






Low-frequency noise affects development and movement patterns of a calanoid copepod

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Abstract

Anthropogenic stressors are omnipresent in marine environments and interfere with organisms of all sizes, from large whales to small organisms. We investigated potential interactive multistressor effects of increased temperature with chronic low-frequency sound on the development and movement patterns of the calanoid copepod *Acartia tonsa*, a model species for small marine zooplankton. Copepods were reared while exposed to chronic low-frequency sound around 15 and 22 dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$ above control sound pressure levels at frequencies between 110 and 120 Hz, at 21°C (increased temperature) and 18°C (control temperature). For each sound and temperature scenario, we assessed after-incubation development rate, stage distribution, and movement patterns. We found that fewer copepods reached the developmental stages copepodites IV to VI in low-frequency sound conditions, even though warmer conditions increased developmental rate. By using high-speed videography in both control and low-frequency sound conditions, we observed that copepods showed more escape behaviors (drops) and fewer feeding-associated behaviors (helical swimming) when exposed to low-frequency sound (~ 42 dB higher than normal at 142 Hz). Copepods reared with added low-frequency sound showed fewer feeding-associated behaviors and did not reverse these trends despite the absence of added sound in their feeding environment. These significant behavioral changes suggest detrimental negative, life-long, consequences for copepods exposed to low-frequency sound.

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NT and GAJ contributed equally to this work.

The stability of aquatic ecosystems is threatened by multiple stressors. Stressors related to global climate change are well-recognized, and their effects on many levels of ecosystem organization are under intense scientific scrutiny. Increased global temperatures impact life histories of numerous aquatic organisms, from altering developmental rates (Byrne et al. 2020) to shifting species distributions (Poloczanska et al. 2016). While studies of large-scale stressors such as increased temperature have become common, local, and regional stressors that act in concert with global change have received considerably less attention. One stressor that is globally spreading, but often originates from a local or regional area is anthropogenic underwater sound from activities such as construction, mining, military activity, energy production, and shipping (Duarte et al. 2021). Anthropogenic sounds are classified as impulsive (high-intensity acoustic signals characterized by a sudden-onset and rapid decay, such as pile driving and underwater explosions) or continuous (lower energy sound of a longer duration, such as shipping and tidal/wind energy production). Low-frequency sound propagates over long distances and tend to raise with

increased shipping (Moore et al. 2012; Duarte et al. 2021; Basan et al. 2024). Whereas there has been considerable interest in the effects of anthropogenic sound on pelagic species such as marine mammals (Erbe et al. 2019; Wang et al. 2024) and fish (Simpson et al. 2019; Cresci et al. 2023; Wang et al. 2024), very little is known about functional, physiological, or demographic effects on invertebrates (summarized in Jerem and Mathews 2021 and Solé et al. 2023). Chronic effects (> 24 h exposure) of anthropogenic sound on lower trophic levels, such as zooplankton, have been largely ignored, probably given the shorter lifespan of many invertebrates (Solé et al. 2023). This is problematic because zooplankton link primary production to higher trophic levels, and have the potential to indirectly influence fisheries yields (Stock et al. 2017).

Within zooplankton, copepods are a group often characterized as “insects of the seas” as they numerically dominate the oceanic metazoan community (Turner 2004). Up to now, only a few studies have examined zooplankton responses to different types of anthropogenic sound, with conflicting results. A field experiment using sonar observations and net sampling found that acute exposure to broadband frequency impulsive sound from air-gun surveys (156 dB re $1 \mu\text{Pa}^2 \text{s}^{-1}$ sound exposure level between 509 and 658 m and 153 dB re $1 \mu\text{Pa}^2 \text{s}^{-1}$ at 1.1–1.2 km range) decreased zooplankton abundance and caused a two- to threefold increase in dead adult and larval zooplankton at a range of > 1 km (McCauley et al. 2017). Changes in nauplii development and survival of *Acartia tonsa* were observed in close vicinity (from 50 to 1.2 km), 6 d after an air-gun exposure of 2.5-h duration (152 dB re $1 \mu\text{Pa}^2 \text{s}^{-1}$ sound exposure level furthest away and 183 dB re $1 \mu\text{Pa}^2 \text{s}^{-1}$ at the closest distance) (Vereide et al. 2023). In contrast, Fields et al. (2019) observed that effects of acute exposure to impulsive sound (221 dB re $1 \mu\text{Pa}^2 \text{s}^{-1}$ sound exposure level at 0 m and 183 dB re $1 \mu\text{Pa}^2 \text{s}^{-1}$ at 25 m) on survival, escape behavior, and gene expression of the copepod *Calanus finmarchicus* were limited to the very close vicinity (< 10 m) to air-gun blasts. Low-frequency sound was investigated as a tactic to combat parasite copepod infestations in the salmon farming industry (Solé et al. 2021). The study showed that over 90% of parasitic copepods *Lepeophtheirus salmonis* displayed fusion of the first antenna setae fusion when exposed to low-frequency tones and combined tones between 350 and 550 Hz (during 4 h, each constant tone was measured at 150 dB re $\mu\text{Pa}^2 \text{Hz}^{-1}$ sound exposure level at a fixed point in the tank), making it difficult for copepods to find fish hosts (Solé et al. 2021).

Intact antennae are essential for copepods, as especially the setae act as velocity and vibration detectors for both prey recognition and predator avoidance (Yen et al. 1992; Kjørboe and Visser 1999). As predators, the copepods' sensory capacity allows them to precisely alter the near-field flow to capture and manipulate particles for ingestion or rejection (Giuffrè et al. 2019), or to determine the position (Yen and Strickler 1996) and quality of food particles (Herstoff et al. 2019). In fact, they can detect vibrations emanating from the second antennae of another

larger copepod one to two body lengths away (Yen and Okubo 2002). As prey, copepods rely on their sensory capacity from 40 to 1000 Hz (Yen et al. 1992) with shear value in lines with their surrounding environment to initiate escape reactions (Fields and Yen 1997). Decreased ingestion and clearance rates of the copepod *A. tonsa* were observed under the exposure of harbor sound (173 dB re $\mu\text{Pa}^2 \text{Hz}^{-1}$) in a laboratory experiment (Kühn et al. 2023), whereas there was no impact on clearance rates for *Eurytemora herdmani* under vessel sound (Aspirault et al. 2023).

