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Assessing the impacts of anthropogenic sounds on early stages of benthic invertebrates: The "*Larvosonic* system"

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Abstract

Noise produced by human activities has increased in the oceans over the last decades. Whereas most studies have focused on the impact of anthropogenic noise on marine mammals and fishes, those focusing on marine invertebrates are rarer and more recent, especially when considering peri-metamorphic benthic stages, highly sensitive to anthropogenic perturbations. A careful review of the literature reveals a simplistic characterization of the acoustics within the containers used to quantify larval and juvenile responses to noise, thus weakening the conclusions of such works. To address this problem, we developed the *Larvosonic* system, a laboratory tank equipped with acoustic assets to assess the impacts of noise on young stages of marine invertebrates. We first provide a careful analysis of the tank sound field using different sound types, and we assess the effects of expanded polystyrene units on the sounds emitted by a professional audio system in order to dampen reverberation and resonance. Then, we apply this acoustic calibration to the effects of both pile driving and drilling noises on postlarvae of the scallop bivalve *Pecten maximus*. Acoustic recordings highlight that diffuser and bass trap components constitute effective underwater sound absorbents, reducing the reflection of the whole frequency bandwidth. Scallop experiments reveal that both type and level of the tested noise influenced postlarval growth, with interactive effects between trophic environment and noise level/spectra. The *Larvosonic* system thus constitutes an efficient tool for bioacoustics research on bentho-planktonic invertebrate species.

Noise produced by human activities has increased in the oceans over the last decades (Duarte et al. 2021). Bioacousticians use a common parameter, named Sound Exposure Level (SEL), to quantify the exposure of an organisms per unit of time to a particular noise of a known source level. SEL can be expressed within a fixed band frequency, usually in dB re 1 μPa s (Martin et al. 2019). Other metrics also are used to quantify various types of sounds. For example, the natural metric to quantify a continuous broadband sound is the root mean square (RMS) SPL, written as $\mathrm{SPL}_{\mathrm{RMS}}$, the metric to quantify transient impulsive signals is the peak-to-peak SPL (Madsen 2005), written as $\mathrm{SPL}_{\mathrm{pp}}$. Importantly, the numerical value of a given metric cannot be compared to the numerical value of another metric (e.g., we cannot compare the SEL of X dB re 1 μPa s with the $\mathrm{SPL}_{\mathrm{pp}}$ of Y dB re 1 μPa s). This difficulty complicates the intercomparison of bioacoustic studies, but is a necessary burden to quantify sound features and their potential impact on the environment.

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Additional Supporting Information may be found in the online version of this article.

Anthropogenic noise in the ocean is created by a myriad of different sound sources. The origins of the loudest noises at the emitting source include explosives (SEL broadband = 237 dB re1 μPa s; Bagočius and Narščius 2019), seismic surveys by airguns (Carroll et al. 2017; SEL broadband ~ 203 dB re 1 μPa s; Day et al. 2016), and drilling (~ 184 dB re 1 μPa RMS; Kyhn et al. 2014) and pile driving operations (SEL broadband ~ 177 dB re 1 μPa s at 750 m; Matuschek and Betke 2009; Amaral et al. 2020; "estimated source level" $\mathrm{SL_E}$ ~ 215–220 dB re 1 μPa s; Ainslie et al. 2012) that have multiplied in shallow waters with the development of marine renewable energy devices. To a lesser extent, vessel noise produced by operating high-energy propellers, gears, and diesel generators can reach more than 200 dB re 1 μPa m at the source for large and powerful ships (Chauvaud et al. 2018; Erbe et al. 2019). Vessels have contributed to a 16-fold increase in low-frequency noise in some parts of the oceans over the last 50 years (McDonald et al. 2006; Malakoff 2010). Within that context, assessing the potential impacts of anthropogenic noise on marine organisms is a major concern for environmental stakeholders. This is attested in the CEE by the adoption of Descriptor 11 in the Marine Strategy Framework Directive Good Environmental Status 2008/56/EC (MSFD-GES; Van der Graaf et al. 2012), which states, "Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment."

Over the last two decades, the bulk of studies have focused on mammals with subsequent implementation of noise exposure criteria (Southall et al. 2007) and underwater acoustic thresholds (National Marine Fisheries Service 2016, 2018). Research focusing on the impacts of underwater noise on fishes (reviewed in Hawkins and Popper 2018) has detailed categories of hearing capabilities related to anatomical features (e.g., presence/lack of a swim bladder; Popper et al. 2014). By contrast, and as outlined by Hawkins and Popper (2017), we recognize a crucial need to increase our knowledge of the potential impacts of anthropogenic noise on marine invertebrate species. Indeed, the sparse available data for any invertebrates mainly focus on adult stages (but *see* references in Bonnel et al. 2022) with very few papers dealing with vibrations effects (Hawkins et al. 2021; Jézéquel et al. 2022) related to some anthropogenic marine operations (Roberts and Elliott 2017). Of paramount importance is the need to specify the biological/ecological responses to man-made noise that impact the components of the animal's fitness including growth, development, longevity, or reproductive success (Hawkins et al. 2020).

