0.89$); delay times were 29% greater when there was SCUBA noise compared to ambient sound (Figure 6A). Acoustic treatment also significantly affected the cleaning rate of *A. pedersoni* (3.4 ± 0.1 events per hour; $V_{22} = 151$, $p = 0.02$, $d = 0.52$), with a 43% lower cleaning rate in the SCUBA-noise treatment compared to the ambient-sound control (Figure 6B). There was, however, no significant treatment difference in either the average clean time (7.4 ± 1.5 s; $V_{22} = 127$, $p = 0.70$) or the rate of cheating by *A. pedersoni* (2.92 ± 1.06 events per min of cleaning; $V_8 = 3$, $p = 0.08$).

4 Discussion

While responses were varied, our experimental findings suggest that noise generated by open-circuit SCUBA diving can impact Caribbean coral reef communities and interspecific cooperation. Specifically, we found that SCUBA-noise playback altered community composition around cleaning stations, and affected cleaning interactions between the common Caribbean cleaner-shrimp species *Ancylomenes pedersoni* and client fishes. At the community level, the prevalence of four out of 15 common Caribbean reef fish species differed when exposed to SCUBA-noise playback compared to ambient-sound playback, with changes in the occurrence of these species driving differences in overall fish community composition between the two acoustic treatments. However, overall species richness was not affected by SCUBA noise. The significant effects on species prevalence at the community level were not uniform, with three species showing a reduction in occurrence during SCUBA noise, but one species showing an increase. Additionally, our results showed altered clientele composition of fishes cleaned by *A. pedersoni*, with two out of eight fish species being cleaned less during the SCUBA-noise treatment. However, these species-specific changes to clientele occurrence did not lead to a change in the overall clientele composition between the two acoustic treatments. Regarding individual cleaning behaviour, SCUBA-noise playback resulted in longer delays in cleaning initiation and fewer cleaning interactions between *A. pedersoni* and client fishes. SCUBA noise did not affect several other behaviours, such as signalling, posing, time spent cleaning and cheating. Overall, we believe that our study provides the first demonstration of the impacts of SCUBA noise on coral reef communities and interspecific interactions, highlighting SCUBA noise as a potentially harmful pollutant in coral reef ecosystems.

When exposed to SCUBA-noise playback, the occurrence of fishes near *A. pedersoni* cleaning stations was 7% lower and the overall community composition of fishes was significantly altered. These results mirror those from terrestrial studies where longer-term experimental playback of traffic noise along 'phantom roads' reduced overall bird abundance and altered community structures (McClure et al., 2013, McClure et al., 2017). The observed interspecific variation in noise effects (i.e., responses observed in some species but not others)

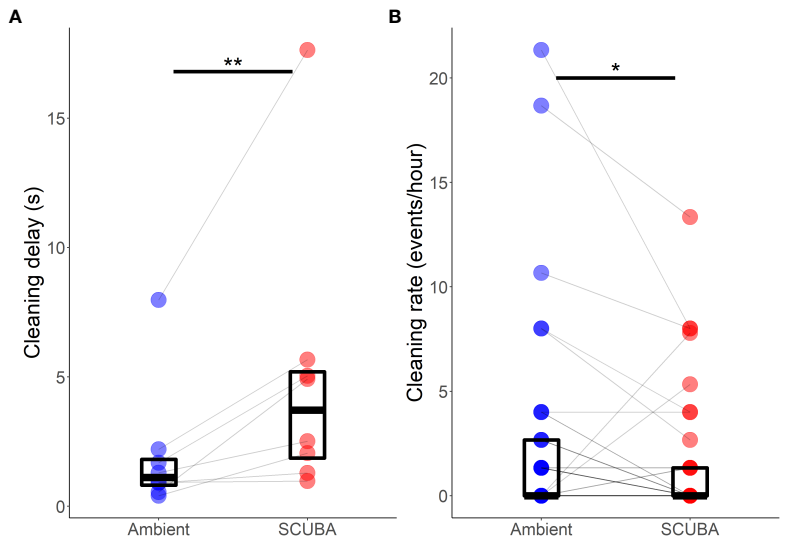

FIGURE 6
Difference in *Ancylomenes pedersoni* **(A)** delay to initiate cleaning and **(B)** cleaning rate between the two acoustic treatments (playback of ambient sound or SCUBA noise). Boxes show median and interquartile range; coloured points show data from individual cleaning stations; grey lines join paired data from the same cleaning stations. *p < 0.05, **p < 0.01. N = 8 cleaning stations for **(A)**; and n = 22 cleaning stations for **(B)**.

is not surprising given that species differ in, for example, ecology (Kunc and Schmidt, 2019), life history (de Jong et al., 2020), prior exposure (Harding et al., 2018), hearing ability (Popper and Hawkins, 2019) and vocal behaviour (Radford et al., 2014), all of which may influence their responses to noise. For instance, noise can induce physiological stress (Wale et al., 2013; Celi et al., 2016; Mills et al., 2020), which may subsequently alter decision-making processes and behaviour during disturbance (Purser and Radford, 2011; Voellmy et al., 2014), but species differ considerably in their susceptibility to stress (Pottinger, 2010). Furthermore, inter- and intra-specific variation in tolerance, sensitisation/desensitisation and/or habituation to anthropogenic noise remain unclear (Harding et al., 2019; Stasso et al., 2023). In fact, previous research using the same study system and location observed a difference in the strength of responses between frequently dived and un-dived locations (Titus et al., 2015a)—a comparison that was logistically beyond the scope of our study. Consequently, local history of diving at this site may have already altered susceptibility to SCUBA noise impacts by some species and/or individuals but not others, potentially resulting in only 27% and 25% of monitored species being affected by noise exposure at the community and clientele levels, respectively. Lastly, because anthropogenic noise has the potential to mask acoustic cues and signals, soniferous species, such as damselfish, may be particularly vulnerable to noise disturbance (Radford et al., 2014; Weilgart, 2018). Interspecific variation in noise effects may also arise through knock-on consequences. We found that three of four species affected by SCUBA noise were members of the same damselfish genus, *Stegastes*: two species (*S. partitus* and *S. variables*) occurred less during SCUBA-noise playback, while a third species (*S. leucostictus*) occurred more. It is possible that *S. partitus* and *S. variables* moved away, sought refuge more or exhibited less territorial behaviour (Benevides et al., 2019) during SCUBA-noise playback, which, in turn, created an opportunity through competitor release for *S. leucostictus* to encroach on territories and resources (Robertson, 1996).

Our finding that SCUBA-noise playback altered cleaning interactions between the cleaner shrimp *A. pedersoni* and its clients, with a 29% longer delay to initiate cleaning and a 43% lower cleaning rate compared to the ambient-sound control, may be due to distraction (Chan et al., 2010) or stress (Pottinger, 2010; Wale et al., 2013; Mills et al., 2020) in cleaners and/or clients. Either way, the results establish acoustic disturbance as a potential mechanism for the previously documented impacts of diver presence on cleaning by *A. pedersoni* (Titus et al., 2015a), and are in line with work showing that motorboat-noise playback can disrupt mutualistic cleaning behaviour by the Indo-Pacific bluestreak cleaner wrasse *Labroides dimidiatus* (Nedelec et al., 2017). Similar to these previous studies, we also found that only some measured behaviours were impacted by exposure to anthropogenic noise; some individual behaviours and social interactions may be more susceptible to disruption than others. Regardless, a decrease in cleaning activity suggests a trade-off, with avoidance of the potential risk and/or cost associated with SCUBA noise occurring at the expense of parasite removal for client fishes and dietary intake for *A. pedersoni* (Cheney and Côté, 2001). While not assessed here, noise negatively affects physiology (Wale et al., 2013; Filiciotto et al., 2014), stress-related behaviour (Filiciotto et al., 2014, Filiciotto et al., 2016) and biochemical regulation (Celi et al., 2015; Filiciotto et al., 2016) in crustaceans, and therefore may be similarly affecting *A. pedersoni*. For clients, cleaning symbioses improve fitness (Grutter, 1999; Becker and Grutter, 2004); therefore, SCUBA noise could lead to a negative impact on the reproductive success and longevity of clients that lose out on cleaning opportunities. Where cleaning stations fail altogether, reef communities can be affected in the form of reduced abundance and species richness, lower growth rates and survivorship, and diminished larval recruitment (Waldie et al., 2011).

Care is needed when extrapolating results from short-term noise experiments to fitness consequences, given that there can be increased tolerance and/or habituation, and compensation during quieter periods (Nedelec et al., 2016b; Radford et al., 2016). However, popular dive sites can receive multiple visits per day, which may equally result in cumulative noise effects. Similar caution is advised about assuming lasting community-level impacts from short-term experiments, although longer-term terrestrial studies have revealed sustained changes in the composition and interactions of species in noisy areas (Francis et al., 2009; Slabbekoorn and Halfwerk, 2009; Barber et al., 2010). Ultimately, extended experimental tests are needed in aquatic ecosystems if we are to understand the full impact of noise pollution.

While our study identifies SCUBA noise as a stressor to coral reef inhabitants, it also suggests a potential avenue for mitigating the impact of SCUBA diving. Managing acoustic disturbance has the potential to reduce the broad-ranging effects of divers on coral reef ecosystems (Lindfield et al., 2014; Titus et al., 2015a; Andradi-Brown et al., 2017; Benevides et al., 2019), without requiring a reduction or cessation of diving activity or the widespread uptake of expensive closed-circuit rebreathers. Instead, divers and the dive industry can adopt simple alterations to dive protocols that reduce the amount of noise exposure to coral reefs, which is a mitigation strategy that has been shown to negate biological responses to other sources of noise (Jain-Schlaepfer et al., 2018; McCormick et al., 2018, McCormick et al., 2019; McCloskey et al., 2020; Nedelec et al., 2022). For example, increasing the distance between a source and the vulnerable site has been shown to be an effective means of reducing the amount of noise exposure to wildlife, alleviating noise-induced behavioural responses (MacLean et al., 2020; McCloskey et al., 2020; Nedelec et al., 2022). Furthermore, tourism and dive operators might consider rotating and/or including more dive sites to avoid concentrating noise exposure and disturbance to a few locations. This concept of managing noise exposure to protect wildlife has been successfully implemented and enforced to safeguard at-risk marine mammal populations, including the critically endangered southern resident killer whale *Orcinus orca* (Williams et al., 2019). While it would require further experimentation to test the benefits of specific temporal, spatial and behavioural management recommendations, we believe that simple guidelines could be developed that mitigate the negative impacts of anthropogenic noise on coral reef habitats, especially given that coral reefs are areas of high biodiversity (Roberts et al., 2002), provide nutrition and livelihoods for millions of people (Cinner, 2014), and have high socio-economic importance and value (de Groot et al., 2012).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://datadryad.org/stash/share/YPdRHTvM6DCGWVVVkMzARh5D61DEjuy4ljYyuc43kNo.

Ethics statement

Experimental protocols were approved by the University of Exeter Animal Ethics Committee (Application, eCLESBio000295). The research was conducted under a research permit (ICF-508-2019) issued to Operation Wallacea by the Honduran government.

Author contributions

KPM, ANR, BMT, DAE and SDS designed the research. KPM, AR, GC, and NL carried out the fieldwork and collected videos and acoustic recordings. KPM extracted data from videos. KPM analysed the data with advice from ANR, EW, BMT, DAE, and SDS. KPM analysed the acoustic data. KPM wrote the manuscript with revisions and advice from ANR, BMT, DAE, and SDS. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by funding from Operation Wallacea, a University of Exeter Vice-Chancellor Scholarship for Postgraduate Research (to KPM), and a Natural Environment Research Council Research Grant (NE/P001572/1 to SDS and ANR).

Acknowledgments

We thank the Operation Wallacea staff and volunteers that enabled this project to be completed, with special thanks to Alec Atherton and Lydia Woods for their assistance in the field; the staff at Coral View Beach Resort & Dive Center (Tonia Woods, Calvin Woods Sr, Calvin Woods Jr, Taylor McField, Adelle Thompson, Rich Astley and Hans Grauer) for logistical support and accommodation during fieldwork; and Harry Harding and Lucille Chapuis for assistance with statistical and acoustic analyses.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1058414/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 05 May 2023
DOI 10.3389/fmars.2023.1111505

OPEN ACCESS

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RECEIVED 29 November 2022
ACCEPTED 17 April 2023
PUBLISHED 05 May 2023

CITATION
Cervello G, Olivier F, Chauvaud L, Winkler G, Mathias D, Juanes F and Tremblay R (2023) Impact of anthropogenic sounds (pile driving, drilling and vessels) on the development of model species involved in marine biofouling. *Front. Mar. Sci.* 10:1111505. doi: 10.3389/fmars.2023.1111505

Impact of anthropogenic sounds (pile driving, drilling and vessels) on the development of model species involved in marine biofouling

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The uncontrolled colonization of benthic organisms on submerged surfaces, also called biofouling, causes severe damage in the shipping and aquaculture industries. Biofouling starts with a benthic biofilm composed of a complex assemblage of microbes, bacteria and benthic diatoms, called microfouling, on which macrofouling invertebrate species settle and grow. Invertebrate larvae may use natural soundscapes to orientate inshore and choose their optimal habitat. Recent studies have demonstrated that ship sounds enhance the larval settlement and growth of several invertebrate species, such as mussels, associated with biofouling. Among invertebrates, effects of sound generated by offshore human activities are still poorly studied. This study aims to assess the effect of pile driving, drilling and vessel sounds on model species associated with micro and macrofouling. First, the biofilm development of *Navicula pelliculosa* and *Amphora coffeaeformis* was assessed, then, the larval development of the blue mussel (*Mytilus edulis*) was evaluated from the D-veliger to the postlarval stage. Mussel larvae and microalgae were exposed 12 h each day in tanks (Larvosonic) adapted to sound experiments under controlled conditions. All anthropogenic sounds induced a thinner *N. pelliculosa* biofilm coupled with a lower microalgae concentration. The drilling sound had a stronger effect on the biofilm thickness. The drilling sound significantly reduced the pediveliger settlement and the postlarvae clearance rate by 70.4% and tended to diminish settler sizes compared to control sound. Contrary to our expectation, pile driving tended to enhance larval recruitment by 22% (P=0.077) and the boat sound did not stimulate larval settlements or recruitment. Drilling sound generated a stressful acoustic environment for pediveliger settlements and postlarvae seem to maintain their shell valves closed to preserve energy. We identify potential causes and mechanisms involved in these impacts of anthropophony on larval ecology and microfouling dynamics.

KEYWORDS

bioacoustics, biofouling, anthropogenic sounds, benthic diatoms, larval development, settlement

1 Introduction

Consideration of ambient underwater sound as an important process of recruitment is growing in marine benthic ecology. Natural ambient underwater sounds act as pelagic cues to orientate fish (Montgomery et al., 2006; Simpson et al., 2016), crustaceans (Radford et al., 2007) and coral (Vermeij et al., 2010) larvae. Sounds emitted by reefs and other natural soundscapes, like waves on rocks seem to indicate beneficial conditions for larval settlement (Montgomery et al., 2006) increasing recruitment success and thus affecting local benthic population dynamics. However, the rapid colonization of macro invertebrates on oceanographic equipment, aquaculture systems, water pumps and particularly on vessel hulls is a big concern for the industry as it generates substantial costs for the cleaning of impacted infrastructure (Schultz et al., 2011). For example, mussels, *Mytilus galloprovincialis* biofouling in New Zealand creates around $16 million yr^{-1} economic loss in *Perna canaliculus* aquaculture production (Forrest and Atalah, 2017).

Biofouling starts with a benthic biofilm composed of a complex assemblage of microbes, bacteria and benthic diatoms, called microfouling, on which macrofouling invertebrate species settle and grow. Briefly, organic compounds and microbes are deposited on a clean surface to form an organic "conditioning layer". This layer acts as a stimulus to bacterial settlement (Dobretsov et al., 2009) and the micro communities develop a quorum sensing communication mechanism (Beitelshees et al., 2018). Bacteria exude a matrix of extracellular polymeric substances (EPS) (Flemming and Wingender, 2010) which facilitate microalgae colonization, usually dominated by diatoms (Bao et al., 2007), followed by fungal and protozoan spores (Callow and Callow, 2011). Mature, thicker and heterogenous biofilm will signal and increase the adhesion abilities of invertebrate larvae or their attachment strength to the substrate (Hadfield, 2011) as shown for the mussel *Mytilus edulis* (Toupoint et al., 2012b). As other marine benthic bivalves, mussels produce pelagic planktotrophic larvae that develop through several veliger stages until the pediveliger, the competent stage to settlement (Bayne, 1965). Pediveliger larvae use environmental stimuli to settle in an optimal habitat and undertake their metamorphosis (Hadfield and Paul, 2001). If settlement conditions are unsuitable, pediveliger larvae can prolong their pelagic dispersal life and delay their metamorphosis for several weeks (Pechenik, 1990; Martel et al., 2014). These larvae can also settle, metamorphosis and carry out a secondary migration process to find a more suitable environment (Günther, 1992; Forêt et al., 2018).

Some anthropogenic noise can mimic natural sounds, like waves on rocks and thereby stimulate the settlement of benthic invertebrates (McDonald et al., 2014). For example, vessels sounds emitted in the laboratory increased by an order of 4 the larval settlement of mussels, *M. edulis*, when combined with a trophic cue (Jolivet et al., 2016). Wilkens et al. (2012) found that loud sounds emitted by a freight ferry reduced the median time to attachment by 40% for the mussel, *Perna canaliculus*. However, the impact seems related to the nature of the anthropogenic sound where louder sounds, like turbine or seismic pulses could interfere with the capacity of larvae to detect trigger settlement cues delaying the metamorphosis of crab megalopae (Pine et al., 2012) or cause direct detrimental effects to the development of scallop veligers (de Soto et al., 2013). Each human marine activity produces its own acoustic signature depending on the gear used and the nature of the bedrock (Hawkins et al., 2015; Carroll et al., 2017; Chauvaud et al., 2018; Solé et al., 2023). Marine shipping constitutes > 90% of the acoustic energy emitted into the sea (Green et al., 1994; McDonald et al., 2014). Vessel and ferry sounds are produced by propellers, motor engines, diesel generators and other equipment involved in the boat machinery producing sound intensities between 140 and 190 dB re 1 µPa m^{-1} depending on vessel size, speed and power engines (Mitson, 1995; Gervaise et al., 2012; Chauvaud et al., 2018). Oil and gas exploration and exploitation, port area maintenance and expansion, or the development of offshore wind farms require construction phases that produce high levels of sound emission (Chauvaud et al., 2018). Pile driving and drilling are commonly used in marine shipyards and belong to the most powerful activities, after seismic surveys (Chauvaud et al., 2018). Modern pile driving consists of striking large diameter piles with a hydraulic hammer into the seabed. The contact between the hammer and piles produce short (~ 0.1s) and loud pulses (Tougaard et al., 2008) ranging from 192 to 270 dB re 1 µPa m^{-1} (Bailey et al., 2010). Drilling sound is generated by the drill bit's high-speed rotation crushing the seabed sediment/rocks. It generates a continuous sound with a relatively loud intensity ranging from 145 to 190 dB re 1 µPa m^{-1} (Chauvaud et al., 2018).

Documentation of the effects of pile driving and drilling sounds on micro and macrofouling development are lacking in the literature. It is important to understand anthropogenic sound effects on the microorganisms that shape and modulate biofilm dynamics and which have a critical role in the recruitment of species from higher benthic trophic levels (Antunes et al., 2019). The main goal of this study is to understand micro- and macrofouling development exposed to different anthropogenic sound sources. Biofilm development is assessed, including benthic bacterial and algae density during the establishment of two benthic diatoms under pile driving, drilling and boat sounds emission. *M. edulis* was used as a macrofouling model species to measure the impact of the same anthropogenic sound emissions on mussel planktonic development and recruitment success on artificial collectors without biofilm. We expect that boat sound will stimulate the development of the diatom biofilm and the recruitment success of the mussel. However, we suggest that louder sounds, particularly pile driving, could have a detrimental effect on micro and macrofouling development.

2 Materials and methods

2.1 Experimental emission system

Experiments were carried out at the ISMER-UQAR wet laboratory facilities (Rimouski, Qc, Canada). To limit sound

reverberation generated in a small tank (Jézéquel et al., 2018) and to obtain sound treatments as similar as possible to the original sound recorded *in situ*, we used *Larvosonic*mesocosms (Figure 1), which included acoustic panels on the internal tank walls, developed and described by Olivier et al. (2023). *Multifuser DC2* panels set at the center of each tank wall provided multi-reflection on both vertical and horizontal planes with maximum efficiency in mid and high frequencies (maximum absorption in air between 0.8-2.5 kHz). *Trap Fuser* set at each corner allowed the sound energy to be trapped in the cavities and/or scattered by the plain surface. The main tank was fully filled with fresh water until the level reached the lid that supported 6 semi-submerged experimental cylinders (5 L) and 6 multiwell plates (6 x 15 mL) (Figure 1). The main structure has the same dimensions as the *Larvosonic*described by Olivier et al. (2023), but was made of plywood coated with epoxy and rested on 4 steel adjustable feet, compared to the *Larvosonic*in plexiglass set on an aluminum frame. Three *Larvosonic*tanks were used for the three sound treatments and another without sound (room ambient sound only) considered to the control treatment. Clark synthesis AQ339 Diluvio™ underwater loudspeakers (8Ohms/20-17000Hz, Littleton, CO, USA) set on the bottom center played the sound treatments (Figure 1). As discussed in Olivier et al. (2023), one tank was not an exposure condition, but a sonorous environment where cylinders are isolated from the main tank as they are also and completely isolated and independent from each other, representing a replication level of 6. During each experiment, abiotic conditions (temperature, salinity, etc.) were monitored in each cylinder to ensure that all cylinders displayed similar conditions.

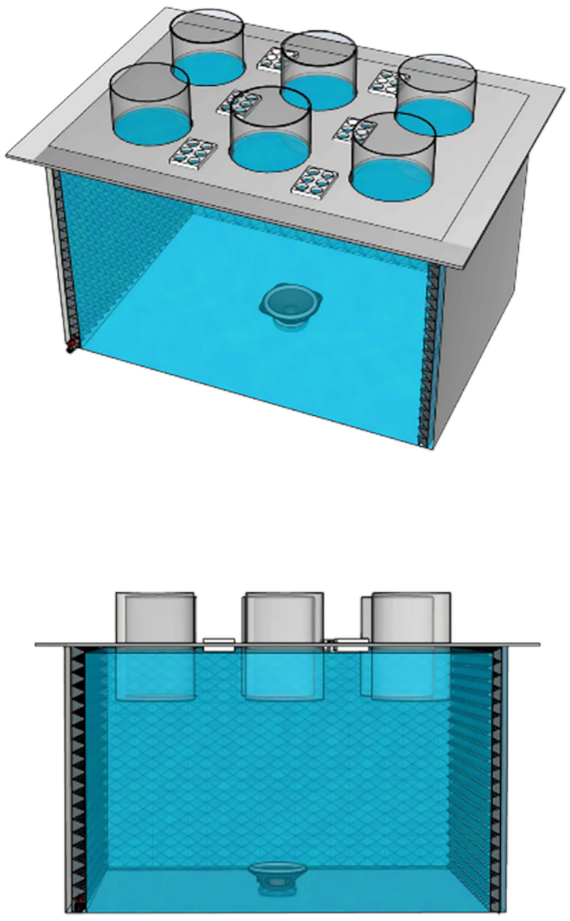

FIGURE 1
Larvosonic tank developed by Olivier et al. (2023) which includes acoustic Multifuser DC2 and Trap Fuser panels on the vertical walls, 6 semi-submerged cylinders (5L) and 6 multiwell plates (6x15mL).

Speakers were connected to an amplifier (DENON/DN-300Z/16–bit/20-20000Hz/44.1KHz, Cumberland, RI, USA), then to a matrix mixer with a signal processor (Yamaha 26x8 MTX3, Buena Park, CA, USA). Pile driving sound was played *via* an SD card, directly inserted into the amplifier set in repeat mode. Drilling and boat sounds were played independently with 2 computers connected to the amplifier using VLC media player software set in repeat mode, with both volumes adjusted to 100%. Sound treatments were recorded for 30s in each 5L experimental cylinders (10 cm above the bottom) with an underwater acoustic recorder (Loggerhead LS1, HTI 96-MIN/3V/LED/-170 dB/44.1 KHz, Sarasota, FL, USA) and calibrated to obtain a similar level to that measured in the field. The sound pressure level (dB re 1µPa) - *peak to peak* - was calculated using the following equation:

$$SPL_{pp} = 20\log[\max(p(t)) - \min(p(t))]$$

where t is the length of the sound and p the pressure units after correcting from volts to µPa. Fourier transformation was applied to each recording to analyze the power spectral density (PSD) using a custom Matlab script (The MathWorks Inc.). Sound treatments were emitted 12 h each day with an alternating sound exposure period of 6 hours followed by 6 hours of silence. A 30 s sequence was looped during experiments. The boat sound used was from a 11 m long D & H Boatbuilding hull equipped with a diesel motor (Cummins 300 hp C series) and was the same originally recorded and used by Jolivet et al. (2016). Drilling and pile driving sounds were recorded during an offshore wind farm installation in the Bay of Saint-Brieuc (France) with a calibrated hydrophone (High Tech, Inc., Mississippi, USA, HTI-99-HF: sensitivity −169.7 dB re 1 V/µ Pa; frequency range 2 Hz to 125 kHz flat response) and the output captured with a calibrated underwater acoustic recorder (RTSYS-Marine Technologies, France, EA-SDA14, 156 kHz, 24-bit resolution). Pile driving is an impulsive sound (one 200 ms impulse every 3 s) dominated by low-frequencies (40 - 800 Hz). The pile driving playback level corresponds to a distance to the source of approximatively 300-400 meters, depending on the project and environmental conditions. Drilling is continuous and its spectrum is characterized by high levels in the 150 - 600 Hz and 4000 - 7000 Hz frequencies range. It corresponds to a distance to the source of approximatively 500 meters.

2.2 Microfouling experiment

Benthic pennate diatoms strains (*Amphora coffeaeformis* CCMP 127 and *Navicula pelliculosa* CCMP 543) were obtained from the Center for Culture of Marine Phytoplankton (CCMP), Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, ME, USA)

and cultivated with an autoclaved medium F/2 with silica (Guillard, 1975). Microalgae were cultured under an LED system (72 mixed blue (24) and white (48) LEDs, 14 W, 6500 Ka) at an intensity corresponding to a photon flux of 200 µmol photons m^{-2} s^{-1} and ultrafiltered (0.02 µm) and UVs treated seawater with a salinity of 27.1, at 20°C. For each diatom species, 20L of culture was prepared until concentrations over 300 000 cell mL^{-1} were obtained. For the sound emission experiments, 2 cylinders $tank^{-1}$ by species illuminated by one LED system (intensity 200 µmol photons m^{-2} s^{-1}; 14h day : 8h night cycle) were inoculated with 5 million cells of *A. coffeaeformis* or *N. pelliculosa*. The volume of the cylinders was completed with ultrafiltered-UVs treated seawater added with F/2 silicate autoclave medium culture added. Into each cylinders, 2 x 3.5 cm-diameter discs roughened by carborundum paper were inserted. Before immersion into the cylinders, discs were washed in a neutral detergent, submerged in 70% aqueous ethanol for 5 min, and then irradiated under UV for 2-h to eliminate any microbial contamination (Leyton and Riquelme, 2008). The use of roughened plexiglass discs promoted benthic biofilm development and facilitated harvesting during sampling. Sounds were emitted for 8 days for *N. pelliculosa* and 9 days for *A. coffeaeformis*, and each of the biofilms on the discs was developed enough to be examined without loss. The first disc in each experimental cylinder was used to measure the biofilm thickness by confocal microscopy (Zeiss inverted microscope Axio observer Z1, Oberkochen, Germany, Figure 2 for example). Discs were stored in individual Petri dishes with the upper face upwards and 5 mL utltrafitered-UVs seawater was added to keep biofilms moist until confocal analysis. The biofilm thickness was measured at 5 random spots on the upper face at a magnification of 10x. On each spot, 3-D images of the biofilm were obtained by mosaic of stitching images at each 10 µm using a laser scanning microscope LSM 700 and analyzed by ZEN 2009 software. The second disc in each experimental cylinder was used to estimate the microalgae and bacteria cell abundance (concentration) in the biofilm with the use of CytoFLEX flow cytometry (Beckman Coulter, IN, USA). Briefly, the biofilms were collected by scraping all the upper face with a razor blade. The samples were placed in a 4.5 mL of 25% glutaraldehyde solution and deionized water for 15 min before storing at -80°C. After thawing, samples were ultrasonicated for 10 min to break down cell agglomerations. For each sample, 500 µl was sieved over a 35 µm filter and heterotrophic bacteria were quantified following staining with SYBR Green I nucleic acid bounder (Molecular Probes Inc., OR, USA). Pigmented microalgae cells were quantified by their natural fluorescence (Belzile et al., 2008; Tremblay et al., 2009).

