



Pile driving and drilling underwater sounds impact the metamorphosis dynamics of *Pecten maximus* (L., 1758) larvae

Mathilde Gigot^{a,*}, Frédéric Olivier^b, Gauthier Cervello^c, Réjean Tremblay^c, Delphine Mathias^d, Tarik Meziane^b, Laurent Chauvaud^a, Julien Bonnel^e

^a Laboratoire des Sciences de l'Environnement Marin (LEMAR) UMR 6539, Université de Brest, CNRS, Institut Universitaire Européen de la Mer (IUEM), Place Nicolas Copernic, 29280 Plouzané, France

^b Laboratoire de Biologie des Organismes et Écosystèmes Aquatiques (BOREA) UMR 8067, MNHN/SU/UNICAEN/UA/CNRS/IRD, 61 Rue Buffon, 75005 Paris, France

^c Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, Québec G5L 2Z9, Canada

^d Société d'Observation Multi-Modale de l'Environnement, 38 rue Jim Sevellec, 29200 Brest, France

^e Woods Hole Oceanographic Institution, Applied Ocean Physics and Engineering Department, Woods Hole, MA 02543, USA

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ABSTRACT

One of the biggest challenges of the 21st century is to reduce carbon emissions and offshore wind turbines seem to be an efficient solution. However, during the installation phase, high levels of noise are emitted whose impacts remain not well known, particularly on benthic marine invertebrates displaying a benthic-planktonic life-cycle. For one century, larval settlement and subsequent recruitment has been considered as a key topic in ecology as it determines largely population renewal. Whereas several recent studies have shown that trophic pelagic but also natural soundscape cues could trigger bivalve settlement, the role of anthropogenic noise remains poorly documented. Therefore, we conducted experiments to assess potential interacting effects of diet and pile driving or drilling sounds on the great scallop (*Pecten maximus*) larval settlement. We demonstrate here that pile driving noise stimulates both growth and metamorphosis as well as it increases the total lipid content of competent larvae. Conversely, drilling noise reduces both survival and metamorphosis rates. For the first time, we provide evidence of noise impacts associated to MREs installation on *P. maximus* larvae and discuss about potential consequences on their recruitment.

1. Introduction

Among Marine Renewable Energy systems (MREs), the offshore wind turbines appear to constitute a valuable option for reducing the use of fossil fuels. When functioning, wind farms generate moderate sound levels, only detectable over underwater ambient noise below 500 Hz (total root-mean-square Sound Pressure Level $SPL_{rms} = 109\text{--}127$ dB re 1 μPa @ 20 m; Tougaard et al., 2009). By contrast, operations associated with the building phase introduce powerful low-frequency noises (20 to 1000 Hz; Greene, 1987; Norro et al., 2013) in shallow water coastal environments, mainly due to pile driving and drilling activities. High-amplitude sound pressure at low frequencies radiates in the water away from the pile and can propagate over kilometers (Amaral et al., 2020; Bailey et al., 2010; Dahl et al., 2015; Robinson et al., 2013). The pile driving noise is defined as impulsive and includes a short sound with

high peak pressure amplitude followed by a fast decay and its spectrum is broadband with most energy below 1 kHz (Leunissen and Dawson, 2018). As an illustration, Robinson et al. (2013) report a SPL_{p-p} of 205 dB re 1 μPa @ 100 m for a 1.8 m diameter pile at 14.5–51.4 m water depth. By contrast, drilling operations produce a continuous broadband sound, with high energy between 100 Hz to 10 kHz (Kyhn et al., 2014). As examples, Jimenez-Arranz et al. (2019) report a $SPL_{rms} = 193.3$ dB re μPa @ 1 m for drilling operations. Depending on the number and the diameter (0.5 to 6.5 m) of piles that are simultaneously used, on the input of energy, and on the properties of the seabed (hard vs soft bottoms), characteristics and level of the sound emitted vary a lot (Vardhan et al., 2009).

Wind farms usually settle in shallow coastal areas (59 km from the coast for 2019 European constructions, Ramirez et al., 2020) which considerably overlap with high biodiversity areas (Gill, 2005). If the

* Corresponding author.

E-mail addresses: mathilde.gigot.pro@gmail.com (M. Gigot), frederic.olivier@mnhn.fr (F. Olivier), gauthier_cervello@uqar.ca (G. Cervello), rejean_tremblay@uqar.ca (R. Tremblay), tarik.meziane@mnhn.fr (T. Meziane), laurent.chauvaud@univ-brest.fr (L. Chauvaud), jbonnel@whoi.edu (J. Bonnel).

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sensitivity of marine mammals and fishes to sound has been studied for decades, focus on marine invertebrates is much more recent (Wale et al., 2021, reviewed in de Soto, 2016; Hawkins and Popper, 2017; Solé et al., 2023). Most of the invertebrates detect the particle motion component of underwater sounds (Nedelec et al., 2016) through statocyst, a chamber of ciliate cells containing at least one dense body (Kaifu et al., 2008) and anthropic sounds can damage this sensory organ (André et al., 2011; Solé et al., 2016). Few studies proved that sound induce stress behavior and overexpression of stress proteins on cephalopods (Samson et al., 2014), bivalves (Charifi et al., 2017; Solan et al., 2016; Vazzana et al., 2016) and crustaceans species (Filiciotto et al., 2014; Lagardère, 1982). The present study focus on a commercially important bivalve species distributed on the Atlantic European coasts, the Pectinid *Pecten maximus*. Bivalves represent an important economic value (Dame and Olenin, 2005; McKindsey et al., 2011). Moreover, as primary consumers in food webs, filter-feeding bivalves constitute a key link between phytoplankton and bottom-dwelling communities (Bridier et al., 2021; Prins et al., 1998). *P. maximus* displays a biphasic life-cycle (Thorson, 1950) including early free swimming veliger larvae that develop into a competent pediveliger which prospects directly on the seafloor (Bayne, 1965) to settle and metamorphose (Hadfield et al., 2001; Pechenik and Heyman, 1987) into a benthic post-larvae. As larvae disperse over kilometers, long-range environmental cues drive the choice of a quality habitat. Sound could be a broad-scale environmental signal for the recruitment of many larvae as recent evidence has been made that those bivalve larvae are sensitive to natural sound cues. *Crassostrea gigas* larvae increase swimming activity in response to natural reef sound (Stocks, 2012) as well as natural oyster reef sound stimulates the settlement of oyster larvae in the field (Lillis et al., 2013) or laboratory experiments (Eggleston et al., 2016). Lillis et al. (2014) hypothesized that acoustics signals could facilitate larval orientation to an appropriate habitat where transition to adult life can be achieved and to maximize performance, survival, and reproduction. Larvae respond also to anthropogenic sound, like vessel noise which increases mussel settlement (*Perna canaliculus*, Wilkens et al., 2012; *Mytilus edulis*, Jolivet et al., 2016), or seismic pulses that delay *Pecten novaezelandiae* development (Aguilar de Soto et al., 2013).