In coastal temperate and subarctic areas, most invertebrates of commercial (fisheries and aquaculture) or functional interest (engineer species, Passarelli et al. 2014; as defined by Jones et al. 1994) display a bentho-planktonic life cycle, including a pelagic larval stage lasting from 1 d to several weeks (Thorson 1950). Colonization of the substratum by competent larvae (i.e., ready to settle and metamorphose) and the subsequent weeks after this primary settlement phase is a critical step during which more than 90% of mortality occurs (Pedersen et al. 2008). This stage is driven by multiple abiotic and biotic factors (but *see* reviews of Butman 1987; Ólafsson et al. 1994; Hunt and Scheibling 1997; Pineda et al. 2009), yet the influence of underwater sound is not well documented.

The effect of sound on some species of marine invertebrate larvae was studied initially more than three decades ago. Branscomb and Rittschof (1984) demonstrated that the primary settlement of wild cyprid stages in the barnacle *Amphibalanus amphitrite* (a major source of fouling in tropical areas) was inhibited by a narrow-band low-frequency 30-Hz sound and that metamorphosis was delayed up to 2 weeks. Working on the same species, Guo et al. (2012) also tested the impact of continuous ultrasounds through substrate vibrations with varying frequencies (23, 63, and 102 kHz) for 24 h on larval ecology. The authors observed that only 23 kHz significantly reduced the primary settlement of cyprid larvae and that the same antifouling effect can be obtained with a discontinuous sound. In another study, Choi et al. (2013) proposed installing collectors (lead-zirconate-titanate ceramic transducers = PZT panels) at sea to estimate fouling dynamics during a 3-month period, and also tested the vibration effect of these panels at various speeds and low frequencies (70–445 Hz). Of the fouling species that recruited on the panels, only barnacle species (*Amphibalanus variegatus* and *Elminius* sp.) were sensitive to the vibrations, with reduced fixation rates beyond 260 Hz. The underwater sound produced by vessels is also an important cue biologically for settlement of the larval stage of species involved in fouling and bioinvasion, such as mussels, ascidians, and barnacles (Wilkens et al. 2012; McDonald et al. 2014; Stanley et al. 2014). Recent evidence suggests that vessel noise generates an interactive nonlinear effect with the trophic environment that acts on larval settlement of *Mytilus edulis* (Jolivet et al. 2016). Prerecorded sounds of natural oyster reefs also can stimulate settlement of *Crassostrea virginica* oysters after a few days (Eggleston et al. 2016). Overall, the positive (e.g., enhanced settlement, metamorphosis, …) and negative (e.g., body malformations, development delays, …) responses from marine invertebrate larvae to anthropogenic noise differ widely among studies (*see* Supplementary Material S1 for details).

The common link binding the studies is the use of tanks, which are required to control development of larvae. However, the sound field in small enclosed seawater tanks is complex and has not been fully characterized in the bioacoustics literature, making data comparisons between studies infeasible. Three major acoustic biases in tanks are described commonly in the literature (Kleiner and Tichy 2014). First, low-frequency sounds are highly attenuated in small tanks when the wavelength of the emitted sound (e.g., a 100 Hz sound has a ~ 15 m wavelength) is larger than the tank size

(Gray et al. 2016; Rogers et al. 2016). This phenomenon can lead to important spatial dissimilarities in the received levels in the tank (even a few centimeters apart), and a global loss of the low-frequency content of the signals (Jézéquel et al. 2019, 2020). Second, depending on the dimensions of the tank, resonant frequencies distort mid-frequency sounds and create holes and peaks in the original sound spectrum and also lengthen the signal at specific resonant frequencies (Akamatsu et al. 2002; Novak et al. 2018). Third, high-frequency signals (with wavelengths much smaller than the tank size) are affected by multiple reflections with the tank walls. This well-known phenomenon in room acoustics lengthens the whole signal and is usually quantified as the "reverberation time" (Kleiner and Tichy 2014).

Overall, acoustic signals recorded in tanks have different duration and frequency content compared to the original sound, which influences bioacoustics studies markedly (Jézéquel et al. 2019). When studying noise impacts on organisms, sound exposure (and thus the resulting potential behavioral response) can vary according to the position in the experimental tank (Jones et al. 2019). As a result, major methodological challenges are associated with proper assessment of the impact of sound on marine animals using laboratory experiments. Meeting these challenges is critical for ecologists to assess the role of anthropogenic noise on larval stages. We recognized a need to develop new standard experimental set-ups that combine both the control of large batches of larvae and an accurate calibration of the sound field. We present here the *Larvosonic* system, which aims to enable laboratory studies to assess potential impacts of anthropogenic broadband noise on young stages of invertebrates. In this article, we first provide a careful analysis of the sound field in the tank using different sound types, and assess the effects of different materials (e.g., diffusers) on the emitted sounds. Then, we apply this acoustic calibration to the effects of both pile driving and drilling noise on postlarvae of the scallop bivalve *Pecten maximus*.