While these previous studies examined copepod responses to acute (short-term, < 24 h) exposure to anthropogenic sound, there is still a critical knowledge gap on how zooplankton are affected by chronic exposure (long-term, > 24 h) to anthropogenic sound. Continuous low-frequency sound (< 500 Hz; Carey and Evans 2011), from for example, operating wind turbines, has not been directly linked to primary or secondary production alterations, even though turbine foundations are related to hydrographical changes that have caused negative cascading effects on numerous organisms, including zooplankton (Wang et al. 2018) and benthic filter feeders (Dannheim et al. 2019; Degraer et al. 2020). Obviously, there are positive aspects of turbine foundations, as they may act as artificial reefs, but careful long-term monitoring of changes to nontarget macrofauna species and meiofauna communities remain necessary (Pardo et al. 2023). Given the anticipated increase in anthropogenic sources of low-frequency sound, we must understand the consequences of chronic exposure for important zooplankton groups such as copepods much better, especially in the context of the general increasing temperature linked to climate change.

In this study, we tested low-frequency sound chronic effects (> 24 h) among two temperatures, control and + 3°C, on developmental rates and movement patterns of the commonly studied calanoid copepod, *A. tonsa*. We hypothesized that chronic exposure to low-frequency sound could alter movement patterns related to environmental sensing resulting in a slower development rate. Chronic low-frequency sound exposure would counteract the usual increase in development rates triggered by warmer temperature (Leandro et al. 2006; Laspoumaderes et al. 2022) because of the copepods higher metabolic demands under warming not fulfilled by prey consumption as a consequence of sensing alterations. We specifically utilized on tonal sound, that is prominent against the ambient sound. The tonal sound source we used was proposed as a standardized laboratory tool, and in the same frequency range as anthropogenic sound created by boat, shipping traffic, or operational wind farms that interferes with broadband natural sounds in natural environments (de Jong et al. 2017). The two temperatures we used reflect the current average summer temperature at our study site (18°C), Helgoland (Germany), and the predicted increase (+ 3°C) under the shared socioeconomic pathways 5–8.5 scenario (Intergovernmental Panel on Climate Change 2023). Both temperatures are within the range that

promotes higher growth rates of *A. tonsa* (Leandro et al. 2006; Laspoumaderes et al. 2022).

Materials and methods

Copepod and alga husbandry

Copepod eggs were obtained from *A. tonsa* cultures at Biologische Anstalt Helgoland, which are maintained at 32 salinity, 18°C, and with a light intensity of $74 \mu\text{mol m}^{-2} \text{s}^{-1}$ in a 12 : 12 h light : dark cycle. Eggs are periodically removed from the 200-L tank culture, collected in a sterile 50-mL tube, suspended in 0.2- μm -sterile natural filtered seawater and kept in the dark at 4°C until use. The number of eggs per tank is not counted, and eggs' size is not measured. Prior to our experiment, cohort hatching rate was determined to estimate the number of nauplii hatched after 48 h per volume of eggs to ensure enough nauplii for the experiment. Hatching conditions were set at 18°C according to previous studies (Meunier et al. 2016; Herstoff et al. 2019). *Rhodomonas salina*, the alga fed to copepods, was maintained in a constantly aerated 5-L batch culture of 0.2- μm -sterile natural filtered seawater at 18°C with a light intensity of $185 \mu\text{mol m}^{-2} \text{s}^{-1}$ in a 12 : 12 h light : dark regime. Subsamples of *R. salina* were measured daily using CASY particle counter (Scharfe Systems) to control the amount fed to copepods.

Rearing copepods under different chronic sound conditions

The long-term culture may be used to a small degree of sound related to the refrigerating system of the controlled temperature room, but not to the tonal sound source used in the current experiment. This background soundscape corresponds to what was measured in the control treatment of a previous study on lobster juveniles (Leiva et al. 2021). After hatching, copepods were transferred to a wood paneled isolated room, where they were cultivated in a full factorial design with two temperatures (18 [culture temperature] and 21°C [shared socio-economic pathways 5–8.5 scenario]) and with two chronic sound conditions (control, i.e., no added sound; and added low-frequency sound; Supporting Information Fig. S1a). Hereafter, these treatments will be called “control” and “added sound,” respectively. Twelve 5-L glass bottles were each inoculated with 4000 48-h-old *A. tonsa* nauplii. The resulting density of 800 ind L^{-1} does not reduce performance, food ingestion, and egg production (Vu et al. 2017). Two refrigerating/heating circulators (CBC 5 Control, IKA) were used to control the temperatures within the bottles ($n = 6$ at 18°C; $n = 6$ at 21°C) with an individual hose system around each bottle. Half of the bottles kept at each temperature were subjected to added sound using a “noise egg” ($n = 3$), while the other half received a non-functional “noise egg” which acted as control ($n = 3$). The “noise egg” setup comprised an electro-vibration motor (Nokia 3310) connected to battery holder; the motor and battery holder were both placed in a “diver egg” (waterproof container;

external dimensions: 11 cm in height and 6.5 cm in diameter; internal dimensions: 10 cm high and 5 cm in diameter) that produced a fundamental frequency of 110 Hz with many strong harmonics (de Jong et al. 2017) (Supporting Information Table S1). Buoyancy counterbalancing was achieved at 10 cm from the bottom by fixing the egg to a sandbag with a thread. The “noise egg” was completely submerged, in suspension, at the center of the 5-L bottle. The “noise egg” was not protected by a net or mesh; the animals could thus have direct contact with the sound source.