Since the last decade, several authors have shown that pelagic food quality and quantity play a major role in bivalve recruitment, especially high levels of phytoplanktonic pulses that strongly trigger settlement ('Trophic settlement trigger' TST) (Androuin et al., 2022; Toupoint et al., 2012a). However trophic and acoustic cues may interact as evidenced by Jolivet et al. (2016) between the picoeukaryotic alga *Nannochloropsis oculata* and vessel noise that both enhance settlement of the blue mussel.

Number and size of offshore wind farms has grown around the world by a mean 22 % per year, with 70 % of total global installations in European waters (Lee and Zhao, 2021) and global capacity could increase 7 fold by 2030 (Global Offshore Wind Report 2021). Population renewal of bivalves largely depend on recruitment process so in a context of extensive wind farm development, there are growing issues about the potential vulnerability of larval stages to pile driving and drilling sounds. Within that context, the aim of our study was to assess for the first time the interactive effect of microalgae diet and either impulsive (pile driving) or continuous (drilling) sounds at increasing levels on great scallop (*Pecten maximus*) larvae. We hypothesize that interacting effects between noise and diet could modulate the settlement dynamics (including metamorphosis). We used mortality, metamorphosis, and growth as settlement success variable and related them to pediveligers lipid content and fatty acid profiles to detect potential energetic effects.

2. Methods

2.1. Larval rearing

Batches of pediveliger larvae were produced at the 'Ecluserie du

Tinduff' (Plougastel-Daoulas, France) following a method inspired by Buestel et al. (1982), as detailed in the supplementary material of Olivier et al. (2023). Briefly, adults were collected by dredge in the Bay of Brest in either Moulin Blanc (48°23.050'N, 004°25.520'W; water depth 10–12 m) or Lanvéoc (48°18.145'N, 004°26.189'W; water depth 11–12 m) the 26th November and the 6th December 2018 and were reared in tanks filled with coarse sediment and 5-µm filtered seawater during 3 months of gametogenesis. Spawning was induced by thermal shock from 14.5 to 19.5 °C on 18 adults chosen for their gonad maturation state. Spawning adults were isolated in beaker and cross-fertilization was performed at a rate of 1 oocyte per 8–10 sperm cells to avoid self-fertilization (Beaumont and Budd, 1983) that reduces the larval viability (Gruffydd and Beaumont, 1972). The resulting eggs were incubated for 48 h at 80 trochophore larvae.ml⁻¹ in 450 and 150 l cylindro-conical tanks. Seawater was 1 µm filtered (salinity = 33 psu; temperature = 19.5 °C), stirred by air-bubbling, treated with U.V. and with 9 ppm of erythromycin to prevent bacterial development. At 2 days post-fertilization (dpf), veliger larvae were sieved and put at 40 larvae.ml⁻¹ density in the tanks filled with 18 °C seawater treated as previously described. Larvae were fed every day with either a DTC diet (*Diacronema lutheri*, *Tisochrysis lutea*, *Chaetoceros neogracilis*) (1:1:1 ratio) at 20 cells.µl concentration from 2 to 8 dpf (day post-fecundation), then with a DTCS diet (DTC + *Skeletonema marinoi*) (DT ²/₃ CS ¹/₃ ratio) at 45 cells.µl concentration up to 23 dpf. Once a day, water was renewed and larvae were sieved on square mesh to eliminate dead and low-growth individuals until decreasing the density until 5 larvae.ml⁻¹ at 23 dpf.

2.2. Sound emissions

To expose the larvae as close as possible to the sonorous field condition, we used the 'Larvosonic' system described in Olivier et al. (2023). Briefly, *Larvosonic* consists of a water bath with a main 800 l tank including 6 cylinders (= units of replication) that are half filled with 5 l of culture solution (filtered seawater, larvae and diet), and 4 multiwell plates (6 × 15 ml) filled with 10 ml of culture solution. Sound is emitted upward from an underwater speaker Clark Synthesis AQ339 Diluvio™ (<https://clarksynthesis.com/aq339/>) placed in the center of the main tank. Sound reverberation is attenuated by Expanded Polystyrene diffusion panels (Vicoustic ©; <http://www.vicoustic.com>) fixed on the internal walls of the main tank. Sound nature and level can be adjusted via a Yamaha MTX3 digital matrix processor coupled to a Powersoft Otto 1204 DSP 8 channels amplifier (8 × 150 W), settings being made by MTX-MRX V4.0.0 Editor interface software. The pile driving sequence was based on a recording during the building phase of an offshore marine wind-farm in the North Sea (depth around 30 m, SOMME database). Each pile driving impulse lasts 200 ms with a repetition every 3 s and the acoustic spectrum was characterized by a high level in the 40–800 Hz frequencies range. The drilling sequence was based on a field recording made in June 2018 at 200 m from the boat operating the geotechnical drilling (SOMME database). Drilling is a continuous sound and its spectrum is dominated by 100–10,000 Hz frequencies with more energy content in the 150–600 Hz and 4000–7000 Hz frequency ranges.