2.3 Macrofouling experiments

Mussels, *M. edulis*, from St. Peters Bay, Prince Edward Island (Canada) were transferred to ISMER-UQAR wet laboratory facilities for larval rearing as described in Rayssac et al. (2010). Spawning was induced on 30 individuals by thermal shock and gametes from different parents were used in a pool-cross design to produce one random larval family. Post-fertilized eggs (66.4 ± 5.3 µm) were transferred to a 100 L bottom flat tank filled with filtered (1 µm) and ultraviolet (UVs) treated seawater at 18-20°C. After 72 h, 25000 D-larvae (113.1 ± 6.5 µm) were transferred into each 5 L experimental cylinder (5 larvae mL^{-1}). During all sound emission experiments, larvae were fed with a mixture of *Pavlova lutheri*, *Tisochrysis lutea*, *Chaetoceros muelleri*, *Tetraselmis suecica* and *Nannochloropsis oculata* (1:1:1:1:1, 60000 cell mL^{-1}). Low intensity cool white lights (2.5 ± 0.4 µmol photon m^{-2} s^{-1}) were aligned and adjusted above each tank with a natural light period of 14h day : 8h night. The temperature during all larvae and postlarvae rearing was maintained between 20 and 22°C.

At 48 h intervals, larvae from each cylinderwere collected on a 35 µm nylon mesh screens and resuspended with 300 mL of 1 µm ultrafiltered and UVs treated seawater to sample 1 mL of larvae for survival and growth estimation. For the growth rates, 30 larvae were measured with the image analysis software Image-Pro Plus coupled to the Evolution VF camera (Media Cybernetics, Silver Spring, MD, USA) and an Olympus BX41 microscope. Survival rates were expressed as the total number of individuals minus the cumulative number of empty shells and based on the first sampling time point. After cleaning the cylinders with Virkon VKS10 disinfectant (LANXESS Deutschland GmbH, Cologne, Germany), the larvae were put back into the growing cylinders with 5 L of 1 µm filtered-UVs treated seawater, with the addition of the microalgae mixture (1:1:1:1:1, 60 000 cell mL^{-1}). When more than 50% of larvae were pediveligers at 14 days post-fertilization (dpf), two collectors consisting of 30 cm polypropylene rope were added to each cylinder. For each following 48 h cleaning session (until the end of the experiment at 24 dpf), the collectors were carefully removed and hung up in the air to avoid juvenile detachment. In parallel, the pelagic larvae were collected on 53 µm nylon mesh screens to estimate survival and growth as described above. At 17 dpf, pelagic larvae were subsampled from each cylinder of the control tank and redistributed randomly into 3 (6 x 20 mL) multiwell plates of all the tanks. In each plate, 3 wells were filled with 10 larvae and 15 mL ultra-filtered-UVs treated seawater with the addition of 60 000 cell mL^{-1} of microalgae mixture as already described. After 72 hours of sound treatments (until 20 dpf), 1 mL of 4% formaldehyde solution was added to each well and the proportion of settled pediveliger larvae (larvae attached to the well surface) and unattached larvae were counted under a binocular microscope. The ratio between settled and the sum of all larvae was considered as the settlement rate (%).

At 24 dpf, the collectors were carefully removed and gently rinsed with a sprayer over a 100 µm nylon mesh screens to collect the settled postlarvae. The cylinders were sieved on 100 µm nylon mesh screens to collect all the pelagic larvae. 10 mL of the water sieved was sampled to estimate the clearance rate (21 to 24 dpf) with a M4e multisizer coulter counter fitted with a 50 µm aperture tube (Beckman, Mississauga, ON, Canada) using a modified formula described in Comeau et al. (2008):

$$CR = (Ln(Ci) - Ln(Cf)) \cdot V \cdot T^{-1} \cdot N^{-1}$$

where *Ci* is the initial microalgal concentration at T0 (cell mL^{-1}), *Cf* is the final concentration, *V* is the volume of seawater

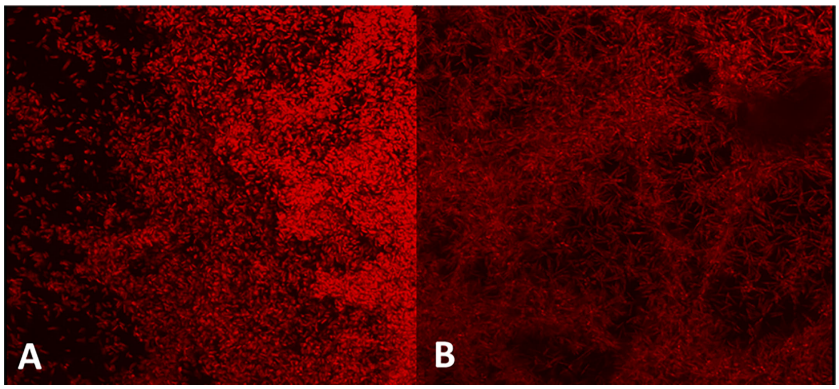

FIGURE 2
3-D images of the biofilm of *Amphora coffeaeformis* **(A)** and *Navicula pelliculosa* **(B)** obtained by confocal microscopy (Zeiss inverted microscope Axio observer Z1, Oberkochen, Germany). Images were obtained by mosaic of stitching images at each 10µm using a laser scanning microscope LSM 700 and analyzed by ZEN 2009 software.

(mL), T is the duration (days) of the experiment and N is the number of postlarvae in each well.

Settled postlarvae on the growing cylinder walls were carefully brushed and pooled with the postlarvae settled on the collectors to estimate the total wet biomass of recruits. Around 50 pelagic larvae and 50 settled postlarvae were collected and kept at -80°C for measurements of the prodissochonch (PII) and larval total length (TL) (Martel et al., 2014) with the use of a Keyence VHX-2000 Series digital microscope with VH-Z100UR objectives (Osaka, Japan, 1µm and HDR resolution). A postlarval growth index (PL) was calculated using the following formula:

$$PL = \frac{TL - PII}{PII}$$

The remaining postlarvae were weighed and stored in 2 mL of dichloromethane (CH_2CL_2) in amber glass vials with Teflon-lined caps at -80°C. Lipid extraction was carried out with dichloromethane and methanol following the method described by Parrish (1999), adapted from Folch et al. (1957). Lipid extracts were separated into neutral and polar fractions using a 6% hydrated silica gel column (Marty et al., 1992). The neutral fraction of each sample was eluted with 10 mL of dichloromethane:methanol (98:2) and the polar fraction with 20 mL of methanol, then the neutral fraction was purified on an activated silica gel with 1 mL of hexane: ethyl acetate (v/v) to eliminate free sterols. Fatty acid methyl esters (FAME) were prepared according to the method described in Lepage and Roy (1984) and analyzed using a multichannel Trace GC ultra (Thermo Scientific) gas chromatograph equipped with a Triplus autosampler, a PTV injector, and a ITQ900 (Thermo Scientific) mass detector, and analyzed with Xcalibur v.2.1 software (ThermoScientific, Mississauga, ON, CA). Methyl nonadecanoate (19:0) was used as an internal standard and FAME were identified and quantified using known standards (Supelco 37 Component FAME Mix and menhaden oil; Supleco) and were further confirmed by mass spectrometry.

2.4 Data analysis

For micro and macrofouling experiments, means (± se) of each variable are presented by sound treatment (tank) and defined as the fixed factor to be tested (4 levels corresponding to control, pile driving, drilling and boat noises). PRIMER (version 7.0.13) was used to perform univariate PERMANOVA (based on Euclidean dissimilarities) analyses to compare differences among sound treatments. Homoscedasticity was previously evaluated with PERMDISP tests. When significant differences were obtained ($\alpha \leq 0.05$), pairwise multiple comparison tests were used to identify differences among sound treatments. For the mussel experiment, neutral and polar fatty acid composition was tested with a multivariate PERMANOVA with the use of sound treatments as fixed factors.

3 Results

3.1 Acoustic

The sound pressure level recorded in all cylinders of each tank is summarized in Table 1. We observed similar measures among cylinders in the same tank with less than 1% variability. The control sound treatment was subjected to 8% contamination from emission from other tanks with a mean control sound pressure enhanced by 9 dB re 1 µPa compared to the room ambient sound.

Pile driving sound recorded in the cylinders reached its maximum power in the 100-500 Hz bandwidth with a maximum peak (200 Hz) around 125 dB re 1 μPa^2/Hz. The 150-800Hz frequencies were amplified by 20-30 dB re 1 μPa^2/Hz versus their open water values. In the highest frequencies [1000-2000 Hz], the spectrum recorded corroborated with the *in situ* spectrum varying from 60 to 80 dB re 1 μPa^2/Hz, characterized by a series of alternating minima and maxima peaks. Sound power at > 5000

TABLE 1 Mean sound pressure level (dB re 1 µPa, pk to pk) of pile driving, drilling, boat and control sound emission recorded in LARVOSONIC cylinders (N = 6) during the sound emissions and before the experiments (room).

Sound treatment	Sound pressure level (dB re 1 µPa)
control	123.8 ± 0.8
boat	139.6 ± 0.4
drilling	128.3 ± 0.4
pile driving	164.2 ± 1.0
room (silent)	114.5 ± 0.1

Hz decreased smoothly to 50-60 dB re 1 µPa^2/Hz and was slightly amplified by 10 dB compared to *in situ* spectrum. The drilling sound emitted by the source (Figure 3) had a low energy content in the 30-80 Hz range, matching with the sound recorded during *in-situ* experiments. Furthermore, the drilling sound emitted by the source displays a slight lower energy content in the 40-60 Hz that could be attributed to temporal variations in the electrical use and pump activities of the experimental wet laboratory. The powerful pile driving sound contaminated the other tank spectra recorded amplifying slightly the 200-800 Hz bandwidth. The nearest tank (drilling) from the pile driving source was the most impacted and exposed from +20 dB to +30 dB re 1 µPa^2/Hz in the 250-800 Hz bandwidth versus its *in situ* intensity. Sound distortion also occurred in the 1000-2000 Hz bandwidth inducing a reduction of about -30 dB (except a peak around 1700 Hz) of the drilling cylinders spectrum. In the 3000-8000 Hz bandwidth, j cylinders ar spectrum power was higher (+5 to + 20dB re 1 µPa^2/Hz) than the *in situ* spectrum. Less distortion occurred due to the boat sound power which maintained its open water soundscape (Figure 3). Frequencies in the 100-1000 Hz were slightly amplified by 5-10 dB re 1 µPa^2/Hz. For frequencies > 1000 Hz, cylinders sound power was reduced from -5 dB to -20 dB re 1 µPa^2/Hz depending on frequency. Maximum mean boat sound power reached 80 dB re 1 µPa^2/Hz< 20Hz. The control sound power was maximum under 50 Hz reaching 60-65 dB re 1 µPa^2/Hz. Increase of power occurred in the 200-850Hz bandwidth with maximum values around 200 and 800 Hz, and smoothly decreasing in higher frequencies around 32 dB re 1 µPa^2/Hz.

3.2 Microfouling experiments

After 8 days of sound treatment, the *N. pelliculosa* biofilm (Figures 4A–C) was thinner when exposed to anthropogenic sounds. Drilling and pile driving had a stronger effect and reduced by 47% and 32% respectively the biofilm thickness (Figure 4A). These reductions could be explained by a lower mean concentration of *N. pelliculosa* cells structuring the biofouling in all anthropogenic sound treatments with a stronger effect when pile driving (-73%) and drilling (-45%) sounds were emitted (Figure 4B). Mean bacteria cell concentration did not differ significantly showing large variations, particularly when boats sound was emitted, as 123% higher bacteria concentrations were measured in the biofilm compared to the control (Figure 4C). The *A. coffeaeformis* biofilm showed strong variation so that no differences between treatments (sound emissions and control) for each variable measured (thickness, microalgae and bacteria cells concentration) in relation to emission of the different anthropogenic sound (Figures 4D–F) were found.

3.3 Macrofouling experiments

Pile driving and drilling sounds tended to reduce by 10% and 11%, respectively, the mean larval survival compared to the control tank, but without a significant effect (Figure 5A). No effect of sound on the larval daily growth was observed (Figure 5B) with mean values > 15 µm day^{-1} until the appearance of the pediveliger stage (14 dpf).

The settlement rate was significantly different among sound treatments (Figure 6A) and was 36.7% lower (P_{MC} = 0.044) than the control treatment for the pediveliger larvae exposed to drilling sound. Pile driving sound also reduced the settlement rate by 20% but the difference with control treatment was not significant as shown by the pairwise test (P_{MC} = 0.123). Boat and drilling sounds also reduced drastically the clearance rate in postlarvae (Figure 6B) with values 70% lower in the drilling sound treatment (Pperm = 0.037). However, the 57% reduction observed in the boat sound treatment was not significant (Pperm = 0.074). After 21 days of sound treatments, pile-driving sound tended to increase by 21.9% of the total wet mass of spats recruited, a result close to the significant threshold with a p-value of 0.077 (Figure 6C). Sizes at metamorphosis (PII) for settled and swimming postlarvae were similar for all sound treatments (Table 2) with no differences in total length (TL) detected for settled and swimming postlarvae. However, drilling sound tended to reduce the TL of both settled (-7.8%) and swimming (-5.9%) postlarvae. These decreases triggered a lower postlarval growth index (PL< 0.4) but was not significant (Table 2). Drilling sound tended to enhance the total neutral (+59.2%) and polar (+63.8%) fatty acid concentrations in settled recruits (Table 3), but due to large variability among the 6 replicates, differences were not significant. The fatty acid composition (Annex 1) of recruits exposed to different sound treatments was similar for each lipid fraction (neutral: pseudo-F = 1.07, Pperm = 0.38 and polar: pseudo-F = 0.54, P-perm = 0.72).

4 Discussion

The experimental platform developed to study the impact of anthropogenic sound on model species structuring biofouling showed high acoustic quality with minimal variability among the sound intensity of the 6 cylinders units in each tank. Thus, the tanks were sonorous environment where cylinders were isolated from the main tank, completely isolated and independent from each other and thus be considered as true replicate as described by Hurlbert (1984). With the use of trap diffusers on the wall of the tanks, reverberation phenomena still occurred in the pile-driving tank amplifying the 200-800 Hz bandwidth frequency and were slightly

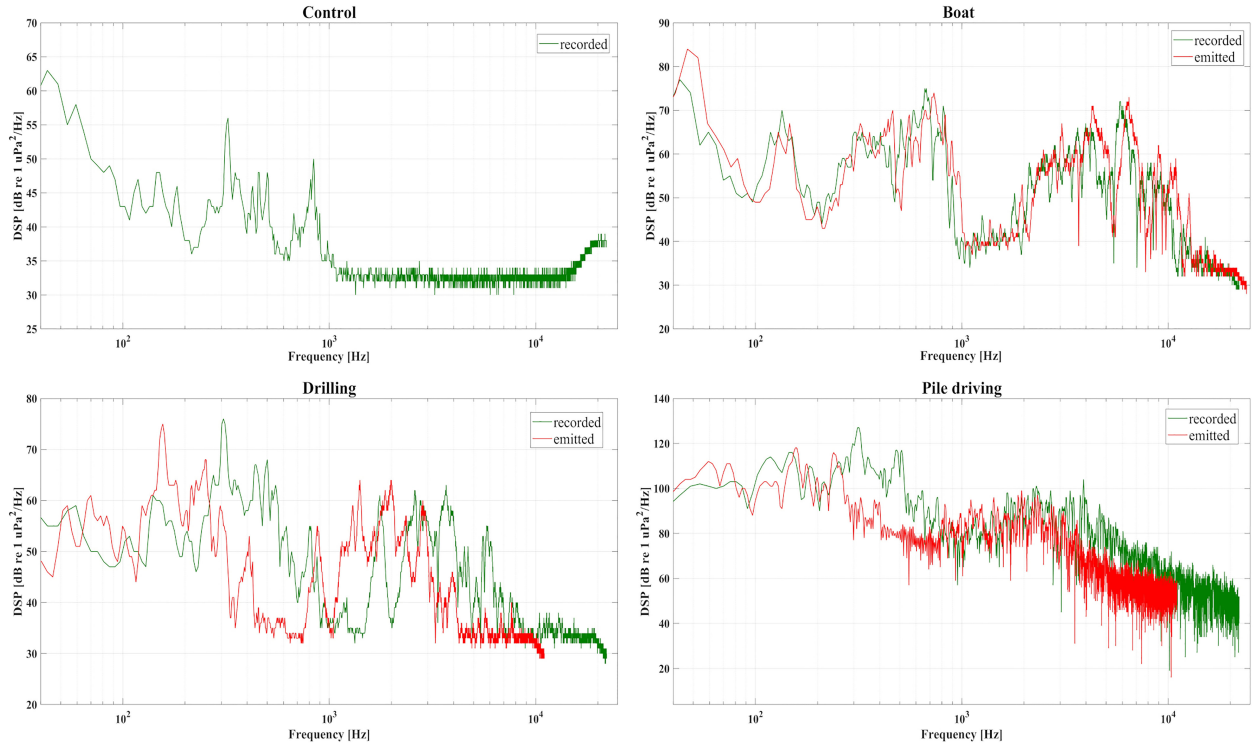

FIGURE 3
Sound power spectra (dB re 1 µPa/Hz) of the control, boat, drilling and pile driving sound emitted (red) into each tank and recorded (green) in each tank's central cylinder.

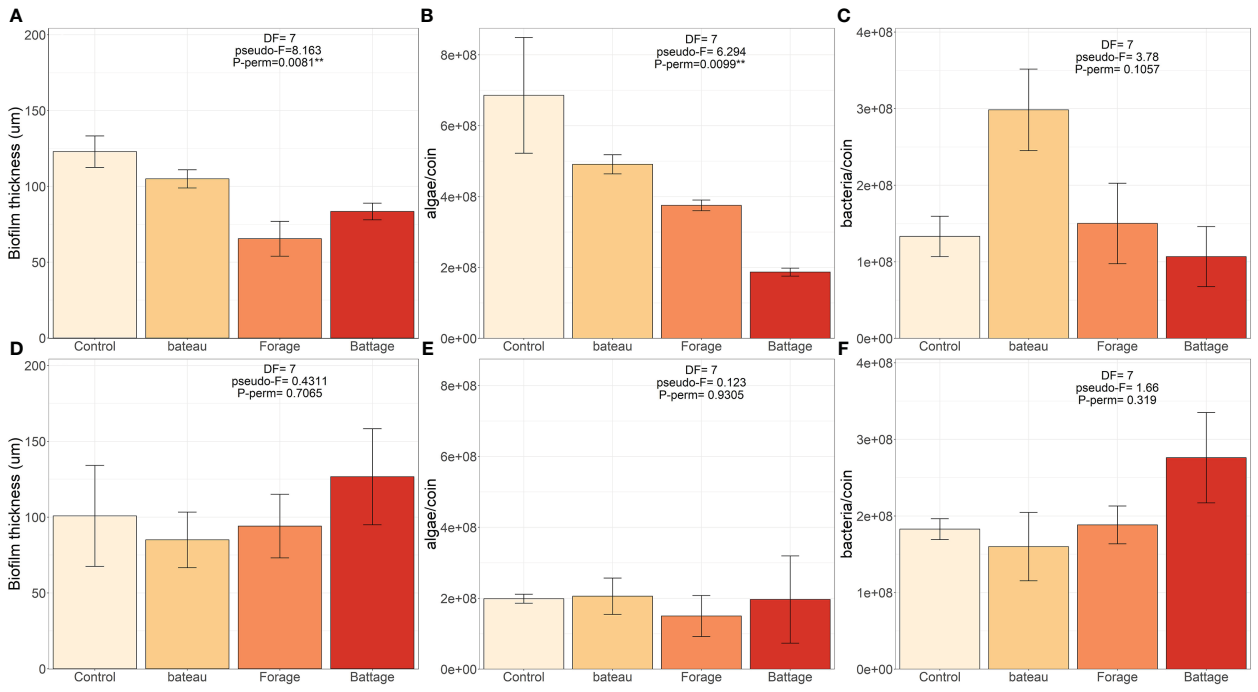

FIGURE 4
Effect of pile driving, drilling and boat sound treatment on the thickness (µm), total microalgae and bacteria cell count of *Navicula pelliculosa* **(A-C)** and *Amphora coffeaeformis* **(D-F)** biofilms.

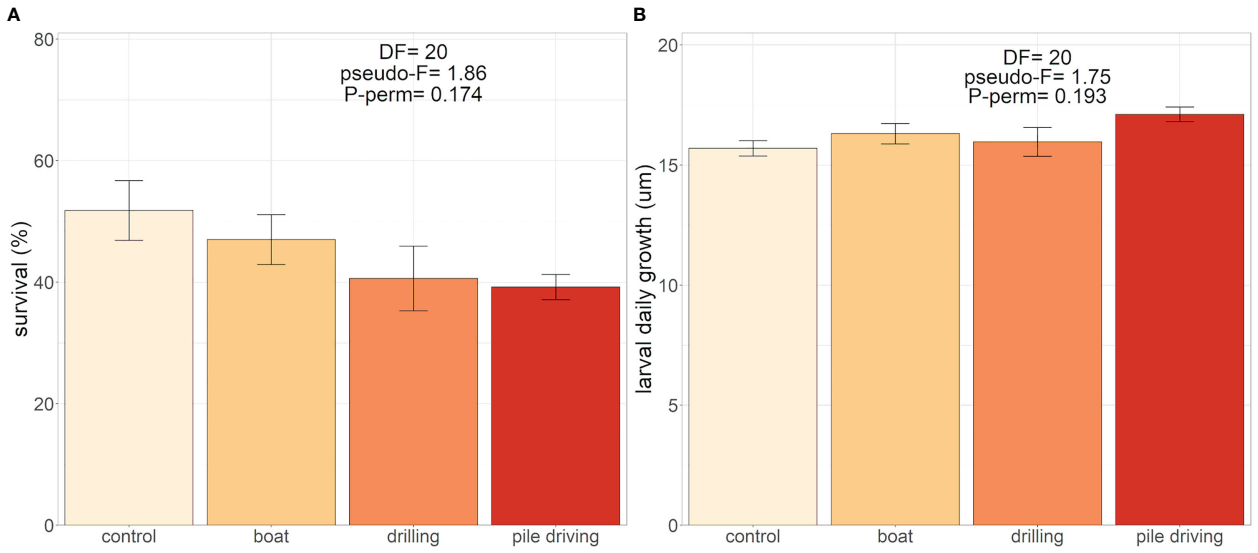

FIGURE 5
Effect of boat, drilling and pile driving sound treatments on **(A)** larval survival (%) (N = 6) and **(B)** the larval daily growth (µm), of *Mytilus edulis* after 12 days of sound treatments (N = 6).

different than sounds measured in the field. The drilling sound power spectrum was also weakly affected by the powerful sound of pile driving in the same frequencies maybe due to its low intensity. All sound treatments induced a thinner *N. pelliculosa* biofilm related to a lower development of these microalgae on the discs, particularly when drilling and pile driving sounds were emitted. In these treatments, biofilm thickness was less than 50% compared to the control. However, this impact of anthropogenic sounds on microfouling development seems species specific, as no impact was measured on the development of *A. coffeaeformis*. Furthermore, we observed that some anthropogenic sounds could also influence the development of the macrofouling. Our study showed for the first time that drilling sound effect (128 $dB_{pk\ to\ pk}$ re 1 µPa) the ontogeny of *M. edulis* and confirmed our hypothesis of a reduction of the settlement rate of the pediveliger larvae (-36.7%) and the clearance rate of post larvae (-70.4%). After 21 days of sound treatments, pile-driving sound (164 $dB_{pk\ to\ pk}$ re 1 µPa) showed an intriguing 21.9% increase in the mass of recruited spats, a tendency close to significance (p-value = 0.077). However, this increasing trend in recruitment was not observed for the boat sound treatment, in contrast to our expectations (Figure 7).

4.1 Drilling sound

All sound treatments tended to induce a thinner *N. pelliculosa* biofilm, but the drilling treatment had the strongest effect inducing a 47% reduction associated with 45% less cell concentration.

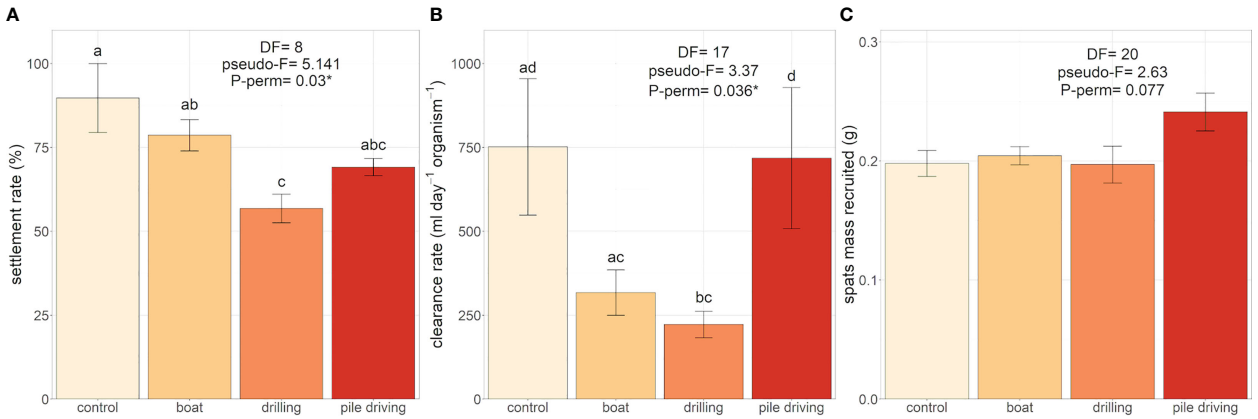

FIGURE 6
Effect of boat, drilling and pile driving sounds on: **(A)** pediveliger (17 to 21 dpf) settlement rate and **(B)** postlarvae (21 to 24 dpf) clearance rate (mL day^{-1} $organism^{-1}$) after 72 h of sound exposure in the multiwell plates (N = 3), **(C)** total mass of spats (g) recruited on collectors and cylinder walls after 21 days of sound treatment (N = 6). "*" means a significant difference detected by the PERMANOVA *post-hoc* pairwise comparison ($\alpha < 0.05$).

TABLE 2 Effect of different anthropogenic sound on the sizes (µm) at metamorphosis PII (N = 6), total length TL (µm) (N = 6) and postlarval growth index PL (N = 6) of *Mytilus edulis* larvae after 21 days of sound treatments.