Sound frequency and pressure were recorded using two calibrated SM3 hydrophones (sensitivity of $-165 \text{ dB re } 1 \text{ V}/\mu\text{Pa}$, Wildlife Acoustics Song Meter Automated Audio Recorder SongMeter, Wildlife Acoustics). Measurements were taken daily in one control and one added sound culture at 44,100 samples per second. The hydrophones were positioned at the bottom of the bottle, at 8 cm from the bottle's center and from the diver egg (Supporting Information Fig. S1a). In the added sound treatments, the mean sound pressure level (SPL) at this position was ~ 22 and $15 \text{ dB re } 1 \mu\text{Pa}^2 \text{ Hz}^{-1}$ above control conditions between frequencies 110 and 120 Hz, at 18°C and 21°C, respectively (Supporting Information Fig. S1a and Table S1). Sound pressure level and the power spectral density (PSD) root mean square level were computed, calibrated, and visualized on 10-s intervals (see Table S1 for the sound recorded in each condition) using the R PAM Guide code (Merchant et al. 2015) (SPL: Fast Fourier Transforms 44,100, Hann window, 50% overlap, average for each file; PSD: Fast Fourier Transforms 44,100, Hann window, 50% overlap, 1 s average). For each treatment, recorded sounds were concatenated to form a single contiguous spectrogram. The correction factor for calibration was calculated using manufacturer's technical specifications, the sensitivity of the transducer, the user-defined gain settings for the deployment in decibels, and the zero-to-peak voltage of the analogue-to-digital converter.

During rearing in the control and added sound conditions, copepods were kept in the dark and fed ad libitum 1×10^4 (nauplii) or 2×10^4 (copepodites) cells animal^{-1} *R. salina*, depending on development (Jones et al. 2002; Meunier et al. 2016; Herstoff et al. 2019). Water in all replicates was changed every other day, after which *A. tonsa* were fed. Because of faster development at warmer temperatures (13.38 ± 0.76 and $8.82 \pm 1.19 \text{ d}$ at 18°C and 22°C to reach copepodite 5, respectively; Leandro et al. 2006), growing time was set to 11 d at 18°C and 9 d at 21°C. This way, we ensured the apparition of C6 stage in the replicates and the detection of developmental differences among control and added sound treatments for each temperature, as well as the presence of a single generation in the replicates (C6 are sexually mature adults, and we wanted to avoid reproduction events within replicates).

Video trials under different sound conditions

On day 9 (21°C) and 11 (18°C), the 12 (2 temperatures \times 2 sound levels \times 3 replicates) bottles from the chronic sound

conditions were processed one by one, alternating between added sound and the control conditions (Supporting Information Fig. S2). Movement patterns were assessed with high-speed videography following previous configuration and protocols (Herstoff et al. 2019). In brief, a mirror-coated 90° prism was placed in one side of a two-chambered acrylic box with interior measurements of 5 cm long × 2.5 cm wide × 2.5 cm deep. The supplemental material offered in Herstoff et al. (2019) is particularly useful to understand how the mirror-coated prism allows the three-dimensional position assessment of the copepods in the chamber. Distances and copepods size were calibrated with a ruler placed at the bottom of the test chamber (0.026 mm pixel⁻¹). Filtered seawater, copepods, and *R. salina* prey were placed in the adjoining test chamber and the acrylic box was illuminated externally using an Olympus KL 1500 LCD at color temperature of 3300 K. This setup allowed tracking of copepod movement in the two planes using a single camera. A monochrome high-speed camera (Phantom MIRO LAB 110) was connected to the Olympus SZX16 stereo microscope with a 12.5X magnification positioned above the chambers.

A modified “noise egg” was used for added sound condition during high-speed videography trials. To generate added sound during trials, the same electro-vibration motor used in the “noise egg” was placed in a 1.5-mL microcentrifuge tube and lowered into the corner of the test chamber containing copepods and their prey (Supporting Information Fig. S1b). The sound source was kept 1 cm from the test chamber walls. For control trials (without added low-frequency sound), the apparatus was left in the test chamber, but the batteries were removed (motor was “off”). Prior to video trials, SPL was measured with a small, calibrated, hydrophone (Aquarian Scientific AS-1 hydrophone with PA4 preamplifier, sensitivity of -208 dB re 1 V/ μ Pa; AFAB Enterprises) connected to an audio recorder (Zoom UAC-2; set to 44,100 samples s⁻¹, 16 bit). The hydrophone was placed in the bottom of the acrylic chamber at 2 cm from the sound source. The added low-frequency sound condition for video trials has SPL ~ 42 dB re 1 μ Pa² Hz⁻¹ above control conditions, at 142 Hz (Supporting Information Table S1 and Fig. S1b).

Shortly before each videography trials, copepods were gently siphoned from 5-L bottles with a long tube on a 40- μ m mesh nylon filter and were flushed back into 200-mL 0.2- μ m-sterile natural filtered seawater at the respective temperature. A sample of 100 individuals was preserved in Lugol's solution at a final concentration of 2% for stage determination and developmental rate (Supporting Information Fig. S2). Two other subsamples of about 30 copepods were gently filtered throughout a 1000- μ m mesh to work with younger copepodites stages for the videography trials under control and added sound conditions. Copepods were transferred with pipette (tip cut to enlarge opening) into the test chamber of the acrylic box, which contained 30 mL of 0.2- μ m-sterile filtered seawater. Shortly thereafter, *R. salina* was added to the test chamber in a final concentration of 1000 cells mL⁻¹, and

the test chamber volume was filled to 50 mL. The copepods were acclimated to the video treatment (control/no added sound or added sound) for 5 min before video capture began (Supporting Information Fig. S2). Both sound conditions were tested for each culture (see Supporting Information Fig. S2 for procedure to determine the order of sound condition assessed for each culture in line to rearing condition). Time to record a set of 10 clips was 23 ± 5 min (mean \pm SD; $n = 24$). In summary, 20 video clips were recorded per replicate ($n = 12$), resulting in analysis of 240 video clips. The observation area covered 1.6875 cm² of the 12.5 cm² chamber surface. A frame rate of 1250 s⁻¹, with an exposure time of 588 μ s and a resolution of 768 × 768 pixels was used (Herstoff et al. 2019). Image capture was initiated with a manual trigger when a copepod was entering the observation area, and a minimum 10-s pause was taken between recordings to ensure that the recorded copepod moved out of the observation area. Only one copepod was swimming in the area at the time video clip was taken. It may have been possible that the same copepod was assessed more than one time in an assay.