The experimental design includes two batches of 4 'Larvosonic' mesocosms which were deployed in 2 independent rooms dedicated either to pile driving (P) or drilling (D) treatments (Fig. 1). Food treatment consisted of 2 different mixtures of microalgae (3 replicates per diet and per Larvosonic system): a classic DTCS diet supplemented with *Nannochloropsis oculata* (DTCS_N+) or not (DTCS_N-). In the first room, we generated three increasing levels of pile driving sound inside three distinct mesocosms during 4 days according to a 6:6 h on:off cycle. The tested SPL_{pp} correspond approximatively to what could be recorded at 78,000, 4000 and 170 m from the pile driving source i.e. P1, P2, and P3 respectively. The transmission loss used to calculate the levels was 15log₁₀(distance), corresponding to *in situ* measured propagation loss (SOMME database). No sound was emitted in the fourth tank that

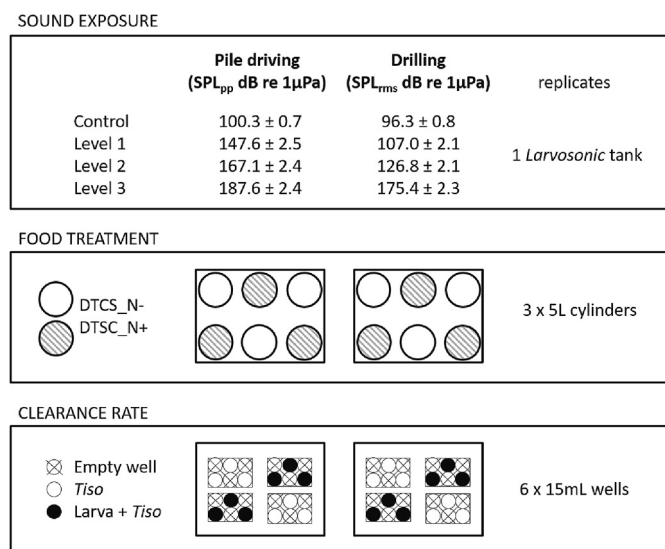


Fig. 1. Schematic representation of the experimental protocol. Larvae were exposed to increasing levels of either pile driving or drilling in Larvosonic tanks compared to control tanks without any sound emitted. DTCS N- and DTCS N+ are the two diets we used, without and with *Nannochloropsis oculata*. Wells contain either nothing, or water with *Tisochrysis lutea*, or *Tisochrysis lutea* and larvae.

constituted the control condition PC. Three sound levels were also used for the drilling sound treatment but distances from the emitting source, as assessed using SPL_{rms}, were estimated to be 21,500, 1000 and <1 m respectively. The on/off cycle was fixed to a 19:5 h and in a control condition DC, no sound was emitted. On/off cycles were chosen to match the foreseen in situ work conditions (Ailes Marines, pers. com.). Sound levels were adjusted by recording 30s of sound emission at the center of each cylinder (10 cm above its bottom) and are expressed in SPL (details in Fig. 1). The SPL_{rms} in controls varied between 96.3 ± 0.8 for PC and 100.3 ± 0.7 dB.re1μPa for DC. Such levels were consistent with ambient sound levels recorded in temperate coastal environments of the western English Channel with contrasting wind conditions (Mathias et al., 2016). Acoustic recordings for calibration were made by using a RTSYS EASDA14 (<https://rtsys.eu/>) underwater acoustic recorder (sampling frequency of 78 kHz, 32-bit resolution) equipped with two HTI-96-min hydrophones with different sensitivities (−165 dB re 1 V/μPa and −210 dB re 1 V/μPa). Recorded files were manually cut using Audacity® (Version 2.4.2; Audacity Team 2018), then analyzed using a custom Matlab script (The MathWorks Inc.) and Sound Pressure Levels (SPL_{pp} for pile driving or SPL_{rms} for drilling) were also calculated using Matlab (but see Olivier et al., 2023).

It is now admitted that invertebrates sense the particle motion component of the sound rather than acoustic pressure (Kaifu et al., 2008; Mooney et al., 2010; Popper and Hawkins, 2018). The relationship between particle motion and pressure is complex: usually particle motion cannot be evaluated from a single pressure measurement. As a result, to characterize particle motion propagation in tanks, direct measures are highly recommended (Jones et al., 2019), particularly in acoustic invertebrates' studies (Nedelec et al., 2016). However, making accurate measures of absolute particle motion level is still challenging, because calibrated off-the-shelf sensors are barely available (Nedelec et al., 2021). As a result, we prefer to resort to pressure only measurements. Those cannot be used to evaluate absolute particle motion impact threshold. However, pressure measurement can be used to evaluate relative impact: if source level increase (respectively decrease) by N dB, then both the pressure level and the particle motion level will also increase (respectively decrease) by N dB (Dahl et al., 2023). This is a well known property of linear acoustics (which describes both pressure and

particle motion fields), and it has been experimentally verified in the Larvosonic (see Olivier et al., 2023).

2.3. Growth, survival and metamorphosis success

Main tanks were filled with 18 °C non-filtered seawater, cylinders were filled with 5 l of 1 μm filtered U.V. treated seawater and 9 ppm of erythromycin. Rooms were maintained with a heater at constant temperature (18 ± 0.6 °C) with a photoperiod of 12:12 h and neon light systems were fixed at the center of each room so that every tank received similar light conditions although small differences may occur at the replicate scale. Also the Larvosonic tanks are made of clear plexiglass so that each cylinders don't received the same lighting without overshadowing each other. Pediveliger larvae were obtained at 23 dpf when 50 % of the population reared in the cylindro-conical tanks reached the double ring stage, that is a proxy of larval competence (Robert et al., 1999). Batches of 20,000 larvae were then introduced in each cylinder/replicate and fed once a day with a concentration of 60 cells/μl (*Tisochrysis lutea* equivalent; Helm and Bourne, 2006) of either DTCS_N+ or DTCS_N- diet in the larval culture (respectively 1:1:1:1 and 3:3:2:2 ratios). Concentrations were adjusted to maintain a similar algal biomass in each cylinder. Cylinders' experiments went on for 4 days from 21 to 25 March 2019 and treated seawater and food were renewed in the middle of exposure by sieving larvae on a 60 μm square mesh, preserving all cylinder population. Temperature and pH were daily monitored in each cylinder to ensure that there was no confounding gradient (± 0.6 °C). At the end of the 4 days experiments, each cylinder was gently rinsed over a 60 μm square mesh sieve to collect swimming larvae, then remaining crawling larvae were detached from the walls and bottom of the cylinder by a gentle water jet and also sieved for collection. Larvae were rinsed to remove feces and sedimented microalgae, and concentrated in 500 ml beakers with filtered seawater. Three 4.13 ml aliquots were sampled in swimmers and crawlers replicates, after homogenization with a plunger to avoid any sampling bias (Helm and Bourne, 2006). Pediveliger larvae were preserved in 4 % formaldehyde before counting and measuring their size. The remaining fractions were filtered on GF/F filters and stored at −80 °C before lipids analysis. The pediveliger larvae were counted using a microscope (Zeiss Axioscope A1, x40 magnification) equipped with a digital camera (Moticam 3.0 10+). Empty shells and larvae with disorganized tissue were considered as dead and counted to assess mortality. The mortality rate was estimated by the ratio between the number of dead individuals and the total number of individuals in each aliquot. We used the presence of demarcation formed by the boundary layer between de Prodissochonch II and the Dissochonch shells as a criterion of metamorphosis (Martel et al., 1995) to determine the number of settlers in each sample. Metamorphosis rate was calculated as the ratio between the number of larvae displaying a dissochonch shell and the total number of larvae in the sample. Recently metamorphosed bivalves post-larvae can disperse after settlement (Gunther, 1992) in response to environmental conditions, like trophic cues related to the phytoplanktonic composition (Forêt et al., 2018). We estimated the secondary migration by the number of larvae presenting a dissochonch shell in the swimming fraction and found <0.4 % so we decided to neglect it. Shell length was measured in accordance with a maximum dorsoventral dimension from the umbo to the most distant part of the shell. Measurements were performed via the Motic Images plus 3.0 software, on 130 individuals before the experiment, 36 swimming and 36 settled individuals per cylinder at the end of the experiment. Daily growth rate was assessed by the difference between mean shell length before the experiment and individual shell length after the experiment, divided by 4, the day length of the experiment.