	Settled postlarvae			Swimming postlarvae		
	PII	TL	PL	PII	TL	PL
P_{perm}	0.784	0.1438	0.139	0.771	0.315	0.304
control	326 ± 1	566 ± 16	0.42 ± 0.02	325 ± 4	560 ± 8	0.42 ± 0.01
boat	326 ± 4	564 ± 16	0.42 ± 0.02	329 ± 3	574 ± 15	0.43 ± 0.02
drilling	324 ± 4	521 ± 12	0.38 ± 0.01	327 ± 3	532 ± 17	0.38 ± 0.02
pile driving	322 ± 4	552 ± 13	0.42 ± 0.01	329 ± 3	560 ± 19	0.41 ± 0.02

"Settled" = spats settled on collectors + cylinder walls.

TABLE 3 Total fatty acid amount of *Mytilus edulis* spats recruited after 21 days of boat, drilling and pile driving sound treatments (N = 6).

	Total fatty acid (µg g^{-1})	
	Neutral	Polar
Pperm	0.104	0.395
control	511 ± 123	167 ± 48
boat	523 ± 89	168 ± 30
drilling	814 ± 1110	271 ± 65
pile driving	432 ± 105	159 ± 42

Diatoms are a major component of microbial slime and of the global primary production of coastal systems (Smetacek, 1999), and dominate the microphytobenthic community in intertidal mudflats (Doghri et al., 2017). They are the prime colonizer with bacterial communities and largely involved in ship hull fouling (Schultz, 2004; Schultz, 2007; Hakim et al., 2019). The presence of a 1 mm thick slime layer increases significantly the hull drag, reducing ship speed by 15% (Lewthwaite et al., 1985). Diatoms are characterized by a unique silicified cell called a frustule, which is a kind of box composed of 2 halves (Wang et al., 2013; Chen et al., 2019). The epitheca (the lid) closes the hypotheca (the box) connected by one or more girdles that facilitate cell expansion and growth (Molino and Wetherbee, 2008; Stefano et al., 2009). *A. coffeaeformis* and *N. pelliculosa* dominate microfouling communities and are pennate diatoms (Mitbavkar and Anil, 2006; Mitbavkar and Anil, 2007; Khandeparker et al., 2014). This group is characterized by the presence of a raphe on both cell valves which is an elongated slit system found on the frustule (Molino and Wetherbee, 2008). This structure allows diatom cells to move or "glide" over a surface to avoid being buried under soft sediments but also to migrate to sufficient light reception and higher nutrient concentrations (Molino and Wetherbee, 2008; Wang et al., 2013; Lachnit et al., 2019). While gliding, diatoms secrete an exopolysaccharide (EPS) mucilage, composed of proteins and carbohydrates with bio-adhesive properties that allow cells to slide but also increase the adhesion of other cells (Higgins et al., 2002; Molino and Wetherbee, 2008; Chen et al., 2019). Diatom adhesion is intimately related to the physico-chemical properties of the submerged surface. It is known that surface roughness, temperature, pH, ionic strength, surface charge, chemical compounds, cell exopolymer, contact time and the nature of the cell are factors involved in the adhesion

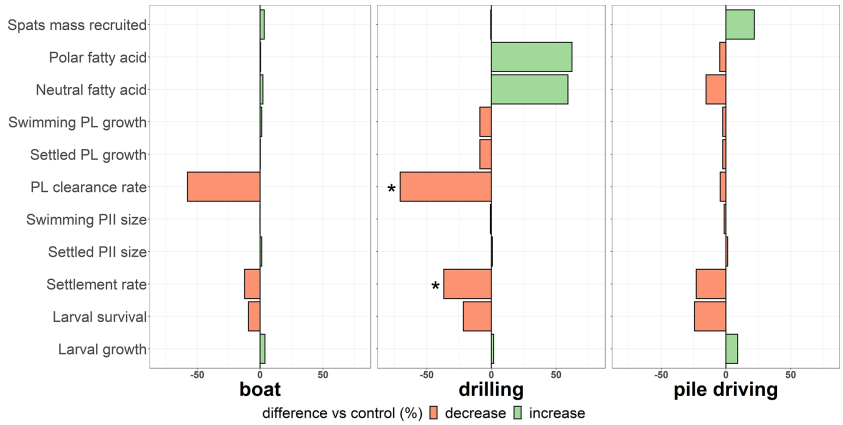

FIGURE 7
Differences (%) between control and anthropogenic sound treatments for all variables measured on the larval development of *Mytilus edulis*. "*" means a significant difference detected by the PERMANOVA *post-hoc* pairwise comparison (α<0.05), "PL" = post larval growth, "PII" = prodissochonch II (pelagic larval shell).

strength of diatom cells (Klein et al., 2014). The concentration and nature of the bacterial communities are also related to the diatoms and plays a major role in the complex interaction developed by all these microorganisms through EPS, also called "Quorum sensing" (Beitelshees et al., 2018). The diatom-bacteria interactions are species-specific, depend on biofilm maturity, diatom community composition and structure, and environmental conditions (Doghri et al., 2017; Koedooder et al., 2019). Bacterial communities can inhibit or accelerate diatom growth. Moreover, bacterial influence differs whether the biofilm is composed of one strain or several diatoms species and can induce a change in the community and diatom-diatom relationships (Koedooder et al., 2019).

Drilling sound strongly inhibited the development of *N. pelliculosa* biofilm but did not affect *A. coffeaformis*. The pressure variation, vibration or particle motion (Popper and Hawkins, 2018) through the viscous-sublayer could generate physical, hydrodynamic conditions that may disturb the ability of *N. pelliculosa* to adhere onto the discs. The pressure variation or vibration of the cylinders and discs could have induced unfavourable surface physico-chemical properties for *N. pelliculosa* development (Ozkan and Berberoglu, 2013). Moreover, the sound wave disturbances could induce negative bacterial-diatom interactions resulting in a negative dynamic for *N. pelliculosa* growth (Koedooder et al., 2019). However, we observed no interaction of drilling sound on bacterial concentration in the biofilm collected. The medium for biofilm growth was Plexiglas discs and only two discs per basin were used for each variable. Therefore, *post-hoc* tests could not be performed. This lack of statistical power makes it impossible to discriminate whether a single or multiple treatments induced a significant reduction effect on microalgal thickness and concentration in *N. pelliculosa*. The slowing effect induced by drilling should therefore be interpreted with caution. The biofilm structure and adhesion strength vary according to hydrodynamic conditions (Zargiel and Swain, 2014) and the nature of the submerged surface. A biofilm developing on a ship's hull will not have the same characteristics as a biofilm developing on a rocky or soft substrate (Klein et al., 2014). In our study, we did not quantify the EPS production accumulated on each disc, nor determine the more precise assembly of the bacterial communities. Furthermore, the biofilms were developed in a static environment without turbulence. Further investigations using different natural surfaces, diatom species, as well as a larger number of replicates are needed to understand if the drilling sound effects the adhesion process of pennate diatoms, their cell physiology or the relationship with bacteria.

According to Rittschof et al. (1998), environmental cues determine the larval settlement process of macrofouling species such as ascidians, barnacles, bryozoans and oysters rather than larval choice (Rittschof and Costlow, 1989; Rittschof et al., 1998). Larval settlement responses differ among species according to surface energy (dispersive polar forces as measured by wettability), light and vibration (Rittschof et al., 1998). Pine et al. (2012) found that sound from both wind and tidal turbines (145 dB re 1 µPa) delay the median time (about 18 hours vs silent treatment) to metamorphosis of crab megalopae. The authors argued that this delay is generated by unfavourable conditions generated by anthropogenic sounds or by the "absence of habitat-specific acoustic settlement cues" (Pine et al., 2012; Stanley et al., 2012). Here, we highlight for the first time that drilling sound affects larvae of aquatic invertebrates. In our experiment, the pediveliger settlement rate was reduced but no effect was detected for the size at metamorphosis compared to the control treatment (Table 2). This reduced settlement rate might be related to shell valve closure, which is usually a response of bivalve species under suboptimal or stressful conditions (Riisgard, 1991; Roberts et al., 2015; Durier et al., 2021). The drilling sound could generate stressful "suboptimal acoustic conditions", potential vibration or particle motion (Popper and Hawkins, 2018) that increase the closure periods of the larval shell valve, decreasing foot activity (Bayne, 1965; Bayne, 1971) and the capacity of competent larvae to explore the substrate and to settle. The decreasing clearance rates observed in postlarvae exposed to drilling sound seem to be in accordance with this hypothesis, as does the 8% decreasing tendency of the postlarval growth (Table 2). Direct physical effects on the mussel epidermal sensory cells or the adductor muscle might also occur and shell valve closure could be a response to stressful neurophysiological stimulation generated by the drilling sound (Lacourse and Northshop, 1978; Roberts et al., 2015). Negative sound effects could also occur in the larval attachment process. Several exogenous factors can affect the byssal attachment of mussel juveniles such as temperature (Lachance et al., 2008), air bubbles or water motion (Alfaro, 2006). Moreover, to reach the substratum, larvae need to cross a potential thin viscous boundary layer present on the substratum surface (Crimaldi et al., 2002; Hendriks et al., 2006; Koehl, 2007). A dysfunction in the byssal thread secretion or complex interactions between the sound wave propagation and vibration with the substratum boundary layer (McDonald et al., 2014) could affect the capacity of mussel larvae to attach. The settlement reduction observed in the 15 mL multiwell plates was not detected in the long-term recruitment process, as the total mass of spat recruited onto the 5 L cylinder collectors and walls was similar to the control. Drilling sound tended to enhance by 60% the total fatty acid amount (neutral and polar) in the juvenile tissue, but these differences were not significant. Fatty acids reflect the physiological condition of an organism (Toupoint, 2012) and correspond to a major source of metabolic energy used for growth, energy storage and other essential physiological maintenance functions (Tremblay et al., 2007; Parrish, 2009; Lee et al., 2018). It remains difficult to understand why these different effects were not detected with the other anthropogenic sounds. Drilling sound was the weakest sound treatment (128 $dB_{pk\ to\ pk}$ re 1 µPa) and no such effect was recorded in the loud pile driving tank (164 $dB_{pk\ to\ pk}$ re 1 µPa). Pile driving sound is associated with powerful but short (0.1s) pulses (Chauvaud et al., 2018) compared to drilling sound which is characterized by continuous sound wave emission. A continuous and constant sound emission, with a lower sound pressure level such as our drilling sound treatment, versus

powerful pile driving pulses, can still have detrimental effects on benthic bivalves.

4.2 Pile-driving sound

In contrast to the drilling treatment, pile driving had less effect on the *N. pelliculosa* biofilm thickness (-32%) but reduced drastically (-73%) the *N. pelliculosa* concentration. Similar to the other anthropogenic sound treatments, bacteria concentration in the *N. pelliculosa* biofilm was not impacted, along with all the indicators of biofilm development of *A. coffeaeformis*. It is therefore difficult to stipulate that pile driving sound impacts the overall dynamics of the biofilm. Clearly, *N. pelliculosa* biofilm development was largely sensitive to high energy, particle motion, pressure variation and/or vibration generated by pile driving sound emitted in our experimental conditions.

The non-significant increase of 21.9% mass of mussel recruits in the pile driving sound treatment is still intriguing. In particular, some cylinders in the pile-driving sound exhibited an increase higher than 40% of mass recruited compared to the control. The pile driving treatment is characterized by a higher power emission in the 100-1000 Hz frequency range compared to the other treatments (Figure 3). Animal activities in coastal habitats produce a wide spectrum of sounds but mostly concentrated in the 100-1000 Hz frequency range. These frequencies are known to attract and indicate favorable conditions (Montgomery et al., 2006) for the settlement of coral (Vermeij et al., 2010), fish (Simpson et al., 2016) and crab (Radford et al., 2007) larvae. The pile driving sound could stimulate in a certain way (physical component) or indicate appropriate acoustic conditions for the settlement of mussels which prefer to settle into noisy habitats such as rocky shores (Wilkens et al., 2012). However, this tendency to stimulate mussel recruitment was not found in the multiwell plates. The variation in settlement response observed between the sound treatment and within the tank could also be explained due to resonance phenomena under specific frequencies (Jézéquel et al., 2018). Further experiments testing the effect of different frequencies from powerful sound could potentially demonstrate the implications of certain frequency ranges on the settlement process of invertebrates.

In our experiments, particle motion and vibration were not measured. Sound waves can be transmitted across the substrate and can also generate waves at the interface of the water and the substrate. Interface waves are characterized by low frequencies (> 30 Hz) associated with large particle motion amplitude (Popper and Hawkins, 2018). Energy from these waves are maximum close to the substrate, which could be of major significance and provide "key information" about the environment to the organisms living close to or in the substrate (Popper and Hawkins, 2018). Potential vibration of the adhesion surfaces (cylinders and collectors) could promote mussel larval recruitment. This hypothesis was also mentioned by McDonald et al. (2014) who suggested that boat hull vibration could influence the settlement behavior or stimulate the adhesive release of fouling species such as the ascidian *C. intestinalis*. These explanations remain hypothetical and raise the complex nature of sound waves propagating into the substrate but also at the interface between the substrate and the water, across the bottom sublayer (Koehl, 2007). Further investigations into the potential effect of particle motion and vibration are required to better understand their implication in the larval settlement process of benthic invertebrates.

De Soto et al. (2013) studied the effect of playback seismic pulses (131_{rms} dB ref 1 µPa) on the New Zealand scallop (*Pecten novaezelandiae*) larvae for 90 hours, immediately after fertilization. D-veliger showed significant developmental delays and 46% of larvae exposed showed body malformations suggesting that physiological stress was induced by this cumulative sound exposure (De Soto et al., 2013). No such effect was observed on *M. edulis* larval development with the pile driving sound emitted, as no differences with the control treatment were observed in larval survival, larval growth (Figure 5) or mean size at metamorphosis (Table 2). Similar results were obtained with flatfish *Solea solea* larvae after 7 days of pile driving (210 dB re 1 μPa^2, peak pressure level, 50-1000Hz) (Bolle et al., 2012). Roberts et al. (2015) observed an increase in shell valve closure in *M. edulis* adults, as a response to sinusoidal vibratory signals in the frequency range of 5 to 410 Hz. This sensitivity increased with lower frequencies (except a response at 410 Hz) leading to potential negative effects on mussel fitness (Roberts et al., 2015). Higher sound wave transmission could be more important in adults due to their bigger size, through external (shell) or internal structures (mantle, foot, statocyst, etc.). Conversely, unsettled larvae devoid of a solid shell (dissochonch) potentially do not interact with these different waveforms. The absence of a short-term effect does not mean that any chronic or sub-lethal effects would not occur over a longer time period. Loud anthropogenic sound exposure during a complete life cycle could potentially highlight chronic or physiological effects on *M. edulis* fitness (Roberts et al., 2015).

4.3 Boat sound

Boat sound showed the least acoustic distortion and was well preserved the *in situ* acoustic signature (Figure 3). Boat sound pressure levels emitted in our experimental system were higher (139 dB re 1 µPa) than that of the drilling treatment (128 dB re 1 µPa). This greater intensity could facilitate a better preservation of the acoustic signature. Wilkens et al. (2012) studied the effect of two sound intensities (high and low) of a ferry sound, on the "time to attachment" of *Perna canaliculus* over 8 hours in 50 mL plastic vials (placed in water baths). Overall, high intensity vessel noise (126 dB_{RMS} re 1 µPa) induced a 40% shorter time for larvae to settle compared to the silent treatment. Larvae exposed to the high intensity noise were attached during the first 72h (Wilkens et al., 2012). Similar results have been observed by Jolivet and colleagues (2016) with the presence of *Nannocloropsis occulata*, a species with a high level of polyunsaturated fatty acids acting as a settlement cues in mussels (Toupoint et al., 2012a). Other biological and chemical cues have

been also determined for the settlement of pediveliger mussel larvae, like mature biofilms (Bao et al., 2007; Toupoint et al., 2012b) and neuroactive compounds (Satuito et al., 1999). However, in our study no enhancement of the settlement was observed as reflected by the absence of increased biomass of recruits at 24 dpf. Without biofilms, trophic triggers and water motion, the sterile substrate of the 15 mL wells was probably not a suitable habitat to stimulate pediveliger settlement. Jolivet et al. (2016) performed settlement experiments in 240 mL cylinders whereas Wilkens et al. (2012) used 50 mL vials. Furthermore, food condition of mussels was not modified during settlement experiments strengthening the results of Jolivet et al. (2016) demonstrating that the boat sound increases settlement of pediveliger mussel larvae when combined to trophic settlement triggers. Each well unit was previously sanded to enhance substrate roughness and facilitate larval adhesion abilities (Abelson and Denny, 1997). Nevertheless, the bottom microstructures apparently did not promote larval attachment in addition to the expected positive boat sound effect. The substratum surface microstructures were smaller than larvae and possibly did not disrupt the potential thin viscous boundary layer (Koehl, 2007). On the other hand, anthropogenic sound waves might also maintain certain hydrodynamic forces in the low water volume that prevent larvae from attaching. Additional water flow analyses at a finer scale in experimental units could provide information on the hydrodynamic properties (Koehl, 2007) and eventual disruption generated by soundwave propagation in the substrate/sublayer interface.

Hydrodynamic forces influence fluid motion and are an essential physical component that determines the larval recruitment success of biofouler larvae (Crimaldi et al., 2002; Koehl, 2007). The larval development of mussels occurs naturally in turbulent shallow waters and pediveliger larvae have strong adhesive abilities (byssus threads) that allow larval settlement under high velocities on hard substrata (Koehl, 2007). Eyster and Pechenik (1987) recorded that water agitation enhanced *M. edulis* larval attachment onto filaments two to eight fold in 2 L beakers. Carrying out settlement assays in a relatively small water volume probably underestimated the number of settled larvae (Pechenik, 1990). The lack of water motion likely generated a mismatch to the larval response under natural physical processes that naturally drive mussel settlement. The use of flowing water and higher-volume experimental units could be a better choice with which to perform future larval settlement experiments. Such an experimental setup would offer potentially more favorable physical conditions for mussel settlements and the implementation of essential small-scale hydrodynamic analyses (Koehl, 2007). Studying the development of micro and macrofouling during the same experiment under different anthropogenic sounds is necessary to assess the effect of anthropophony on the complete establishment of biofouling.

5 Conclusion

We observed that anthropogenic sound induced different effects on the biofilm development dependent on species involved and specific sounds. With respect to microfouling, all types of sounds tested showed no impact on bacteria concentration constituting the biofilm and on the development of *A. coffeaeformis*, but drilling and pile driving sounds impacted negatively the development of *N. pelliculosa* biofilm. These results suggest that these sound emissions were characterized by intensity and/or power spectrum generating substrate interference and unfavourable conditions for the establishment of the *N. pelliculosa* diatom slime layer. More research is needed to understand the sensitivity mechanisms of diatoms species in relation to substrate interference related to sound emission. Evaluating the sound effect on macrofouling development, we selected the blue mussel as a model species for this study. It was negatively impacted by the drilling sound characterized by emission of 128 dB re 1 µPa, particularly at the competent stage to settle and after metamorphosis and settlement on the substrate. Thus, negative impacts have been measured only when mussels were in contact with the substrate. We suggest that the sound treatment could induce a stressful acoustic environment for the development of postlarvae which prefer to reduce their metabolism and conserve their energy. The variation in settlement response between the experimental units raises some questions about resonance and distortion of sound spectra in the tanks and might explain why a 21.9% increase in recruitment success in the pile-driving treatment was non-significant. Bivalves start their life in the water column, then swim and crawl at the interface of the substrate and the bottom layer to finally attach and connect to the seabed. This transition from pelagic life to a benthic environment should be considered a sensitive stage for anthropogenic activities interacting with the seabed. However, in accordance to Slabbekoorn and Bouton (2008), the responses could be certainly best tested, not only by using replicate set of individuals, but also as well as a replicate set of call recordings. Also, further studies on the potential effect of noise on the complex interactions between substrate sound propagation/vibration and particle motion with the viscous sublayer and particularly the larval perception of sound propagation or substrate borne vibration are required to better understand the larval settlement process on a finer scale.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GC carried out the experiments, performed statistical analysis and has written the manuscript. RT supervised all the study, established the methodology and provided technical support. FO and LC developed the LARVOSONIC basins, provided the underwater acoustic recorder, acoustic support and participate to data analyses. DM performed the acoustic analysis. FJ and GW participated in data analyses. All authors contributed to the article and approved the submitted version.

Funding

We are grateful for the financial support provided for two years by the Natural Sciences and Engineering Research Council of Canada's (NSERC) Strategic Network, Canadian Healthy Oceans Network (CHONe) (Grant ID: 468437), and its partners: Fisheries and Oceans Canada and the Northern Institute for Research in Environment and Occupational Health and Safety (INREST), representing the Port de Sept-Îles and Ville de Sept-Îles. This work was also supported by complementary funding provided by Ressources Aquatiques Québec (RAQ), a strategic research network funded by the Fonds de recherche du Québec – Nature et technologies (FRQNT, #2014-RS-171172).

Acknowledgments

Our sincere thanks to Nathalie Gauthier and Élodie Bouchard for their help at the Aquaculture Station of UQAR-ISMER. Marine Sciences Institute The study is a contribution to the international laboratory BeBEST.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1111505/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 15 May 2023
DOI 10.3389/fmars.2023.1111466

OPEN ACCESS

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RECEIVED 29 November 2022
ACCEPTED 02 May 2023
PUBLISHED 15 May 2023

CITATION
Aspirault A, Winkler G, Jolivet A, Audet C, Chauvaud L, Juanes F, Olivier F and Tremblay R (2023) Impact of vessel noise on feeding behavior and growth of zooplanktonic species.
Front. Mar. Sci. 10:1111466.
doi: 10.3389/fmars.2023.1111466

Impact of vessel noise on feeding behavior and growth of zooplanktonic species

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Anthropogenic noise is a pervasive feature of the coastal ocean soundscape and is intensifying as vessel traffic activity increases. Low-frequency sounds from wave action on coastal reefs or anthropogenic noise have been shown to initiate larval settlement of marine invertebrates and accelerate metamorphosis to juvenile stages. These results suggest that some planktonic species can perceive and be impacted by anthropogenic sound. Hence, we tested the hypothesis that vessel noise has an impact on the feeding behavior of blue mussel (*Mytilus edulis*) veligers and of the copepod *Eurytemora herdmani* as well as on the growth of the rotifer *Brachionus plicatilis*. The results show that microalgae and feeding behavior of early life stages of mussels and copepods are not influenced by the presence of vessel noise. The growth of the rotifers was similar between the two sound treatments, but rotifers' egg production in the absence of vessel noise was higher and eggs were also larger. Our results suggest that the effects of noise on plankton are complex; much more work is needed to unravel these often subtle effects.

KEYWORDS

bioacoustic, zooplankton, clearance rate, growth, vessel noise emission

1 Introduction

Acoustics are an emerging field of research in coastal ecology. Scientists use underwater acoustic technologies not only to determine the sound composition of the aquatic environment (the "soundscape"), but also to study wildlife responses to natural and anthropogenic sounds (Rountree et al., 2006; Gannon, 2008; Jolivet et al., 2016). There has been an expansion in using ocean environments by humans over the last 50 years (Simard et al., 2016), and low-frequency noise has increased by 32-fold and is now dominated by anthropogenic noise, particularly in coastal environments (McDonald et al., 2008). Studies on the impact of anthropogenic noise on marine life have mostly focussed on marine

mammals and fishes (Popper, 2003; Barlow and Gisiner, 2006; Popper and Hawkins, 2016), but few data are available for zooplankton species (Day et al., 2016; McCauley et al., 2017; Fields et al., 2019). The importance of zooplankton in marine food webs is well known (Sameoto et al., 1994). They sustain major fisheries and aquaculture industries, and any factor modifying their diversity or productivity can have important environmental impacts. Noisy environments may also affect the behavior of invertebrates (Olivier et al., 2023; Solé et al., 2023), for example mussel larvae settle more rapidly and at a higher rate when they are exposed to vessel noise leading to smaller settlers (Wilkens et al., 2012; Jolivet et al., 2016). Other invertebrate larvae also change their behavior when exposed to vessel noise including the ascidian, *Ciona intestinalis*, which shows more intensive settling in the presence of vessel noise (McDonald et al., 2014). However, little information is available on the effect of vessel noise on feeding, growth and survival of zooplankton.

In this study, we tested the hypothesis that the level of vessel noise measured in coastal environments by Jolivet et al. (2016) negatively impacts feeding behavior, growth and egg production of different zooplankton species. We used different biological models, such as larvae of the blue mussel (*Mytilys edulis*), rotifers and copepods to get a better understanding of the impact of vessel noise on organisms with different life cycles and the presence of feeding appendages for copepods. Mussel larvae feed with a ciliate velum and copepods with feeding appendages (Koehl and Strickier, 1981) and we suggest that cilia from the velum could be perturbed by water vibration or particle motion generated by vessel noise, decreasing feeding success. The blue mussel, a major aquaculture species around the world, has been mainly grown in protected nearshore areas, like bays and estuaries (Camacho et al., 1991; Drapeau et al., 2006) corresponding to environments that are exposed to important levels of vessel noise. Pelagic stages of the blue mussel include the D-stage veliger up to the pediveliger stage, representing the competent stage to explore the substrate, settle and metamorphose into juveniles. In contrast to mussels, copepods spend their entire life cycle in the water column. They are a major zooplankton component and present in all oceans. *Eurytemora herdmani*, is a neritic species and dominates the coastal and estuarine zooplanktonic community (Runge and Simard, 1990). As their feeding behavior is mainly influenced by abiotic factors (Escribano and McLaren, 1992), it could also be affected by vessel noise. Modification of feeding behavior could negatively impact growth and reproduction of copepods as well as the other species that are dependent on them. The non-crustacean zooplankton, *Brachionus plicatilis* is a rotifer, which is easy to rear in large quantities and to harvest. It is the most commonly used species for live feed in aquaculture hatcheries all over the world. *B. plicatilis* can reproduce by parthenogenesis (Gilbert, 1977), so the number of individuals in a population can double in 24 h (Hirayama and Kusano, 1972). When conditions are suboptimal, rotifers may use sexual reproduction (Gilbert, 1977) and population density may decrease. Its small size (less than 400 µm) and its cruising swimming behavior in the water column makes it a suitable first live prey for first feeding stages of fish larvae.

In this study, our objectives were to determine the impact of vessel noise on: i) the feeding behavior of blue mussels (D-larvae and veligers) and copepods (*E. herdmani*) and ii) the growth and egg production of rotifers (*B. plicatilis*) under optimal and suboptimal physiological conditions obtained by different feeding treatments. Clearance rates were used to measure feeding behavior while counts and size measurements were used to quantify rotifer growth and egg production. No information is available on potential perception of noise in zooplankton species. However, generally these species have ciliated mechanosensory cells, in their statocyst or corona depending on species, suggesting potential perception and negative impacts of anthropogenic sounds considered now as emergent pollutants. We then hypothesized that vessel noise would modify reproductive behavior and number/size of eggs.

2 Materials and methods

2.1 Underwater sound

As described in Jolivet et al. (2016), the vessel noise emitted in the experimental tanks was originally recorded at a mussel aquaculture site at St. Peter's Bay on Prince Edward Island (Canada, 46° 25.963 N; 62° 39.914 W). A hydrophone (High Tech, Inc., Mississippi, USA, HTI-99-HF: sensitivity −169.7 dB re 1 V/µ Pa; frequency range 2 Hz to 125 kHz flat response) connected to an underwater acoustic recorder (RTSYS-Marine Technologies, France, EA-SDA14, 156 kHz, 24-bit resolution) was placed 25 cm from the bottom, near the anchor of the mussel line. The boat (11 meters long, D & H Boatbuilding hull with diesel motors, Cummins 300 hp C series) passed three times above the recording hydrophone during calm natural conditions characterized by a wave height of 0.2 m and wind speed of 3.8 m s^{-1} (http://climat.meteo.gc.ca/). Source sound levels were determined with MATLAB (The MathWorks, Inc.) to obtain a 30 s sequence corresponding to vessel noise at maximum sound intensity which was looped during experiments.