Materials and procedures

Larvosonic system

The structure consists of one main tank of ~ 800 liters (Fig. 1; external dimensions: length × width × height = 1360 × 935 × 680 mm) made of transparent plexiglass (10 mm in thickness), assembled with Altuglas© Adhesive S2002 methyl methacrylate glue. The tank is designed to be settled on an aluminum frame on which a PVC plate is fixed (not included in Fig. 1). The main tank is filled completely so that the sealed cover is in direct contact with seawater to avoid any air–water interface (except few microbubbles) that is known to alter sound propagation (Rogers et al. 2016). Horizontal level can be adjusted through inox screws in the frame and in plastic disks bases that maximize the decoupling of sound emission with the floor. At the center of this main tank is a removable plate on which various type of underwater speakers are fastened, their selection being related to the type of sound tested: the Clark Synthesis AQ339 Diluvio™ (https://clarksynthesis.com/aq339/) was the most adequate speaker for noises related to marine renewable energy devices operations but both DNH Aqua30 units (https://www.dnh.no/uploads/filer/365-AQUA-30.pdf) and Lubell LL916 (http://www.lubell.com/LL916.pdf) can also be used (*see* below). To attenuate sound reverberation and resonant frequencies in the *Larvosonic* system, we used two types of expanded polystyrene diffusion panels (*Vicoustic*©; http://www.vicoustic.com) fixed on additional plexiglass plates that can be settled on the internal walls of the main tank. *Trap Fuser* unidirectional panels were positioned at each corner of the main tank to allow sound energy to be trapped in the cavities and/or scattered by the plain surfaces. The bi-dimensional *Multifuser DC2* panels were positioned at the center of each wall of the main tank providing multireflection on both vertical and horizontal planes with maximum efficiency in mid and high frequencies (maximum absorption in air between 0.8 and 2.5 kHz). The underwater speaker AQ339 was screwed on a removable plexiglass plate also screwed to the floor of the main tank at its center. This speaker was adapted to reproduce the natural spectrum and level of drilling and/or pile driving sounds due to its broadband high-level response (*see* SPL diagram vs. frequency here: http://www.lubell.com/AQ339_SPL.pdf). The main tank holds several containers of larval batches. Each container is composed of a Plexiglas cylinder (internal dimensions: diameter = 192 mm, total height = 382 mm), half-filled (water height = 175 mm) with a mixture of diet, larvae, and filtered seawater. Because we intended to conduct fatty acid analyses of the larvae, and large batches of peri-metamorphic stages are required for that purpose, we adjusted the total volume within the 5-liter cylinder to maintain 40,000 individuals at the veliger stage and 10,000 at the postlarval stage (larval concentration < 1 larva mL^{-1} to avoid any density-dependent effects) during the experiment. This was the main constraint of the actual design of the *Larvosonic* system. To study variations in the filter-feeding activity of larvae, microplates can be also used, each of 6 wells being filled at the start of short experiments with batches of 20 larvae and a solution of a monoculture of microalgae (total volume = 20 mL; Cervello et al. unpubl. data). For a given *Larvosonic* system, we were thus able to settle six cylinders and six microplates fitting in a removable lid fixed to the main tank (Fig. 1A,B). We used a Yamaha MTX3 digital matrix processor, coupled to a Powersoft Otto 1204 DSP eight-channel amplifier (8 × 150 W), to allow simultaneous sound emissions of various types and levels per *Larvosonic* unit. Sound files can be played through two Denon DN300Z CD/media players that each includes Bluetooth®/USB/SD/Aux and an (https://www.denonpro.com/index.php/products/view3/dn-300z) AM/FM tuner reader. Settings were determined using the MTX-MRX Editor V4.0.0 for Win10 (https://usa.yamaha.com/products/proaudio/processors/mtx/downloads.html#product-tabs) freely available on the following website.