Copepod stage distribution and developmental rate after rearing

An Olympus SZX16 stereo microscope with a calibrated eyepiece micrometer was used to measure the Lugol's preserved copepods' lengths, and to identify their stage. Copepods were observed under a magnification of 40X (copepods and adults) or 100X (nauplii), and categorized to stage using number of swimming leg pairs and the number of urosome segments (Lovegrove 1956; Trujillo-Ortiz 1986; Sabatini 1990). As organisms shrink in Lugol (Jaspers and Carstensen 2009), total length was not considered here. To determine the stage distribution, the percentage of each developmental stage per replicate was determined. Developmental rate was calculated by labeling all nauplii and copepod stages from 2 to 12 (no nauplii at stage NI were observed), such that 2 = naupliar stage NII, and 12 = adult copepod (copepod stage CVI) (Harris et al. 2000; Malzahn and Boersma 2012). Developmental rate was calculated as molt rate per day by dividing the sum of all developmental stage relative contribution by the duration of the growing phase (9 d at 21°C and 11 d at 18°C).

Analysis of swimming speed, net displacement, and movement patterns

Phantom's camera software was used to analyze the videos. The length of the copepods in the video was measured to assess their development stage based on the length range from literature (Sabatini 1990). Copepods used in the 240 video pathways analyzed were between stages II and V (at 97%) according to size (CI = 1%, > CI < CII = 2%, CII = 6%, > CII < CIII = 14%, CIII = 13%, CIV = 37%, > CIV < CV = 16%, CV = 11%, CVI = 0%). Copepod movement was tracked in both horizontal (X, Y) and vertical dimensions (Y, Z) using the image projected on the mirror. The X, Y, and Z positions of the focal animals

were determined every 10th frame of the video clip, yielding a three-dimensional analysis of movement at least 0.17 s long, where the copepod's position was recorded every 0.0008 s.

Displacement patterns were quantified following (Herstoff et al. 2019). In brief, to calculate swimming speed (v_{swim} , mm s⁻¹), displacement distance was divided by the fraction of the time spent swimming, f_{swim} , which was in turn set as the fraction of analyzed intervals where the animals were detected to be moving (Bianco et al. 2013).

$$v_{\text{swim}} = D \div (f_{\text{swim}} \times t)$$

Net displacement (D_{net} , mm) was calculated based on the start and end coordinates of the copepod given the X, Y, and Z coordinates referenced from a fixed point at times 0 and t (Seuront et al. 2004; Dur et al. 2011; Herstoff et al. 2019) using first Euclidean distance formula:

$$D_{\text{net}} = \sqrt{(X_t - X_0)^2 + (Y_t - Y_0)^2 + (Z_t - Z_0)^2}$$

The D_{net} was then normalized to the duration (t , s) of the clip, where F is the number of frames analyzed and FR is the frame rate.

$$t = F \times \frac{1}{\text{FR}}$$

Movement patterns exhibited by copepods during video trials were associated with displacement (unidirectional jumps, drops, and turns) or feeding (sawtooths and helices). Drops are fast negative vertical displacement (Bradley et al. 2013) of more than one body length between two sequentially analyzed frames within a clip. Drops do not return copepods to the original plane. Helical swimming is highly correlated with feeding (Henriksen et al. 2007) and has been seen in multiple life-history stages (Herstoff et al. 2019), while drops are associated with escape behavior (Bradley et al. 2013). The digitized copepod movement tracks were plotted in R (R Core Team 2024) using a three-dimensional scatterplot (Sanftmann and Weiskopf 2012), and the patterns quantified for each of the clips taken during video trials.

Statistical analyses

All analyses were done with R (R Core Team 2024), and a significant difference was set at $p < 0.050$ for all statistical tests. Data were tested for normality (Shapiro test) and homogeneity (Bartlett test) and transformed if necessary. We analyzed development rate using a two-way ANOVA with two main fixed effects (temperature and chronic sound condition).

For v_{swim} and $\log(D_{\text{net}})$, data were analyzed through linear mixed model effects with package "nlme" (Pinheiro et al. 2024). This approach captures the structure of the data where the pseudoreplicates (240 analyzed copepod paths) are

nested under the genuine replicates (12 culture bottles). The full model included: (1) bottle replicate as a random factor; (2) video sound condition nested in chronic sound condition as fixed factor; (3) chronic sound condition as fixed factor; (4) temperature as fixed factor; and (5) interactions among fixed factors. Several models were created starting from the most complex, the full model, then the additive model (no interaction of fixed factors), then simpler models without the video's sound condition, and with single factor chronic sound condition and temperature. Model selection took place through maximum likelihood fitting on the fixed terms. Backward model selection for linear mixed models were carried through the Akaike information criterion (AIC) score. Models were ranked according to their AIC score, and the one with the lowest AIC was chosen. If the difference between the lowest AIC score and the second lowest score (ΔAIC) was greater than 3, the model with lower AIC was selected regardless of differences in complexity. The likelihood ratio test was applied as hypothesis testing only when ΔAIC was less than 3. When models differed significantly ($p < 0.05$), the one with lower AIC score was selected. If models did not differ, the simplest model was chosen. The linear mixed-effects models retained for both response variables were additive (no interaction of fixed factors) (Supporting Information Table S2).

Movement patterns were analyzed using the package "mvabund" (Wang et al. 2022) to fit generalized linear models using a negative binomial distribution with a log-link function (Herstoff et al. 2019). This approach has the advantage of accounting for confounding mean–variance relationships often associated with count data. The use of negative binomial distribution minimizes type I error by bootstrapping the residuals with replacement via ANOVA to test correlations in the data (Warton et al. 2016; Wang et al. 2021). Statistical significance was tested using an ANOVA and a likelihood ratio test.