2.2 Organism maintenance

All experiments were carried out at the UQAR-ISMER wet laboratory facilities (Rimouski, Qc, Canada). Mussels, *Mytilus edulis*, from St. Peter's Bays, Prince Edward Island (Canada) were spawned and reared according to Rayssac et al. (2010). Briefly, spawning was induced by thermal shock and the larvae were reared in three 60 L conical tanks. Water was changed every 2-3 days before the addition of a food mixture of *Diacronema lutheri*, *Tetraselmis suecica* and *Chaetoceros gracilis* at 30 000 cells ml^{-1}. When larvae were competent to settle (development of eyespot and foot), they were transferred to three downweller systems to facilitate metamorphosis. For experiments, we used D-larvae (7-day post-fertilization, 120.5 ± 0.2 µm) and veligers (16-day post-fertilization, 150.5 ± 0.38 µm).

Adult copepods (*E. herdmani*) without sex differentiation were sampled in the St. Lawrence Estuary (Rimouski: 48° 28' 51.0"N 68° 31' 03.4"W) on October 24 and November 8, 2017. Zooplankton was obtained by 100 m horizontal tows from a pier repeated 6 times using a ring plankton net of 0.5 m diameter and 250 µm mesh size. Samples were preserved in a cooler with air bubbling for transport to the wet laboratory within one hour. Zooplankton was maintained in 40 L tanks at 15°C with air bubbling and fed *Tetraselmis suecica* (a green alga) at a concentration of 30 000 cells ml^{-1} until the start of the experiments. The mean prosomen length of the copepods in the experiments was 689 ± 5.19 µm.

Rotifers (*B. plicatilis*) were reared in an 18 L tank using filtered (0.2 µm) seawater in a greenhouse under natural photoperiod conditions and at temperatures >20°C following methodology described in Martinez-Silva et al. (2018). Each morning, the number of individuals in rearing tanks was estimated to adjust food concentration according to culture density. Rotifers were fed three times a day. Two batches of rotifers were reared to obtain rotifers with two physiological conditions. One was fed with the commercial formulation SELCO® (Sparkle, INVE Aquaculture Ltd., Thailand), corresponding to the optimal conditions, and the second with the microalgae concentrate REED (1:1:1 *Nannochloropsis occulata*: *Isochrysis galbana*: *Diacronema lutherii*, Instant algae, REED Mariculture, CA, USA), corresponding to the suboptimal conditions. Rotifers of 159 ± 2.3 µm were used for experiments. Lipid analysis was used to obtain the physiological conditions of those rotifers fed with different foods, SELCO® or REED, as lipids represent their main energetic reserves (Seychelles et al., 2009).

2.3 Experimental design

All experiments were conducted in a similar system described in Jolivet et al. (2016) consisting of an isolated quiet room with four 40 L tanks, each one containing 30 L of water and two multiwell plates (6 x 20 mL) placed on a platform 18.5 cm from the tank bottom to keep plates' rims 1 cm above the surface (Figure 1). Each tank was placed individually on 13 cm of isolating foam (Foamular C-300, Owens Corning, Toledo, OH, USA). Tanks were used to emit underwater sound and to maintain constant temperature (19 ± 2° C) monitored with HOBOware (Hobo Pendant Temperature/Light 64K Data logger UA-002-64, Onset, Bourne, MA, USA). Low intensity lights (133.18 ± 24.02 lux) were aligned and adjusted above each tank with a photometer (Q201 Quantum PAR Radiometer, Irradian Limited, East Lothian, Scotland) with a natural light period (12:12 h). Each tank corresponded to an acoustic treatment (two sound treatments tanks and two control tanks). Each experiment was replicated twice for each species on different rearing batches. Filtered (until 0.2 µm mesh) and UV treated sea water (23.7 PSU to 27.6 PSU between experiments) was used in the experimental chambers and organisms were fed with microalgae culture at a final concentration of 30 000 cell ml^{-1} per chamber. The microalgae, *D. lutheri* was used in the experiments with the mussels (D-larvae and veligers) and the rotifers, whereas *Tetraselmis suecica* in the experiments with the copepods.

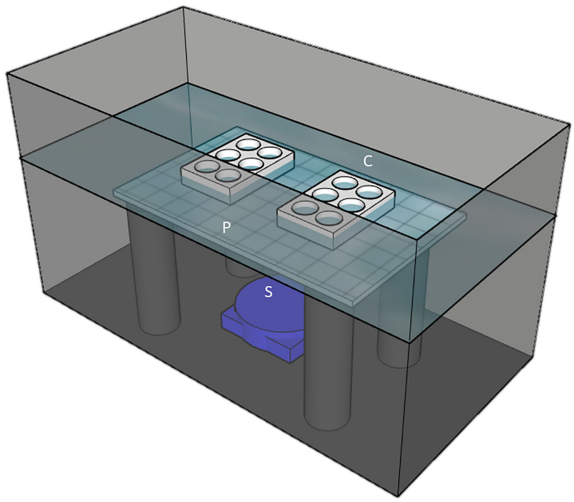

FIGURE 1
Experimental design for one aquarium with sound exposure.

Microalgae species were selected for their optimal retention efficiency. Motile flagellate species were selected to decrease sedimentation potential during the 24 h experiment and preliminary tests on two plates (12 chambers) by phytoplankton species (*T. suecc*ica and *D. lutheri*) showed less than 10% of sedimentation for both species. Sedimentation was estimated by cell concentration measured on the 5 ml surface seawater in each 6 chambers. Initial and final microalgae concentrations were measured using a coulter particle analyzer (Multisizer 4e, Beckman Coulter, Indianapolis, IN, USA).

For each vessel noise tank, underwater loud speakers (AQUA 30, 8 Ohms, 80–20,000 Hz, DNH, Sharon Hill, PA, USA) were placed in the middle of two sound treatment tanks and were connected to an amplifier (Brio-R, Rega, UK) and a computer that continuously replayed vessel noise using VLC software. Consequently, each multiwell plate was located 10 cm from the centre of the source. The sound under experimental conditions was calibrated to replicate as best as possible the shape of the *in situ* spectrum of vessel noise with a digital recorder (Song Meter SM4 Acoustic Recorder, Wildlife acoustics, Maynard, MA, USA) connected to a hydrophone (SM3/SM4, Wildlife Acoustics) recording frequencies from 2 Hz to 48 kHz with a sensitivity of -165 dB re 1 V/uPa. To realize calibration, the two multiwell plates were replaced by 250-ml jar on the platform, as the hydrophone was too large for the 20-ml well, and sound level analysed using MATLAB (The MathWorks, Inc.). Sound measurements can't be made directly within one cell, but we reasonably expect similar sound transmission between the underwater speaker and the jar as fluid characteristics are similar on both sides of plexiglass walls of either jar or wells. Thus, two measures were obtained per tank, two tanks per treatment for a total of 4 measures per treatment. The results allowed us to adjust the sound level in the tank by changing the gain from the amplifier and the sound level in the VLC software to match the sound conditions measured in the field. As noted by Jolivet et al. (2016) with the use of the same system, the multiple reflections off the glass sides of the tanks produced relatively

homogeneous sound conditions (SEM: ± 1.5 dB) over the jars. Recordings in the control tanks were also made to validate the presence of "silent" conditions where sound without vessel noise was played.

2.4 Feeding experiments

For the experiments with the copepods, the organisms were individually selected and three of them were placed per chamber, each one containing 5 ml of filtered sea water. For the experiments with the mussel D-larvae or veligers, a prior count of larval concentration in the tank was obtained to use around 7.5 mussels ml^{-1} per chamber. When all the chambers were filled with organisms, food (30 000 cell ml^{-1} of microalgae), and the last 5 ml of filtered seawater were added and animals were exposed to sound treatments. In each tank, one plate of 6 X 10 ml chambers with the organisms and one control plate (6 chambers) with only the microalgae were used (Figure 1). Individuals in one chamber being independent from other chambers, each chamber was considered as a replicate. Thus, for each treatment, the n= 12 (6 chamber X 2 tanks). Control plates with only microalgae were used to estimate if vessel noise impacted survival of microalgae. After 24 hours, 50 µl of Lugol fixative was added to each chamber to fix the microalgae and the organisms. The remaining liquid was then passed through a 20 µm filter to remove experimental organisms (mussels, copepods, and rotifers) and then microalgae concentration was measured using a coulter counter. Organisms were counted and identification of sex, stage, species, and length of the copepods was done using an Olympus SZ61 binocular microscope (4.5-20X; model SZ2-ST; Olympus Corporation, Tokyo, Japan). Mussel larvae were measured with an Olympus BX41 microscope (100X). Pictures were taken using an Evolution VF colour camera and the software Image-Pro Express 5.1.0.12 (Media Cybernetics, Inc., USA).

The clearance rate (CR) was calculated using a modified formula described in Comeau et al. (2008):

$$CR = [(lnC_1 - lnC_2) - (lnC_3 - lnC_2)] \cdot V \cdot T^{-1} \cdot N^{-1}$$

where C_1 is the microalgae concentration (cells ml^{-1}) in the control chamber after 24h; C_2 is the microalgae concentration in each chamber at T0; $C_3$is the microalgae concentration in the experimental chamber after 24h; V is the volume (ml) of filtered sea water in chambers; T is the duration (days) of the experiment; and N the number of organisms per chamber.

2.5 Growth experiments

To estimate growth, twenty small rotifers were selected and placed in a cell with 5 ml of filtered sea water, and as already described, microalgae and the last 5 ml of filtered sea water were added when all chambers had been filled with rotifers. In each tank, two plates of 6 X 10 ml chambers were used (one with microalgae and organisms, and one control with only microalgae). After 24 hours, each cell received 50 µl of lugol and the number of rotifers was counted. Total numbers in each cell and plate were pooled together to obtain the total per aquarium (two tanks replicates per sound treatment). The body length of each individual was measured with a microscope (Olympus BX41) as described above and the number of eggs attached to each individual also counted.

2.6 Lipids analysis

Two samples of 20 000 rotifers were collected from each replicate rearing tank and rinsed with filtered sea water (0.2 µm) with a 50 µm net. The samples were filtered onto precombusted (450°C) 25 mm GF/C filters. One filter was stored in 1 ml chloroform in amber glass vials with Teflon-lined caps at -80°C until lipid analyses, and the other was rinsed with ammonium formate (3%) and used for dry weight determination (70°C for 24 h). Lipids were extracted in dichloromethane–methanol using the modified Folch procedure (Folch et al., 1957) described in Parrish (1987). Fatty acid methyl esters (FAME) were prepared by transesterification as described in Lepage and Roy (1984) and eluted on an activated silica gel with hexane and diethyl ether to eliminate the free sterols. Fatty acids were analysed using a multichannel Trace GC ultra (Thermo Scientific) gas chromatograph equipped with a Triplus autosampler, a PTV injector, and a ITQ900 (Thermo Scientific) mass detector, and analyzed with Xcalibur v.2.1 software (ThermoScientific, Mississauga, ON, CA). FAMEs were identified with known standards (Supelco 37 Component FAME Mix and menhaden oil; Supleco Inc., Belfonte, PA, USA) after manual verification of the fatty acids integration.

2.7 Data analysis

We used Rstudio v.1.1.368 for data analysis. The levels of sound emitted in tanks in the presence or absence of vessel noise were compared using t-tests for each of the three frequency groups (100-10 000, 100-1 000 and 1 000-10 000 Hz). All analyses on feeding and growth experiments were done using linear mixed-effect models (lmer in R). For feeding, clearance rate in each species was compared with sound (presence or absence of vessel noise) as a fixed factor, and batch (two batches for each experiment) and tanks (two tanks per sound treatment) as random factors. Effects of sound exposure on microalgae used as food were tested on all experiments combined together. We used a linear mixed-effect model (lmer in R) with sound effect (presence or absence of vessel noise) as a fixed factor, and experiments (6 experiments represented by two batches of copepods, D-larvae and veliger larvae) and tanks (two tanks per sound treatment) as random factors. For growth experiments on rotifers, t-tests were used for each rotifer experiment (fed with SELCO or REED) to compare sizes of rotifers exposed or not to vessel noise. Similar analyses were used for egg production by rotifers. Total fatty acid content in rotifers fed with SELCO and REED were compared with Student t-tests.

Homoscedasticity and normality were tested using Levene and Kolmogorov-Smirnov tests respectively. When necessary, data were transformed using logarithm functions. PRIMER software (version

7.0.13) was used to perform multivariate PERMANOVA analyses to compare fatty acid composition of each rotifer feeding treatment (REED and SELCO) based on Euclidean dissimilarities following validation of the assumptions of homoscedasticity using PERMDISP tests. The SIMPER procedure was performed to identify FA explaining the most important dissimilarity between treatments.

3 Results

The results observed for each frequency group indicates that the sound level was relatively homogeneous through all the experiments for each sound treatment (Table 1, Figure 2). Sound levels in the tank in the presence of vessel noise corresponded to the *in situ* source signal for the three different frequency groups. For the two other tanks – treatments without sound emission – sound levels differed sharply from the two tanks exposed to vessel noise. The differences were significant for the three frequency groups (all comparisons: t = 0, p< 0.001).

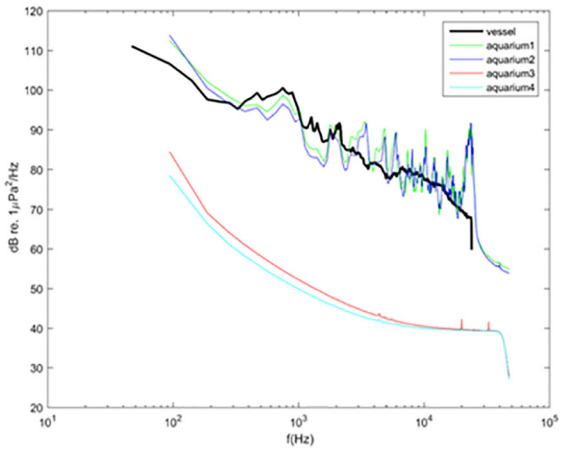

FIGURE 2
Mean sound spectra (dB re 1 µPa 2 Hz $^{-1}$) for the different experiments. Bold black line represents the vessel noise recorded *in situ* and the other lines of the spectra of sounds recorded in the four tanks used. Green and blue lines are from tanks 1 and 2 for the sound treatment and red and light-blue lines are from tanks 3 and 4 for the silent treatment.

3.1 Phytoplankton

Microalgae concentration at the end of the control experiments (without zooplankton species) was not modified by vessel noise (df = 1 and 11, F = 0.15, p = 0.74) and there was no tank effect (df = 1, X^2 = 2.67, p = 0.1). However, we observed a difference in the initial concentration between experiments (df = 1, X^2 = 80.64, p< 0.001) related to the estimation of microalgae concentration at the beginning of each experiment. Copepods were fed initially with an average of 23 338 ± 76 cell ml^{-1}, mussel D-larvae with 24 156 ± 127 cell ml^{-1} and mussel veligers with 21 830 ± 115 cell ml^{-1}.

3.2 Mussel larvae

The clearance rates of the one-week-old D-larvae were similar for individuals exposed or not to vessel noise (df = 1 and 11, F = 0.02, p = 0.90) with no aquarium effect (df = 1, X^2 = 3.34, p = 0.07) and no batch effects (df = 1, X^2 = 0.0, p = 1.0) (Figure 3). The two-week-old veliger mussels also showed clearance rates independent of the presence or absence of the vessel noise (df = 1 and 11, F = 2.08, p = 0.16), no tank (df = 1, X^2 = 0.0, p = 1.0) or batch (df = 1, X^2 = 0.0, p = 1.0) effects (Figure 3).

TABLE 1 Sound levels (dB re 1 µPa) measured *in situ* and in the experimental tanks: two tanks in presence of vessels sound and two tanks in absence of vessels sound.

	100-10 000 Hz	100-1 000 Hz	1 000-10 000 Hz
In situ vessel noise	130.7	129.9	122.7
Tanks in presence of sound	129.2 ± 2.6	127.1 ± 3.1	124.9 ± 1.7
Tanks in absence of sound	91.0 ± 2.2	90.2 ± 2.3	83.4 ± 1.4

3.3 Copepods

Vessel noise showed no impact on feeding behavior of the copepod *E. herdmani* (df 1 and 11, F = 0.119, p = 0.74) and no tank effect was noted (df = 1, X^2 = 2.84^{-14}, p = 1). Each of the two batches of copepods (each one with 12 replication levels) showed different clearance rates (df = 1, X^2 = 0.33, p< 0.001), but each batch showed no impact of vessel noise on their respective feeding behavior (df = 1 and 11, F = 1.15, p = 0.29 and df = 1 and 11, F = 0.28, p = 0.65) (Figure 3).

3.4 Rotifers

The sum of total fatty acid concentrations of rotifers fed the SELCO formulation (347 ± 41 µg·mg^{-1}) was higher (t = 10.883, p< 0.0001) than those of rotifers fed the REED microalgae concentrate (76 ± 6 µg·mg^{-1}) (Table 2). Their fatty acids composition was also significantly different (df=1 and 9, pseudo-F = 15.48, p = 0.007). The SIMPER analysis showed that 16:0 and 18:0 saturated fatty acids explained over 43.8% of the differences in fatty acids composition of rotifers fed with REED and SELCO. Rotifers fed the REED microalgae concentrate showed higher levels of saturated fatty acids. Rotifers fed SELCO formulation accumulated 2 to 3 times more essential polyunsaturated fatty acids (20:5n3, 22:6n3 and 20:4n6) than those fed microalgae.

For experiments using rotifers fed with REED, no impact of vessel noise was observed on total length (n = 718, t = 1.72, p = 0.09) with a mean (± SD) of 164.6 ± 18.5 µm (presence and absence of

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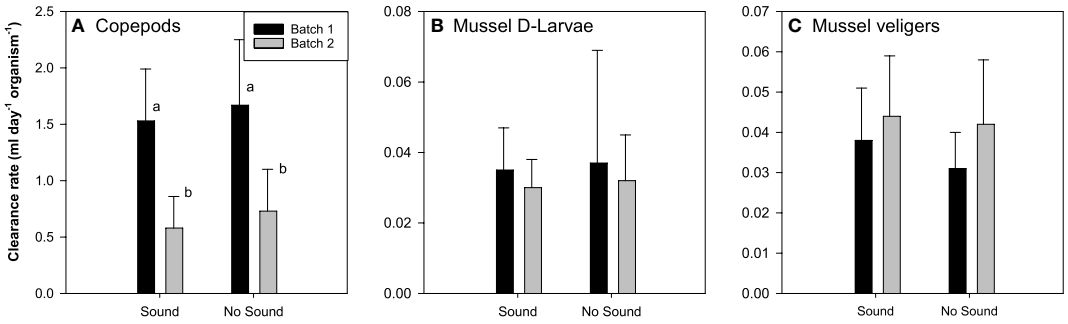

FIGURE 3
Mean clearance rates (ml day^{-1} organism^{-1}) ± standard deviation of zooplanktonic species [**(A)** Copepods, **(B)** Mussel D-larvae, **(C)** Mussel veligers] submitted or not to vessels noise exposure. Different letters indicate a significant difference. N = 12.

vessel noise treatments together). Similarly, for rotifers fed SELCO, there was no effect of vessel sound (n = 793, t = 1.654, p = 0.10). However those fed SELCO were slightly longer (9%, but not significantly) than the REED fed rotifers (n = 718, t = 0.27, p = 0.63), resulting in a mean rotifer length of 178.2 ± 14.5 µm. Females with one egg occurred in experiments with the SELCO feeding regime but, females with 2 eggs were not observed in any of the experiments. Vessel noise had a significant effect on the egg

TABLE 2 Fatty acid composition and total fatty acid concentration of rotifers fed SELCO formulation or REED microalgae concentrate.

	Rotifers SELCO	Rotifers REED
Fatty acid		
14:0	3.0 ± 0.1	2.6 ± 0.1
15:0	0.7 ± 0.0	0.7 ± 0.0
16:0	33.4 ± 0.3	40.7 ± 0.6
17:0	0.6 ± 0.0	0.9 ± 0.0
18:0	29.2 ± 0.7	41.2 ± 1.0
20:0	1.0 ± 0.0	0.7 ± 0.0
21:0	0.3 ± 0.0	0.1 ± 0.0
22:0	0.6 ± 0.0	0.3 ± 0.0
24:0	0.8 ± 0.0	0.3 ± 0.0
17:1w	0.8 ± 0.1	0.6 ± 0.1
18:1w9	5.4 ± 0.1	4.3 ± 1.0
20:1w9	2.3 ± 0.4	0.5 ± 0.0
22:1w9	1.6 ± 0.1	0.6 ± 0.1
24:1w9	0.7 ± 0.0	0.2 ± 0.0
18:2w6	1.5 ± 0.1	0.7 ± 0.1
18:3w6	0.4 ± 0.0	0.1 ± 0.0
18:3w3	0.7 ± 0.0	0.3 ± 0.0
18:4w3	0.8 ± 0.0	0.3 ± 0.0
20:3w6	0.5 ± 0.0	0.2 ± 0.0
20:4w6 (AA)	0.6 ± 0.0	0.2 ± 0.0
20:3w3	0.7 ± 0.0	0.2 ± 0.0
20:5w3 (EPA)	3.7 ± 0.2	1.1 ± 0.1
22:6w3 (DHA)	2.0 ± 0.1	0.7 ± 0.0
TFA µg mg^{-1} dry mass	346.7 ± 18.4	76.4 ± 2.6

production and the egg size of SELCO fed rotifers. In the control treatment (absence of vessel noise), 44% more eggs were produced (U = 0.011, p = 0.029) and eggs were slightly larger (t = 2.154, p = 0.034) compared to the sound treatment (Figure 4).

4 Discussion

Our results do not support the hypothesis that vessel noise negatively affects feeding behavior and growth of different zooplankton species. However, vessel noise negatively impacted rotifers egg production in those fed with higher fatty acid content. Our study represents a rare example of experimental studies focusing on the impact of anthropogenic noise on planktonic marine life (Chauvaud et al., 2018). We obtained data in small tanks characterized by the presence of reverberation, absent in field conditions. However, we replicated the sound level measured in the field and the sound spectrum exposure was similar to the natural exposure of zooplankton. In the present experiment the water volume was small, so that particle motion could not be measured. In the absence of technology to measure particle motion in small volumes of 200 ml, as used in the present study, only the pressure component of sound has been measured.

4.1 Phytoplankton

Microalgae concentrations from the control cells (no consumer/predator) at the beginning and at the end of each experiment showed no differences in presence or absence of vessel noise. Thus, vessel noise did not stimulate microalgae culture growth or cell death. In our experiments, the light level was too limiting to stimulate important culture growth. Concentrations were therefore stable during the 24h experiment and microalgae were still in suspension and available for zooplankton feeding.

4.2 Feeding behavior

We still do not completely understand how small invertebrates like mussel larvae and copepods detect marine sounds. However, McCauley et al. (2017), observed that low-frequency acoustic air gun impulse used at high level during seismic surveys decreased zooplankton abundance, by a level over two-fold. Copepods showed higher mortality within 10m distance of to an air gun impulse but no effect further form the sound source (Fields et al., 2019). Whereas, Jolivet et al. (2016) showed a positive impact of vessel noise on the settlement of mussel larvae which strongly suggests that mussel larvae might be able to sense the water vibration or the particle motion generated by vessel noise, similar to adults sensing

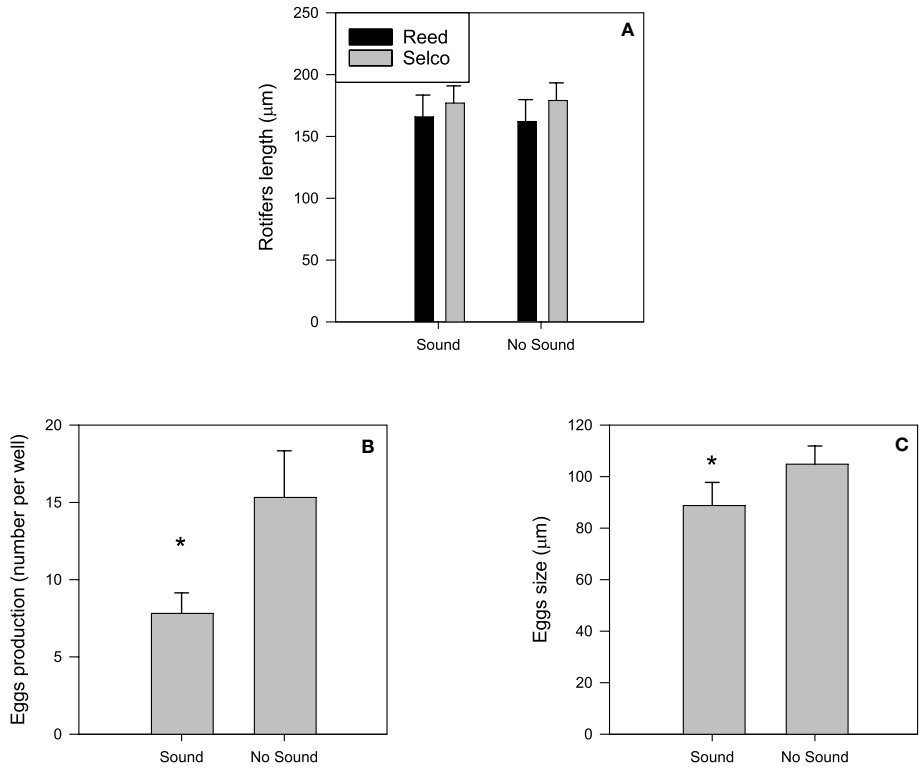

FIGURE 4
Impact of vessels noise exposure on length of rotifers **(A)** fed on two feeding diets (Reed and Selco) and on eggs productivity **(B)** and their length **(C)** produced by rotifers feed on Selco. No egg production was observed for rotifers feed on Reed. Asterix indicates a significant difference. Mean ± standard deviation, N= 12.

substrate-borne vibration in the range of 5 Hz to 400 Hz (Roberts et al., 2015; Olivier et al., 2023). This perception may be due to the presence of a pair of statocysts at the base of the foot, as observed in pediveliger of different bivalve species (Cragg and Nott, 1977; Bellolio et al., 1993). Statocysts are formed by invagination of the foot epithelium forming a spherical sac connected to the mantle cavity by a cylindrical ciliated canal and are used to orientate crawling. This ability to perceive noise and use it as a cue may also explain why we did not see any effect of noise on feeding behavior. As suggested by Jolivet et al. (2016), the natural habitat of the blue mussel is the near shore which is characterized by wave crashes on rocks producing a large range of underwater sound, including the range of intensity and frequency produced by the vessel noise used in our study. Thus, if we consider that vessel noise mimics natural noise present in the near shore, it was not surprising that mussel feeding behavior was not affected by it. Thus, our results suggest that mussels exposed to vessel noise maintain their ability to gain the energy needed for their future settlement and metamorphosis. The absence of an impact of vessel noise on the clearance rates of mussels was observed on two ontogenetic larval stages.