2.4. Clearance rates

In each main larvosonic tank, 3 wells per plate were filled with 10 ml

of similar treated water that cylinders and 50 pediveliger larvae were added into the wells of two of the plates, as the third plates was used to estimated microalgae sedimentation. Larvae in the multiwell plates were fed with a concentration of 60 cells/ μl of *Tisochrysis lutea* and after 24 h exposure, a lugol solution was added to 1 % into each well to fix the microalgae. Each well was then filtered through a 40 μm filter to collect the larvae and stored in 5 ml Eppendorfs in a freezer (-20°C).

The concentration of microalgae in the well was estimated manually under microscope using a hemocytometer following the method described in Helm and Bourne (2006). The clearance rates (CR) in each well were calculated using a modified formula described by Comeau et al. (2008):

$$CR = ((Ln(C_{0i}) - Ln(C_{0f}) - (Ln(C_{1i}) - Ln(C_{1f}))) \cdot V \cdot T^{-1} \cdot N^{-1}$$

where C_i is the initial microalgae concentration and C_f as the final concentration of each well, C_0 means the well devoid of larvae whereas C_1 are the wells contained the larvae, V is the well volume (10 ml), T as the duration of the experiment (24 h) and N as the number of larvae counted in each well.

2.5. Fatty acids analyses

Fatty acids extractions were performed following a modified method from (Bligh and Dyer, 1959) described in (Leroy et al., 2013), on either control or D3 and P3 sound. Briefly, pure borosilicate glass 47 mm Whatman GF/F filters (0.7 μm porosity) were combusted at 450°C for 4 h and pre-weighed. Then food diet, seawater with erythromycin and pediveliger larvae were filtered onto the resulting filters and stored at -80°C until their transfer to the laboratory (Paris, BOREA Laboratory, MNHN). Filters were lyophilized and weighed and a defined amount (20 or 30 μg) of Tricosanoic acid (23:0) was added to every sample as an internal standard. After extraction, fatty acids in hexane were injected in a gas chromatograph (GC, Varian CP-3800) equipped with a flame ionization detector, to be separated and quantified. FAMES peaks were identified by comparison of their retention time with those of analytical standards (Supelco™ 37, PUFA-1 Marine Source, and Bacterial Mix, Supelco Inc., USA). For each FA, we report percentage of total FA (TFA) or absolute concentrations ($\text{mg}\cdot\text{g}^{-1}$). FAs are designated as X:Y ω Z, where X is the number of carbons, Y the number of double bonds, and Z the position of the ultimate double bond from the terminal methyl group.

2.6. Statistical analyses

All others data analyses were performed on PRIMER7 software, using analyses of variance with 10,000 permutations (Legendre and Legendre, 2012) of residuals under a reduced model (PERMANOVA). Permutational method is a kind of randomization which allows us to get rid of the distributional assumptions as normality and can be applied to very small samples (Legendre and Legendre, 2012). Similarities matrices based on ratios were produced using the Euclidean distance.

A one-way Permanova tested the effect of crawlers and swimmers' separation (2 levels) on daily growth rate of all larvae. Following growth rate examination was then applied only on crawlers' fraction whereas mortality and metamorphosis analysis include both fractions together. For mortality, metamorphosis, and daily growth rates, the effects of pile driving (4 levels: PC, P1, P2, and P3) or drilling (4 levels: DC, D1, D2, and D3) in interaction with diet (2 levels: DTCS_N+ and DTCS_N-) were tested separately with two-way Permanova. For CR and larvae size in well plates, a univariate PERMANOVA was performed testing this impact of pile driving and drilling separately. When differences were significant ($p\text{-perm} \leq 0.05$), multiple comparison pairwise tests were used to determine which groups significantly differed. Distance-based tests for homogeneity of multivariate dispersions (PERMDISP) were carried out to evaluate the homogeneity of ratios in each condition.

For FA datasets, only 2 levels of sound (control and L3) were compared. Euclidean coefficient is considered as inappropriate for analyzing abundance data because of it is affected by null values (Legendre and Legendre, 2012), so Bray-Curtis similarity index is usually used for abundances data (Barbier et al., 2017; Leroy et al., 2013). FA abundance, that includes null values that must not contribute much to the samples similarity, were used to produce triangular similarity matrices, based on Bray-Curtis similarity index. FA ratios were clustered into FA families: Σ branched, Σ SFA, Σ MUFA, Σ PUFA, ω 3, and ω 6, respectively the sum of respectively branched, saturated, mono-unsaturated, polyunsaturated, ω 3, and ω 6 FA. Separately for FA abundance, each FA sums and TFA concentration ($\text{mg}\cdot\text{g}^{-1}$), effect of pile driving (2 levels: PC, and P3) or drilling (2 levels: DC, and D3) in interaction with diet (2 levels: PTCS_N- and PTCS_N+) and fractions (2 levels: crawlers and swimmers) were tested with 3-crossed factors Permanova. Then effect of pile driving (2 levels: PC, and P3) or drilling (2 levels: DC, and D3) in interaction with diet (2 levels: PTCS_N- and PTCS_N+) on TFA concentration were tested by a 2-crossed factors Permanova for swimmers and crawlers fractions separately. When significant difference was detected among FA abundance, similarity percentage breakdown (SIMPER, Clarke, 1993) were computed to determine which FA were more responsible for the difference between groups and difference between factors was tested for each FA responsible for the dissimilarity with a PERMANOVA.

