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Musseling through: *Mytilus* byssal thread production is unaffected by continuous noise

Sheng V. Wang [a,b,*], Julius A. Ellrich [a], Jan Beermann [b], Bernadette Pogoda [c], Maarten Boersma [a,d]

[a] *Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Biologische Anstalt Helgoland, Shelf Sea System Ecology, Helgoland, Germany*
[b] *Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Benthic Ecology, Bremerhaven, Germany*
[c] *Alfred Wegener Institute (AWI), Helmholtz Centre for Polar and Marine Research, Shelf Sea System Ecology, Bremerhaven, Germany*
[d] *University of Bremen, FB2, Bremen, Germany*

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ABSTRACT

Anthropogenic low-frequency noise (ALFN) is a rising pollutant in the world oceans. Despite the ubiquity of ALFN, its effect on marine invertebrates is still poorly understood. Here, we tested how continuous low-frequency noise (CLFN), a substantial component of ALFN, affects the byssal thread production of *Mytilus*, a cosmopolitan genus of mussels with high ecological and economic importance. The effects of acute CLFN exposure and predator cues on byssogenesis by *Mytilus* spp. were explored in both the presence and absence of predator cues. While predator effluents increased thread production, CLFN had seemingly no effect on thread counts. Further, trends suggested a synergistic effect of CLFN and predator cues. The behavioral indifference of *Mytilus* spp. toward CLFN could contribute to the observed prevalence of these animals in inherently disturbed habitats. This would partly explain their success in colonizing and persisting on artificial substrata rife with disturbances.

1. Introduction

Anthropogenic low-frequency noise (ALFN), or man-made noise with frequencies between 10 and 500 Hz (Carey and Evans, 2011), is a rising pollutant in our oceans. Low-frequency noise levels in the open oceans have increased approximately 3.3 dB, or doubled in power, every decade since at least the year 1950 to at least 2007 (Frisk, 2012). Such rates of increase have not been calculated for the years beyond, however ocean noise has been shown to still be, concomitant with ship traffic, generally on the rise (Jalkanen et al., 2022; Miksis-Olds et al., 2013; Possenti et al., 2024). While commercial shipping (the noise emissions of which are predicted to double every 11.5 years) has been the major contributor to this steady increase (Hildebrand, 2004; Jalkanen et al., 2022; Mustonen et al., 2019; Possenti et al., 2024; Ross, 1979), it is by no means the sole significant source of ALFN; offshore construction, ocean exploration, and energy production such as wind farms and oil rigs also contribute substantially to the increasing noise levels in marine environments (Duarte et al., 2021; Hildebrand, 2004). Offshore wind farms in particular have surged in prevalence internationally and are projected to continue to do so to meet green energy goals (DeCastro et al., 2019; IEA, 2022; WindEurope, 2023; Wiser et al., 2015). The main noise-emitting structure in wind turbines is the gearbox; the vibrations from its operation conduct down the pillar and radiate outward into the water and can vary depending on wind-driven parameters (Lindell, 2003; Pangerc et al., 2016). In spite of how much ALFN is being produced, research on how such noise affects most marine life is still scarce and the potential effects thus poorly understood.

Bivalves (and marine invertebrates as a whole) are one such group of understudied organisms despite their economic and ecological significance, sometimes as key species, in their respective marine ecosystems. Commercially relevant mussels, oysters, and scallops have received more attention, but knowledge of ALFN effects is still sporadic at best (Carroll et al., 2017; Solé et al., 2023). Published studies to date documented some responses of bivalves to anthropogenic noise, reaching from reduced bioirrigation behavior and anti-burrowing behaviors in clams (Solan et al., 2016; Wang et al., 2022) to stunted growth and increased mortality in oysters and scallops (Charifi et al., 2018; Day et al., 2016; de Soto et al., 2013).

* Corresponding author. Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Biologische Anstalt Helgoland, Shelf Sea System Ecology, Helgoland, Germany.
E-mail address: Sheng.Wang@awi.de (S.V. Wang).

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Mytilus is a cosmopolitan genus of mussels that plays a large role in many ecosystems and fisheries. The high biomass of their aggregations combined with their suspension feeding make them a potent biofilter and bioremediator (organism that removes environmental pollutants via storage, burial, and recycling) for large volumes of water (Beaumont et al., 2007; Birkbeck and McHenery, 1982; Broszeit et al., 2016; Jørgensen, 1990; Lindahl et al., 2005). Their filter feeding activity also facilitates the transfer of nutrients from the water column to the benthic substrate: the fecal matter produced feeds deposit feeders while the mussels themselves serve as important food sources for predators such as crabs and sea stars (Bergström et al., 2019; Kotta et al., 2009; Zhou et al., 2006). Thus, *Mytilus* play an important role in benthic-pelagic coupling. Mussel beds also provide biogenic substrate and otherwise-limiting resources for algae, invertebrates, and different life-stages of organisms (Albrecht and Reise, 1994; Norling and Kautsky, 2007) and as such are important hotspots of biodiversity (Craeymeersch and Jansen, 2019). Their modification of the environment and influence on local community composition thus deem them ecosystem engineers (Borthagaray and Carranza, 2007; Dürr and Wahl, 2004; Norling and Kautsky, 2008; Tsuchiya and Nishihira, 1986).

Mytilus spp. are frequently associated with manmade structures such as offshore wind farms where they can even dominate communities in terms of abundance and/or biomass (e.g., Coolen et al., 2022; De Mesel et al., 2015; Krone et al., 2017; Krone et al., 2013). Accordingly, the effect of LFN on *Mytilus* has been relatively more investigated than most other bivalves and invertebrates in general, although the research is still at an earlier stage and thus the documentation comprises largely of scattered details and partly contrasting leads. *Mytilus edulis,* when exposed to ship noise playback (continuous LFN, henceforth “CLFN”), exhibited significantly more DNA damage and severely reduced algae filtration rates compared to controls (Wale et al., 2019). However, boat noise combined with food presence as settlement triggers yielded a large increase in the settlement rate of *M. edulis* larvae*,* whereas boat noise or food alone resulted in much lower settlement rates (Jolivet et al., 2016). Evidence is sometimes even contradictory: the clearance rates of *Mytilus* spp. appeared unaffected by sound pulse trains (impulsive LFN, henceforth “ILFN”) at various pulse rates (Hubert et al., 2022b), but *M. edulis* exposed to experimental pile-driving (also ILFN, match in pulse rate to one treatment in Hubert et al. (2022b)) displayed higher clearance rates compared to controls (Spiga et al., 2016).