Results

Copepod stage distribution and developmental rate after rearing

Higher temperature significantly increased development rate by 13% (two-way ANOVA, $F_{1,8} = 21.81$, $p = 0.002$; Fig. 1). In control sound condition, a significantly greater proportion of copepods reached the older stages (stages CIV–CVI) compared to added sound condition, + 55.6% and + 22.8% at 18°C and 21°C, respectively (Fig. 1) (ANOVA, $F_{1,8} = 16.63$, $p = 0.004$). There was a higher number of less developed individuals (nauplii or first copepodite stages) in added sound condition, + 9% and + 4% at 18°C and 21°C, respectively (Fig. 1). There was no interaction between temperature and sound condition for developmental rate.

Swimming speed, net displacement, and movement patterns

Copepods that grew in control sound conditions swam faster than those in added sound condition ($F_{1,9} = 5.075$,

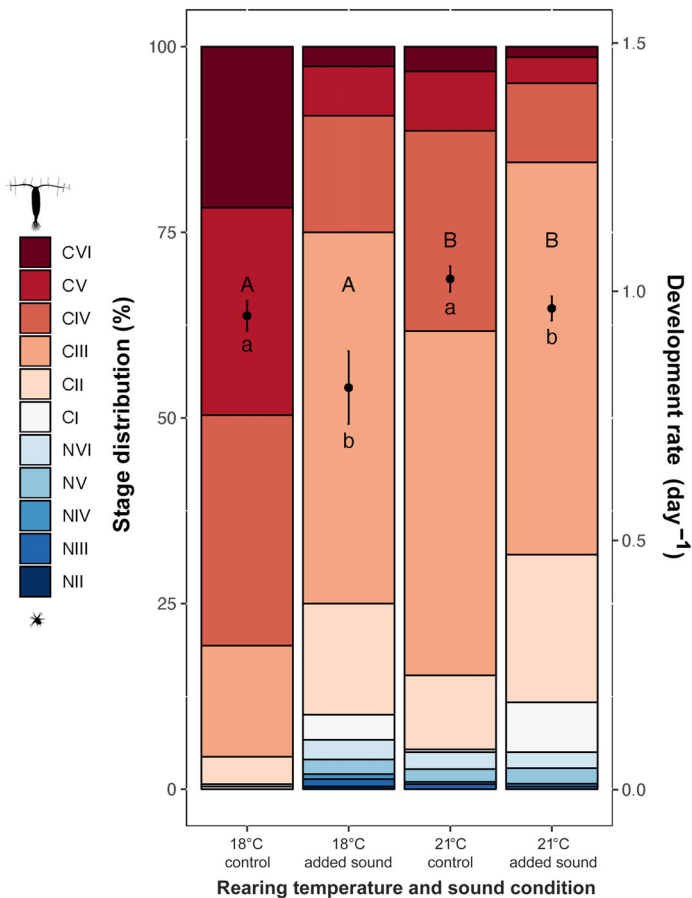


Fig. 1. Copepods' stage distribution and development in relation to temperature and sound condition after rearing. One hundred individuals were identified for stage distribution (in %; left axis; stacked colored boxes, where N = nauplii and C = copepods) and developmental rate (molt d^{-1} ; right axis; dots and whiskers; mean \pm standard deviation); For letters indicating statistically significant differences, uppercase refer to fixed factor temperature while lowercase refer to fixed factor sound condition.

$p = 0.051$; Supporting Information Fig. S3), and copepods reared at 21°C swam slower than those reared at 18°C ($F_{1,9} = 8.415$, $p = 0.018$; Supporting Information Fig. S4), with no significant interaction between the two factors. Thus, added sound and higher temperature both slowed down copepod movements. Regardless of the sound condition during rearing, copepods' swimming speed under video added sound condition was significantly higher than copepods from video control condition ($F_{2,226} = 39.090$, $p < 0.0001$; Fig. 2). The higher swimming speed observed during video added sound coincided with higher log-transformed mean net displacement ($F_{2,224} = 13.336$, $p < 0.0001$; Supporting Information Fig. S5).

Temperature did not have a significant effect on any of the movement patterns analyzed, and so results from both temperature conditions were pooled for this analysis. Overall, the recorded videos under added sound condition present

significant changes in movement patterns; turns (*Deviance* or *Dev* = 24.341, $p = 0.001$; Fig. 3a), unidirectional jumps (*Dev* = 44.563, $p = 0.001$; Fig. 3b), helices (*Dev* = 9.321, $p = 0.002$; Fig. 3c), and sawtooths (*Dev* = 37.013, $p = 0.001$; Fig. 3e) were all performed less frequently, while drops (*Dev* = 4.316, $p = 0.043$; Fig. 3d) were performed more frequently. For helical swimming, there was an interaction between the video sound condition and chronic sound condition (*Dev* = 7.638, $p = 0.029$; Fig. 3c). In the video control condition of copepods from the culture-added sound condition, helical swimming was lower than copepods from the culture control condition.

Discussion

Low-frequency tonal sound slows down developmental rates and alters movement patterns of the calanoid copepod, *A. tonsa*. These results highlight the need to consider noise when assessing copepod life histories and population dynamics, particularly when predicting ecosystem responses to anthropogenic disturbances. Increased energy demands caused by more escape behaviors (Marrasé et al. 1990) and resulting lower recruitment will unavoidably lead to a lower abundance of copepods. Hence, we propose that the negative impact of noise on copepod fitness may have negative cascading effects through marine food webs as they may alter the quality and quantity of prey available for higher trophic levels. Copepods not reaching the later stages of development will not transfer essential biomolecules needed by their predators (Malzahn et al. 2007). Because many higher trophic levels feed on later developmental stages of copepods, these results are especially important to consider, for example, in commercial fisheries developing in new available areas such as the Arctic, where increased shipping will bring more low-frequency noise (< 1000 Hz) to the acoustic habitat (Moore et al. 2012), and for protecting endangered marine mammals near offshore wind farm projects, like right whales.