When comparing the veliger clearance rates with literature values for larvae of similar size (156 µm mussel larvae, Sprung, 1984), our results show lower values despite similar food concentration and temperature conditions. Sprung (1984) used a food concentration of *Isochrysis galbana* of 20,000 cells ml^{-1} and obtained a clearance rate of 0.1056 ml day^{-1} $larva^{-1}$ with a decrease to 0.0504 ml day^{-1} $larva^{-1}$ when algal concentration was 40,000 cells ml^{-1}. The microalgae size of *D. lutheri* (4–6µm), used here, was slightly larger than the size of *I. galbana* (4.5 µm) used in Sprung's (1984) experiment. Since retention efficiency of mussel larvae is maximal for phytoplankton of 3.5 µm in diameter, the size of *D. lutheri* could explain the lower clearance rate we observed. Food availability can also affect the filtration rate of bivalves (Hawkins et al., 1998), but in our experimental conditions, no food limitation was observed at the level of 30,000 cells ml^{-1}.

We also found no impact of vessel noise on the clearance rate of the copepod *Eurytemora herdmani*. Previous studies have found that the clearance rate of different species of copepods is dependent on algal concentration and can be adjusted until a maximum rate is reached (Conover, 1956; Mullin, 1963). Tackx et al. (2003) obtained clearance rates of *E. affinis* ranging from 0.24 ml to 0.36 ml day^{-1} $copepod^{-1}$, similar to clearance rates in our experiment. There is few information on the effects of noise on copepods as emphasized in comprehensive reviews, such as those by Popper and Hawkins (2016); Chauvaud et al. (2018) and more recently Bonnel et al. (2022) and Solé et al. (2023). Copepods can perceive underwater sound at the adult (Yen et al., 1992) or copepodite stage (Solé et al., 2021b) through mechanoreceptors (sensory setae) found on the first antenna (Weatherby and Lenz, 2000). Yen et al. (1992) showed that the effective range of stimulation was 40-1000 Hz and that spikes could be triggered with displacement velocities as small as 10 nm that fits within the range of particle motion associated to underwater sounds. The response to these mechanical stimuli were variable among the 15 copepod species tested (Yen et al., 1992). The absence of a response in feeding rate when exposed to vessel noise is thus surprising but might be related to the small model species *Eurytemora herdmani* tested in the present study. Indeed, McCauley et al. (2017) and Fields et al. (2019) assessed *via in situ* sampling and experiments, respectively, the seismic air gun impacts on copepods. Results were contradictory as low mortality was observed after seismic surveys exposure for *Calanus finmarchicus* (Fields et al., 2019) whereas major impacts were shown on diverse zooplankton assemblage including copepods (McCauley et al., 2017). Solé et al. (submitted) suggest that such opposite results can be explained by the size of the plankton species as the less impacted *C. finmarchicus* has a much larger size than the small copepod species that were mostly affected by the seismic air gun impulses in the study of McCauley et al. (2017). Solé et al. (2023) suggests that the impact of noise on marine organisms might be species-specific.

Our study used different invertebrate organisms that each feed with morphologically different apparatus. Mussel larvae feed with a velum and copepods with feeding appendages (Koehl and Strickier, 1981). In spite of those differences in the feeding appendages, we did not find an impact of vessels noise on any of these organisms.

4.3 Growth and egg production

The higher total fatty acid concentration and higher content in essential polyunsaturated fatty acids in the rotifers fed the SELCO formulation explains at least partially their better growth and egg production compared to those fed REED (Srivastava et al., 2006). The REED fed rotifers accumulated high levels of saturated fatty acids (Lubzens et al., 1985). SELCO is a commercial formulation specifically designed for the production and rearing of rotifers. Rotifers fed with SELCO contained sufficient essential fatty acids (EPA: 20:5n-3, DHA: 22:6n-3 and AA: 20:4n6) to stimulate high levels of growth and reproduction (Fernandez-Reiriz et al., 1993; Dhert et al., 2001). For example, EPA is known to be a fatty acid that is essentially required to sustain growth and reproduction of different invertebrates (Ravet et al., 2003; Guo et al., 2016) such as *Daphnia* (Müller-Navarra et al., 2000; Gladyshev et al., 2008), or purple sea urchin (Sanna et al., 2017), insects (Stanley-Samuelson, 1994a), and other invertebrates (Stanley-Samuelson, 1994b). The rotifer *B. plicatilis* is one of the very few organisms able to biosynthesize PUFA in conditions of food deficiency (Lubzens et al., 1985; Bell and Tocher, 2009). However, the rate of this biosynthesis is low and food deficiency in essential fatty acids does not support high levels of growth and reproduction (Lubzens et al., 1985). Thus, due to the use of REED and SELCO in different rotifer batches, it was possible to obtain rotifers with different physiological conditions. The poor condition of rotifers fed REED did not allow us to detect an impact of vessel noise. In the absence of vessel noise, rotifers fed REED did not produce eggs suggesting that their condition was not good enough to invest energy in their reproduction. However, we observed an impact of vessel noise in rotifers fed SELCO. Since the rotifers in the absence of sound and fed with SELCO produced eggs, their physiological condition was able to sustain energy investment in reproduction. When exposed to sound, rotifers were probably more stressed, leading to a decrease

in their energy investment in egg production which resulted in low numbers of smaller eggs. No information is available on organs in rotifers that would allow them to perceive underwater sound. However, the ciliated mechanosensory cells in their corona could be involved, as suggested in cnidarian medusae by Solé et al. (2016). The corona is a ciliated crown of the apical region of the body helping to acquire food and is used for locomotion.

5 Conclusion

No impact of vessels noise was observed on the feeding behavior of the mussel larvae or the copepods. Our study only found an impact of vessel noise on the egg production of rotifers. This information is important for the understanding of the effect of anthropogenic noise on marine life, as zooplanktonic species are at the basis of the marine food web. Thus, this study contributes to fill the gaps in knowledge on the impacts of anthropogenic noise on zooplankton for which little is known.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AA carried out the experiments, performed statistical analysis and has written the manuscript. RT supervised the entire study, established the methodology and provided technical support. FO and LC, provided the underwater acoustic recorder, acoustic support and participated in data analyses. AJ performed the acoustic analysis. CA, GW, and FJ participated in data analyses. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Fonds de Recherche du Québec - Nature et Technologies (FRQNT; team project 2016-PR-190063) and Ressources Aquatiques Québec Research Network (FQRNT, strategic network program #2014-RS-171172).

Acknowledgments

The authors wish to thank Nathalie Gauthier for logistical support during the experiment, Jean-Bruno Nadalini and Mathieu Babin for assistance in lipid analyses. The study is a contribution to the international laboratory BeBEST.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Marine Science

TYPE Mini Review
PUBLISHED 30 May 2023
DOI 10.3389/fmars.2023.1111599

OPEN ACCESS

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RECEIVED 29 November 2022
ACCEPTED 20 February 2023
PUBLISHED 30 May 2023

CITATION
Pysanczyn JW, Williams EA, Brodrick E, Robert D, Craggs J, Marhaver KL and Simpson SD (2023) The role of acoustics within the sensory landscape of coral larval settlement.
Front. Mar. Sci. 10:1111599.
doi: 10.3389/fmars.2023.1111599

The role of acoustics within the sensory landscape of coral larval settlement

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Recruitment of coral larvae on reefs is crucial for individual survival and ecosystem integrity alike. Coral larvae can detect and respond to a wide range of biotic and abiotic cues, including acoustic cues, to locate suitable sites for settlement and metamorphosis. However, the acoustic ecology of coral larvae, including how they perceive auditory cues, remains poorly understood. In this mini-review we consider both *ex situ* physiology and behavior, and *in situ* ecological and behavioral studies, to first provide an updated overview of the abiotic and biotic cues used by coral larvae to guide settlement. We then explore in detail the use of acoustic cues and the current literature on behavioral responses to acoustic stimuli. Finally, we discuss gaps in our understanding of the mechanisms by which coral larvae detect acoustic cues, highlighting a novel application of technology to explore these sensory capabilities. We also address how larval phonotaxis, i.e., the ability to orient to a sound cue, can be applied to coral reef conservation. Current research suggests that acoustic cues are likely used at small spatial scales, and that coral larvae may have directional acoustic sensitivity enabling phonotactic behavior. Recruitment of coral larvae on reefs is significantly influenced by habitat-specific soundscape variation and likely affected by anthropogenic disturbance. We propose a novel application of the remote sensing technology, micro-scanning laser Doppler vibrometry (LDV), to quantify the micromechanical responses of putative acoustically sensitive epidermal microstructures. We then highlight the potential for incorporation of acoustic enrichment techniques in coral reef conservation and restoration interventions.

KEYWORDS

coral reefs, bioacoustics, phonotaxis, laser doppler vibrometry, restoration, acoustic enrichment, larvae

1 Introduction

Marine invertebrate larvae were once considered passive particles lacking the ability to detect or respond to their environment (G. Thorson, 1950; Chia et al., 1984) but it is now widely accepted that interactions between both environmental conditions and biologically-generated cues affect larval behavior and physiology across many marine invertebrate taxa, including corals (Rodriguez et al., 1993; Shanks, 2009; Gleason and Hofmann, 2011) (Table 1). The ocean was once described as 'The Silent World' by Cousteau and Dumas in 1953, but we now know that coral reefs are bioacoustically rich. Many reef inhabitants produce sound during a wide array of behaviors which together contribute to the ambient soundscape of the "choral" reef (Schmitz, 2002; Lobel et al., 2010; Lobel, 2013; Radford et al., 2014a). This ambient soundscape has been shown to act as an orientation cue for the pelagic larvae of many fish, decapod crustaceans and reef-building corals, assisting their orientation towards suitable settlement sites (Tolimieri et al., 2000; Tolimieri et al., 2002; Jeffs et al., 2003; Simpson et al., 2004; Leis and Lockett, 2005; Simpson et al., 2005; Montgomery et al., 2006; Vermeij et al., 2010; Radford et al., 2011).

Reef-building corals represent keystone species in coral reef ecosystems, providing valuable ecosystem goods and services to 100s of millions of people (Woodhead et al., 2019). However, the behavioral responses of coral larvae (planulae) to acoustic stimuli and the sensory mechanisms by which they detect acoustic cues remain poorly understood. Yet, these are of increasing importance, especially in the context of growing anthropogenic pressures on coral reefs, including climate change, overfishing, sewage and fertilizer runoff and noise pollution (Lecchini et al., 2018; Richmond et al., 2018; Jones, 2019; Duarte et al., 2021). Coral larvae can respond to an array of environmental cues that guide their settlement. We review these, with a particular emphasis on acoustics and soundscapes, the importance of which is just recently coming to light.

1.1 Environmental cues influencing coral larval settlement

1.1.1 Water flow and local currents

Local water currents play an extremely important role in the connectivity between coral reefs, influencing species diversity, dispersal and recruitment of coral larvae across local to regional spatial scales (Roberts, 1997; Veron, 2000; Veron, 2011; Veron et al., 2015; Hata et al., 2017). Currents connecting reefs seldom fall below 100 mms^{-1} (Baird and Morse, 2014). As coral larvae swim at speeds of <5 mms^{-1} (Szmant and Meadows, 2006; Gleason et al., 2009; Hata et al., 2017), directed swimming from the open ocean to reefs is limited. Nevertheless, modelling using data obtained from fish has shown that vertical migration of larvae during ontogeny reduces interactions with ocean currents, thus altering recruitment and connectivity among reefs (Paris et al., 2007). Wave action has also been shown to accelerate development in purple sea urchin (*Strongylocentrotus purpuratus*) and Pacific sand dollar (*Dendraster excentricus*), where increased turbulence, associated with shallower coastal waters, induced larval competence and enhanced larval settlement (Gaylord et al., 2013; Hodin et al., 2018).

1.1.2 Light intensity

Corals need sufficient levels of solar radiation to support the photosynthetic requirements of their symbionts (Chalker et al., 1988). Ambient light levels, spectral quality and substratum color significantly influence larval settlement across many species of coral larvae (Babcock and Mundy, 1996; Mundy and Babcock, 1998; Mason et al., 2011; Strader et al., 2015; Foster and Gilmour, 2016; Sakai et al., 2020). However, the strength and directionality of larval phototaxis varies with species, age, water temperature, light intensity and wavelength of light (Lewis, 1974; Bassim and Sammarco, 2003; Brooke and Young, 2005; Gleason et al., 2006; Sakai et al., 2020; Mulla et al., 2021).

During settlement experiments, coral larvae of many species preferentially settle onto the undersides of substrates in shallower water, altering their settlement preferences to vertical and upward facing surfaces at greater depths (Birkeland, 1977; Bak and Engel, 1979; Birkeland et al., 1981; Wallace and Bull, 1981; Rogers et al., 1984; Harriott, 1985; Wallace, 1985; Babcock and Mundy, 1996; Strader et al., 2015). Several species, however, aggregate in darker regions, representing a trade-off between required photosynthetically active radiation (PAR) and intensified levels of ultraviolet radiation (UVR). At irradiance levels found in near-surface waters, light has been shown to increase avoidance behaviour (Gleason et al., 2006), prolong settlement (Baker, 1995; Kuffner, 2001) and cause higher levels of mortality of larvae (Gleason and Wellington, 1995; Wellington and Fitt, 2003).

1.1.3 Hydrostatic pressure

Hydrostatic pressure causes directional changes in swimming orientation (barotaxis) in a range of aquatic invertebrate taxa (Forward, 1990; Kingsford et al., 2002; Goldsteins and Butler, 2009). However, to our knowledge, only one study on the brooding coral *Porites astreoides* (Stake and Sammarco, 2003) has examined barotaxis in cnidarians. In this study, booded larvae were exposed to pressures ranging from surface conditions (103.4 kPa) to those at ~40 m below the surface. When exposed to surface pressure, larvae displayed positive barotaxis and swam downwards, but at greater pressures, larvae swam upwards (Stake and Sammarco, 2003). Although evidence of barotaxis in coral larvae is limited, these findings reflect those demonstrated by other zooplankton (Morgan, 1984; Forward, 1989; Forward, 1990; Kingsford et al., 2002). Furthermore, barotaxis enables corals to sense and settle in their species-specific optimal irradiance environments, even when irradiance information is lacking, e.g., during diurnal/nocturnal shifts or periods of shading (Stake and Sammarco, 2003; Gleason and Hofmann, 2011).

1.1.4 Temperature variation

Stressful sublethal temperatures interfere with normal settlement behavior in coral larvae. In studies on two broadcast-spawning corals, warmer water temperatures negatively affected

TABLE 1 Collated research outlining the abiotic and biotic environmental factors and cues that induce behavioral, physiological and ecological changes associated with enhanced or disrupted settlement in coral larvae.

	Environmental Factor/Cue	Behavioural, Physiological & Ecological changes	References
Abiotic	Light Intensity	1. Step-down photophobic response (marked decrease in swimming speed in response to an attenuation of light intensity). 2. Determination of settlement orientation 3. Avoidance of biological harmful levels of UVR 4. Delay in settlement 5. Increase in mortality	1. Sakai et al., 2020 2. Birkeland, 1977; Bak and Engel, 1979; Birkeland et al., 1981; Wallace and Bull, 1981; Rogers et al., 1984; Harriott, 1985; Wallace, 1985; Babcock and Mundy, 1996 3. Gleason et al., 2006 4. Baker, 1995; Kuffner, 2001 5. Gleason and Wellington, 1995; Wellington and Fitt, 2003
	Hydrostatic Pressure	1. Barotaxis	1. Stake and Sammarco, 2003
	Sedimentation	1. Reduction in net settlement 2. Induction of settlement on suboptimal surfaces	1. Lewis, 1974; Hodgson, 1990; Gilmour, 1999; Goh and Lee, 2008; Perez et al., 2014; Humanes et al., 2017 2. Babcock and Davies, 1991; Gilmour, 1999; Babcock and Smith, 2000; Birrell et al., 2005; Ricardo et al., 2017
	Temperature	1. Increased mortality 2. Reduction of pre-competency period 3. Reduction in settlement success 4. Increased respiration 5. Reduced photosynthesis 6. Reduced number of algal symbionts 7. Reduced longevity 8. Interference with the detection of other cues	1. Edmunds et al., 2001; Bassim and Sammarco, 2003; Randall and Szmant, 2009a; Randall and Szmant, 2009b 2. Nozawa and Harrison, 2005; Randall and Szmant, 2009a; Heyward and Negri, 2010 3. Jokiel and Guinther, 1978; Bassim et al., 2002; Bassim and Sammarco, 2003; Randall and Szmant, 2009a; Ritson-Williams et al., 2016 4. Edmunds et al., 2001; Edmunds et al., 2005 5. Edmunds et al., 2001; Edmunds et al., 2005 6. Edmunds et al., 2001; Edmunds et al., 2005 7. Edmunds et al., 2001; Putnam et al., 2008 8. Bassim and Sammarco, 2003; Putnam et al., 2008; Winkler et al., 2015
	Water Current/ Flow	1. Increased dispersal and reef connectivity	1. Roberts, 1997; Veron, 2000; Gleason and Hofmann, 2011; Veron, 2011; Veron et al., 2015; Hata et al., 2017
Biotic	Biochemical cues	1. CCA-induced settlement/ metamorphosis 2. Species specific and generalist attraction to CCA 3. Biofilm induced settlement/ metamorphosis 4. Response to CCA-associated microbial communities 5. Avoidance of repellent chemical cues produced by coralline algae and epithelial sloughing	1. Morse et al., 1988; Morse and Morse, 1991; Morse et al., 1994; Heyward and Negri, 1999; Hadfield and Paul, 2001; Negri et al., 2001; Baird and Morse, 2004; Golbuu and Richmond, 2007; Erwin et al., 2008; Vermeij and Sandin, 2008; Hay, 2009; Ritson-Williams et al., 2009; Diaz-Pulido et al., 2010; Ritson-Williams et al., 2010; Ritson-Williams et al., 2014; Ritson-Williams et al., 2016; Gómez-Lemos et al., 2018 2. Harrington et al., 2004; Ritson-Williams et al., 2009; Ritson-Williams et al., 2010; Tebben et al., 2015; Gómez-Lemos et al., 2018; Jorissen et al., 2021 3. Negri et al., 2001; Erwin et al., 2008; Tebben et al., 2011; Tran and Hadfield, 2011; Siboni et al., 2012; Sneed et al., 2014; Gómez-Lemos et al., 2018; Dobretsov and Rittschof, 2020; Siboni et al., 2020; Jorissen et al., 2021 4. Harrington et al., 2004; Ritson-Williams et al., 2010; Gómez-Lemos et al., 2018; Jorissen et al., 2021 5. Masaki et al., 1984; Keats et al., 1997; Suzuki et al., 1998; Degnan and Johnson, 1999; Harrington et al., 2004
	Acoustic cues and Soundscape	1. Positive phonotaxis 2. Increased settlement due to louder acoustic levels and higher levels of low-frequency sound 3. Interference of anthropogenic noise on settlement choice	1. Vermeij et al., 2010 2. Lillis et al., 2016; Lillis et al., 2018 3. Lecchini et al., 2018

larval physiology, dispersal and settlement *via* increased larval mortality (Bassim and Sammarco, 2003; Randall and Szmant, 2009a), increased swimming/searching behaviors (Bassim and Sammarco, 2003), reduced pre-competency period (Nozawa and Harrison, 2005; Heyward and Negri, 2010) and reduced settlement success (Jokiel and Guinther, 1978; Bassim et al., 2002; Bassim and Sammarco, 2003). Similarly, in studies on brooding corals, as water temperatures dropped below or exceeded the ambient temperatures from where they were collected, planulae exhibited increased mortality (Edmunds et al., 2001; Randall and Szmant, 2009b; Ritson-Williams et al., 2016), reduced longevity (Edmunds et al., 2001; Putnam et al., 2008), reduced net settlement (Hartmann et al.,

2013; Ritson-Williams et al., 2016), increased metamorphosis, reduced photosynthesis and diminished algal symbiont density (Edmunds et al., 2001; Edmunds et al., 2005).

1.1.5 Suspended and deposited sediment

Sedimentation has negative effects on both adult and larval-stage coral (Reviewed in Jones et al., 2015; Tuttle and Donahue, 2022). In observational studies of the brooding species *Favia fragum* (Lewis, 1974) and *Pocillopora damicornis* (Hodgson, 1990; Goh and Lee, 2008; Perez et al., 2014), net larval settlement was significantly reduced when suspended sedimentation was higher. Likewise, in field and laboratory studies, both high (~100 mg l^{-1}) and low (~50 mg l^{-1}) levels of suspended sediment adversely affected larval settlement and survival in the broadcast-spawning species *Acropora digitifera* and *A. tenuis* (Gilmour, 1999; Humanes et al., 2017). In both *in situ* and aquaria studies using larvae of the broadcast-spawning *A. millepora*, increased deposited sedimentation both reduced larval settlement and prevented larval settlement on upward facing substrates, with larvae settling only on vertical surfaces and the undersides of substrates (Babcock and Davies, 1991; Gilmour, 1999; Babcock and Smith, 2000; Birrell et al., 2005; Ricardo et al., 2017). Sedimentation most likely interferes with larval settlement by disrupting other sensory mechanisms, e.g., by masking chemical cues and impairing phototaxis (Ricardo et al., 2017). However, because it is difficult to track sediment dynamics on reef surfaces through time, it remains difficult to predict how the effects of sedimentation on short-term settlement will affect longer-term recruitment and survival.

1.1.6 Biochemical cues

In numerous studies of both brooding and broadcast-spawning coral species, crustose coralline algae (CCA) and its cell wall-associated compounds have been widely found to attract coral larvae and induce coral larval attachment (Morse et al., 1988; Morse and Morse, 1991; Morse et al., 1994; Morse et al., 1996; Heyward and Negri, 1999; Hadfield and Paul, 2001; Negri et al., 2001; Baird and Morse, 2004; Harrington et al., 2004; Golbuu and Richmond, 2007; Erwin et al., 2008; Vermeij and Sandin, 2008; Hay, 2009; Ritson-Williams et al., 2009; Diaz-Pulido et al., 2010; Ritson-Williams et al., 2010; Ritson-Williams et al., 2014; Tebben et al., 2015; Ritson-Williams et al., 2016; Gómez-Lemos et al., 2018; Jorissen et al., 2021). While CCA has also been found to induce settlement and metamorphosis across many different invertebrate taxa (Pawlik, 1992; Hadfield and Paul, 2001; Whalan et al., 2012; Sneed et al., 2015), the inducing capacity of CCA is highly variable, with complex interspecific interactions between corals and CCA. In two critically endangered species of broadcast-spawning Caribbean Acroporids (*A. palmata* & A. *cervicornis*), different species of CCA each induce varied amounts of larval settlement, with two relatively rare species of CCA being the most effective (Ritson-Williams et al., 2010). Interestingly, the cosmopolitan encrusting coralline algae *Titanoderma prototypumm*, found across both Caribbean and Pacific reefs, appears to be more attractive to larvae of reef-building Acroporids, inducing greater rates of settlement compared with other, more common, co-inhabiting CCA species (Harrington et al., 2004; Ritson-Williams et al., 2010; Gómez-Lemos et al., 2018). Furthermore, *T. prototypumm* significantly promoted settlement on the CCA surface compared with neighboring dead coral or plastic surfaces (Jorissen et al., 2021). In addition, some studies have found that specific microbial biofilms can also induce larval settlement in the absence of the CCA (Negri et al., 2001; Erwin et al., 2008; Tebben et al., 2011; Sneed et al., 2014; Gómez-Lemos et al., 2018; Dobretsov and Rittschof, 2020; Jorissen et al., 2021). Marine microbial biofilms are composed of many species of bacteria, unicellular algae (including diatoms) and protozoa. These produce an array of extracellular polymeric substances and signaling proteins shown to impact larval settlement and metamorphosis (reviewed in Dobretsov & Rittschof, 2020). Several studies have identified *Pseudoalteromonas* spp., a marine bacterium found in both Caribbean and Pacific CCA species, as a strong inducer of metamorphosis in larvae from both brooding and broadcast-spawning corals, including the important reef-building families Acroporidae and Pocilloporidae (Negri et al., 2001; Tebben et al., 2011; Siboni et al., 2012; Tebben et al., 2015) as well as an inducer of complete settlement (i.e., attachment to the substrate and metamorphosis) (Tran and Hadfield, 2011; Sneed et al., 2014; Tebben et al., 2015). It is worth noting that many CCA species have also evolved strategies to deter or prevent larval settlement, such as allelopathy (Suzuki et al., 1998; Degnan and Johnson, 1999) and sloughing (shedding of upper epithelial layers) (Masaki et al., 1984; Keats et al., 1997).

Thus, it is likely that CCA-induced coral settlement results from cues produced both by the CCA itself and by the associated microbial biofilm (Webster et al., 2004; Gómez-Lemos et al., 2018; Jorissen et al., 2021).

2 Acoustic cues and soundscapes

The grinding and popping of foraging echinoids, grazing scarids, vocalizing fish and snapping shrimp all contribute to the biophony of coral reefs (Simpson et al., 2004; Simpson et al., 2008; Lobel et al., 2010; Lobel, 2013; McWilliam et al., 2017). Thus, higher quality, healthy coral reefs are significantly louder, richer in acoustic events and more acoustically complex than degraded reefs (Piercy et al., 2014; Bertucci et al., 2016; Freeman and Freeman, 2016; Gordon et al., 2018). Acoustic cues are particularly useful for aquatic animals as sound travels faster and further underwater relative to other sensory cues, irrespective of directional currents (Urick, 1983; Ainslie, 2010; Duarte et al., 2021). Many marine invertebrates, therefore, have evolved the ability to detect and respond to acoustic cues, most likely by using specialized receptors (Salmon and Horch, 1973; Popper et al., 2001; Schmitz, 2002; Kaifu et al., 2008; Mooney et al., 2010; Vermeij et al., 2010; Wilkens et al., 2012; Lillis et al., 2013; Edmonds et al., 2016; Lillis et al., 2016; Solé et al., 2016; Vazzana et al., 2016; Charifi et al., 2017; Wale, 2017; Jézéquel et al., 2018; Lillis et al., 2018), and many taxa demonstrate increased rates of larval settlement in the presence of acoustic cues and during louder levels of acoustic cues (Jeffs et al.,

2003; Simpson et al., 2004; Simpson et al., 2005; Stanley et al., 2010; Simpson et al., 2011; Stocks, 2012; Stanley et al., 2012a; Stanley et al., 2012b; Lillis et al., 2013; Lillis et al., 2015; Hinojosa et al., 2016).

Acoustic cues can also influence the swimming orientation and settlement behavior of coral larvae. In an *in situ* settlement chamber experiment, larvae of the Caribbean scleractinian coral *Orbicella faveolata* (previously *Montastraea faveolata*) exhibited directed phonotaxis, with larvae moving towards the source of a broadcasted coral reef soundscape irrespective of chamber orientation (Vermeij et al., 2010). In a separate study, *O. faveolata* larvae exhibited higher settlement rates when exposed to soundscapes from louder, more diverse coral reefs when compared to soundscapes from two quieter reefs characterized by either sponges and coral rubble or industrial debris and algal growth. (Lillis et al., 2016). These findings imply that the elevated acoustic power associated with more diverse habitats, or the absence or presence of specific frequencies within healthier habitats, may lead to increased larval settlement. The same authors found that settlement rates in larvae of the reef-building coral *Porites astreoides* doubled in an acoustic environment with higher levels of low-frequency sound, which are typical of a healthier reef with higher coral cover and higher densities of fish (Lillis et al., 2018). This suggests that low-frequency sounds are the predominant drivers of response in this species, and that the absence of these low frequencies may reduce settlement.