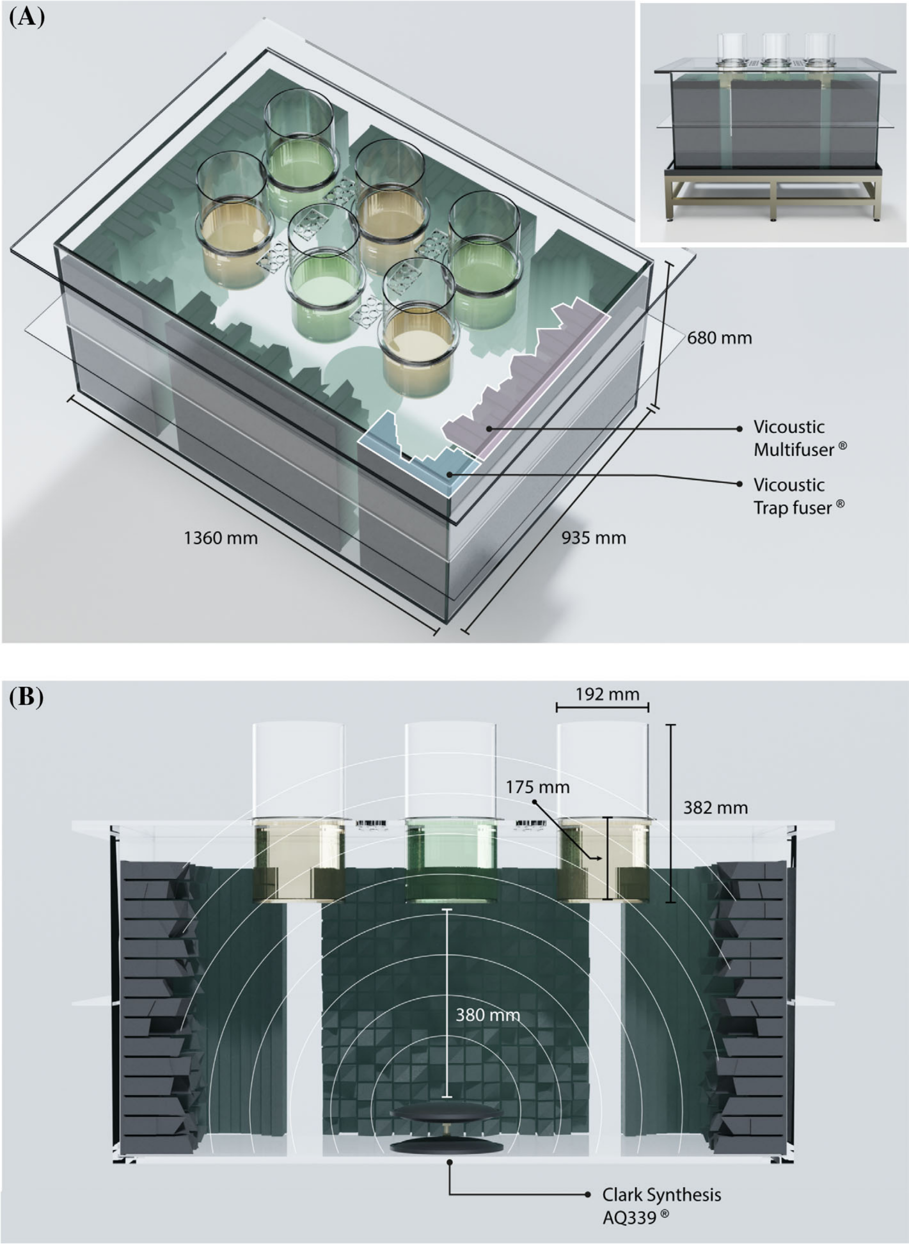


Fig. 1. General description of the *Larvosonic* system in (A) 3D view and (B) cross-section.

Sound field of the *Larvosonic*

Sound recordings and analyses

All acoustic recordings were made with a RTSYS EA-SDA14 (https://rtsys.eu/) underwater acoustic recorder (sampling frequency of 78 kHz, 32-bit resolution) equipped with two calibrated HTI-92-WB (http://www.hightechincusa.com/products/hydrophones/hti92wb.html; High Tech, Inc.) hydrophones (length × diameter = 102 × 38 mm; sensitivity of either −165.2 dB re 1 V μPa^{-1} or – 210 dB re 1 V μPa^{-1}) located at the center of each cylinder. After recording, sound files were selected manually using Audacity® (Version 2.4.2; Audacity Team 2018), and then analyzed using a custom Matlab script (The MathWorks, Inc.). Sound time series ($S(t)$) were first corrected from volts to μPa pressure units ($p(t)$) by multiplying voltage data by sensitivity values (accounting for gain applied) provided by the recorder's constructor using the following equation:

$$p(t) = S(t) \times 10^{-G/20} \times D \times 10^{-\mathrm{SH}/20}, \tag{1}$$

where G (dB) is the recorder gain (G = 0.72 dB), D is the dynamic response of the recorder (2.5 V), and SH is the sensitivity of the hydrophone. SPLs (in dB re 1 μPa; Supplementary Material S2) for each subsample were calculated in a time window corresponding to the length of the recorded sound (T) (Erbe 2010), where $T = 5$ (sweep and white noise), 7 (drilling), 10 (pile driving) or 28 s (boat). Power spectral densities (PSDs, dB re 1 μPa Hz^{-1}) and spectrograms (dB re 1 μPa Hz^{-1}) were computed on Matlab software. PSD representations were produced by dividing the recording sound into windows (5-s duration) and conducting a discrete Fourier transformation on each window. Spectrograms of the chirp sounds (*see* "Sound field calibration" section) were restricted to the 0–3-kHz range to focus on resonant frequencies. Chirp signals were first decimated by a factor of 7 to center the figures on the low part of the specter where most of the resonant frequencies occurs. Then, spectrograms were computed using a hamming window of 47-ms duration, a fast Fourier transform length of 3000 samples, and a 99% overlap.