Copepods are already negatively affected by climate-associated changes (Richardson 2008) and our study demonstrates that low-frequency sound (+ 15–22 dB re $1 \mu Pa^2 Hz^{-1}$) may exacerbate these effects. Added SPLs used in our experiment are comparable to SPLs that occur 100 m around an operating single turbine (between 105 and 125 dB re $1 \mu Pa$) (Tougaard et al. 2020), and to median ship traffic noise levels exceeding 120 dB re $1 \mu Pa$ (20 dB above modeled natural background sound) (Farcas et al. 2020). Wind turbines generating up to 6 MW produce broadband root mean square SPLs between 129 and 166 dB re $1 \mu Pa m$ (at 1 m from the turbine) (Stöber and Thomsen 2021). Wind turbines in the future will likely be more powerful (> 10 MW) with broadband SPLs of > 170 dB re $1 \mu Pa m$ (at close range) (Stöber and Thomsen 2021). The SPLs between 122 and 129 dB re $1 \mu Pa m$ between 109 and 142 Hz fundamental frequency used in this study were in the frequency range of current operational noise

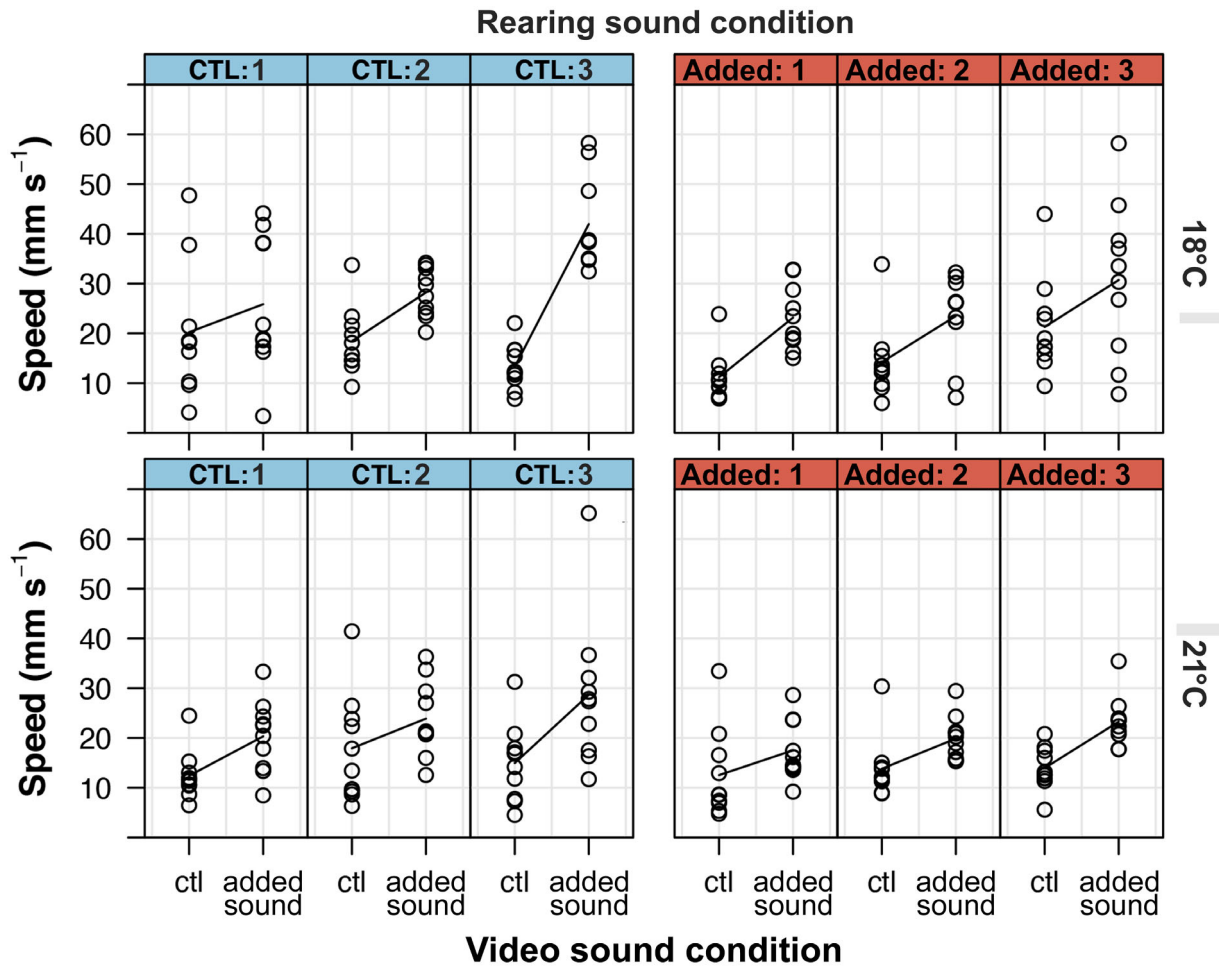


Fig. 2. Copepods' swimming speed in video control and added sound conditions for each rearing treatment. Approximately 30 copepods from all cultures (CTL = control, ADDED = added sound) were introduced in an acrylic chamber, separately, for video trials in control (ctl; $n = 10$) and added sound (added sound; $n = 10$) conditions. Swimming speed (mm s^{-1}) was calculated using the distance displaced over the fraction of the time spent swimming.

of wind farms which produce the most energy (between 2 and 200 Hz) (Popper and Hawkins 2019; Tougaard et al. 2020). Therefore, the behavioral response of copepods to the sound signal used in this study is valid within the framework of current wind energy powered projects and calls for careful environmental studies regarding more potent offshore wind farms.