High-frequency sounds attenuate more rapidly underwater, but lower-frequency sounds emanating from reefs are theoretically detectable to invertebrates within 500 m from the source (Rogers and Cox, 1988; Anderson et al., 2021). However, currents and fluid flows may limit the ability of larvae to successfully navigate to cues 500 m away; therefore in practice, the range of detection and successful response may be closer still to 10 – 100 m (Gleason and Hofmann, 2011). Although *O. faveolata* larvae exhibit directional phonotaxis *in situ* (Vermeij et al., 2010), the experimental confinement to an acrylic chamber likely restricted fluid flow, allowing larvae to move unimpeded by currents. Therefore, our understanding of the spatial scale at which coral larvae are able to detect acoustic stimuli in their natural environment is still limited. The difficulties associated with *in situ* settlement experiments in complex topographical and hydrodynamic environments both highlights the challenge of interpreting the ecological significance and restoration utility of experimental results (Hata et al., 2017; Mayorga-Adame et al., 2017; Randall et al., 2020; Levenstein et al., 2022) as well as the many considerations that must be made when deisgning future acoustic larval settlement experiments.

To date, most studies of phonotaxis in coral planulae have been conducted with larvae from broadcast spawners (but see Lillis et al., 2018), therefore larvae from brooding corals are relatively understudied. However, it is proposed that mechanosensory epidermal cilia are responsible for auditory perception in coral (Vermeij et al., 2010). Therefore, given the abundance of dense cilia found on their surface, brooded larvae are also expected to possess the sensory mechanisms to detect and respond to acoustic stimuli (Gleason and Hofmann, 2011). This hypothesis requires further testing.

3 Mechanisms for acoustic detection in coral larvae

Sonic vibrations in water have both pressure and particle motion components (Reviewed in Nedelec et al., 2016). In their adult stages, most aquatic invertebrates can detect the particle motion component of sound, using specialized organs such as mechanosensory setae, chordotonal stretch receptors between the joints of appendages and statocyst and statolith receptor systems (Popper and Fay, 1999; Popper and Lu, 2000; Popper et al., 2001; Bleckmann, 2004; Nedelec et al., 2016). Many invertebrate larvae, including those of cnidarians, have a diversity of cilia-based mechanosensory systems that function during feeding, locomotion, tactic response, predator–prey interactions and settlement (Chia and Crawford, 1977; Chia and Koss, 1979; Freeman and Ridgway, 1990; Marlow et al., 2009; Bezares-Calderón et al., 2020), with many of these systems sensitive to acoustic particle motion (Tranter et al., 1982; Rogers and Cox, 1988; Budelmann, 1992; Kennedy et al., 1996; Zhadan, 2005; Tran and Hadfield, 2013; Lillis et al., 2015).

The sensory mechanisms employed by coral larvae to detect acoustic stimuli, however, remain unknown. Early studies of the temperate reef-building coral-species *Balanophyllia regia* and the tropical coral species *Pocillopora damicornis* demonstrated that the larval ectoderm is primarily composed of flagellated collar cells - a single flagellum surrounded by a ring of microvilli (Lyons, 1973; Vandermeulen, 1975). While the main function of these cells are primarily thought to be calcification, phagocytosis of food particles and motility, it has been suggested that these cells may also have a sensory function. This assumption was based on their similarities with statocyst systems used in the detection of acoustic cues in other invertebrate taxa (Lyons, 1973).

The laser Doppler vibrometry (LDV) method relies on the detection of the Doppler frequency shift that occurs when light is dispersed by a moving surface (Rothberg et al., 2017). In a study exploring particle motion detection in marine invertebrates, LDV was used to measure whole body vibrations (displacement, velocity and acceleration) as a putative stimulus of statocyst organs in cuttlefish (Family Sepiidae) and scallops (Family Pectinidae) (André et al., 2016). This experiment piloted the use of LDV techniques in an underwater bioacoustics study and highlights its potential value for use across other marine invertebrate taxa. LDV has also been successfully used to measure the mechanical response of microstructures such as antennae and sensory hairs to electrical and sound stimuli in several terrestrial invertebrates (Göpfert et al., 1999; Göpfert and Robert, 2002; Sutton et al., 2016). Although it is evident that coral larvae both respond to acoustic cues and possess the mechanosensory structures capable of detecting particle motion (Vermeij et al., 2010; Lillis et al., 2016; 2018) (Figures 1C, D), to date there have not been any attempts to measure the mechanical responses of their exterior cilia-based sensory systems to acoustic cues in a bioacoustics study, nor has this been done for the larvae of any marine invertebrate. We propose that laser Doppler vibrometry could be broadly applied to investigate the mechanosensory ability

of coral larvae epidermal cilia, including quantifying both cilia beat dynamics and frequency-specific sensitivity to incident particle velocity. Using analytical signals capturing the spectral diversity of samples from coral reef sound recordings, and playbacks of the recordings themselves, it will be possible to determine the auditory sensitivity and bandwidth of coral larvae, offering a mechanistic basis for their phonotactic behavior (Figures 1A, B).

4 Ecological significance and applying acoustic enrichment to reef conservation and restoration

Coral reef soundscapes play an important role in coral larval orientation, habitat location, settlement and recruitment, ultimately affecting reef growth and resilience (Vermeij et al., 2010; Lillis et al., 2016; Lillis et al., 2018). However, with many coral reefs subject to degradation through climate change, overfishing and pollution, reef soundscapes are changing (Spalding and Brown, 2015; Hughes et al., 2017; Hughes et al., 2018; Duarte et al., 2021). For example, between 2012 and 2016, cyclones and intense bleaching meant the Great Barrier Reef experienced the most severe degradation period in recorded history (Hughes et al., 2017). As a result, soundscapes were negatively impacted across four complementary ecoacoustic indices; they were on average 15 dB SPL re 1 µPa quieter and had significantly reduced acoustic complexity, richness and rates of snapping shrimp (Family Alpheidae) snaps (Gordon et al., 2018).

In light of the ecological crisis on coral reefs, novel restoration techniques are becoming increasingly important in the conservation and restoration of these ecosystems. One promising new tool is acoustic enrichment, whereby recordings from relatively healthy coral reefs are played back through underwater speakers (Gordon et al., 2019). This approach has been demonstrated to improve metrics of fish community health in degraded coral reef habitat on an experimental scale (Gordon et al., 2019). Over the natural fish breeding season on the Great Barrier Reef (November-December), this study showed that reefs with acoustic enrichment had increases in fish recruitment across multiple trophic guilds, a doubling in overall fish abundance, and a 50% increase in species richness (Gordon et al., 2019). A subsequent study found that successful management and restoration of coral reefs leads to the recovery of the natural soundscape; maturing restoration projects in Sulawesi exhibited similar levels of acoustic richness to healthy reefs (Lamont et al., 2021).

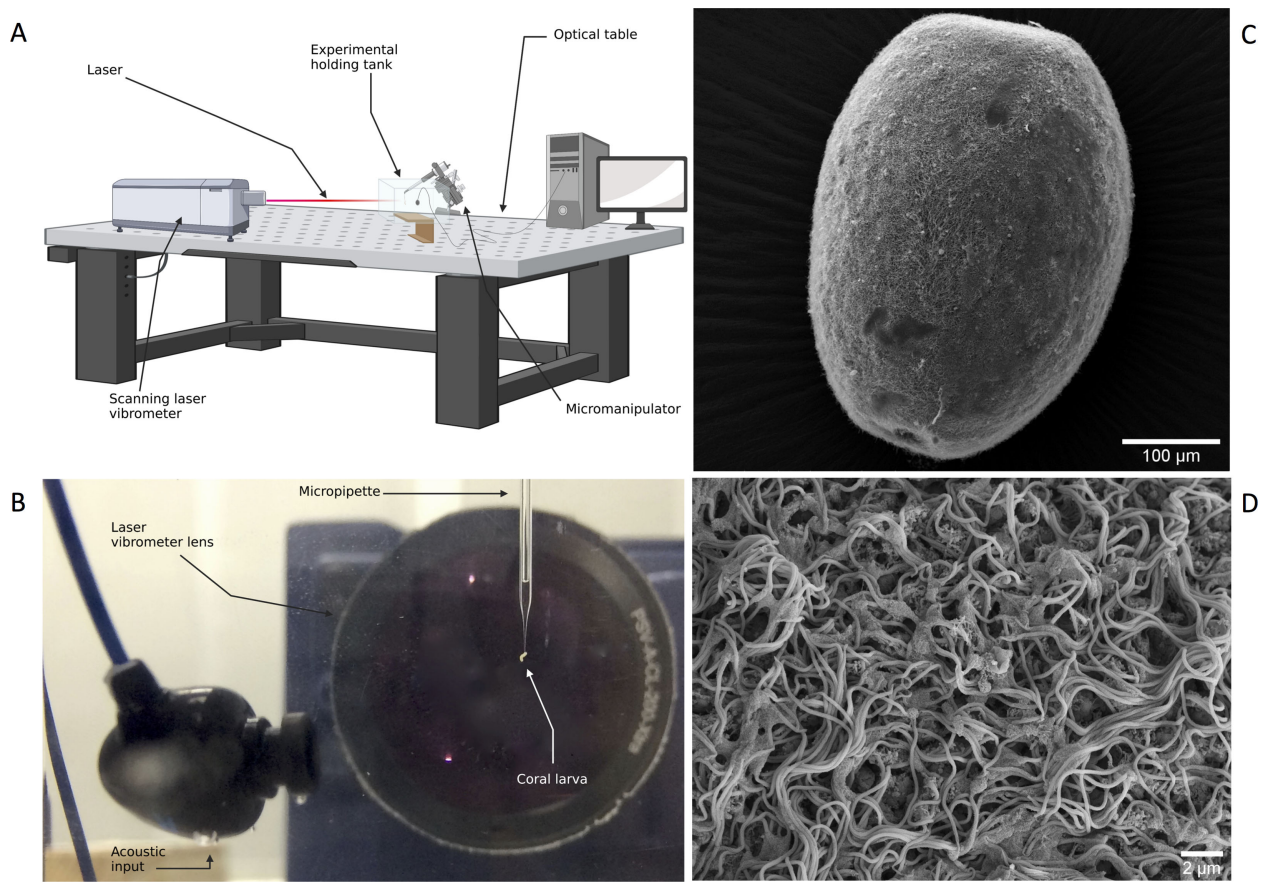

FIGURE 1
(A) Proposed set-up for coral larvae laser Doppler vibrometry experiment. **(B)** Close-up of set-up for tethering *Acropora millepora* larvae in laser Doppler vibrometry experiment **(C)** Scanning Electron Microscopy (SEM) image of an *Acropora millepora* planula larva. **(D)** Magnified larval epiderm highlighting cilia. SEM images: Emelie Brodrick. Laser Doppler Vibrometry schematic **(A)** created with BioRender.com.

Recent coral reef restoration efforts have focused on increasing population sizes, genetic diversity and the natural adaptive capacity of corals, for example, through fragment rescue, asexual propagation, *in situ* and *ex situ* coral nurseries and sexual propagation in order to mitigate reef degradation caused by climate change and local stressors (Heyward et al., 2002; Cruz and Harrison, 2017, dela Cruz and Harrison, 2020; Suzuki et al., 2020; Randall et al., 2020; Vardi et al., 2021; Harrison et al., 2021; Baums et al., 2019, 2022). In addition, coral breeding efforts in land-based facilities continue to scale up (Craggs et al., 2017; Craggs et al., 2020; O'Neil et al., 2021) while virtually all coral propagation programs seek more efficient ways to induce coral settlement in large numbers without introducing potentially detrimental competing organisms (Randall et al., 2020). Acoustic enrichment can be used in conjunction with all of these newer, breeding-based restoration techniques to help increase settlement rates, population growth and species diversity. By boosting coral settlement at restoration sites, short term acoustic enrichment will also help to restore natural acoustic complexity and phonic richness, thus further accelerating and reinforcing reef recovery.

Current examples of acoustic enhancement in reef restoration include 'The Reef Song Project', an Australian Coral Reef Resilience Initiative (ACRRI) undertaken in association with the Australian Institute of Marine Science (AIMS). This project is the first to investigate the efficacy of acoustic enrichment *in situ*. Using healthy reef recordings to attract fish communities to sixty patch reefs made of coral rubble and live fragments at Ningaloo Reef and the Great Barrier Reef in Australia, this five-year initiative is primarily exploring the roles of fish husbandry and herbivory on coral growth and reef recovery. Using photogrammetry, coral growth will be monitored over time (Australian Institute of Marine Science, 2023). Additionally, the Woods Hole Oceanographic Institute (WHOI) have developed the 'Reef Solutions Initiative'. Following the discovery by WHOI scientists that coral larvae are attracted to the soundscapes of healthy reefs (Lillis et al., 2016; Lillis et al., 2018), this initiative seeks to incorporate acoustic enrichment into intervention strategies to help corals repopulate degraded reefs (Woods Hole Oceanographic Institute, 2023). To improve our understanding of the reef recovery process and the impact of reef restoration, the application of low-cost, low specification passive acoustic monitoring in combination with machine-learning analysis may be applied to improve the analysis of ecoacoustic indices and successfully track coral reef restoration (Lamont et al., 2022; Williams et al., 2022).

In sum, acoustic enrichment is a promising tool for coral reef restoration due to its demonstrated efficacy across multiple taxa, yet its potential is still largely untested. Restoring keystone species and re-establishing complex interspecific interactions can promote successful management and restoration of coral reef ecosystems. Reef-building scleractinian corals are keystone species and it is their three-dimensional structure on which all coral reef life forms depend for food, sanctuary and survival. In order to fully assess the potential of acoustic enrichment and effectively apply this method as a reef restoration tool, we must continue to explore how different coral taxa respond to acoustic cues while gaining a better understanding of the mechanisms by which coral larvae sense their acoustic environment. This will also allow us to effectively place acoustics within the hierarchy of sensory cues that coral larvae integrate to locate an optimal site for settlement and recruitment to the reef.

Author contributions

JP, EW and SDS conceived the idea for this mini-review. JP wrote the manuscript. All authors listed made substantial contribution to the discussion of ideas outlined in the work and the development of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This material is based in part on work supported by NSF grants IOS-1848671 and CBET2133675 (to KLM). EWA is supported by a BBSRC David Phillips Fellowship BB/T00990X/1. SDS is supported by NERC Grant NE/P001572/1.

Acknowledgments

We/The authors would like to thank Prof. Andrew Radford and the extended University of Exeter and University of Bristol Bioacoustics Research Group for their ongoing stimulating discussions, support and insight.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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TYPE Original Research
PUBLISHED 19 June 2023
DOI 10.3389/fmars.2023.1117431

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RECEIVED 06 December 2022
ACCEPTED 10 April 2023
PUBLISHED 19 June 2023

CITATION
Gigot M, Tremblay R, Bonnel J, Chauvaud L and Olivier F (2023) Physiological condition of the warty venus (*Venus verrucosa* L. 1758) larvae modulates response to pile driving and drilling underwater sounds.
Front. Mar. Sci. 10:1117431.
doi: 10.3389/fmars.2023.1117431

Physiological condition of the warty venus (*Venus verrucosa* L. 1758) larvae modulates response to pile driving and drilling underwater sounds

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Noise is now recognized as a new form of pollution in marine coastal habitats. The development of marine renewable energies has introduced new sonorous perturbations, as the wind farm installation requires pile driving and drilling operations producing low frequency sounds at high sound pressure levels. Exponential expansion of offshore wind farms is occurring worldwide, making impact studies, particularly on benthic species highly abundant and diverse in the coastal area used for wind farming, a necessity. As larval recruitment is the basis for establishing a population, we conducted an experimental study to assess the interactive effects of pile driving or drilling sounds and larval rearing temperature on the endobenthic bivalve *Venus verrucosa*. In ectothermic animals, temperature modifies the organism's physiology, resulting in performance variability. We hypothesize that temperature modulation could change larval responses to noise and explore the potential interacting effects of temperature and noise. Using two distinct rearing temperatures, physiologically different batches of larvae were produced with contrasting fatty acid content and composition in the neutral and polar lipid fractions. Without defining any absolute audition threshold for the larvae, we demonstrate that the effects of temperature and noise were ontogenic-dependent and modulated larval performance at the peri-metamorphic stage, acting on the metamorphosis dynamic. At the pediveligers stage, a strong interaction between both factors indicated that the response to noise was highly related to the physiological condition of the larvae. Finally, we suggest that underwater noise reduces the compensatory mechanisms established to balance the temperature increase.

KEYWORDS

anthropophony, energetic metabolism, larval recruitment, metamorphosis trigger, fatty acids

1 Introduction

Thermal tolerance is species specific (Rayssac et al., 2010), with each species occupying a particular thermal niche of optimal functioning outside which it may fail to survive. Within a thermal range, temperature controls various essential features of an ectothermic organism's physiology, as it alters chemical and enzymatic reactions, rates of diffusion, membrane fluidity, and protein structure (reviewed in Sokolova, 2021), resulting in performance variability. The present study focused on an ectothermic infaunal bivalve species, the warty venus *Venus verrucosa*, which lives on seagrass habitats, detrital sandy, or coralline rhodolith bottoms to a depth down to 30 m and has a great commercial interest (Arneri et al., 1998). As other filter-feeding bivalve, it provides ecosystem services as reviewed in Vaughn and Hoellein (2018) and Smaal et al. (2019). *V. verrucosa* has broad thermal tolerance that explains its large distribution in the Atlantic from Norway to South Africa, and in the Mediterranean Sea (Poppe and Goto, 1993). Recently, Forêt et al. (2020) showed that rearing temperature modulates the fatty acid profile of *V. verrucosa*, as juveniles reared at 20°C contained largely less energetic (neutral) lipids than those reared at 15°C. As the main energetic reserve in marine bivalve larvae are the neutral lipids (Holland and Spencer, 1973; Gallager et al., 1986; Whyte et al., 1991), they positively correlate with their survival (Rayssac et al., 2010). Thus, temperature modulation could have long-term impacts on fitness. Moreover, energy metabolism modulates the responses to multiple stressors (Sokolova, 2021), and temperature is known to interact with many other factors. For example, Cherkasov et al. (2007) showed that temperature amplifies the toxicity of cadmium, leading to elevated oxidative stress in mitochondria, which may have important implications for the survival of *Magallana gigas*. Reciprocally, cadmium pollution reduces the thermal tolerance of *M. gigas* (Lannig et al., 2006).

Aquatic anthropogenic noise was recently recognized as a new form of pollution (Barber et al., 2010; Slabbekoorn et al., 2010) and, as it increases annually (Chapman and Price, 2011; Tournadre, 2014), several authors have emphasized its impact on adult marine organisms, including behavior (Fewtrell and McCauley, 2012), oxygen intake (Regnault and Lagardere, 1983; Wale et al., 2013a), food uptake (Wale et al., 2013b; Charifi et al., 2017), growth (Lagardère, 1982), and gene expression (Peng et al., 2016), and could even induce severe injuries (André et al., 2011). Noise also impacts larval development of invertebrates, as some studies have revealed significantly deep effects, particularly on growth, survival, and settlement success (Branscomb and Rittschof, 1984; de Soto et al., 2013; Gigot et al., in revision; Wilkens et al., 2012; Lillis et al., 2015; Jolivet et al., 2016). While many authors agree that the main sensory organ involved in sound perception in bivalve larvae are statocysts (ciliated cells containing statolith or statoconia; Budelmann, 1992), that are observed at the pediveliger stage (Cragg and Nott, 1997; but see extensive review on invertebrates in Solé et al., 2023), their audition thresholds or sensitivity to particular frequencies remains largely unknown. Such research is particularly pertinent in the context of renewable energy device installations, such as wind farms, which usually settle in shallow coastal water overlapping areas of rich biodiversity (Ramirez et al., 2020). Offshore wind farms are growing in size and number, with a global capacity that could increase 7-fold by 2030 (Lee and Zhao, 2021) and involves drilling and pile driving operations that generate high levels of anthropophony (Norro et al., 2013). Pile driving noise results in short impulses with high sound pressure and broadband spectrum below 1 kHz (SPL_{p-p} = 205 dB re 1 µPa @ 100 m) (Robinson et al., 2013). Drilling is characterized by a continuous broadband sound, with maximum energy between 100 Hz and 10 kHz (SPL_{rms} =184 dB re 1 µPa @ 1m) (Kyhn et al., 2014).

Within this context, we tested whether temperature could modulate the response of *V. verrucosa* larvae to anthropogenic noise. Most bivalve species display a biphasic life-cycle with early swimming pelagic veliger larvae developing into a competent pediveliger stage able of settling and metamorphosing into a benthic post-larva (Figure 1). Pediveliger larvae select their benthic habitat upon several environmental biotic and abiotic variables (Toupoint et al., 2012) including soundscape (Lillis et al., 2013). We decided to study this particular transient phase and exposed larvae at pre-metamorphic veliger and peri-metamorphic pediveliger stages.

As sound impact on *V. verrucosa* has never been investigated, we hypothesize that drilling and pile driving playback modifies the settlement dynamics of competent pediveliger (Eggleston et al., 2016), as observed in epifauna species, such as blue mussel *Mytilus edulis* (Jolivet et al., 2016) and great scallop *Pecten maximus* (Gigot

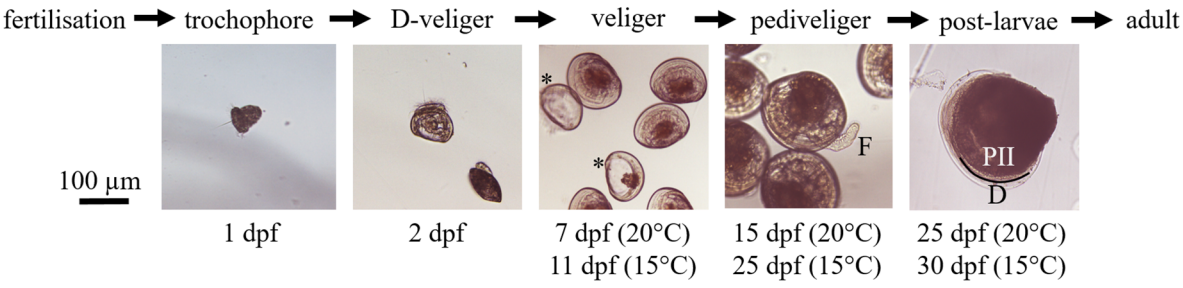

FIGURE 1
Larval development of *V. verrucosa* during the experiment. Corresponding days post-fertilization (dpf) are indicated below each picture for the two rearing temperatures. Stars (*) highlight two empty shells considered as dead veliger larvae. On pediveligers picture we can distinguish the 'foot' specific to this stage, indicated by (F). The demarcation between prodissoconch II (PII) and the dissoconch (D) shells, which is a criterion of metamorphosis, is materialized by a black line.

et al., 2023). Temperature mainly influences the physiological state of bivalve larvae by modifying the lipid composition (Pernet et al., 2007; Rayssac et al., 2010; Barret et al., 2016) and thereby could impact larval development, particularly the success of metamorphosis. Lipid accumulation (neutral) and membrane fatty acid (polar) composition acting on membrane fluidity are modified by temperature exposure and modulate the responses to stressors. We tested the hypothesis that response of warty venus to anthropogenic noise highly relates on their physiological state, both in terms of energetic reserves (neutral lipids) and fatty acid tissue composition (polar lipids). Thus, two larval batches were produced at different rearing temperature in the thermal niche of the warty venus to obtain contrasting total fatty acid (TFA) content and profiles, as already observed by Forêt et al. (2020) in young juveniles of *V. verrucosa*, before their exposure to pile driving and drilling noises.

2 Materials and methods

2.1 Thermal modulation of physiological state

Larvae were obtained following a modified protocol (Buestel et al., 1982) detailed by Forêt et al. (2020). Adults were collected by dredging in the Bay of Brest in January 2021 and fed continuously during gametogenesis at the 'Ecloserie du Tinduff' (Plougastel-Daoulas, France) with a DTCS diet (*Diacronema lutheri*, *Tisochrysis luthea*, *Chaetoceros neogracilis, Skeletonema marinoi*; ⅔ DT, ⅓ CS). Spawning was induced by thermal shock in 30 adults and cross-fertilization performed as described by Beaumont and Budd (1983). The resulting eggs were incubated for 48 h at 18°C in cylindro-conical tanks filled with 1-µm filtered, UV-treated seawater treated with 9 ppm erythromycin to avoid bacterial development (salinity = 33 psu; temperature = 19.5°C). Use of erythromycin treatment on scallop larvae has been demonstrated do not impact the long-term *P. maximus* larval performance (Holbach et al., 2015). Two days after fertilization (2 dpf), trochophore larvae were sieved and transferred to two larval tanks at a temperature of either 15°C or 20°C (Figure 2), and at 40 larvae/ml. Each day, the water was renewed and dead individuals counted and removed by sieving. Larvae were fed daily with a 3:3:2:2 ratio of DTCN diet (N for *Nannochloropsis occulata*) at 40 cells/µl adjusted to the biovolume of *T. luthea* (Helm and Bourne, 2006). At the pediveliger stage, larvae were fed with a DTCSN diet (1:1:1:1:1). We conducted two experiments, one at the veliger stage and the second at the pediveliger stage (Figure 2). During the first day (d0) of each experiment, larval samples were fixed in 4% formaldehyde until further abundance counting. Sampled larvae were counted and measured under a microscope (Zeiss Axioscope A1, x40 magnification) equipped with a digital camera (Moticam 3.0 10+). To assess the mortality rate at d0 in each tank sample

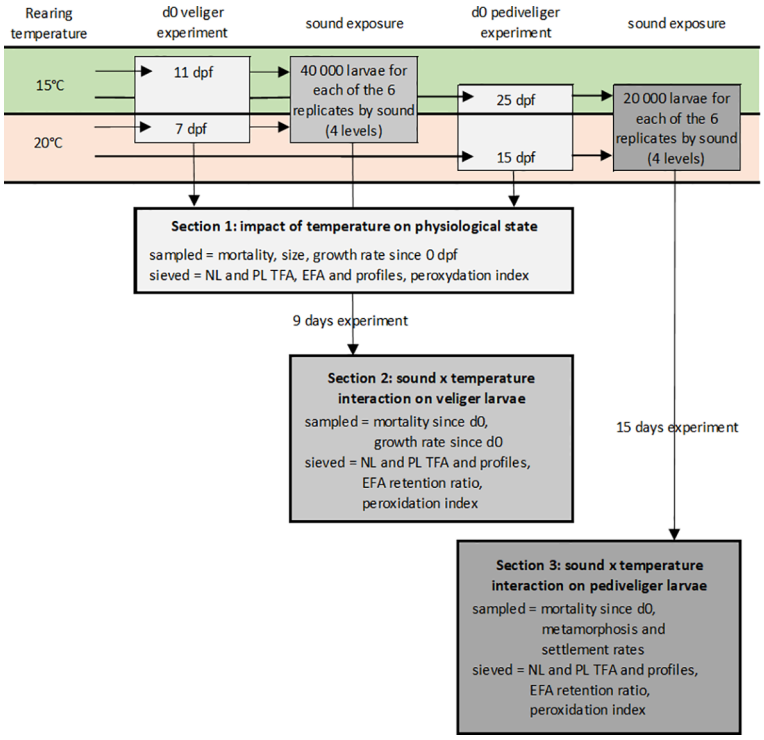

FIGURE 2
Schematic representation of the experimental protocol. Two batches of *Venus verrucosa* larvae were reared at 15 and 20°C. After 11 or 7 dpf and 25 or 15 dpf, depending on the batch, veliger and pediveliger larvae replicates were sampled and sieved to determine the impact of temperature on their physiological state. The veliger and pediveliger replicates were separately exposed to two temperatures (15 and 20°C) and four sound levels (control, low and high pile driving, and high drilling) for 9 and 15 days. At the end of the experiments, larvae were sampled and sieved to determine the potential interaction between the two factors.