Resonance calculation

Reverberation is a well-known effect of sound fields in enclosures, and is described in detail in room acoustics literature (e.g., chapter 2 of Kleiner and Tichy 2014). At high frequencies, tank walls cause multiple reflections leading to the persistence of a sound after its emission. At mid-frequencies, the sound field exhibits a series of resonant frequencies depending on the tank dimensions and wall properties, whereas at low-frequencies the sound speed decays exponentially with the source–receiver range. Simple equations for an experimental tank are given in Akamatsu et al. (2002), which summarizes the main effects by showing empirical examples. Although Akamatsu's acoustic model has been used previously to understand sound fields in tanks (Jézéquel et al. 2018, 2019; Jones et al. 2019), such a model remains quite simplistic because it does not integrate properties of the tank walls, and so tends to overestimate the resonant frequencies. A better model, more complex but still easily tractable, is proposed in Novak et al. (2018), who also provided a companion Matlab code to estimate the sound field in a tank and its associated resonant frequencies. We used this more tractable model to assess the resonant frequency of the first three modes in the *Larvosonic* system. The model inputs include tank size (*see* Fig. 1A), water properties (sound speed c_{wat}, density ρ_{wat}), air properties (sound speed c_{air}, density ρ_{air}), as well as wall properties (thickness h, sound speed c_{wall}, and density ρ_{wall}) (*see* Table 1). The variable c_{wat} can be related to temperature T, salinity S, and pressure P by the equation $c_{\mathrm{wat}} = 1449.2 + 4.6\ T - 0.0557\ T^2 + 0.00029\ T^3 + (1.34–0.0107\ T)\ (S - 35) + 1.58 * 10^{-6}\ P$ (Medwin and Clay 1998). In our experimental conditions, we have $c_{\mathrm{wat}} = 1493$ m s^{-1} and $\rho_{\mathrm{wat}} = 1027$ g cm^{-3}. The wall properties are $c_{\mathrm{wall}} = 1700$ m s^{-1}, $\rho_{\mathrm{wall}} = 1180$ g cm^{-3}, and $h = 10$ mm, with c_{wall} and ρ_{wall} nominal values for Plexiglas (https://www.classltd.com/sound-velocity-table/).

Table 1. Parameters used to calculate theoretical resonant frequencies using the model from Novak et al. (2018).

Parameter		Notation	Value	Unit
Wall properties	Thickness	h	10^{-3}	m
	Sound speed	c_{wall}	1700	m s^{-1}
	Density	ρ_{wall}	1180	kg m^{-3}
Water properties	Temperature	T	11.3	°C
	Salinity	S	34.1	‰
	Pressure	P	1013.25	Pa
	Sound speed	c_{wat}	1493	m s^{-1}
	Density	ρ_{wat}	1027	kg m^{-3}
Air properties	Sound speed	c_{air}	340	m s^{-1}
	Density	ρ_{air}	1255	kg m^{-3}

Sound field calibration

To calibrate the sound field in the *Larvosonic* system, we conducted a series of tests in February 2021 by playing a 90-s long sequence including different broadband and transient (i.e., pile driving, spiny lobster antennal rasps, and chirps) or continuous (i.e., drilling, white noise, and fisherman boat) sounds (*see* Supplementary Material S2 for associated PSDs and SPLs). The chirp sounds were swept linearly from 20 to 10 kHz over a 5-s period. The white noise was spread equally over a wide frequency band (between 10 Hz and 10 kHz). Sound recordings were made when temperature and salinity of seawater were equal to 11.4°C and 34.2 psu, respectively. No larvae were present in the cylinders. Both pressure and particle velocity are two components of the acoustic field that are obtained by solving the linear wave equation. However, in a tank, for a given frequency, the impedance (ratio between KE and PE) evolves nonlinearly as a function of the source–receiver distance. Similarly, for a given source–receiver configuration, the impedance evolves nonlinearly as a function of frequency. As a result, KE cannot be easily assessed from PE, which is often used to justify the measurement of particle velocity for bioacoustic studies. However, when source level increases by N dB, both PE and KE will increase by N dB. Similarly, if SL decreases by N dB, both PE and KE will decrease by N dB. As a result, a pressure-only measurement cannot be used to evaluate impact in terms of absolute particle motion threshold; still, it can be used to assess impact in terms of trends, that is, showing that a specific response increases (or decreases) when both PE and KE increase (or decrease). To empirically illustrate this behavior, we acquired particle motion and pressure data associated with decreasing source-level drilling sound in December 2021, by using a vector sensor of the Sercel company based on the micro electro-mechanical system QuietSeis™ accelerometer sensor (Fougerat et al. 2018) and a traditional hydrophone, respectively. We considered a series of five drilling sounds that were emitted in the tank, with source level (SL) decreasing by about 1 dB from one signal to the next, leading to a 4 dB decrease over the whole sequence. For a given source position, particle motion and pressure were measured in either central or external cylinders. PE and KE were evaluated in frequency bands centered on 50, 100, 250, 500, and 750 Hz, with a 10 Hz bandwidth, arbitrarily chosen within the bandwidth of the particle motion sensor (about 5–800 Hz). Sound recordings were made when temperature and salinity of seawater were around to 17°C and 34 psu, respectively. No larvae were present in the cylinders.

We also quantified acoustic tank impacts on different sound features (frequency content and SPLs) that were used to assess (1) the intracylinder (records from various locations within a given cylinder) and intercylinder (records from all cylinders) acoustic variability; (2) the efficiency of diffusers on sound features (with or without diffusers); and (3) the influence of the presence of cylinders (with or without cylinders). The SPL at resonant frequencies is theoretically mostly independent of the distance between source and receiver (Akamatsu et al. 2002; Novak et al. 2018). We thus calculated the $\mathrm{SPL_{RMS}}$ for each resonant frequency and for several non-resonant frequencies in both central and lateral cylinders, situated at different distances from the speaker.