Copepods showed a greater frequency of anti-predator defense behaviors in noisy conditions, meaning that potentially less energy was available for secondary production, which led to the slower copepod developmental rates observed in this study. We postulate that the behavioral changes observed under video added sound conditions indicated that the copepods were stressed and attempted to escape. For example, increased copepod swimming speed was considered a stress response in several studies investigating pollutants (Cailleaud et al. 2011), and high rearing densities (Nilsson et al. 2017). The SPLs in the video-added sound condition were to threefold higher than those in the rearing sound condition, which is possibly the reason why we

observed an overall increase in swimming speed among video sounds conditions and decreased swimming speed among rearing sound conditions. In rearing conditions, acclimation and potentially metabolic suppression might have happened in the added sound treatment to decrease energy demands during period of stress as observed in copepods exposed to hypoxia and acidification (Wyeth et al. 2022), or as consequence of lower encounters with food particles because of masking (leading to metabolic suppression). Rapid pressure drops caused by impulsive sounds also resulted in lower swimming speed just after and 5 h after pressure drop in *A. tonsa* (Vereide et al. 2024). One limitation of our study was that we could not measure particle motion due to the challenges of working in small containers (Nedelec et al. 2016). Particle motion has received little attention to date, even though it is the sound component particularly perceived by organisms like fish and invertebrates as they detect their predator/prey/environment using vibrations sensed by body cavities or specialized receptors (Mooney et al. 2020). The potential effect of particle

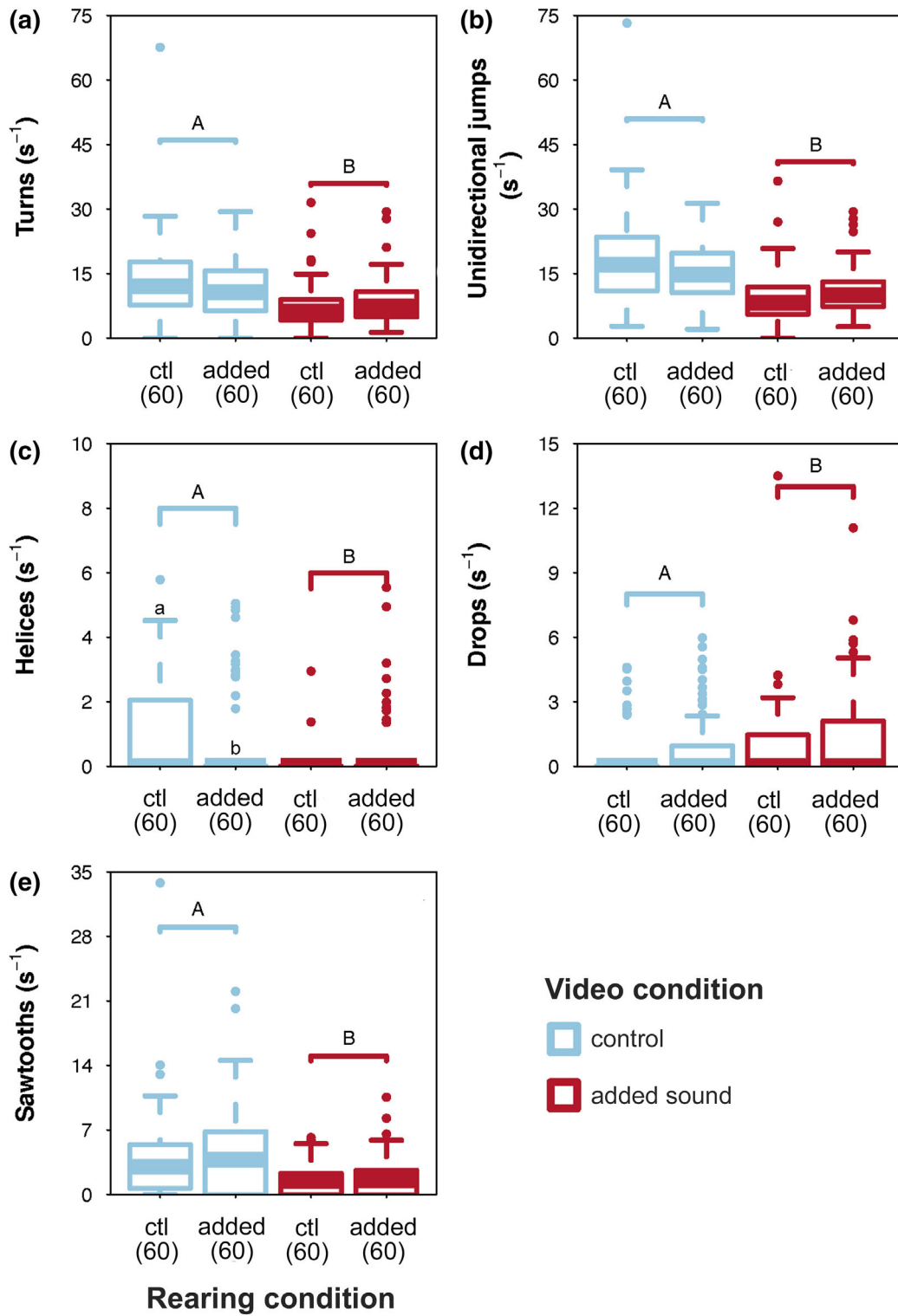


Fig. 3. Copepods behaviors in video control and added sound conditions. Approximately 30 copepods from all cultures (ctl = control; added = added sound) were introduced in an acrylic chamber, separately, for video trials in control and added sound conditions. As temperature was not a driver in this analysis, results were pooled. Behaviors assessed were: (a) turns, (b) unidirectional jumps, (c) helices, (d) drops, and (e) sawtooths; all reported in s^{-1} . For letters indicating statistically significant differences, uppercase letters refer to video sound conditions during trials; lowercase letters refer to rearing sound conditions; numbers are given in parentheses.

motion, and especially in such small glass bottle setups, needs to be tackled in future studies to understand the actual sound exposure and its effect on copepods and their prey.