3. Results

3.1. Mortality rate

Mortality rates ($\text{MoR} \pm \text{standard error}$) of scallop pediveliger larvae in the pile driving experiment were very low ($\text{mean MoR} = 2.15 \pm 0.41 \%$) and no difference were detected between both sound ($p\text{-perm} = 0.5023$) nor diet ($p\text{-perm} = 0.2634$) treatments. In contrast, there were significant differences in the mortality rate between drilling sound treatments ($\text{df} = 3$, $\text{pseudo-F} = 7.1495$, $p\text{-perm} = 0.003$) without any diet effect ($p\text{-perm} = 0.9223$). Multiple comparison tests showed that the mortality rate in drilling treatments ($\text{mean MoR}_{\text{D1,D2,D3}} = 3.2 \pm 0.21 \%$) was 2.3 times higher than in control ($\text{mean MoR}_{\text{DC}} = 1.4 \pm 0.35 \%$), but always very low (below 4 %).

3.2. Metamorphosis rate

Metamorphosis rate ($\text{MR} \pm \text{standard error}$) ranged either between 6.5 and 17.7 % for the whole experimental population. Metamorphosis rate significantly varied among pile driving treatments ($\text{df} = 3$, $\text{pseudo-F} = 3.5556$, $p\text{-perm} = 0.0428$) (Fig. 2), without any diet effect ($p\text{-perm} = 0.4166$). MR were $\sim 50 \%$ higher in the P2 ($\text{mean MR}_{\text{P2}} = 17.7 \pm 2.3 \%$) than in the other treatments ($\text{mean MR}_{\text{PC, P1, P3}} = 11.9 \pm 1.6 \%$).

For the drilling exposure data, metamorphosis rate was not related to diet ($p\text{-perm} = 0.6235$), but significantly varied between sound treatments, ($\text{df} = 3$, $\text{pseudo-F} = 7.9187$, $p\text{-perm} = 0.003$) (Fig. 2). The metamorphosis rates in D2 and D3 were 43 % lower ($\text{mean MR}_{\text{D2, D3}} = 6.9 \pm 0.8 \%$) than those of C and D1 ($\text{mean MR}_{\text{DC, D1}} = 12.1 \pm 1.4$).

3.3. Growth rate

Mean growth rate ($G \pm \text{standard error}$) differed significantly ($\text{df} = 1$, $\text{pseudo-F} = 513.54$, $p\text{-perm} = 0.0001$) between settled larvae ($\text{mean } 4.36 \pm 0.2 \mu\text{m}/\text{day}$) and larvae collected in the water column (no growth during 4 days). For crawler fractions of the pile driving experiment, larval growth significantly varied between sound ($\text{df} = 3$, $\text{pseudo-F} = 11.379$, $p\text{-perm} = 0.0001$) and diet treatments ($\text{df} = 1$, $\text{pseudo-F} = 4.1402$, $p\text{-perm} = 0.0437$). The growth rate for larvae exposed to P3 ($\text{mean } G_{\text{P3}} = 6.44 \pm 0.7 \mu\text{m}/\text{day}$) was 1.8-fold higher than for control and P2 ($\text{mean } G_{\text{PC, P2}} = 3.5 \pm 0.5 \mu\text{m}/\text{day}$), and 3-fold higher than P1 growth rate ($\text{mean } G_{\text{P1}} = 2.12 \pm 0.4 \mu\text{m}/\text{day}$) (Fig. 3). Growth rate for

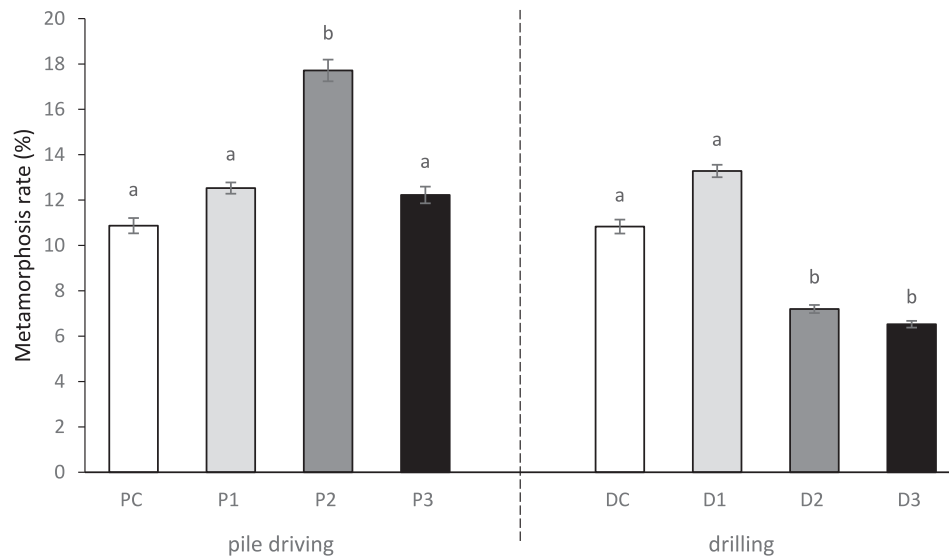


Fig. 2. Mean metamorphosis rate $\pm$ standard error (%) of larvae exposed to the different intensities of pile driving (PC, P1, P2 and P3) and drilling (DC, D1, D2 and D3) for total fraction (crawlers + swimmers). Only effect of sound intensities is presented, as no interaction (diet $\times$ sound) and diet effect were significant. Groups annotated with the same letter do not differ significantly at p -perm = 0.05.