It is important, however, to differentiate the noise sources used in these studies and their purposes. Currently, most of the laboratory experiments investigating the effects of noise on marine invertebrates use noise sources that fall into one of two categories: recorded and generated (Solé et al., 2023). Recorded noises are usually hydrophone recordings featuring the treatment of interest (e.g., ships, pile-driving, natural soundscape) played back using underwater speakers. Generated noises are usually sounds with specific acoustic attributes created either digitally and played back through underwater speakers (e.g., sound pulses, sweeps, pure tones) or physically in real-time using a device (such as the noise egg utilized in this study). The former category prioritizes a more “realistic” representation of the treatment or soundscape *in situ* but becomes difficult to reproduce as a result. The latter category prioritizes the establishment of basic cause-effect relationships using controlled, reproducible sounds, however is less “realistic” as the sounds have no true *in situ* equivalent. The choice of the experimental noise source ultimately depends on the research question and experimental design. A direct comparison of results across noise categories must therefore be done with caution.

Mytilus individuals secrete fibrous structures, so-called byssal threads (a bundle of which is called a “byssus”), to tether themselves to hard substrate and to one another. Through this, several individuals form aggregates that resist wave action, offer protection against predation, and provide the aforementioned substrate for other organisms (Bell and Gosline, 1997; Moeser et al., 2006; Reimer and Tedengren, 1997). The mussels’ byssal thread production can be stimulated by external drivers such as the presence of predators (Côté, 1995; Garner and Litvaitis, 2013; Li et al., 2015; Rickaby and Sinclair, 2018), while anthropogenic disturbance such as pile-driving playback (ILFN) can lead to a reduced production of byssal threads and threads with lower mechanical strength, reducing overall mussel attachment strength (Zhao et al., 2021). Many studies used *Mytilus* individuals collected from anthropogenic environments, which are typically subjected to ALFN. However, *Mytilus* seems to be tolerant of these noisy environments as the mussels typically thrive on artificial substrata in areas with human activity and have been shown to habituate to ILFN (Hubert et al., 2022a; Krone et al., 2013). Here, we tested how the exposure to acute CLFN affects the byssus production of *Mytilus* spp. in response to predator presence. For this purpose, we tested for interactive and potentially cumulative effects between the CLFN and predator presence, using *Mytilus* individuals sampled from a small harbor, typically characterized by intermittent ALFN.

2. Methods

2.1. Animal collection and general experimental settings

Individuals of *Mytilus* spp. were collected in September 2022 from a pontoon inside the South Harbor of the island of Helgoland (German Bight, North Sea; 54°10′36.8″N, 7°53′36.1″E). The site is fully protected from waves by the walls of the harbor complex and shows only weak tidal currents of up to 0.1 m s^{-1} (Beermann, 2014). The mussels were immediately sorted in the laboratory of the Biologische Anstalt Helgoland (BAH) then placed into a bivalve cage suspended in a 5800 L flow-through outdoor tank for a minimum of one week before being used in the experimental setups. Fresh, local seawater for the flow-through was continuously provided via the seawater pumping system of the BAH. The sampled population likely consisted of pure *Mytilus edulis* Linnaeus, 1758 and hybrids of *M. edulis* with *Mytilus galloprovincialis* Lamarck, 1819*,* while pure *M. galloprovincialis* were absent (Coolen et al., 2020); determining the exact species requires molecular techniques that were not feasible to perform here as part of a behavioral experiment with live individuals. For predator presence and preparation of waterborne cues, a brown crab (*Cancer pagurus* Linnaeus, 1758*,* 10.65 cm carapace width) was caught in the Kringel rocky intertidal zone of Helgoland (54°10′37.65″N, 7°53′06.95″E) during high tide using a fish-baited trap.

The experiment was conducted in clear 1 L glass jars (105 mm × 105 mm × 145 mm; L × W × H) placed in a temperature-controlled chamber providing constant conditions (17 °C average to simulate temperature at time of sampling, 12 h light: 12 h dark). Each jar was filled with 1 L of filtered seawater or seawater with waterborne predator cue treatment. Squares of vibration-reducing rubber pads (110 mm × 110 mm × 20 mm) were placed under each jar to reduce the conduction of ambient room vibrations directly to the jar. Water temperature and noise were monitored daily to confirm identical conditions across the different treatments. A single *Mytilus* individual was placed in each jar for the duration of the experiment.

2.2. Low-frequency noise treatment and monitoring

So-called “noise eggs” were used to produce continuous low-frequency noise (CLFN) treatment (see de Jong et al. (2017)). Identical to the setup of Wang et al. (2022), the noise eggs employed diverged from the original design by using two nickel-metal hydride (NiMH) rechargeable batteries in parallel. Two batteries extended the noise egg uptime and the NiMH nature of the batteries supplied power at astable 1.2 V for most of each discharge cycle; as the frequency of the motor is modulated by the voltage provided, a flat discharge curve is desired. Differing from Wang et al. (2022), coin motors were used to drive the eggs instead of pager motors for slightly lower dominant frequencies (90–100 Hz). There was a peak at approximately 50 Hz present in all treatments likely due to ambient climate room and machinery noise; as the control was not truly silent in the low-frequency range, the terms “noise” and “control” will henceforth imply “added noise” and “no added noise,” respectively, for the current study. The noise eggs were half-submerged in each jar using racks.