Although our study used two temperatures to simulate summer current and predicted ocean surface temperatures under warming conditions (Intergovernmental Panel on Climate Change 2023) in temperate latitudes such as the North Sea, these temperatures were not intended to be harmful for the copepods. Indeed, warmer temperatures usually benefit *A. tonsa* egg production and hatching success (Holste and Peck 2006). This species is thermally resistant across life stages, and copepods can readily acclimate to numerous temperatures, allowing survival during cold and hot events (Sunar and Kir 2021). While we expected warmer temperatures to promote faster development (Laspoumaderes et al. 2022) and swimming speed (Moison et al. 2012; Svetlichny and Oberegger 2022), we did not see this in the warmer temperature cultures with added sound. In contrast, in our study, we observed lower swimming speeds at warmer temperatures, which may be related to shifts in energy budgets (and potentially, elemental requirements) as copepods compensate for increased energy demands triggered by +3°C temperature increase. In particular, and in contrast to all other behaviors considered in this study, copepods reared in added sound conditions and exposed to control sound conditions during video trials did not demonstrate a return to the feeding-related behavior of helical swimming. Supporting our suggestion of decreased feeding when copepods are exposed to sound during development, a recent experiment with the same copepod species found decreased ingestion and clearance rates under exposure to harbor noise (173 dB re $\mu\text{Pa}^2 \text{Hz}^{-1}$) in a laboratory experiment (Kühn et al. 2023). Taken together, we suggest that physiological benefits usually expected at warmer temperature (i.e., higher growth rates and greater swimming speed) were overcome by the added sound condition during rearing. The effect of added sound on development was less strong at 21°C, potentially because increasing temperatures lead to a decrease of the speed at which sound travels (Mackenzie 1981; Kunc et al. 2016), as seen from the lower observed SPLs at 21 compared to 18°C. Future studies must investigate the narrowing of the optimal thermal windows caused by the effect of added sound on prey-predators' encounter rates.

The mechanisms that resulted in decreased ingestion and feeding behaviors under low-frequency sound exposure could be related to a disruption of the function of the setae, which detect slow-moving, low-frequency disturbances, which allows copepods to sense small amounts of fluid displacement (Shen et al. 2020), from 1 to 150 Hz (Yen and Strickler 1996). Added low-frequency sound conditions during rearing and videography could have resulted in two possible, nonexclusive mechanisms that reduced the rate of certain behaviors observed in our experiment. Predators perceive copepods from the fluid disturbances that copepods create when they feed (Kjørboe et al. 2014), and thus predator presence tends to reduce the

amount of time copepods exploit food patches in laboratory experiments (Saiz et al. 1993). Because copepods also sense predator presence by the predator's disturbance of the water (Kjørboe 2013), added sound might have been confused with predator presence, reducing copepod swimming and feeding (Kvile et al. 2021). Further, low-frequency sound can damage cells and organelles like mitochondria, as observed in cephalopods (André et al. 2011). Thus, the overall slower swimming speed in copepods reared in added sound conditions may reflect mechanically or metabolically induced injuries. Hence, we propose that future work should use cell markers to scrutinize stress responses under added low-frequency sound conditions.

Low-frequency sound likely has different impacts on key copepods behaviors compared to turbulence, a natural oceanographic perturbation, even though the resulting impairments could look alike (Kühn et al. 2023). Many laboratory studies have shown that moderate turbulence affects a copepod's ability to detect hydrodynamic signals (Gilbert and Buskey 2005; Elmi et al. 2021), which may be compensated for by an increased encounter rate with food (Rothschild and Osborn 1988). While ocean turbulence is usually rather weak in terms of duration in a specific area (Franks et al. 2022), anthropogenic low-frequency sound is omnipresent and may vary in intensity according to wind speed (e.g., wind acting on turbines), wind direction, and sound source (Yoon et al. 2023). At first glance, our observation of higher swimming speed and more hopping under video added sound conditions aligns with some previous observations of copepod swimming in vortexes that mimic turbulent conditions (Webster et al. 2015; Elmi et al. 2021). To better stay within the vortexes, copepods displayed higher swimming speeds and increased hopping, while no escape behaviors are observed (Webster et al. 2015; Elmi et al. 2021). However, in our experiment, we observed many escape behaviors, like drops, which may have important, negative energetic consequences for the animal. While turbulent conditions can benefit copepods by increasing a copepod's encounter rate with food (Rothschild and Osborn 1988) or mates (Michalec et al. 2020), low-frequency sound does not have similar benefits. Furthermore, anthropogenic sound also acts as an additional sensory input that copepods need to deal with in their day-to-day predation risk assessment; previous work has found that other sensory input, as simple as the presence of light, primes greater escape responses while predation pressure remained unchanged, probably because they are more visible for predators (Fields et al. 2012). Anthropogenic sound exposure may thus act as an unidentified threat for copepods that mistakenly treat this information as if it was coming from a predator.

We recognize that observed behavioral effects of low-frequency sound in our experiments may have differed between video trials and rearing sound conditions. Copepods from rearing added sound conditions experienced a weaker magnitude of low-frequency noise exposure (+15–22 dB re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$) compared to added sound video trials (+42 dB

re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$). Although the noise source used to generate added low-frequency sound was the same, the sound level and harmonic periodicity was higher in the video trials due to the smaller volume of the video chamber as compared to the rearing vessels (50 vs. 5000 mL). Unfortunately, it was not possible to assess behavioral patterns while copepods were developing in the culture bottles because the experimental setting did not allow video recording at a sufficient resolution to observe behaviors. Standardized aquatic laboratory systems to study anthropogenic sound effect on benthic larvae have been developed (Olivier et al. 2023), from which parameters such as growth and survival can be directly obtained. Similar systems could be developed for plankton, and with the inclusion of specialized cameras to study metabolic and physiological traits via developmental stage and feeding rate during rearing added sound condition, without needing to move specimens and altering the noise field.

In summary, we found that copepods developed more slowly when exposed to low-frequency sound, regardless of temperature. Because exposure to low-frequency sound resulted in fewer feeding-associated behaviors, we suggest that we must consider these effects when predicting ecosystem responses in future planned areas for shipping routes or offshore wind parks, especially because copepods are important prey for so many larger marine consumers. Plankton concentrated in upwelling areas, oceans fronts and other oceanographic features that are quite stable in time and can be observed, modeled, and predicted. Areas with high zooplankton abundance should be properly assessed when marine industry development is prospected. Although offshore wind farms are of importance to society, we have largely neglected studying how the operation phase of wind farms may influence organisms of all trophic levels, beyond marine mammals or economically important fauna. Based on our findings, we propose that the negative effects on smaller key organisms like copepods should also be considered in future research on wind farms, and that assessment of low-frequency sound on marine biota should take a whole ecosystem approach when considering benthic-pelagic secondary production. Our work is an important first step toward understanding the mechanisms that may influence marine zooplankton and sets the stage for establishing tolerance threshold levels of anthropogenic noise for all the biological components of marine ecosystems.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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