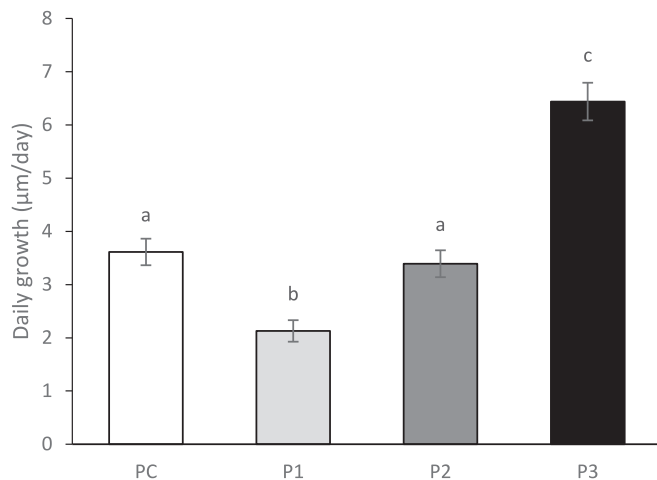


Fig. 3. Mean daily growth $\pm$ standard error ($\mu\text{m}/\text{day}$) of larvae exposed to increasing levels of pile driving sound (PC, P1, P2 and P3) among crawlers fraction. Only effect of sound intensities is presented, as no interaction (diet $\times$ sound) and diet effect were significant in the pile driving group. Groups annotated with the same letter do not differ significantly at p -perm = 0.05.

pile driving experiment crawlers fed with PTCS_N+ was 32 % higher (mean $G_{\text{PN}+} = 4.44 \pm 0.4 \mu\text{m}/\text{day}$) than for larvae fed with PTCS_N- (mean $G_{\text{PN}-} = 3.34 \pm 0.4 \mu\text{m}/\text{day}$) (Fig. 4).

For crawlers of the drilling experiment, larval growth significantly varied between diet treatment ($df = 1$, pseudo- $F = 8.4493$, p -perm = 0.0028) without any effect of the sound treatments (p -perm = 0.7344). Growth rate of crawlers fed with PTCS_N+ was 33 % lower (mean $G_{\text{PN}+} = 3.88 \pm 0.4 \mu\text{m}/\text{day}$) than for larvae fed with PTCS_N- (mean $G_{\text{PN}-} = 5.78 \pm 0.5 \mu\text{m}/\text{day}$) (Fig. 4).

3.4. Clearance rate

Mean pediveliger clearance rate (CR) varied from 0.177 to 0.411 $\text{ml} \cdot \text{day}^{-1} \cdot \text{organism}^{-1}$ with a mean $\text{CR}_p = 0.311 \pm 0.04 \text{ ml} \cdot \text{day}^{-1} \cdot \text{organism}^{-1}$, without any significant differences among pile driving treatments

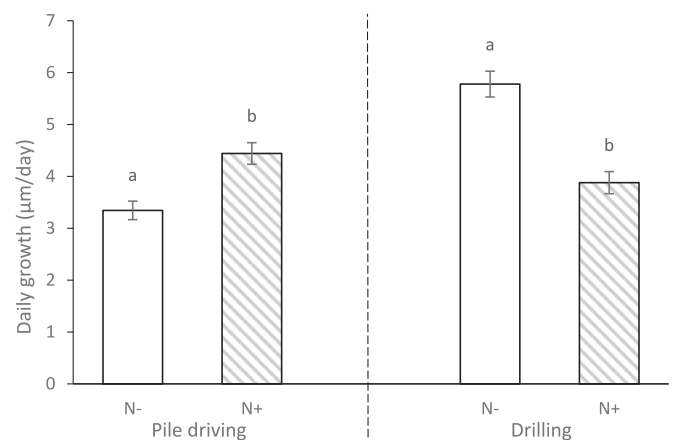


Fig. 4. Mean daily growth $\pm$ standard error ($\mu\text{m}/\text{day}$) of larvae exposed to two diets among crawlers fraction. Groups annotated with the same letter do not differ significantly at p -perm = 0.05.

Table 1

Mean daily clearance rate (CR) $\pm$ standard error ($\text{ml} \cdot \text{day}^{-1} \cdot \text{organism}^{-1}$) of *Pecten maximus* pediveliger larvae exposed to increasing levels of drilling (DC, D1, D2, D3) or pile driving (PC, P1, P2, P3) sounds. Groups annotated with the same letter do not differ significantly at p -perm = 0.05. Mean values of CR_p are included although no significant differences were observed at $\alpha = 0.05$.

	Drilling			Pile driving		
p-perm	0,0483			0,3381		
DC / PC	0,296	$\pm$	0,08 a	0,411	$\pm$	0,08
D1 / P1	0,177	$\pm$	0,03 b	0,214	$\pm$	0,06
D2 / P2	0,195	$\pm$	0,07 ab	0,303	$\pm$	0,07
D3 / P3	0,213	$\pm$	0,03 b	0,383	$\pm$	0,08

($p = 0.3381$; Table 1). Drilling significantly reduced larval clearance rate ($df = 3$, pseudo- $F = 3.2959$, $p = 0.0483$; Table 1) by 28 or 30 % between control (mean $\text{CR}_{\text{DC}} = 0.296 \pm 0.08 \text{ ml} \cdot \text{day}^{-1} \cdot \text{organism}^{-1}$) and either D3 (mean $\text{CR}_{\text{D3}} = 0.213 \pm 0.03 \text{ ml} \cdot \text{day}^{-1} \cdot \text{organism}^{-1}$) or D1 (mean $\text{CR}_{\text{D1}} = 0.177 \pm 0.03 \text{ ml} \cdot \text{day}^{-1} \cdot \text{organism}^{-1}$).

Table 2

Fatty acid composition (mean % of the total identified FAs $\pm$ standard deviation) of scallop pediveliger larvae control (C) and exposed to drilling sound (D3). Σ branched, Σ SFA, Σ MUFA and Σ PUFA are the sum of respectively branched, saturated, monounsaturated and polyunsaturated fatty acids. $\omega 3$ and $\omega 6$ are respectively the sum of $\omega 3$ and $\omega 6$ fatty acids. TFA is the total fatty acids concentration in mg.g^{-1} . Groups annotate with different letters differ significantly at $p\text{-perm} = 0.05$.