Frequencies in each jar were monitored via an Aquarian Scientific AS-1 hydrophone suspended in the center of the jar. The hydrophone, coupled with a PA-6 preamplifier (+26 dB), was plugged into an audio interface (ZOOM UAC-2). The interface was then connected to and powered by a laptop via USB. Thirty-second recordings of each jar were taken once per experimental trial and analyzed in R (3.5.1) (R Core Team, 2022) with PAMGuide code (Merchant et al., 2015) to generate aggregate power spectral density (PSD) graphs (Fig. 1). The following settings were used: Fs(Hz) = 44,100, Window = Han 50% for SPL; Fs (Hz) = 44,100, Window = Hann 50%, and Average = 1 s for PSD. The calibration correction factor was calculated using manufacturer technical specifications for the AS-1 hydrophone, gain values from the PA-6 preamplifier and UAC-2 audio interface, and the zero-to-peak voltage of the UAC-2.

2.3. Experimental setup and data collection

The byssal thread production of *Mytilus* was tested in two different seawater treatments: untreated filtered seawater and filtered seawater that contained waterborne predator cues. The two seawater treatments were run under both added noise and control conditions. For the predator cue treatment, a single male *C. pagurus* was placed in a bucket with 16 L of filtered seawater and two individuals of *Mytilus* spp. as feed (both of which were always successfully consumed). An air stone was inserted for oxygenation, then the bucket was covered and left overnight. After 24 h, the crab was removed and the water was mixed, filtered through a 20 μm mesh, then poured into the respective treatment jars. The crab was held in a separate tank until the process was repeated for subsequent experimental trials. Five replicate jars for each treatment combination (four combinations: cues/noise, cues/control, no cues/noise, no cues/control) were implemented and the experimental trial was repeated five times in succession, resulting in a total of 25 replicates per treatment combination (4 combinations × 25 replicates = 100 individuals tested in total).

After filling the jars with their respective water treatments, batteries were inserted into the noise eggs of noise treatments. The water in each jar was constantly bubbled via pipette tips connected to air tubing for finer (and thus quieter) bubbles. Twenty *Mytilus* spp. of similar size (means: 24.85 ± 1.64 (SD) mm length, 13.76 ± 1.04 mm width, 8.53 ± 0.68 mm height) were randomly selected from the holding cage, measured, then randomly distributed across all jars. Each experimental trial was run for 24 h. Predator presence effects on byssogenesis has been reported to be (or become) apparent at approximately 6 h (e.g., Côté, 1995; Rickaby and Sinclair, 2018) while effects were also observed at 24 h in a preliminary experiment (unpublished observation). Therefore, byssal thread and byssus counts were registered after 6 h and 24 h by counting all thread attachment points on the glass.

2.4. Statistical analyses

2.4.1. Thread production after 6 h

As many individuals had not yet initiated thread-building activities after 6 h, zeros were abundant in the data and barred meaningful quantitative analyses of the raw 6 h thread counts. To describe the influence of each predictor on the probability that a mussel produced at least one thread at 6 h, a mixed effect logistic regression (MELR) was performed after transforming byssal thread counts into binary “Activity” data. Counts with no byssal threads at all were treated as “0″s while counts of one or more byssal threads were treated as “1″s. “Activity” was designated as the response variable (reference level = 0, or no activity) and “Noise” (2 levels: added noise, no added noise) and “Predator cues” (2 levels: present, absent) as the predictors. “Block” (5 levels, one for every trial) was included as a random effect to account for the possible random effects of different experimental runs. An additive MELR and an interactive MELR were performed at a 95% confidence level. Odds ratios for significant predictors were calculated by taking the exponential of their beta coefficient, and 95% confidence intervals were calculated using the following equation (Hailpern and Visintainer, 2003):

$$\textit{Odds ratio } 95\% \ CI = e^{\beta \pm (1.96 * \textit{Std. error})}$$

For every predictor, a likelihood ratio test (LRT) was performed between its null model (model without predictor in question) and the respective full model at a 95% confidence level. The resulting P-value indicated the significance of the relationship between the predictor and byssal thread production. Likelihood ratio tests were also performed for the interaction between “Noise” and “Predator cues” as well as for “Block.”

2.4.2. Thread production after 24 h

Five replicates lost their noise treatment overnight due to malfunctions of their respective noise eggs and were thus excluded from the dataset of counts after 24 h. “Threads” was designated as the response variable and like with the 6 h data, “Noise” and “Predator cues” were designated as predictors and “Block” as the random effect. An additive negative binomial generalized linear mixed-effects model (NBGLMM) and an interactive NBGLMM were performed at a 95% confidence level.

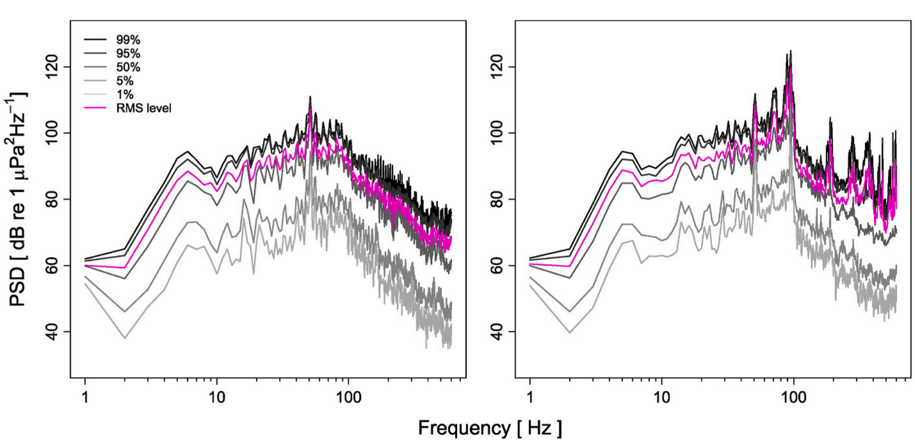

Fig. 1. Power spectral density (PSD) comparison of the control (left) and noise (right) treatment conditions. The pink line represents the root mean square level (RMS) while the other lines depict five different percentiles (1%, 5%, 50%, 95%, 99%), all measured in decibels (dB). Higher decibel numbers mean higher sound intensity.