(Figure 2 Section 1), we calculated the ratio between empty shells (Figure 1) and alive larvae. Mean shell length, from the umbo to the most distant part of the shell, was measured using Motic Images plus 3.0 software for 100 individuals in each tank. Growth rates were then calculated separately for veliger/pediveliger and 15/20°C batches by dividing the mean size deducted from the length at d0 by the number of days since fertilization (dpf).

At d0 of the veliger and pediveliger experiments (Figure 2 Section 1), batches of 40 000 and 20 000 larvae, respectively, from each of the 15 and 20°C populations, and DTCSN and DTCN diets (4 replicates of each) were sieved on pre-burned glass microfiber filters (GF/F) and stored at -80°C until fatty acid analyses. The GF/F filters were first lyophilized, weighed, and lipids extracted following the procedure in Folch et al. (1957) using dichloromethane–methanol instead of chloroform as modified by Parrish (1987). Extracts were separated into neutral and polar fractions by chromatography on silica gel micro-columns (30×5 mm i.d., packed with Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) (Marty et al., 1992). Neutral lipids represent energetic lipids mainly Triacylglycerids (TAG) and polar lipids are structural lipids mainly Phospholipids (PL). Each fraction was methylated in fatty acid methyl esters (FAMEs) following the modified method from Lepage and Roy (1984), and the NL samples were purified on an activated silica gel with 1 mL of hexane:ethyl acetate (v/v) to eliminate free sterols. FAMEs were analyzed in the full scan mode (ionic range: 50–650 m/z) on a Polaris Q ion trap coupled multichannel gas chromatograph (Trace GC ultra, Thermo Scientific, MA, USA) equipped with an autosampler (model Triplus), PTV injector, and mass detector (model ITQ900, Thermo Scientific, MA, USA). Separation was performed through a Supelco Omegawax 250 capillary column (30 m × 250 µm × 0.25 µm film thickness). The initial oven temperature was 100°C for 2 min, then 140°C for 1 min, and was increased at a rate of 10°C/min until it reached 270°C, where it was held for 15 min. The injector temperature was 90°C and a constant helium flow of 1.0 ml/min was used. A volume of 1 µl was injected. Fatty acids were identified and quantified by comparing retention times and mass spectra with a calibration curve of known standards with concentrations ranging from 0.5 to 20 µg/ml (Supelco 37 Component FAME Mix Supelco Inc., Belfonte, PA, USA) using Xcalibur v.2.1 software (Thermo Scientific, Mississauga, ON, CA). Fatty acids are designated as X: YwZ, where X is the number of carbons, Y the number of double bonds, and Z the position of the ultimate double bond from the terminal methyl group. We report the values of each fatty acid as absolute concentrations (mg/g) or percentage of TFA (for profile composition comparisons) separately for NL and PL fractions. We present the concentrations of three essential fatty acids (EFAs): arachidonic acid (ARA, 20:4w6), eicosapentoic acid (EPA, 20:5w3), and docosahexaenoic acid (DHA, 22:6w3).

All data analyses were performed in PRIMER7 software using the PERMANOVA+ package. We performed analysis of variance for univariate or multivariate data on resemblance matrices using 10 000 permutations (Legendre and Legendre, 2012) under a reduced model. Similarity matrices were produced using Bray-Curtis or Euclidean distance on fatty acid profiles and all other data, respectively. For both veliger and pediveliger stages, univariate 1-factor PERMANOVA were performed on d0 data, including size, growth rate, and TFA and EFA concentrations separately in NL and PL fractions to test the impact of rearing temperature (15°C and 20° C). We also conducted a multivariate PERMANOVA on the whole fatty acid profile for both lipid fractions (NL/PL) and both larval stages (veliger/pediveliger). When a significant difference was detected in the fatty acid profiles (p-perm < 0.05), a similarity percentage breakdown (SIMPER) (Clarke, 1993) was computed to determine which fatty acid contributes the most to the difference.

2.2 Sound × temperature interaction on veliger larvae

Exposure to drilling and pile driving sounds was realized using the *Larvosonic* system (Olivier et al., 2023), which includes a main 800-l tank, and a Clark Synthesis AQ339 Diluvio™ underwater speaker (https://clarksynthesis.com/aq339/) connected to a power amplifier allows sound emission to six 5-l cylinders (independent replication units above the speaker) half-immersed in this water bath. Because invertebrates lack gas-filled organs classically used to sense the pressure component of sound, they are sensitive to the motion of water particles *via* statocysts (Mooney et al., 2012; Popper and Hawkins, 2018). However, in the *Larvosonic* system, we demonstrate experimentally that when the sound level decreases, both acoustic pressure and particle motion decrease by exactly the same level (Olivier et al., 2023). Audition thresholds of the larvae are unknown and our experiment is not intended to define them but to explore initially the potential responses of the larvae to anthropic sound at levels comparable to those emitted in the natural environment. Emission levels were calculated by recording 30 s of sound at the center of each tank using an RTSYS EA-SDA14 (https://rtsys.eu/) underwater acoustic recorder (sampling frequency 78 kHz, 32-bit resolution) equipped with an HTI-96-min hydrophone (sensitivity = -165 dB re 1 V/µPa). Then emission levels were adjusted to match our experimental design. The pile driving sound sequence was recorded during the building phase of an offshore marine wind farm in the North Sea (depth ~30 m, SOMME database), and the drilling sound sequence corresponds to a recording of geotechnical drilling made in June 2018 at a distance of 200 m from the boat (SOMME database). Both sounds were the same than those characterized in Olivier et al. (2023). Pile driving is an impulsive sound (one 200-ms impulse every 3 s) dominated by low frequencies (40 - 800 Hz) (Supplementary Material S1). Drilling is continuous, and its spectrum is characterized by a high level in the 150 - 600 Hz and 4000 - 7000 Hz frequency ranges (Supplementary Material S1). Different *Larvosonic* tanks (n=8) with non-filtered seawater were deployed equitably in two controlled rooms (15°C and 20°C) under a 12:12h photoperiod. In each room, we generated drilling at high intensity (called D) in one tank (SPL_{rms} = 175.4 ± 2.3 dB re 1 μPa^{-1}), and two increasing levels of pile driving (P and P+) in two other experimental tanks (SPL_{pp} = 147.6 ± 2.5 and 187.6 ± 2.4 dB re 1 μPa^{-1}). As no sound was emitted in the fourth control tank, it characterized the ambient sound of the experimental room. The frequency content was maximum under 1000 Hz (low-frequencies) and levels (SPLrms =

98.8 ± 0.8 dB re 1 µPa-1) and spectrum were consistent with ambient sound levels recorded in temperate coastal environments of the western English Channel with contrasting wind conditions (Mathias et al., 2016) (Supplementary Material S1). However control condition do not reproduce natural acoustic conditions and the objective is to investigate the effect of the addition of anthropic sounds.

Veliger experiments started when mean larval length reached 124 µm (i.e., 7 or 11 dpf for larvae reared at 20 or 15°C, respectively). On the first day of the experiment, cylinders were filled with 5 l of 1-µm filtered, UV-treated seawater and 9 ppm erythromycin. Approximately 40 000 veliger larvae were introduced into each of the 48 cylinders. Drilling and pile driving sounds were emitted following 19:5 h and 6:6 h on:off cycles, matching the on-site work conditions (Ailes Marines pers. com.) for 9 days (Figure 2 Section 2). Larvae were fed once a day with mix algae at a concentration of 40 cells/µl as already described. Every 3 days, dead and alive larvae were sieved and the water renewed. At the end of veliger exposure (day 9), three larval samples were taken in each cylinder and fixed with 4% formaldehyde. Mortality and growth rates were assessed as the difference between the means on day 9 (N = 48) and d0. The daily growth rate was measured on 33 individuals by replicate, then divided by the number of days (i.e., 9 days). The remaining larvae were sieved on GF/F filters and stored at -80°C until further analysis of the fatty acids as described previously. We calculated absolute concentrations of TFA, fatty acid profiles (%), and the EFA selective retention ratio (ratio between PL fatty acids contained in larvae and the concentration of the total fraction of the same fatty acid in diet to investigate potential selective retention) separately for the NL and PL fractions. If the relative proportion of a fatty acid in the larvae/diet was >1, it was selectively incorporated and could suggest potential dietary deficiency under this rearing condition.

Separately for each parameter (mortality and daily growth rates, TFA concentrations, fatty acid profiles, EFA ratios, and peroxidation index), two-way PERMANOVA was performed to assess the impact and potential interaction between temperature (15°C and 20°C) and sound (C, D, P, and P+) treatments. Significant differences were analyzed by multiple comparison pairwise tests, and fatty acids that contribute the most to the significant difference between fatty acid profiles were assessed by similarity percentage breakdown (SIMPER).

2.3 Sound × temperature interaction on pediveliger larvae

Pediveligers were exposed to similar sound and temperature treatments as veligers except for the following points. The experiment was started when pediveligers reached a mean length of 190 µm at 15 and 25 dpf for 20°C and 15°C batches, respectively. Approximately 20 000 pediveligers were introduced in each replicate cylinder and exposed to sound treatments for 15 days (Figure 2 Section 3). At each seawater renewal (days 3, 6, 9, 12, or 15), each cylinder was gently rinsed over a 60-µm square mesh sieve to collect swimming larvae. Crawling larvae were detached from the walls and bottom of each cylinder by a gentle water jet and set apart. Three samples were taken in both the swimming and crawling larval fraction for further counting. On days 9 and 12, the remaining crawlers were sieved on GF/F filters and swimmers were put back into the cylinders. On day 15, both fractions were sieved on GF/F filters and stored at -80°C. The NL and PL fatty acid content of larvae collected on day 15 in each replicate cylinder were assessed on pooled swimmer and crawler fractions using previously described methods.

Mortality rates were assessed for each of the samples (N = 48) on days 9, 12, and 15 by subtracting the d0 mortality rate. We used the presence of demarcation between prodissoconch II and the dissoconch shells as a criterion of metamorphosis (Martel et al., 1995) to determine metamorphosis rates. As on days 9 and 12, the crawler fraction was removed from the cylinders, and we integrated the mortality and metamorphosis rates of those fractions into the mortality rate of the following samples. M_{9C} is the number of metamorphosed larvae in the crawler fraction on day 9, M_{12C} is the number of metamorphosed larvae in the crawler fraction on day 12, M_{15CS} is the number of metamorphosed larvae in the crawler and swimmer fractions on day 15, X_{9C} is the number of alive larvae in the crawler fraction on day 9, X_{12C} is the number of alive larvae in the crawler fraction on day 12, and X_{15CS} is the number of alive larvae in the crawler and swimmer fractions on day 15. The cumulative metamorphosed rate on day 15 (C_{15})as defined as:

$$C_{15} = \frac{M_{9C} + M_{12C} + M_{15CS}}{X_{9C} + X_{12C} + X_{15CS}}$$

Calculation of the mortality rate followed the same pattern, with the number of dead larvae instead of metamorphosed and total larvae instead of alive.

Separately for each parameter (mortality, metamorphosis and settlement rate, TFA concentrations, fatty acid profiles, EFA ratios, and peroxidation index), two-way PERMANOVA was performed to assess the impact and potential interaction between temperature (15°C and 20°C) and sound (C, D, P, P+). Significant differences were analyzed by multiple comparison pairwise tests and SIMPER analyses.

3 Results

3.1 Thermal modulation of physiological state

Daily growth rates varied according to rearing temperature at both the veliger and pediveliger stage (Table 1B). Growth was 55% and 67% higher for veliger and pediveliger larvae reared at 20°C compared to 15°C (Table 1A). To avoid a length difference between thermal batches at the start of both the veliger and pediveliger experiments (Table 1B), larvae were collected at different rearing times (7 and 11 dpf at 20 and 15°C, respectively).

In veligers, the fatty acid profiles in NL and PL fractions varied according to temperature (Table 1B, see SIMPER analyses S1(a) and S1(b) for fatty acid contributing to the differences). At 15°C, veligers accumulated 1.9- and 1.5-fold more 20:5w3 and 22:6w3 than

TABLE 1 Thermal modulation of physiological state.

(A)

stage	temperature (°C)	mortality (%)	size (µm)	growth (µm/day)	TFA (mg/g)		20:4n6 (mg/g)		20:5n3 (mg/g)		22:6n3 (mg/g)		peroxidation index
					NL	PL	NL	PL	NL	PL	NL	PL	
veliger	15	6.75 ± 0.3	124.44 ± 0.76	**11.31 ± 0.07 a**	**8.15 ± 0.98 a**	2.73 ± 0.34	*0.05 ± 0.01*	0.03 ± 0.001	**0.87 ± 0.18 a**	0.19 ± 0.05	**0.87 ± 0.23 a**	0.45 ± 0.15	5.51 ± 0.77
	20	2.34 ± 0.3	122.96 ± 1.04	**17.56 ± 0.15 b**	**5.20 ± 0.15 b**	2.68 ± 0.38	*0.03 ± 0.01*	0.03 ± 0.001	**0.45 ± 0.00 b**	0.17 ± 0.06	**0.56 ± 0.03 b**	0.48 ± 0.15	5.62 ± 0.88
pediveliger	15	3.57 ± 0.7	189.93 ± 1.39	**7.60 ± 0.05 α**	9.13 ± 2.22	3.56 ± 0.60	0.02 ± 0.005	0.02 ± 0.01	1.17 ± 0.62	0.31 ± 0.1	0.74 ± 0.39	0.79 ± 0.29	8.83 ± 1.60
	20	1.08 ± 0.4	190.24 ± 1.27	**12.68 ± 0.08 ε**	11.50 ± 2.41	4.42 ± 0.30	0.02 ± 0.004	0.02 ± 0.01	1.25 ± 0.57	0.32 ± 0.04	0.83 ± 0.37	0.90 ± 0.16	10.03 ± 0.76

(B)

stage	statistical values	size	growth	TFA		20:4n6		20:5n3		22:6n3		peroxidation index	profiles	
				NL	PL	NL	PL	NL	PL	NL	PL		NL	PL
veliger	df	1	1	1	1	1	1	1	1	1	1	1	1	1
	pseudo-F	1.333	1457.8	8.834	0.009	4.871	0.039	21.258	0.386	7.399	0.062	0.009	16.108	3.332
	p-perm or p (MC)	0.250	**0.0001**	**0.028**	0.934	*0.065*	0.844	**0.0034**	0.556	**0.0336**	0.818	0.927	**0.0006**	**0.0358**
pediveliger	df	1	1	1	1	1	1	1	1	1	1	1	1	1
	pseudo-F	0.027	2580.5	0.513	1.785	0.001	0.000009	0.042	0.021	0.092	0.435	1.624	14.016	2.244
	p-perm or p (MC)	0.869	**0.0001**	0.501	0.231	0.969	0.998	0.846	0.888	0.774	0.538	0.246	**0.0006**	0.148

(A) Mean mortality rate, size, growth rate, TFA, 20:4w6, 20:5w3, and 22:6w3 concentrations in neutral and polar fractions and peroxidation index ± standard error for Venus verrucosa veliger or pediveliger larvae reared at 15 or 20°C before starting sound experiments. Veliger and pediveliger data were analyzed separately; Values annotated with different letters differed significantly at p-perm< 0.05 (in bold). Veliger and pediveliger data were analyzed separately; "a" and "b" represent significant difference of the values for the veliger experience; "α" and "ε" represent significant difference of the values for the pediveliger experiment. (B) Results of the statistical analyses performed on Table 1A data. The degrees of freedom (df), ratio of between-cluster variance to within-cluster variance (pseudo-F), and the probability value (p-perm or p (MC)) are indicated for each PERMANOVA, testing the impact of temperature on veliger's or pediveliger's parameters. Statistical values for PERMANOVA of the FA profiles of the neutral and polar fractions are also indicated. Significant p-perm values are in bold.

10.3389/fmars.2023.1117431

rearing at 20°C (Table 1A); this difference was close to significant for 20:4w6 (Table 1B), but only in the NL fraction. These results reflect the accumulation of TFAs observed only in the NL fraction, with 57% more TFA at 15°C than 20°C (Tables 1A, B). Without changes to the fatty acid composition in the PL fraction, the membrane peroxidation index showed no difference between rearing temperatures.

At the pediveliger stage, the fatty acid profile varied according to temperature only in the NL fraction (Table 1B, see SIMPER analysis S1(c)) without changes in TFA or EFA concentrations. In the PL fraction, no differences were observed according to rearing temperature (Tables 1A, B).

3.2 Sound × temperature interaction on veliger larvae

In veliger larvae, no interaction between the two stressors was observed for each variable measured, but effects related to temperature were observed for the majority of variables and the effect of sound for fewer variables. Mortality, daily growth, and TFA concentration in the NL fraction were related to temperature change only without the impact of sound (Table 2B). Thus, mortality rates and daily growth rates at 20°C were 5.8- and 1.2-fold higher than in the 15°C batches, respectively (Table 2A; Figure 3C). Larvae reared at 15°C contained 35% more NL fatty acids. Rearing temperature also impacted the fatty acid composition of the NL fraction of larvae (Table 2B), with higher levels of 20:5w3 at 15°C (see SIMPER analyses S2(b) for fatty acids contributing to the difference). In this case, the sound treatment also showed a significant effect, with variation only between drilling (D) and high-level pile driving (P+) (p-perm pairwise = 0.0467). In the PL fraction, differences were related to temperature and sound treatment. Fatty acid profiles varied according to temperature and pile driving but not to drilling noise in the PL (p-perm = 0.0196 and 0.0021 for C vs. P and C vs. P+ pairwise test, respectively) The 20:5w3, 16:0, and 16:1w5 were higher in larvae reared at 15°C, but with a lower value for 22:6w3. The two pile driving treatments were associated with higher accumulation of 22:6w3, 20:5w3, and 16:1w5 than control larvae (see SIMPER analyses S2(c) and S2(d)). The TFA concentration varied according to temperature, with 14% higher fatty acid concentration in the PL fraction of 15°C larvae, and according to sound, as larvae exposed to pile driving concentrated 18% more TFA in their PL fraction (Tables 2A, B; p-perm = 0.0065 and 0.001 for C vs. P and C vs. P+ pairwise test, respectively). Consequently, these changes in TFA and fatty acid composition modified the peroxidation index in the PL fraction according to rearing temperature and sound exposure, with values 16.5% higher for larvae reared at 15°C and 23% higher for larvae exposed to pile driving (Table 2A; p-perm = 0.0034 and 0.0006 for C vs. P and C vs. P+ pairwise test, respectively).

The EFA selective retention ratios of 20:5w3 and 22:6w3 varied according to temperature and sound exposure, with levels 25% and 12% higher in larvae exposed to 15°C for 20:5w3 (df = 1; p-perm = 0.0001) and 22:6w3 (df = 1; p-perm = 0.0231), respectively (Figures 3A, B). These two fatty acids were also 18% and 26% higher in larvae exposed to pile driving sounds compared to the control (Table 2A) for 20:5w3 (df = 3; p-perm = 0.0026) and 22:6w3 (df = 3; p-perm = 0.0026), respectively (Figures 3A, B). However, all ratios were systemically less than or approximately 1. However, 20:4w6 showed no variation (p-perm = 0.3321 and 0.6703 for temperature and sound, respectively), with a mean ratio<1. Thus, a potential dietary deficiency of EFA was not observed for veliger larvae for any of the tested treatments.

3.3 Sound × temperature interaction on pediveliger larvae

In pediveliger larvae, interactions between temperature and sound were significantly observed only for some variables associated with fatty acids. Mortality rates varied according to rearing temperature and sound treatment (Table 3B); they were 9-fold higher when larvae were reared at 15°C (Table 3A) and reduced by 33% and 29% when larvae were exposed to drilling and pile driving sounds, respectively (Table 3A; Figure 4C). Metamorphosis and settlement rates varied only according to temperature, with higher values observed at 15°C compared to 20°C (Table 3B), with a 33% and 30% increase for metamorphosis and settlement, respectively (Table 3A). Settlement rates varied according to temperature; when reared at 20°C, larvae settled 30% lower than when reared at 15°C (Table 3A). The interaction between sound and temperature was near significant (p-perm=0.06; Table 3B), with settlement 29% lower in larvae reared at 20°C and exposed to pile driving sounds compared to the control condition (Table 3A).

The fatty acid profiles of pediveliger larvae varied according to temperature in both the NL and PL fractions without any interaction or effect of sound (Table 3B) and was mainly associated with higher accumulation of 22:5w3 and 16:1w5 and lower level of 22:6w3 in the NL fraction. In the PL fraction, we observed a higher accumulation of 22:6w3 and 16:0 in combination with lower levels of 18:0 at 20°C (see SIMPER analyses S3(a) and S3(b)). An interaction between both factors was observed in TFA concentration (Table 3B), but only for the NL fraction, with higher values in larvae reared at 20°C, particularly for the control, with nearly twice the TFA concentration than larvae exposed to anthropogenic sounds (Table 3A). In the PL fraction, only temperature affected the TFA concentration, with a value 2.2-fold higher at 20°C than at 15°C (Table 3A). The higher fatty acid concentration in the PL fraction of larvae reared at 20°C in combination with higher accumulation of 22:6w3, resulting in an increased peroxidation index (Table 3B).

The EFA selective retention ratio of 20:4w6 varied according to temperature (df = 1; p-perm = 0.0001) and was 1.4-fold lower for larvae reared at 20°C compared to 15°C (1.78 vs. 1.28, respectively). The selective retention ratio of 20:5w3 and 22:6w3 varied according to an interaction between sound and temperature (df = 3 and p-perm = 0.0056 for 20:5w3; df = 3 and p-perm = 0.0106 for 22:6w3). Larvae reared at 20°C had a 2.3- or 1.75-fold reduction of the 20:5w3 ratio when exposed to low level

TABLE 2 Sound × temperature interaction on veliger larvae.

(A)

factor(s)	level	mortality (%)	growth (µm/day)	TFA (mg/g)		20:4n6 ratio	20:5n3 ratio	22:6n3 ratio	peroxidation index
				NL	PL				
temperature	15	**0.64 ± 0.45** }	**6.2 ± 0.05** }	**8.58 ± 0.55** }	**3.60 ± 0.11** }	0.91 ± 0.04	**0.24 ± 0.01** }	**1.38 ± 0.06** }	**8.74 ± 0.34** }
	20	**3.73 ± 0.42** {{	**7.2 ± 0.06** {{	**6.36 ± 0.36** {{	**3.15 ± 0.12** {{	0.80 ± 0.09	**0.18 ± 0.06** {{	**1.22 ± 0.06** {{	**7.50 ± 0.35** {{
sound	C	2.63 ± 0.76	6.76 ± 0.08	7.01 ± 0.42	**3.08 ± 0.10 a**	0.78 ± 0.05	**0.19 ± 0.01 a**	**1.15 ± 0.03 a**	**7.26 ± 0.23 a**
	P	3.08 ± 0.89	6.69 ± 0.07	7.84 ± 1.07	**3.49 ± 0.19 bc**	0.79 ± 0.04	**0.22 ± 0.02 bc**	**1.38 ± 0.08 b**	**8.53 ± 0.52 bc**
	P+	2.53 ± 0.73	6.66 ± 0.08	8.38 ± 0.90	**3.77 ± 0.13 b**	0.90 ± 0.1	**0.23 ± 0.01 b**	**1.52 ± 0.07 b**	**9.38 ± 0.38 b**
	D	2.39 ± 0.69	6.70 ± 0.09	6.56 ± 0.51	3.15 ± 0.17 ac	0.95 ± 0.16	0.19 ± 0.01 ac	1.15 ± 0.08 a	7.31 ± 0.46
temperature x sound	15 x C	1.04 ± 0.94	6.18 ± 0.09	7.82 ± 0.42	3.29 ± 0.09	0.92 ± 0.03	0.21 ± 0.00	1.22 ± 0.04	7.81 ± 0.20
	15 x P	0.002 ± 0.86	6.3 ± 0.08	10.38 ± 0.98	3.88 ± 0.23	0.87 ± 0.02	0.26 ± 0.13	1.53 ± 0.13	9.59 ± 0.73
	15 x P+	0.15 ± 0.86	6.09 ± 0.1	9.62 ± 1.35	3.82 ± 0.27	0.91 ± 0.18	0.26 ± 0.14	1.52 ± 0.14	9.55 ± 0.81
	15 x D	1.38 ± 1.01	6.23 ± 0.10	6.95 ± 0.80	3.42 ± 0.12	0.93 ± 0.02	0.22 ± 0.06	1.26 ± 0.06	8.03 ± 0.37
	20 x C	3.76 ± 0.95	7.4 ± 0.11	6.21 ± 0.47	2.87 ± 0.08	0.64 ± 0.01	0.16 ± 0.04	1.09 ± 0.04	6.71 ± 0.22
	20 x P	3.68 ± 1.17	7.12 ± 0.12	5.93 ± 0.85	3.10 ± 0.13	0.71 ± 0.05	0.18 ± 0.07	1.22 ± 0.07	7.48 ± 0.39
	20 x P+	3.31 ± 0.78	7.16 ± 0.12	7.14 ± 98	3.73 ± 0.09	0.88 ± 0.13	0.21 ± 0.06	1.52 ± 0.06	9.21 ± 0.31
	20 x D	4.16 ± 0.54	7.13 ± 0.13	6.17 ± 0.69	2.88 ± 0.27	0.97 ± 0.34	0.16 ± 0.14	1.05 ± 0.14	6.60 ± 0.41

(B)

factor(s)	statistical values	mortality	growth	TFA		20:4n6 ratio	20:5n3 ratio	22:6n3 ratio	peroxidation index	profiles	
				NL	PL					PL	NL
temperature	df	1	1	1	1	1	1	1	1	1	1
	pseudo-F	23.116	168.54	12.526	13.328	1.111	41.856	5.865	11.235	40.126	60.583
	p-perm	**0.0001**	**0.0001**	**0.001**	**0.0007**	0.3321	**0.0001**	**0.0231**	**0.0021**	**0.0001**	**0.0001**
sound	df	3	3	3	3	3	3	3	3	3	3
	pseudo-F	0.577	0.791	1.522	6.227	0.619	6.529	7.032	7.112	3.039	2.03
	p-perm	0.630	0.507	0.222	**0.0032**	0.670	**0.0026**	**0.0026**	**0.0011**	**0.0067**	**0.037**

(Continued)

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TABLE 2 Continued

(B)

factor(s)	statistical values	mortality	growth	TFA		20:4n6 ratio	20:5n3 ratio	22:6n3 ratio	peroxidation index	profiles	
				NL	PL					PL	NL
temperature x sound	df	3	3	3	3	3	3	3	3	3	3
	pseudo-F	0.117	1.206	1.201	1.492	0.467	0.864	0.876	1.202	0.397	1.451
	p-perm	0.949	0.300	0.319	0.233	0.765	0.481	0.475	0.325	0.925	0.152

(A) Mean mortality rate, growth rate, TFA concentrations, 20:4w6, 20:5w3, and 22:6w3 ratio in neutral and polar fractions, and peroxidation index ± standard error for Venus verrucosa veliger larvae reared at 15 or 20°C and exposed to pile driving or drilling sound. Values annotated with different letters or different symbols differed significantly at p-perm< 0.05 (in bold). "}" and "{{" represent significant difference of the values due to a temperature effect. "a", "b" and "c" represent significant difference and similarity of the values due to sound effect. (B) Results of the statistical analyses performed on Table 2A data. The degrees of freedom (df), ratio of between-cluster variance to within-cluster variance (pseudo-F), and the probability value (p-perm) are indicated for each PERMANOVA, testing the impact of temperature, then the temperature interaction with pile driving or drilling, on veliger's parameters. Statistical values for PERMANOVA of the FA profiles of the neutral and polar fractions are also indicated. Significant p-perm values are in bold.