	C			D3		
16.0	15.09	$\pm$	0.77	15.04	$\pm$	0.81
16.1 ω 7	12.16	$\pm$	0.51	12.29	$\pm$	0.54
20.5 ω 3	11.6	$\pm$	1.01	11.92	$\pm$	0.97
22.6 ω 3	8.24	$\pm$	1.22	8.70	$\pm$	1.08
18.1 ω 9	6.46	$\pm$	0.24	6.50	$\pm$	0.27
18.4 ω 3	6.21	$\pm$	0.3	6.33	$\pm$	0.33
14.0	6.18	$\pm$	0.28	6.27	$\pm$	0.25
18.1 ω 7	6.03	$\pm$	0.25	6.08	$\pm$	0.24
18.0	5.32	$\pm$	1.15 a	3.84	$\pm$	0.48 b
18.3 ω 3	3.66	$\pm$	0.12	3.73	$\pm$	0.05
18.2 ω 6	3.08	$\pm$	0.06	3.12	$\pm$	0.05
16.3 ω 4	1.83	$\pm$	0.1	1.86	$\pm$	0.09
20.4 ω 6	1.80	$\pm$	0.12	1.83	$\pm$	0.13
14.1 ω 5	1.56	$\pm$	0.07	1.57	$\pm$	0.07
16.2 ω 6	1.16	$\pm$	0.06	1.15	$\pm$	0.05
20.1 ω 9	1.13	$\pm$	0.04	1.14	$\pm$	0.03
22.5 ω 6	1.08	$\pm$	0.14	1.22	$\pm$	0.31
Σ branched	0.39	$\pm$	0.18	0.40	$\pm$	0.14
Σ SFA	28.05	$\pm$	1.77 a	26.53	$\pm$	1.39 b
Σ MUFA	28.98	$\pm$	1.11	29.20	$\pm$	1.13
Σ PUFA	42.58	$\pm$	2.73	43.88	$\pm$	2.56
PUFA/SFA	1.53	$\pm$	0.18	1.66	$\pm$	0.18
ω 3	30.86	$\pm$	2.42	31.86	$\pm$	2.32
ω 6	8.74	$\pm$	0.3	8.99	$\pm$	0.35
TFA (mg.g^{-1})	30.64	$\pm$	7.76	31.60	$\pm$	7.44

3.5. Fatty acids

A total of 47 fatty acids were found in our samples, with 17 fatty acids in proportion $> 1\%$ of TFA which collectively contribute to 92.6 % of the TFA (Table 2). Dominant fatty acids were 16:0 (15.09 $\pm$ 0.22 %), 16:1 ω 7 (12.16 $\pm$ 0.15 %), 20:5 ω 3 (11.6 $\pm$ 0.29 %) and 22.6 ω 3 (8.24 $\pm$ 0.35 %). The PERMANOVA analysis did not show any significant dissimilarities between fatty acid profiles of different conditions, sound ($p\text{-perm}$ (D) = 0.1715, $p\text{-perm}$ (P) = 0.6851), diet ($p\text{-perm}$ (D) = 0.6518, $p\text{-perm}$ (P) = 0.5516) or fraction ($p\text{-perm}$ (D) = 0.2247, $p\text{-perm}$ (P) = 0.2495). The percentage of Σ SFA ($\pm$ standard deviation) content was lower in D3 drilling (mean Σ SFA_{D3} = 26.5 $\pm$ 1.4 %) compared to the control samples (mean Σ SFA_{DC} = 28 $\pm$ 1.8 %) (Table 2; df = 1, pseudo-F = 4.9557, $p\text{-perm}$ = 0.0421), without any diet ($p\text{-perm}$ = 0.6563) nor fraction ($p\text{-perm}$ = 0.169) effect. SIMPER analyses revealed that this difference was mainly due to the 18:0 pattern. There was a significant interaction (df = 1, pseudo-F = 11.096, $p\text{-perm}$ = 0.0106) between sound and fraction factors for the TFA concentration in the pile driving group. The TFA ($\pm$ standard deviation) concentration was 13 % higher for the P3 (mean TFA_{P3} = 42.4 $\pm$ 8.2 mg.g^{-1}) than for the control treatment (mean TFA_{PC} = 37.6 $\pm$ 4.1 mg.g^{-1}) only for swimming larvae (df = 1, pseudo-F = 15.893, $p\text{-perm}$ = 0.019).

4. Discussion

Our study, based on a set of experiments conducted on *Pecten maximus* pediveligers, reveals complex effects of noises that are related to the phase of installation of EMRs (pile driving and drilling operations) with a low influence of the trophic environment.

4.1. Direct impact of anthropogenic noise on larval mortality and growth

While pile-driving noise did not significantly impact the larval survival rates, drilling noise was the sole to induce a significant but low

overmortality from 1.38 % to 3.23 %. Even for the most elevated level of anthropogenic noise tested, larval survival remained high ($> 96.5\%$) after four days of exposure, so that we conclude about a limited impact on scallop larvae mortality. Those results are in accordance with Aguilar de Soto et al. (2013) who showed no mortality on *Pecten novaezelandiae* D-veliger larvae exposed to seismic pulses during 90 h-short-term experiments. The addition of *Nannochloropsis oculata* in the diet stimulated the growth of competent larvae exposed to pile driving (from 2 to 6 $\mu\text{m/day}$ for control and exposed larvae respectively). Inversely, Olivier et al. (2023) observed a similar growth stimulation on post-larvae but for the sole drilling sound (from 3 to 4 $\mu\text{m/day}$) showing that growth responses to anthropogenic noise could be ontogenetic related.

4.2. Constrating effects of drilling and pile driving

We show here that a pile driving sound of 167.1 dB re 1 μPa stimulated both metamorphosis (+49 %) and growth (+2.5 %) of *P. maximus* larvae while drilling sound decreased metamorphosis (−43 %) without any effect on growth, suggesting that those two sounds modulate scallop development in opposite ways. This is in accordance with the results of other previous studies demonstrating either positive or negative impact of sound on the settlement of marine invertebrate larvae (Jolivet et al., 2016; Lillis et al., 2013; Wilkens et al., 2012). We first hypothesize that larvae could be sensitive to the temporal characteristics of sounds, either impulsive (pile driving) or continuous (drilling) as some studies demonstrate their contrasting effects on the adult stages. *M. edulis* adults show higher clearance rates when exposed to pile driving impulsive sound (Spiga et al., 2016), and lower when submitted to a continuous ship noise (Wale et al., 2019). Second, the drilling sound contains higher energy in the 1000–10,000 Hz frequency band than the pile driving sound, and larval responses could differ according to the spectrum composition of sound. High selectivity in the response to sound spectrum was already demonstrated by Lillis et al. (2013) who showed that oyster larvae settle more rapidly when exposed to a reef than to an offshore sound, dominated either by high or low frequencies respectively. The hypothesis advanced by Lillis et al. (2013) is that the soundscape may provide a potential cue of suitable benthic habitats, that has to be selected preferentially by competent planktonic larvae (Eggleston et al., 2016; Lillis et al., 2014) to favor post-settlement survival (Hunt and Scheibling, 1997; Olafsson et al., 1994).