Likelihood ratio tests were then performed at a 95% confidence level between the full and null model for each predictor, interaction, and random effect to test the significance of their relationship with byssal thread production.

Negative binomial GLMMs were chosen for quantitative analysis as the thread counts exhibited right-skewedness and heavy overdispersion, the former of which was evident through histograms of the raw data. Overdispersion was detected in the Poisson GLMMs (interactive model: $\chi^2(90) = 1211.62$, p = 8.53e-196; additive model: $\chi^2(91) = 1257.14$, p = 2.11e-204) by comparing the sum of squared Pearson residuals with the residual degrees of freedom (Bolker et al., 2009; Venables and Ripley, 2013). The standardized residuals for each GLMM were also plotted and visually assessed; nearly all residuals of the NBGLMMs fell within two standard deviations of the mean while those of the Poisson GLMMs lay mostly outside this range. To confirm that NBGLMMs fit the data significantly better than Poisson GLMMs, LRTs were run between each NBGLMM and its respective Poisson counterpart (interactive model: $\chi^2(1) = 828.90$, p = 2.2e-16; additive model: $\chi^2(1) = 853.99$, p = 2.2e-16).

An estimated marginal means (EMMs) post-hoc analysis using the Tukey method was performed on the interactive NBGLMM using the R package *emmeans* (Lenth, 2023). Descriptive statistics and graphical figures were calculated and produced using GraphPad Prism (8.0.2).

All statistical analyses were performed in RStudio (2022.07.1 + 554) with R (4.2.1) using the packages *stats* (R Core Team, 2022) and *lme4* (Bates et al., 2015) unless otherwise specified.

3. Results

The interaction between noise and predator cues tested non-significant and did not seem to affect the probability of *Mytilus* spp. producing at least one byssal thread after 6 h (interactive model: $\chi^2(1) = 0.8412$, p = 0.3591). Noise as a main effect, holding predator treatment constant, also did not seem to affect this probability (additive model: $\chi^2(1) = 0.1809$, p = 0.6706). However, *Mytilus* spp. in predator cue water, holding noise treatment constant, were 2.85 times (95% CI = 1.23, 6.62 times) more likely to have produced at least one byssal thread in 6 h than those in filtered seawater (additive model: $\chi^2(1) = 6.2035$, p = 0.01275) (Fig. 2).

Similar to the 6 h data, the interaction between noise and predator cues tested non-significant and did not seem to affect byssal thread counts at 24 h (interactive model: $\chi^2(1) = 1.7918$, p = 0.1807). Noise as a main effect also did not seem to significantly affect thread counts (additive model: $\chi^2(1) = 0.5791$, p = 0.4467) while predator cues as a main effect did (additive model: $\chi^2(1) = 3.9863$, p = 0.04587) (Fig. 3). *Mytilus* spp. in predator cue water, noise effect held constant, produced a mean of 24.32 ± 18.76 (SD) byssal threads while those in water without predator cues produced a mean of 17.04 ± 15.86 threads.

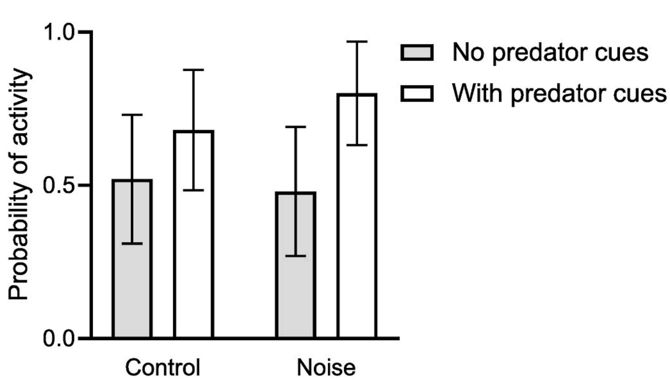

Fig. 2. Mean probabilities of *Mytilus* spp. activity after 6 h of exposure to continuous low-frequency noise and waterborne predator cues (*Cancer pagurus* effluents). The production of at least one thread by an individual signifies activity. The error bars depict the 95% confidence intervals for probability of activity under each treatment.

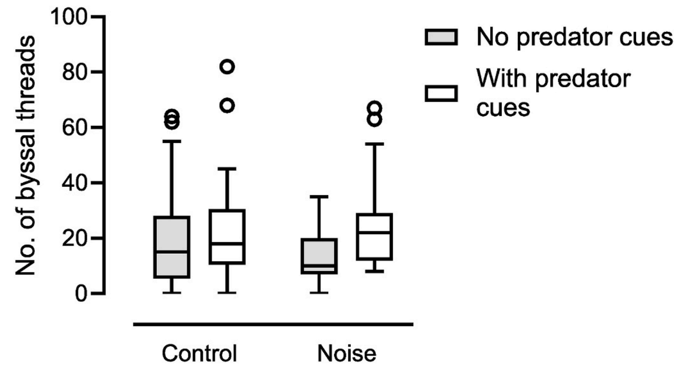

Fig. 3. Number of byssal threads produced by *Mytilus* spp. individuals after 24 h of exposure to continuous low-frequency noise and waterborne predator cues (*Cancer pagurus* effluents). The median, first and third quartile, and 1.5 IQR Tukey fences are shown. Outliers are represented by circles.

While the overall interaction between noise and predator cues on byssal thread production at 24 h tested non-significant, there was a noticeable (albeit non-significant) disparity between the cue and cue-free mean thread count differences as well as variability under noisy and control conditions. In the presence of noise, *Mytilus* spp. in predator cue water and in cue-free water produced means of 25.64 ± 17.64 (SD) and 13.70 ± 9.67 threads, respectively (mean difference of 11.94 threads; coefficient of variation = 68.79% and 70.57%, respectively). However, in the absence of noise, *Mytilus* spp. in cue water and in cue-free water produced means of 23.16 ± 19.98 and 20.12 ± 19.66 threads, respectively (mean difference of 3.04 threads, coefficient of variation = 86.28% and 97.72%, respectively). The same pattern was also reflected in the median thread counts (Fig. 3).