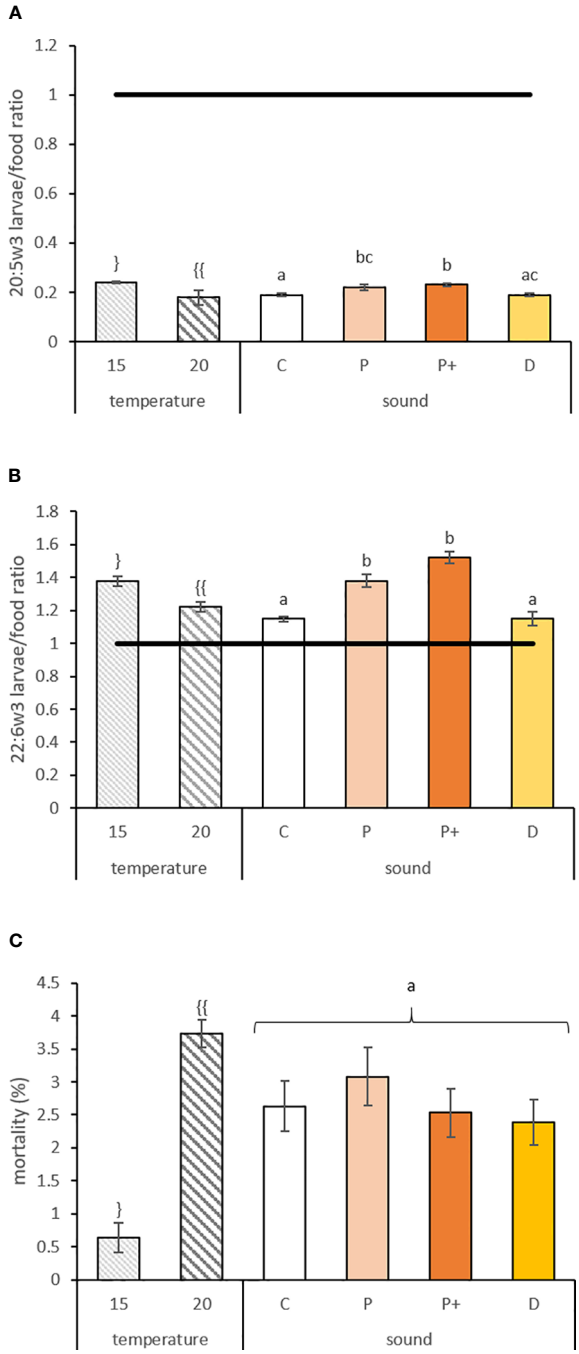

FIGURE 3
(A) 20:5w3 and **(B)** 22:6w3 larvae/food ratio and **(C)** mortality (%) ± standard error for veligers larvae reared at different temperatures (15 and 20°C) and exposed to sounds for 9 days (C, P, P+, and D represent control, low level pile driving, high level pile driving, and drilling, respectively). The line indicates equal amounts of fatty acids in the larvae and in the diet. Values annotated with different letters or symbols differed significantly at p-perm< 0.05. "}" and "{{" represent significant difference of the values due to a temperature effect. "a", "b" and "c" represent significant difference and similarity of the values due to sound effect.

pile driving or drilling sounds compared to the control condition, but all ratios were<1 (Figure 4A). The retention ratio of 22:6w3 showed selective retention for all treatments and was 2.2-fold reduced by exposure to low-level pile driving sounds and 1.7-fold

TABLE 3 Sound × temperature interaction on pediveliger larvae.

(A)

factor(s)	level	mortality (%)	metamorphosis (%)	settlement (%)	TFA (mg/g)		20:4n6 ratio	20:5n3 ratio	22:6n3 ratio	peroxidation index
					NL	PL				
temperature	15	**16.59 ± 0.98 }**	**21.50 ± 1.57 }**	**85.12 ± 1.32 }**	**7.48 ± 0.62 }**	**2.67 ± 0.23 }**	**1.78 ± 0.25 }**	**0.18 ± 0.02 }**	**1.26 ± 0.12 }**	**5.91 ± 0.57 }**
	20	**1.79 ± 0.40 {{**	**16.11 ± 1.01 {{**	**59.91 ± 3.53 {{**	**19.21 ± 2.79 {{**	**5.76 ± 0.62 {{**	**1.28 ± 0.03 {{**	**0.47 ± 0.03 {{**	**2.94 ± 0.19 {{**	**13.96 ± 1.57 {{**
sound	C	**11.77 ± 2.86 a**	20.64 ± 1.47	78.41 ± 2.72	15.75 ± 4.15	4.61 ± 1.07	1.77 ± 0.30	0.37 ± 0.10	2.29 ± 0.61	8.18 ± 2.59
	P	**7.49 ± 2.17 b**	16.20 ± 1.81	68.87 ± 6.14	10.84 ± 1.11	3.56 ± 0.20	1.65 ± 0.27	0.26 ± 0.02	1.76 ± 0.10	1.34 ± 0.47
	P+	**8.40 ± 2.24 b**	17.61 ± 2.06	68.95 ± 5.52	14.01 ± 4.06	4.34 ± 0.97	1.46 ± 0.13	0.33 ± 0.08	2.21 ± 0.50	6.58 ± 2.33
	D	**9.09 ± 2.40 b**	20.77 ± 2.49	73.84 ± 5.99	10.06 ± 2.33	3.63 ± 0.61	1.35 ± 0.11	0.27 ± 0.06	1.75 ± 0.34	4.49 ± 1.42
temperature x sound	15 x C	20.82 ± 1.66	23.90 ± 1.36	83.20 ± 2.94	6.05 ± 1.25	2.16 ± 0.47	1.89 ± 0.24	0.15 ± 0.01	**0.98 ± 0.09 λ**	4.73 ± 1.11
	15 x P	14.13 ± 1.69	16.36 ± 2.73	85.69 ± 3.46	8.44 ± 1.00	3.30 ± 0.33	2.21 ± 0.10	0.22 ± 0.02	**1.56 ± 0.15 β**	7.31 ± 0.56
	15 x P+	14.81 ± 2.14	20.42 ± 3.78	85.92 ± 1.43	8.47 ± 0.87	2.76 ± 0.24	1.56 ± 0.11	0.19 ± 0.04	1.37 ± 0.35 λβ	6.39 ± 0.76
	15 x D	16.59 ± 1.47	25.33 ± 3.52	85.68 ± 2.86	7.61 ± 1.40	2.70 ± 0.56	1.52 ± 0.16	0.18 ± 0.08	1.26 ± 0.53 λβ	5.85 ± 1.37
	20 x C	2.73 ± 0.69	17.38 ± 1.84	*73.63 ± 3.85 a*	**30.30 ± 2.75 α**	8.28 ± 0.70	1.60 ± 0.21	**0.70 ± 0.01 α**	**4.26 ± 0.09 α**	20.34 ± 1.98
	20 x P	0.84 ± 0.55	16.04 ± 2.62	*52.04 ± 6.36 b*	**13.24 ± 0.96 ε**	3.82 ± 0.19	1.09 ± 0.25	**0.31 ± 0.11 ε**	**1.96 ± 0.80 ε**	9.30 ± 0.47
	20 x P+	2 ± 1.02	14.80 ± 1.12	*51.98 ± 4.08 b*	19.56 ± 7.46 αε	5.92 ± 1.64	1.36 ± 0.14	0.48 ± 0.07 αε	3.05 ± 0.40 αε	14.35 ± 4.21
	20 x D	1.59 ± 0.82	16.22 ± 2.57	*61.99 ± 9.68 a*	**13.73 ± 5.32 ε**	5.01 ± 1.01	1.09 ± 0.31	**0.40 ± 0.10 ε**	**2.48 ± 0.64 ε**	11.84 ± 2.22

(B)

factor(s)	statistical values	mortality	metamorphosis	settlement	TFA		20:4n6 ratio	20:5n3 ratio	22:6n3 ratio	peroxidation index	profiles	
					NL	PL					PL	NL
temperature	df	1	1	1	1	1	1	1	1	1	1	1
	pseudo-F	235.9	8.5974	51.737	15.489	13.624	7.0122	46.934	32.422	13.744	20.945	69.937
	p-perm	**0.0001**	**0.006**	**0.0001**	**0.0001**	**0.0001**	**0.0107**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
sound	df	3	3	3	3	3	3	3	3	3	3	3
	pseudo-F	3.6674	1.5244	1.6963	1.7286	1.1484	1.1207	3.0304	1.8547	1.2265	0.73534	0.7184
	p-perm	**0.0205**	0.2256	0.1818	0.1174	0.3442	0.3541	**0.0453**	0.1593	0.2962	0.7002	0.7391

(Continued)

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TABLE 3 Continued

(B)

factor(s)	statistical values	mortality	metamorphosis	settlement	TFA		20:4n6 ratio	20:5n3 ratio	22:6n3 ratio	peroxidation index	profiles	
					NL	PL					PL	NL
sound x temperature	df	3	3	3	3	3	3	3	3	3	3	3
	pseudo-F	1.5339	1.0074	2.6752	2.3223	1.8367	1.1023	5.5294	9.957	1.5613	0.84986	0.6978
	p-perm	0.2219	0.3919	*0.0618*	**0.0467**	0.1019	0.3673	**0.0056**	**0.0106**	0.1763	0.5814	0.7531

(A) Mean mortality, metamorphosis and settlement rate, TFA concentrations, 20:4w6, 20:5w3, and 22:6w3 ratio in neutral and polar fractions, and peroxidation index ± standard error for Venus verrucosa pediveliger larvae reared at 15 or 20°C and exposed to pile driving or drilling sound. Values annotated with different letters or different symbols differed significantly at p-perm< 0.05 (in bold). "*" and "{{" represent significant difference of the values due to a temperature effect. "a" and "b" represent significant difference and similarity of the values due to sound effect." Greek letters represent significant effect of temperature and sound interaction, with "λ" and "β" representing significant difference and similarity due to sound effect for the 15°C exposed larvae and "α" and "ε" representing significant difference and similarity due to sound effect for the 20°C exposed larvae. (B) Results of the statistical analyses performed on Table 2 data. The degrees of freedom (df), ratio of between-cluster variance to within-cluster variance (pseudo-F), and the probability value (p-perm) are indicated for each PERMANOVA, testing the impact of temperature, then the temperature interaction with pile driving or drilling, on pediveliger's parameters. Statistical values for PERMANOVA of the FA profiles of the neutral and polar fractions are also indicated. Significant p-perm values are in bold.

increased by exposure to drilling sounds, but only for larvae exposed to 20°C (Figure 4B). Thus, a potential dietary deficiency was observed for 22:6w3, mainly in pediveliger larvae reared at 20°C (control value >4), and anthropogenic sounds seem to decrease the level of selective retention and, thus, the dietary deficiency with ratio values ≤3.

4 Discussion

As expected, the present study showed that the physiological state of veliger and pediveliger stages of *V. verrucosa* was highly impacted by the temperature experienced by larvae during their development. In the veliger stage, larvae accumulated more neutral lipids when exposed at 15°C, and pediveliger showed a potential dietary deficiency of EFAs at 20°C, specifically 22:6n3. We show complex interactions between rearing temperature and anthropogenic sound exposure associated with the installation of offshore wind turbines that clearly impact larvae. The response of warty venus to noise appears to be highly dependent on both developmental stage and physiological state.

4.1 Impact of rearing temperature on larval physiology

The physiological state of bivalve larvae is based on their lipid content (Pernet et al., 2005), which relies on biotic and abiotic environmental factors, such as diet quality (Delaunay et al., 1993; Pernet and Tremblay, 2004) and temperature (Pernet et al., 2007). Lipids play a central role in supporting larval development (Glencross, 2009). By rearing larvae at two temperatures, we were able to produce two physiologically contrasting larval batches with distinct performances, fatty acid content, and profiles, mainly for NL fractions. As expected, larval growth was faster at 20°C (Bayne, 1965; Pechenik, 1990), which explains why veliger and pediveliger experiments with 15 and 20°C batches started at different times post-fertilization based on size criteria. This size threshold we adopted allows larval experiments to start at a similar developmental stage (Forêt et al., 2020). Lipids are essential for the development of bivalves, specifically polar lipids, which are mainly phospholipids incorporated in membranes and maintain cell membrane integrity in invertebrate species (Gallager et al., 1986). Because all fatty acids have different properties, the fatty acid composition of the PL fraction influences the membrane fluidity and peroxidation index, which is a proxy for the membrane susceptibility to peroxidation. For example, the membrane is prone to peroxidative damage as higher proportions of polyunsaturated fatty acids are found in the PL fraction (Hulbert et al., 2007). Conversely, a membrane with higher proportions of saturated and monounsaturated fatty acids will be more resistant to lipid peroxidation, which produces highly reactive molecules that can also cause damage to membrane DNA and proteins (Sukhotin et al., 2002). By modulating the rearing temperature from 15°C to 20°C, we modified the fatty acid composition of larval membrane in pediveligers, increasing lipid peroxidation 2.4-times and subsequent

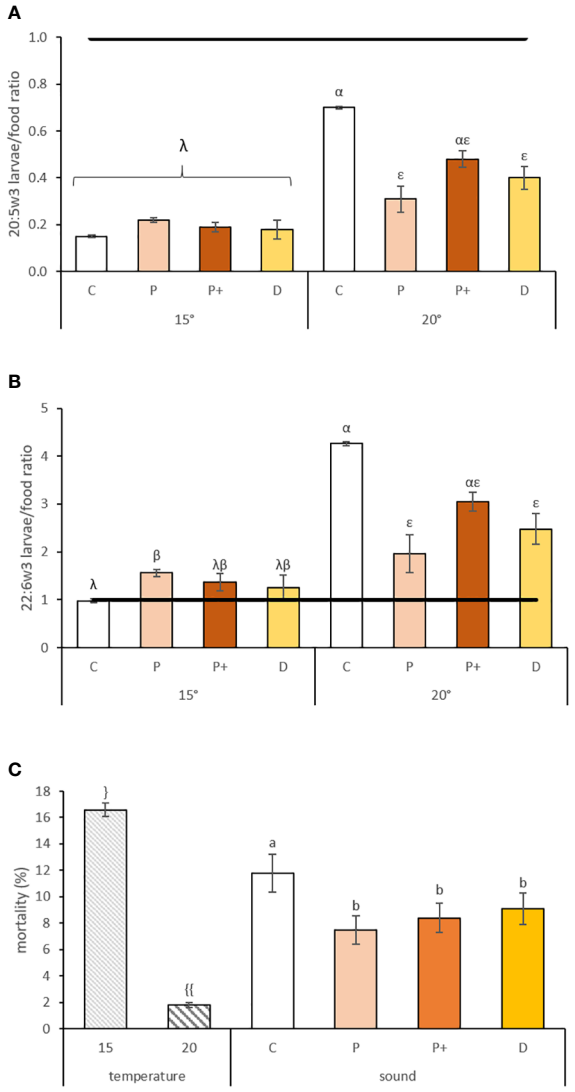

FIGURE 4
(A) 20:5w3 and **(B)** 22:6w3 larvae/food ratio and **(C)** mortality (%) ± standard error for pediveligers larvae reared at different temperatures (15 and 20°C) and exposed to sounds for 15 days (C, P, P+ and D represent control, low level pile driving, high level pile driving, and drilling, respectively). The line indicates equal amounts of fatty acids in the larvae and in the diet. Values annotated with different letters or symbols differed significantly at p-perm< 0.05. "}" and "{{" represent significant difference of the values due to a temperature effect. "a" and "b" represent significant difference and similarity of the values due to sound effect." Greek letters represent significant effect of temperature and sound interaction, with "λ" and "β" representing significant difference and similarity due to sound effect for the 15°C exposed larvae and "α" and "ε" representing significant difference and similarity due to sound effect for the 20°C exposed larvae.

sensitivity to membrane lipids to potential oxidative damage. However, this pattern was not observed in veliger larvae.

The lipid content and composition of the NL fraction traduced the larval energetic storage (Gallager et al., 1986). Veligers reared at 20°C had lower energetic reserves (TFA in NL fraction) and concentrations of EFAs in the NL fraction, as well as a higher mortality rate at the end of the experiment. At the subsequent pediveliger stage, 20°C larvae accumulated more energetic reserves and no significant differences from larvae reared at 15°C were detected when starting pediveliger experiments. However, at the end of the experiment, pediveligers reared at 20°C had 2.6-fold more energetic lipids and 2.2-fold more membrane lipids than those reared at 15°C, which was coupled with a highly reduced mortality rate. However, lipid accumulation in the NL fraction was different between sound treatments, with higher values in the control. The positive correlation between higher temperature and increased lipid content at the pediveliger stage highly depends on the thermal optimum, which is species-specific (Rayssac et al., 2010; Pörtner et al., 2017). Fatty acid content is highly correlated with larval performance (Delaunay et al., 1992; Pernet and Tremblay, 2004), with a positive relationship between energy reserves and survival rates (Rayssac et al., 2010). Previous work on young stages of *V. verrucosa* showed that 60 dpf juveniles reared at 20°C accumulated 2 to 3 fewer lipids, as well as less lower triacylglycerols content, a main component of energetic storage, than those reared at 15°C (Forêt et al., 2020). Such thermal influence diverges from our data acquired on pediveligers but is in agreement with the veliger data affording for ontogenic variations (Pernet et al., 2007). High variation among larval stages has been reported in the literature; Marty et al. (1992) described 10-fold higher TFA content in great scallop pediveligers than in veligers. Previous studies suggest that the selective pressure of temperature is highly ontogenic (Pörtner et al., 2017) and predominant during early ontogeny (Rayssac et al., 2010), which is in accordance with the inverted effect of rearing temperature observed on veliger and pediveliger larvae. Thus, *V. verrucosa* seems to accumulate less energetic reserves and structural lipids at pre-metamorphic (our results) and post-metamorphic (Forêt et al., 2020) stages when reared at high temperature, whereas the peri-metamorphic stage (our results) stores more energetic and structural lipids. As increasing temperature usually raises the metabolism, this lipid accumulation at the pediveliger stage could be related to higher metabolic and energetic needs during metamorphosis. The costs associated with acclimatizing to thermal stress during metamorphosis seems to be offset by higher fatty acid accumulation (Zippay and Helmuth, 2012).

The strong increase in lipid concentration and selective storage of dietary lipids 20:4w6 and 22:6w3 at the pediveliger stage compared to the veliger stage indicates a transition from endogenous to exogenous nutrition (Delaunay et al., 1992; Pernet et al., 2005). At the early veliger stage, the energetic content of larvae is mostly based on lipid reserves transferred from the mother to the egg (Yamamoto et al., 1999). We hypothesize that, at the young veliger stage, higher temperature causes a high metabolic and energetic demand that larvae cannot compensate through feeding with the selected diet, inducing higher mortality rates. As larvae age, feeding capacities and activity increase to compensate for the higher metabolic and energetic needs of the metamorphosis process. Larvae can selectively accumulate fatty acids (Pernet and Tremblay, 2004) and the ratio of EFA in the larva to the same fatty acid originating from the diet indicates whether larvae selectively incorporate a specific EFA from microalgae (Cabrol et al., 2015). A ratio > 1 means that the proportion of EFAs in the PL fraction is higher than in the diet, suggesting selective incorporation into membrane phospholipids. Higher selective

retention highlights potential deficiencies in diet to meet the physiological needs. Our results at the pediveliger stage included higher DHA (22:6w3) retention ratios (> 3) at a rearing temperature of 20°C, suggesting that food quantity or quality seems too low to satisfy the metabolic needs at 20°C and that larvae could compensate by increasing feeding. Although there is no comparative study on filtration rate in *V. verrucosa*, Bayne (1965) showed an increase in the clearance rate of *M. edulis* with increasing temperature. Our data highlight that higher lipid content and EFA retention ratios are associated with a lower metamorphosis rate. The physiological status of competent larvae determines the active substrate prospection and selectivity during settlement (Pernet et al., 2005). Pediveligers accumulating more lipids would be more selective, potentially delaying their metamorphosis if the habitat for settlement is unsuitable (Tremblay et al., 2007). During the competence phase, the larva consumes its energetic reserves until reaching a threshold below which "the desperate larvae" can no longer delay metamorphosis and settle anywhere (Toonen and Pawlik, 2001). We hypothesize that higher temperature increases the larval selectivity capacity by increasing energetic lipid accumulation.

To prevent mortality in small experimental tanks with high biomass larvae (Holbach et al., 2015), antibiotics were used to avoid any bacterial contamination. However, antibiotics also prevented the development of a biofilm, which constitutes a positive settlement cue for bivalve larvae (Leyton and Riquelme, 2008), inducing a negative effect on their settlement (Pernet et al., 2006). Furthermore, in the absence of air injection and water agitation in experimental tanks to avoid sound perturbation for anthropogenic noise emission, no positive settlement cue was related to hydrodynamics/turbulence (Tremblay et al., 2020). Finally, with the absence of artificial collectors in the tanks, larvae could only settle on the smooth walls of the cylinders, which is less suitable than filamentous or rough surfaces (Le Tourneux and Bourget, 1988; Harvey et al., 1993). We suggest that these experimental conditions used to maintain better soundscape conditions were not optimal for larval settlement, stimulating metamorphosis delay and potential "desperate" conditions. However, such conditions are often encountered in the natural environment (Toonen and Pawlik, 2001). We conclude that higher metabolism and feeding at 20°C delayed metamorphosis and raised the selectivity of pediveliger larvae.

4.2 Sound reduces larvae settlement, mortality, and thermic compensatory mechanisms

The present study on an endobenthic bivalve demonstrated the ontogenic effect of anthropogenic sounds on larvae. Pile driving sounds slightly modified the energetic state of veliger larvae without inducing any effect on their mortality or growth. Under pile driving exposure, the veliger fatty acid profile changed in the sole PL fraction, particularly for EPA and DHA. The fatty acid content in the PL fraction and EFA retention ratio gradually increased with pile driving sound levels, suggesting that larvae accumulated more membranous fatty acids, particularly EFAs. Such results could relate to settler growth stimulation or traduce an increase in the metabolic level due to stress (Spiga et al., 2016), but further experiments are needed.

Our results highlight an ontogenic interaction between the physiological consequences of rearing temperature and the acoustic response of larvae, but only at the pediveliger stage. Both sounds reduce fatty acid content in the NL fraction of larvae reared at 20°C, but not at 15°C. Such observations concur with similar studies showing that temperature amplifies the effect of another stressor (Cherkasov et al., 2007) because physiological stress induced by one factor reduces the resistance of another (Zippay and Helmuth, 2012). For example, Lannig et al. (2006) concluded that cadmium pollution reduces the thermal tolerance of the oyster *Crassostrea virginica*. The present results also indicate that sound reduces the compensatory mechanisms established to balance the temperature increase.

However, the retention ratio indicated another pattern, as the DHA (22:6w3) retention ratio for larvae reared at 20°C decreased in response to sound (from > 4 to < 3) exposure. Thus, anthropogenic sounds could stimulate feeding or assimilation of pediveliger larvae at 20°C, decreasing the impact of the potential diet deficiency. However, this stimulation does not seem to be enough to compensate for the temperature impact in the context of sound exposure, as the TFA content in NL fractions was nearly 2-time less with sound treatments compared to control.

If energetic fatty acid accumulation (TFA in NL fraction) in response to increased temperature enhances larval selectivity and delays metamorphosis in the context of a non-optimal habitat for settlement, no acceleration of metamorphosis would be observed. Higher TFA content in the NL fraction was associated with a higher settlement rate but without changes in the success of metamorphosis. As described by Delaunay et al. (1992), there is not necessarily a direct relationship between lipids and larval growth. Despite the lower energetic content, larvae do not adopt a "desperate" behavior. Inversely, the settlement process was slowed down by pile driving sounds at 20°C, as indicated by the slightly lower proportion of larvae crawling on the cylinder surface. We then hypothesized that such anthropogenic noise is a negative settlement cue for *V. verrucosa* larvae. Our results agree with those from *Balanus amphitrite*, in which metamorphosis was delayed in response to low-frequency sounds (Branscomb and Rittschof, 1984). However, it also contrasts with other acoustic impact studies on bivalve species showing increased settlement in response to low-frequency anthropogenic sound for mytilids *M. edulis* (Jolivet et al., 2016) and *P. canaliculus* (Wilkens et al., 2012). Thus, the response to sound is highly species-specific. As it was demonstrated on adults bivalves (Zhao et al., 2021) further experiments are needed to determine if sound reduce attachment performances of larvae. In contrast to 20°C larvae, the fatty acid content of 15°C-reared larvae did not decrease with sound exposure, as it was already low. The effect of sound diverges between the 15 and 20°C rearing conditions, showing that the response to sound is highly dependent on the larval physiological state. Our study agrees with previous studies showing strong interactions between the physiological state of larvae and the response to an environmental stressor (Lannig et al., 2006; Freuchet et al., 2015; Torres et al., 2021). This study gives

precursory results on the effects of sound on marine invertebrates larvae but it is important to keep in mind that this study carried out in the laboratory does not reproduce the real natural conditions. Its goal is to standardize as many parameters as possible to make only the factors tested vary and being reproducible. Therefore the results demonstrated here cannot be directly extrapolated to the natural environment but still give answers on the acoustic sensitivity of invertebrate larvae. Although, given that thermal variations in the marine environment can modulate the acoustic response of bivalve larvae, there is an urgent need to integrate multiple factor interactions into future anthropogenic noise studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MG conducted the experiments, performed data analysis, and wrote the original draft. RT supervised fatty acid analysis, and contributed to results interpretation and manuscript review. JB supervised the acoustics analyses and reviewed the manuscript. LC conceived the study, led the project administration, and funding acquisition. FO conceived the experimental design and methods, supervised the experiments, and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The work reported in this paper was a part of the PhD of Mathilde Gigot, funded by the CNRS. Additional financial support was provided by the IMPAIC project ('*IMPacts Acoustiques sur les Invertébrés de la baie de Saint-BrieuC*') supported by Ailes Marines. This study is a contribution to the BeBEST2 International Research Project (CNRS INEE/LEMAR and UQAR/ISMER).

Acknowledgments

We deeply thank all of the Tinduff hatchery team for their technical support, their useful advice, and their remarkable skill in larval production. We thank Guillaume Bridier for conducting FA identification. We thank Joséphine Broussin for helping during the experimentations.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1117431/full#supplementary-material

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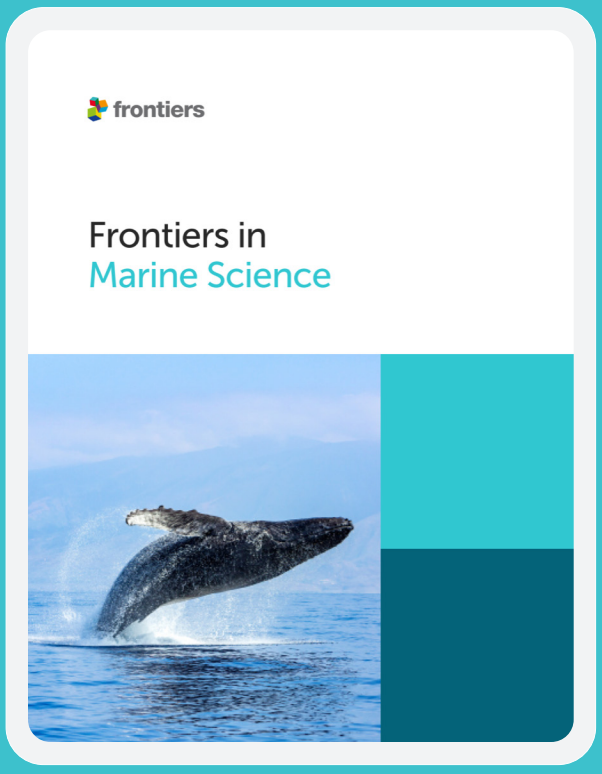

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