4.3. Impact on settlement and metamorphosis

In mollusks, larvae can extend the duration of their pelagic phase in absence of positive settlement cue (Bayne, 1965; Pechenik, 1990; Culliney, 1974), resulting in a decrease in the energetic reserves (Gebauer et al., 2003; Pechenik, 1990). We show that drilling decreased slightly the SFAs percentage of *P. maximus* larvae in parallel to a 5 % settlement inhibition. Because the β -oxidation of SFAs produces more efficiently ATP than of PUFAs at equal chain length (Langdon and Waldock, 1981), lower SFAs content could result from a higher energy demand, like extending the prospecting pelagic phase. Data on clearance rates confirm this hypothesis, as drilling inhibits larval feeding. On the contrary, we observe that pile driving exposure led to an increase of the total fatty acid content of swimming larvae without a higher clearance rate and PUFA content, which are related to algal assimilation. We thus hypothesize that pile driving, by reducing the time to metamorphosis, could favor competent larvae to settle more rapidly with higher lipid reserves (Stanley et al., 2009).

When a larva delays its metamorphosis, energetic reserves will decrease to a critical threshold that allows successful metamorphosis. At this stage, larva can settle spontaneously without any positive settlement cue even on an unfavorable substratum (Gribben et al., 2006; Olivier et al., 2000; Tremblay et al., 2007), also known as the “desperate larvae hypothesis” (Knight-Jones, 1951; Lagarde et al., 2018; Pechenik, 1990; Toonen and Pawlik, 1994). Within that context, drilling noise

potentially increases habitat selectivity and so delays of metamorphosis, which could lead larvae to a desperate behavior. Inversely, acceleration of the metamorphosis process by pile driving reduces habitat selectivity and could lead to environment mis-choice.

In the present work, antibiotics were used to prevent *Vibrio* spp. infection (Nicolas et al., 1996), but they also block the development of bacterial benthic biofilms that are significant cues to trigger metamorphosis (Hadfield, 2011; Hudon and Bourget, 1981; Toupoint et al., 2012b). Similarly, experiments were conducted without any flow rate, which is proved to improve the settlement of pectinids (Tremblay et al., 2020). Without those important cues metamorphosis rates were low in our control tanks (around 10.8 % in 4 days), but because pile driving enhanced it, we suggest that exposed settlers could decrease their habitat selectivity and metamorphosis in an unsuitable environment. By contrast, drilling sound exposure delays metamorphosis and may thus increase larvae selectivity in non-optimal conditions.

4.4. TST influence on settlement and metamorphosis of *Pecten maximus*

In the last decades, evidence has been made that the phytoplankton abundance and composition in term of species or fatty acids content could trigger settlement of pelagic larvae (*Trophic Settlement Trigger*, TST; Androuin et al., 2022; Toupoint et al., 2012a). In Jolivet et al. (2016), the TST induced by the picoeukaryotic algae *Nannochloropsis oculata* acted in synergy with the vessel noise thus increasing the settlement rate of *Mytilus edulis* larvae by 70 % in only 67 h. In the present work, we did not detect a clear trophic influence of *N. oculata* on *P. maximus* larvae metamorphosis. Lipid content and FA profiles were similar between larvae fed with diets enriched or not with *N. oculata*, with tiny variation in 20:4ω6 whereas *N. oculata* is rich of this particular FA. Such result is confirmed by Olivier et al. (2023) who did not detect differences in FA profiles linked to addition of *N. oculata* to the diet on post-larvae of the same species. We then hypothesize that pediveligers and post-larvae of *P. maximus* do not assimilate *N. oculata*, and that this microalga cannot be considered as a TST cue for this species. Such contrasting responses between mussels and scallops confirm that larval sensitivity to TST should be species-specific (Androuin et al., 2022).

5. Conclusion

The present study gives evidence offshore installations, via the noise they introduce in the marine environment, impact bivalve recruitment through the metamorphosis dynamics and could by cascade influence post-larval survival and population renewal. We show here that noise impact is level dependent but also non-linear with the level. Indeed, a significant effect of both sounds from the intermediate sound level used was observed in our study. Sound levels and distances calculation were based on acoustic pressure measurements to be comparable with existing literature and available recording data. In the field, such level correspond to either 330 m or 170 m distance from the source of pile driving or drilling operations. Because dose dependency information is crucial for stakeholders to manage such EMRs operations and protect marine biota, the present results coupled to sound propagation studies could allow to determine impact areas. Whereas human activities in the marine environment are growing rapidly, the knowledge relative to the impacts of sound, and particularly drilling, on the different stages of marine organisms (embryos, larvae, juveniles and adults) is critically lacking and there is thus a crucial need to develop dedicated research on invertebrate species (Hawkins and Popper, 2017).

CRediT authorship contribution statement

Mathilde Gigot: conduct the experiments, performed data analysis, wrote the original draft. **Frédéric Olivier**: conceived the experimental design and methods, supervised the experiments and reviewed the manuscript. **Gauthier Cervello**: conduct clearance rate experiments,

analysis, and description in the draft. **Rejean Tremblay**: contributed to results interpretation and manuscript review. **Delphine Mathias**: did the acquisition and parts of the acoustic signal analysis. **Tarik Meziane**: supervised fatty acid analysis and contributed to manuscript review. **Laurent Chauvaud**: conceived the study, led the project administration and funding acquisition. **Julien Bonnel**: supervised the acoustics analyses and reviewed the manuscript.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Laurent Chauvaud reports financial support was provided by Iberdrola SA.

Data availability

Original data are available on demand to the corresponding author.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2023.114969>.

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