4. Discussion

The presence of waterborne predator cues increased the probability of *Mytilus* spp. building byssal threads within the first 6 h, suggesting that mussels that sensed predators were in an alerted state and produced threads more readily than those in the absence of predators. Correspondingly, mussels that were exposed to predator cues also produced more threads in 24 h than mussels in cue-free water. *Mytilus* species are known to increase their byssus production in the presence of predators, especially in response to crabs (Côté, 1995; Garner and Litvaitis, 2013; Leonard et al., 1999). This allows the mussels to fasten themselves more tightly to substrate as well as congregate into larger aggregates where individuals are less easily isolated by predators, thereby increasing their chances of defense and survival (Côté and Jelnikar, 1999; Elner, 1978; Reimer and Tedengren, 1997). The results observed here were thus expected and are in line with existing literature. Surprisingly, however, predator cues appeared to only slightly affect byssal thread production at 24 h in the absence of added noise. This may have been because the standardized waterborne cues gradually weakened over time without replenishment. Côté (1995) and Rickaby and Sinclair (2018) observed similar phenomena: predator cue effects on byssal thread production are evident after just a few hours, but may become much weaker or non-significant altogether by 24 h.

The exposure to continuous low-frequency noise (CLFN) did not seem to affect the byssal thread-building activity of *Mytilus* spp. although there was a trend toward higher probabilities of thread production at 6 h in the noisy predator cue treatment compared to the no-noise predator cue treatment. In addition, both the lowest and highest mean thread counts (as well as a much larger mean difference between cue and cue-free thread counts) were observed under noise conditions at 24 h. This suggests that noise possibly synergizes with predator cues to a small extent detectable when compared to no-cue noise treatments. Although speculative, predator cues may have chemically triggered the anti-predator response while the vibrations from ALFN added an additional mechanical stimulus simulating predator or threat presence, reinforcing the initial response. A study by Zhao et al. (2021) found that after individuals of *Mytilus coruscus* were exposed to ten days of pile-driving playback, the average number of newly secreted threads in 24 h was reduced by 6.83–12.02% depending on playback sound pressure level, although this effect was ultimately not significant. Impulsive LFN (ILFN; in this case, from pile-driving playback) potentially affects *Mytilus* spp. more than CLFN does. For example, impulsive noises have been shown to be more hazardous than continuous noises at comparable sound pressure levels for marine mammals (Gordon et al., 2003; Madsen et al., 2006; Southall et al., 2019), but such comparisons have rarely been investigated or made for marine invertebrates (Carroll et al., 2017; Solé et al., 2023). Our findings suggest that the effects of CLFN might manifest in ways and circumstances different from those of ILFN, which should be considered when designing future experiments.

There are a few potential reasons why the *Mytilus* spp. in the present study were not clearly affected by CLFN. The possible mix of *Mytilus* species could have added interspecific variability (e.g., differing sensitivities to noise, slightly differing responses) to the experimental trials. In addition, the mussels were harvested from a pontoon complex that is seasonally frequented by commercial and recreational vessels. These mussels could have, therefore, been acclimated to a certain level of acoustic disturbance. Hubert et al. (2022a) demonstrated that *Mytilus* spp. (also collected from a disturbed habitat) reduced their responsiveness to repeated sound exposures, albeit using periodic LFN instead of CLFN. These findings suggests that *Mytilus* spp. may be, at least on a behavioral level, actually robust against or adaptable to ALFN. This would explain how they successfully foul and thrive on surfaces that continuously conduct operational noise such as ship hulls, oil rigs, and offshore wind farm pillars (Coolen et al., 2022; Degraer et al., 2020; Krone et al., 2013; Wilhelmsson and Malm, 2008).

Mytilus spp. appear to be indifferent to LFN or even be attracted to CLFN. When exposed to CLFN from ships alone, pediveliger larvae of *M. edulis* exhibited a 27% increase in settlement rate. However, exposure to both CLFN and *Nannochloropsis oculata* (microalgae; food cue) produced a synergistic 50.7% increase in settlement rate (Jolivet et al., 2016). A similar trend was also observed for *Perna canaliculus*, the New Zealand mussel (Stanley et al., 2016; Wilkens et al., 2012). Conversely, Cervello et al. (2023) found that boat noise playback did not enhance larval settlement whereas pile-driving playback did in a setup with much smaller settlement chambers. The navigation and promotion of settlement by acoustic cues have also been observed in oyster larvae and are hypothesized to be methods of scouting optimal settlement sites with conspecifics (Lillis et al., 2013, 2014, 2015; Schmidlin et al., 2024; Williams et al., 2022). A similar mechanism may be at play with *Mytilus* spp. as they are also gregarious settlers (animals that settle next to their own kind) and their conspecifics often colonize structures characterized by environmental disturbance such as anthropogenic noise.

Interestingly, this indifference to or even potential preference for CLFN does not seem to be limited to bivalve larval stages. Wilhelmsson and Malm (2008) observed that many mussels on sampled monopiles were several years older than the monopiles themselves, suggesting active adult migration toward the turbines and many meters upward. This inter-habitat movement is likely spurred by better feeding conditions and relief from benthic predators. While structures such as offshore wind farms appear auspicious for *Mytilus* spp., the potential combined effects of CLFN and additional vertical substrate on local ecosystems are still unknown.

5. Conclusion

Mytilus spp. seemed to be behaviorally indifferent toward acute continuous low-frequency noise exposure regardless of waterborne predator cue presence. This insensitivity to certain types of anthropogenic disturbance may contribute to their colonization success in anthropogenically disturbed habitats such as offshore wind turbines and ship hulls (Carlton and Vlasic, 2005; Dannheim et al., 2020). Thus, despite the ongoing and ever-expanding anthropogenic modification of marine habitats, *Mytilus* can be expected to persist in these disturbed habitats, if not profit from the additional substrate provided by man-made structures.