Because the frequency response of our underwater speaker was between 10 and 1000 Hz only (Lubell Labs Inc.®), we deployed the speaker in a large outdoor pool (length × width × height = 12 × 13 × 1.4 m) to assess its higher frequency band response (seawater 8°C and 34.5 psu).

Experimental sounds

The main experiment was conducted using pile driving (P) and drilling (D) sound signals. Those signals, described below, are different from the calibration sequence presented in "Sound field calibration" section. Prior to noise emissions, we characterized the ambient noise levels of our experimental setup in all six cylinders of the two control tanks. The $\mathrm{SPL_{RMS}}$ varied between 93.2 and 94.6 dB re 1 μPa. Such noise levels are comparable to ambient noise levels found in temperate coastal environments with variable wind conditions (Mathias et al. 2016).

The pile driving sequence is based on a *.wav file recorded by an RTSys system during the building phase of an offshore marine wind-farm of the North Sea (depth around 30 m, confidential data) including 6 h of sound emission followed by 6 h of silence, and then repeated. The drilling sequence (field recording in June 2018 at 200 m from the boat operating the geotechnical drilling; SOMME database) is based on a sound file of 19 h of sound emission followed by 5 h of silence, also repeated. These scenarios were chosen to match *in situ* work conditions (Ailes Marines pers. comm.). Experiments were run in two isolated rooms, each with four *Larvosonic* systems. In one room, pile driving noise was emitted in three *Larvosonic* systems using a different source level (P1, P2, P3) in each tank and the fourth tank was used as a control (PC, no pile driving sound). Similarly, in the other room, drilling noise was emitted in three *Larvosonic* systems using a different source level (D1, D2, D3) in each tank and the fourth tank was used as a control (DC, no drilling sound). In all tanks, source levels were tuned so that measured noise exposure levels corresponded to what would be measured *in situ* at various distances from the source. The source levels were adjusted by recording 30 s of sound emission at the center of each cylinder (10 cm above its bottom) and are detailed in Table 2.

Experiments in *Larvosonic* biological trials with postlarvae of *P. maximus*

A noise impact study was performed on postlarvae of the scallop *Pecten maximus*. Batches of 10,000 postlarvae (34 d postfertilization [dpf]) were introduced in each cylinder previously filled with 5 liters of filtered seawater with 9 ppm of erythromycin. The main tank of each *Larvosonic* systems ($N = 8$) were filled with 10-μm filtered seawater. Temperature in the room was controlled with a heater at 18°C (± 0.4°C) and a photoperiod of 12 : 12 h (L : D) was maintained. Food was added once a day at a final concentration of 60 cells μL^{-1} in the postlarval culture. We included a diet treatment ($N = 2$, 3 replicates per diet) consisting of a DTCS (*Diacronema lutheri*, *Tisochrysis luthea*, *Chaetoceros neogracilis*, *Skeletonema marinoï*) diet with (DTCS_N+) or without addition (DTCS_N−) of the microalgae *Nannochloropsis oculata* and concentrations were adjusted to maintain a similar biomass in each cylinder. Experiments started at midday by simultaneously reading sound *.wav files according to a 6:6-h or a 19:5-h on/off cycle for pile driving or drilling sounds, respectively (*see*

Table 2. Level of noise emitted in the *Larvosonic* system during the great scallop experiments as a function of either drilling (D) or pile driving (P) treatments expressed as $\mathrm{SPL_{pp}}$ or RMS as well as $\mathrm{SEL_{24h}}$.

	Ambient room noise $\mathrm{SPL_{RMS}}$ re 1 μPa ± SE	P1 or D1	P2 or D2	P3 or D3	Theoretical *in situ* SL at 1 m
Pile driving (P)					
$\mathrm{SPL_{pp}}$ re 1 μPa	100.3 + 0.7	147.6 + 2.5	167.1 + 2.4	187.6 + 2.4	221
$\mathrm{SEL_{24h}}$ re 1 μPa s ± standard error (SE)	144.1 + 0.9	186.9 + 1.5	197.5 + 2.5	215.8 + 2.2	
Drilling (D)					
$\mathrm{SPL_{RMS}}$ re 1 μPa	96.3 + 0.8	107.0 + 2.1	126.8 + 2.1	175.4 + 2.3	172
$\mathrm{SEL_{24h}}$ re 1 μPa s ± standard error (SE)	143.1 + 0.7	153.4 + 2.1	173.1 + 2.1	221.7 + 2.3	

"Experimental sounds" section), and were conducted for 4 d. At the hallway point, batches of postlarvae from each cylinder were collected through a 60-μm^2 mesh and water and diet were renewed. The same method was used at the end of the experiments to collect postlarvae that were rinsed and concentrated in a 300-mL beaker with filtered seawater until subsampling. The larvae-rearing design, methods related to counting and length measurements or to fatty acid and statistical analyses are detailed in Supplementary Material S3.

Results

Larvosonic acoustics

SPL variability

Within the cylinders, the SPL$_{\mathrm{RMS}}$ values for white noise recorded at two depths and different horizontal positions were stable and ranged from 144.9 to 147.9 dB re 1 μPa. The SPL$_{\mathrm{RMS}}$ values recorded at the bottom of the cylinder (15-cm depth) were higher than those at the top (5-cm depth), probably due to the direct proximity of the speaker, but the associated frequency spectra were highly similar between recording positions (*see* Supplementary Material S3).