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CRediT authorship contribution statement

Sheng V. Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Julius A. Ellrich:** Writing – review & editing, Validation, Resources, Methodology, Formal analysis, Conceptualization. **Jan Beermann:** Writing – review & editing, Validation, Supervision. **Bernadette Pogoda:** Writing – review & editing, Resources, Conceptualization. **Maarten Boersma:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing 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.

Data availability

Data will be made available on request.

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References

Albrecht, A., Reise, K., 1994. Effects ofFucus vesiculosus covering intertidal mussel beds in the Wadden Sea. Helgol. Meeresunters. 48, 243–256.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67, 1–48.
Beaumont, N.J., Austen, M.C., Atkins, J.P., Burdon, D., Degraer, S., Dentinho, T.P., Derous, S., Holm, P., Horton, T., van Ierland, E., Marboe, A.H., Starkey, D.J., Townsend, M., Zarzycki, T., 2007. Identification, definition and quantification of goods and services provided by marine biodiversity: implications for the ecosystem approach. Mar. Pollut. Bull. 54, 253–265.
Beermann, J., 2014. Spatial and seasonal population dynamics of sympatric Jassa species (Crustacea, Amphipoda). J. Exp. Mar. Biol. Ecol. 459, 8–16.
Bell, E.C., Gosline, J.M., 1997. Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two Mytilus species. Mar. Ecol. Prog. Ser. 159, 197–208.

Bergström, P., Hällmark, N., Larsson, K.-J., Lindegarth, M., 2019. Biodeposits from Mytilus edulis: a potentially high-quality food source for the polychaete, Hediste diversicolor. Aquacult. Int. 27, 89–104.

Birkbeck, T.H., McHenery, J.G., 1982. Degradation of bacteria by Mytilus edulis. Mar. Biol. 72, 7–15.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135.

Borthagaray, A.I., Carranza, A., 2007. Mussels as ecosystem engineers: their contribution to species richness in a rocky littoral community. Acta Oecol. 31, 243–250.

Broszeit, S., Hattam, C., Beaumont, N., 2016. Bioremediation of waste under ocean acidification: reviewing the role of Mytilus edulis. Mar. Pollut. Bull. 103, 5–14.

Carey, W.M., Evans, R.B., 2011. Ocean Ambient Noise: Measurement and Theory. Springer Science & Business Media.

Carlton, J., Vlasic, D., 2005. Ship vibration and noise: some topical aspects. In: 1st International Ship Noise and Vibration Conference. Citeseer, pp. 1–11.

Carroll, A., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B., 2017. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Cervello, G., Olivier, F., Chauvaud, L., Winkler, G., Mathias, D., Juanes, F., 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.

Coolen, J.W., Vanaverbeke, J., Dannheim, J., Garcia, C., Birchenough, S.N., Krone, R., Beermann, J., 2022. Generalized changes of benthic communities after construction of wind farms in the southern North Sea. J. Environ. Manag. 315, 115173.

Charifi, M., Miserazzi, A., Sow, M., Perrigault, M., Gonzalez, P., Ciret, P., Benomar, S., Massabuau, J.-C., 2018. Noise pollution limits metal bioaccumulation and growth rate in a filter feeder, the Pacific oyster Magallana gigas. PloS one 13, e0194174.

Coolen, J.W.P., Boon, A.R., Crooijmans, R., van Pelt, H., Kleissen, F., Gerla, D., Beermann, J., Birchenough, S.N.R., Becking, L.E., Luttikhuizen, P.C., 2020. Marine stepping-stones: connectivity of Mytilus edulis populations between offshore energy installations. Mol. Ecol. 29, 686–703.

Côté, I.M., 1995. Effects of predatory crab effluent on byssus production in mussels. J. Exp. Mar. Biol. Ecol. 188, 233–241.

Côté, I.M., Jelnikar, E., 1999. Predator-induced clumping behaviour in mussels (Mytilus edulis Linnaeus). J. Exp. Mar. Biol. Ecol. 235, 201–211.

Craeymeersch, J., Jansen, H., 2019. Bivalve assemblages as hotspots for biodiversity. Goods and Services of Marine Bivalves 275–294.

Dannheim, J., Bergström, L., Birchenough, S.N., Brzana, R., Boon, A.R., Coolen, J.W., Dauvin, J.-C., De Mesel, I., Derweduwen, J., Gill, A.B., 2020. Benthic effects of offshore renewables: identification of knowledge gaps and urgently needed research. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 77, 1092–1108.

Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Hartmann, K., Semmens, J.M., 2016. Assessing the impact of marine seismic surveys on southeast Australian scallop and lobster fisheries (FRDC Report 2012/008). University of Tasmania, Hobart.

de Jong, K., Schulte, G., Heubel, K.U., 2017. The noise egg: a cheap and simple device to produce low-frequency underwater noise for laboratory and field experiments. Methods Ecol. Evol. 8, 268–274.

De Mesel, I., Kerckhof, F., Norro, A., Rumes, B., Degraer, S., 2015. Succession and seasonal dynamics of the epifauna community on offshore wind farm foundations and their role as stepping stones for non-indigenous species. Hydrobiologia 756, 37–50.

de Soto, N.A., Delorme, N., Atkins, J., Howard, S., Williams, J., Johnson, M., 2013. Anthropogenic noise causes body malformations and delays development in marine larvae. Sci. Rep. 3, 1–5.

DeCastro, M., Salvador, S., Gómez-Gesteira, M., Costoya, X., Carvalho, D., Sanz-Larruga, F., Gimeno, L., 2019. Europe, China and the United States: three different approaches to the development of offshore wind energy. Renew. Sustain. Energy Rev. 109, 55–70.

Degraer, S., Carey, D.A., Coolen, J.W., Hutchison, Z.L., Kerckhof, F., Rumes, B., Vanaverbeke, J., 2020. Offshore wind farm artificial reefs affect ecosystem structure and functioning. Oceanography 33, 48–57.

Duarte, C.M., Chapuis, L., Collin, S.P., Costa, D.P., Devassy, R.P., Eguiluz, V.M., Erbe, C., Gordon, T.A., Halpern, B.S., Harding, H.R., 2021. The soundscape of the Anthropocene ocean. Science 371, eaba4658.

Dürr, S., Wahl, M., 2004. Isolated and combined impacts of blue mussels (Mytilus edulis) and barnacles (Balanus improvisus) on structure and diversity of a fouling community. J. Exp. Mar. Biol. Ecol. 306, 181–195.

Elner, R., 1978. The mechanics of predation by the shore crab, Carcinus maenas (L.), on the edible mussel, Mytilus edulis L. Oecologia 36, 333–344.

Frisk, G.V., 2012. Noiseonomics: the relationship between ambient noise levels in the sea and global economic trends. Sci. Rep. 2, 437.

Garner, Y.L., Litvaitis, M.K., 2013. Effects of injured conspecifics and predators on byssogenesis, attachment strength and movement in the blue mussel, Mytilus edulis. J. Exp. Mar. Biol. Ecol. 448, 136–140.

Gordon, J., Gillespie, D., Potter, J., Frantzis, A., Simmonds, M.P., Swift, R., Thompson, D., 2003. A review of the effects of seismic surveys on marine mammals. Mar. Technol. Soc. J. 37, 16–34.

Hailpern, S.M., Visintainer, P.F., 2003. Odds ratios and logistic regression: further examples of their use and interpretation. STATA J. 3, 213–225.

Hildebrand, J., 2004. Sources of Anthropogenic Sound in the Marine Environment, Report to the Policy on Sound and Marine Mammals: an International Workshop. US Marine Mammal Commission and Joint Nature Conservation Committee, London, England. UK.

Hubert, J., Booms, E., Witbaard, R., Slabbekoorn, H., 2022a. Responsiveness and habituation to repeated sound exposures and pulse trains in blue mussels. J. Exp. Mar. Biol. Ecol. 547, 151668.

Hubert, J., Moens, R., Witbaard, R., Slabbekoorn, H., 2022b. Acoustic disturbance in blue mussels: sound-induced valve closure varies with pulse train speed but does not affect phytoplankton clearance rate. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 79, 2540–2551.

IEA, 2022. Wind Electricity. IEA, Paris.

Jalkanen, J.-P., Johansson, L., Andersson, M.H., Majamäki, E., Sigray, P., 2022. Underwater noise emissions from ships during 2014–2020. Environ. Pollut. 311, 119766.

Jolivet, A., Tremblay, R., Olivier, F., Gervaise, C., Sonier, R., Genard, B., Chauvaud, L., 2016. Validation of trophic and anthropic underwater noise as settlement trigger in blue mussels. Sci. Rep. 6, 33829.

Jørgensen, C.B., 1990. Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology. Olsen & Olsen.

Kotta, J., Herkül, K., Kotta, I., Orav-Kotta, H., Lauringson, V., 2009. Effects of the suspension feeding mussel Mytilus trossulus on a brackish water macroalgal and associated invertebrate community. Mar. Ecol. 30, 56–64.

Krone, R., Dederer, G., Kanstinger, P., Krämer, P., Schneider, C., Schmalenbach, I., 2017. Mobile demersal megafauna at common offshore wind turbine foundations in the German Bight (North Sea) two years after deployment - increased production rate of Cancer pagurus. Mar. Environ. Res. 123, 53–61.

Krone, R., Gutow, L., Joschko, T.J., Schröder, A., 2013. Epifauna dynamics at an offshore foundation–implications of future wind power farming in the North Sea. Mar. Environ. Res. 85, 1–12.

Lenth, R.V., 2023. Emmeans: estimated marginal means, aka least-squares means. R package version 1.8 (5). https://CRAN.R-project.org/package=emmeans.

Leonard, G.H., Bertness, M.D., Yund, P.O., 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, MYTILUS edulis. Ecology 80, 1–14.

Li, L., Lu, W., Sui, Y., Wang, Y., Gul, Y., Dupont, S., 2015. Conflicting effects of predator cue and ocean acidification on the mussel Mytilus coruscus byssus production. J. Shellfish Res. 34, 393–400.

Lillis, A., Bohnenstiehl, D.R., Eggleston, D.B., 2015. Soundscape manipulation enhances larval recruitment of a reef-building mollusk. PeerJ 3, e999.

Lillis, A., Eggleston, D.B., Bohnenstiehl, D.R., 2013. Oyster larvae settle in response to habitat-associated underwater sounds. PLoS One 8, e79337.

Lillis, A., Eggleston, D.B., Bohnenstiehl, D.R., 2014. Soundscape variation from a larval perspective: the case for habitat-associated sound as a settlement cue for weakly swimming estuarine larvae. Mar. Ecol. Prog. Ser. 509, 57–70.

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.-O., Olrog, L., Rehnstam-Holm, A.-S., Svensson, J., Svensson, S., Syversen, U., 2005. Improving marine water quality by mussel farming: a profitable solution for Swedish society. AMBIO A J. Hum. Environ. 34, 131–138.

Lindell, H., 2003. Utgrunden Off-Shore Wind Farm - Measurements of Underwater Noise, p. 30. Sweden.

Madsen, P.T., Wahlberg, M., Tougaard, J., Lucke, K., Tyack, P., 2006. Wind turbine underwater noise and marine mammals: implications of current knowledge and data needs. Mar. Ecol. Prog. Ser. 309, 279–295.

Merchant, N.D., Fristrup, K.M., Johnson, M.P., Tyack, P.L., Witt, M.J., Blondel, P., Parks, S.E., 2015. Measuring acoustic habitats. Methods Ecol. Evol. 6, 257–265.

Miksis-Olds, J.L., Bradley, D.L., Maggie Niu, X., 2013. Decadal trends in Indian Ocean ambient sound. J. Acoust. Soc. Am. 134, 3464–3475.