In addition, the SPLs of other tested sounds were often higher in the two cylinders located at the center of the main tank compared to lateral cylinders, with very similar associated frequency spectra (*see* Supplementary Material S3). The mean SPL$_{\mathrm{pp}}$ ($\pm$ standard deviation) of pile driving noise and mean SPL$_{\mathrm{RMS}}$ of drilling noise received in the two central cylinders were calculated at 166.1 $\pm$ 0.3 and 153.1 $\pm$ 0.2 dB re 1 μPa, respectively, whereas these levels decreased to 159.5 $\pm$ 1.0 and 147.4 $\pm$ 0.4 dB re 1 μPa for the four lateral cylinders, respectively. No significant difference ($p > 0.05$) was found in SPLs between front and back cylinders located at the same central or lateral position. Interestingly, noise levels were higher when adding 1 liter of seawater within cylinders (final volume of 6 liters) than the mean SPL$_{\mathrm{pp}}$ of pile driving, initially equal to

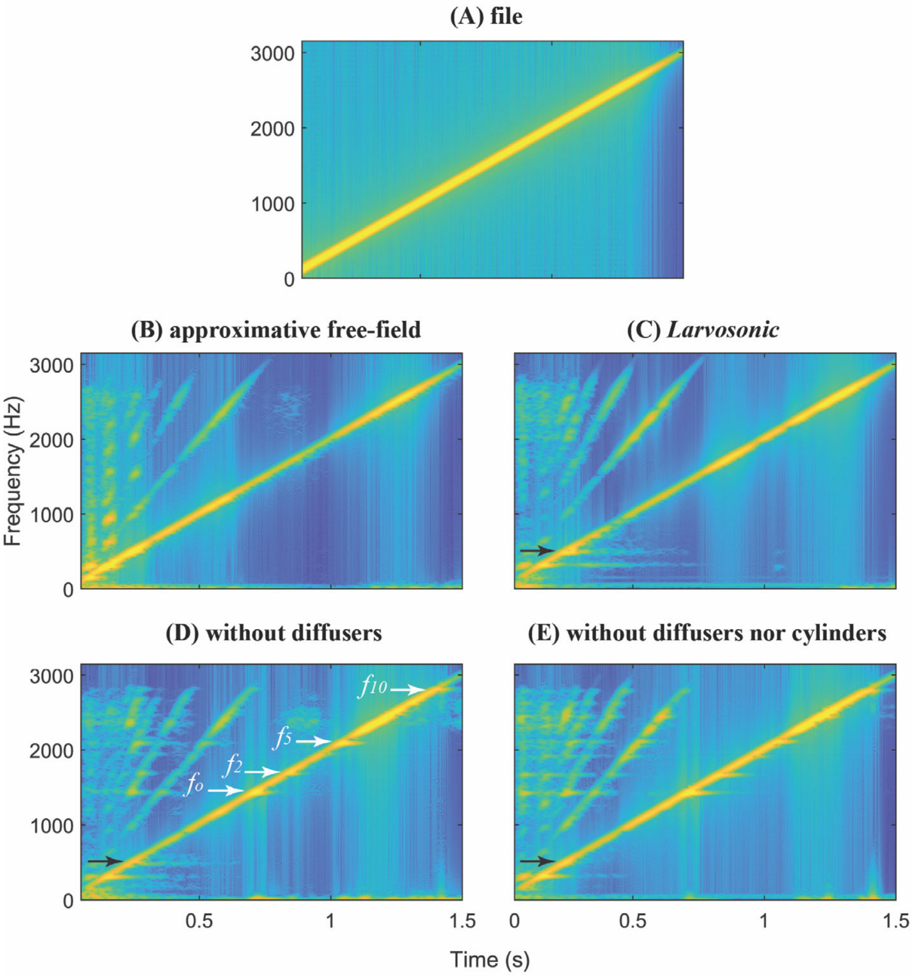


Fig. 2. Spectrograms (sampling frequency = 44.1 kHz, FFT size = 3000; hamming window = 47 ms; 99% overlap) of the chirp (**A**) recorded either in a large pool (**B**) or in the *Larvosonic* system including all components (**C**) or without diffusers (**D**) and without diffusers nor cylinders (**E**). The chirp swept linearly from 20 Hz to 10 kHz over a 5-s period. White arrows indicate the four most obvious resonant frequencies identified. Black arrows indicate the 500-Hz persistent band in (**C**) and (**D**) which disappears when removing cylinders in (**E**) and does not exist in the large pool (**B**).

166.4 dB re 1 μPa, an increase of 3 dB re 1 μPa. Similarly for drilling noise, the mean SPL_{RMS} increased from 149.1 to 151.5 to 153.8 dB re 1 μPa for 4-, 5-, and 6-liter water volumes.