Moeser, G.M., Leba, H., Carrington, E., 2006. Seasonal influence of wave action on thread production in Mytilus edulis. J. Exp. Biol. 209, 881–890.

Mustonen, M., Klauson, A., Andersson, M., Clorennec, D., Folegot, T., Koza, R., Pajala, J., Persson, L., Tegowski, J., Tougaard, J., Wahlberg, M., Sigray, P., 2019. Spatial and temporal variability of ambient underwater sound in the baltic sea. Sci. Rep. 9, 13237.

Norling, P., Kautsky, N., 2007. Structural and functional effects of Mytilus edulis on diversity of associated species and ecosystem functioning. Mar. Ecol. Prog. Ser. 351, 163–175.

Norling, P., Kautsky, N., 2008. Patches of the mussel Mytilus sp. are islands of high biodiversity in subtidal sediment habitats in the Baltic Sea. Aquat. Biol. 4, 75–87.

Pangerc, T., Theobald, P.D., Wang, L.S., Robinson, S.P., Lepper, P.A., 2016. Measurement and characterisation of radiated underwater sound from a 3.6 MW monopile wind turbine. J. Acoust. Soc. Am. 140, 2913–2922.

Possenti, L., de Nooijer, L., de Jong, C., Lam, F.-P., Beelen, S., Bosschers, J., van Terwisga, T., Stigter, R., Reichart, G.-J., 2024. The present and future contribution of ships to the underwater soundscape. Front. Mar. Sci. 11.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Reimer, O., Tedengren, M., 1997. Predator-induced changes in byssal attachment, aggregation and migration in the blue mussel, Mytilus edulis. Mar. Freshw. Behav. Physiol. 30, 251–266.

Rickaby, R., Sinclair, J., 2018. Native versus invasive crab effluent effects on byssal thread production in the mussel, Mytilus trossulus (Gould, 1950). Arbutus Rev. 9, 20–31.

Ross, D., 1979. Mechanics of Underwater Noise (No Title).

Schmidlin, S., Parcerisas, C., Hubert, J., Watson, M.S., Mees, J., Botteldooren, D., Devos, P., Debusschere, E., Hablützel, P.I., 2024. Comparison of the effects of reef and anthropogenic soundscapes on oyster larvae settlement. Sci. Rep. 14, 12580.

Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., White, P., 2016. Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6, 1–9.

Solé, M., Kaifu, K., Mooney, T.A., Nedelec, S.L., Olivier, F., Radford, A.N., Vazzana, M., Wale, M.A., Semmens, J.M., Simpson, S.D., Buscaino, G., Hawkins, A., Aguilar de Soto, N., Akamatsu, T., Chauvaud, L., Day, R.D., Fitzgibbon, Q., McCauley, R.D., André, M., 2023. Marine invertebrates and noise. Front. Mar. Sci. 10.
Southall, B.L., Finneran, J.J., Reichmuth, C., Nachtigall, P.E., Ketten, D.R., Bowles, A.E., Ellison, W.T., Nowacek, D.P., Tyack, P.L., 2019. Marine mammal noise exposure criteria: updated scientific recommendations for residual hearing effects. Aquat. Mamm. 45.
Spiga, I., Caldwell, G.S., Bruintjes, R., 2016. Influence of pile driving on the clearance rate of the blue mussel, Mytilus edulis (L.). Proceedings of Meetings on Acoustics 27, 040005.
Stanley, J.A., Wilkens, S., McDonald, J.I., Jeffs, A.G., 2016. Vessel noise promotes hull fouling. In: Popper, A.N., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life II. Springer, New York, New York, NY, pp. 1097–1104.
Tsuchiya, M., Nishihira, M., 1986. Islands of Mytilus edulis as a habitat for small intertidal animals: effect of Mytilus age structure on the species composition of the associated fauna and community organization. Mar. Ecol. Prog. Ser. 171–178.
Venables, W.N., Ripley, B.D., 2013. Modern Applied Statistics with S-PLUS. Springer Science & Business Media.
Wale, M.A., Briers, R.A., Hartl, M.G.J., Bryson, D., Diele, K., 2019. From DNA to ecological performance: effects of anthropogenic noise on a reef-building mussel. Sci. Total Environ. 689, 126–132.
Wang, S.V., Wrede, A., Tremblay, N., Beermann, J., 2022. Low-frequency noise pollution impairs burrowing activities of marine benthic invertebrates. Environ. Pollut. 310, 119899.
Wilhelmsson, D., Malm, T., 2008. Fouling assemblages on offshore wind power plants and adjacent substrata. Estuar. Coast Shelf Sci. 79, 459–466.
Wilkens, S., Stanley, J., Jeffs, A., 2012. Induction of settlement in mussel (Perna canaliculus) larvae by vessel noise. Biofouling 28, 65–72.
Williams, B.R., McAfee, D., Connell, S.D., 2022. Oyster larvae swim along gradients of sound. J. Appl. Ecol. 59, 1815–1824.
WindEurope, 2023. Wind energy in europe: 2022 Statistics and the outlook for 2023-2027. Available online: https://windeurope.org/intelligence-platform/product/wind-energy-in-europe-2022-statistics-and-the-outlook-for-2023-2027/ (accessed on 09 March 2023).
Wiser, R., Lantz, E., Mai, T., Zayas, J., DeMeo, E., Eugeni, E., Lin-Powers, J., Tusing, R., 2015. Wind vision: a new era for wind power in the United States. Electr. J. 28, 120–132.
Zhao, X., Sun, S., Shi, W., Sun, X., Zhang, Y., Zhu, L., Sui, Q., Xia, B., Qu, K., Chen, B., 2021. Mussel byssal attachment weakened by anthropogenic noise. Front. Mar. Sci. 1957.
Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Liu, Y., Xu, X., Zhang, F., 2006. Feeding and growth on bivalve biodeposits by the deposit feeder Stichopus japonicus Selenka (Echinodermata: holothuroidea) co-cultured in lantern nets. Aquaculture 256, 510–520.