Reverberation and spectral analysis

The PSDs of drilling, pile driving (Fig. 4), and the four other tested sounds (*see* Supplementary Material S1) displayed a relative dip at 800 Hz as well as a complex pattern between 2 and 20 kHz, with frequencies between 4 and 6 kHz being overexpressed. Although the chirp recorded in the large pool matched the shape of the original wav file (Fig. 2A), some frequencies from the chirp recorded in the *Larvosonic* were overexpressed and persisted in time without diffusers (Fig. 2C) compared to with diffusers (Fig. 2B). One must also notice that time course of pile driving signal emitted in the *Larvosonic* differs moderately from what is observed in the field due to speaker limitations (Fig. 6A). Because time persistence is typical of resonance phenomena, we compared observed frequencies to those predicted by the theoretical model. We visually identified 10 resonant frequencies (Table 3) and the four most obvious being pointed out in Fig. 2C (white arrows). We found that the SPL associated with resonant frequencies varied less between cylinders than for nonresonant frequencies. For example, the SPL_{RMS} values at 1000 and 1200 Hz (nonresonant frequencies) were 5.3 and 8 dB higher, respectively, in central cylinders than the lateral cylinders, whereas SPL_{RMS} values for resonant frequencies (Table 3) varied little (from 0.1 to 3.8 dB) between all cylinders. The positive role of our diffusers in reducing resonant effects in the *Larvosonic* was particularly evident on impulsive sounds (which are highly impacted by persistence), for which the diffusers decreased the amplitude of reverberation-generated oscillations that can be observed after the impulsions (Fig. 3). We also noticed that diffusers reduced low-frequency (below 50 Hz) background noise (*see* Fig. 2).

However, in the *Larvosonic*, some low frequencies at 300 and 500 Hz were overexpressed and persisted in time with or without diffusers and their associated sound level was higher in the central cylinders. When considering the chirp recorded in the pool, 300- and 500-Hz frequencies were still overexpressed but not persistent. This phenomenon is consistent with the speaker frequency response, which has peaks at these frequencies, but that does not explain the persistence in the *Larvosonic*. When all *Larvosonic*'s cylinders were removed, the persistence associated with the 500-Hz band disappeared (*see* black arrows in Fig. 2B–D), which suggests a complex acoustic effect related to the sound interaction with the cylinders. On the other hand, the persistence at 300 Hz did not disappear when cylinders were removed. The origin of the 300-Hz band in the *Larvasonic* is an open question.

Kinetic energy vs. potential energy

In each frequency band, the source level (SL) decreases by about 1 dB when the signal number increases, resulting in an overall ~ 4 dB decrease from signals 1 to 5 (Fig. 4, left panel). For the two receiver locations, both potential energy (PE) and kinetic energy (KE) decrease in the very same way, with an overall decrease of ~ 4 dB from signals 1 to 5 (Fig. 4, middle and right panels). Furthermore, the difference between KE and PE relates either on frequency for a given source location or for a given frequency on source location. As expected, the acoustic impedance (PE/KE in dB) calculated at a given frequency and one of the two measurements locations is constant (independent from source level) but it drastically changes with location and/or with the frequency considered (Fig. 5). All the measurements show also consistently negative impedance traducing that KE > > PE (Fig. 4).

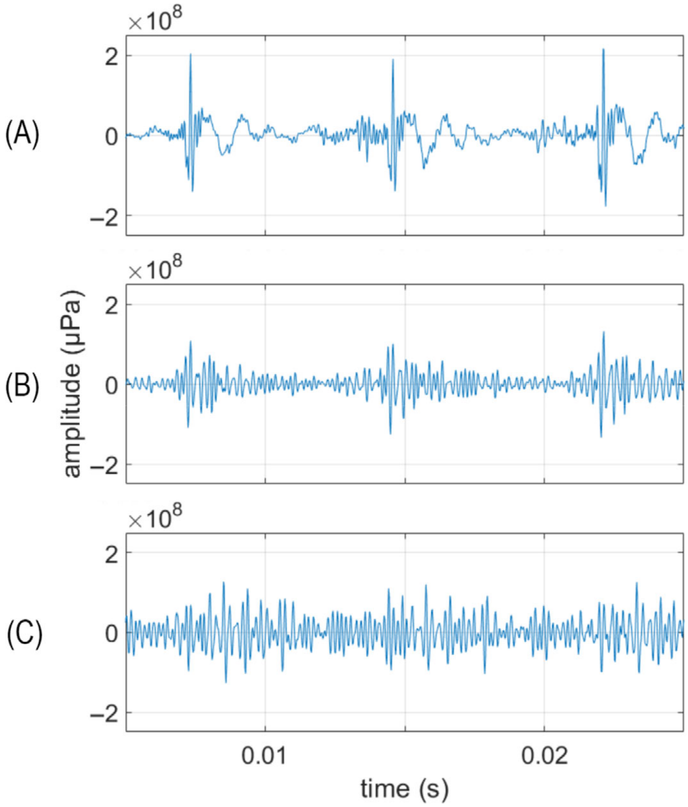


Fig. 3. Waveform of a spiny lobster antennal rasps recorded either (**A**) in situ (Jézéquel et al. 2019) or in the *Larvosonic* system with (**B**) or without diffusers (**C**).

Impact of pile-driving and drilling sounds on *P. maximus* postlarvae

Pile driving (P) and drilling (D) acoustic treatments and ambient noise

The PSDs of pile driving and drilling noise recorded in the *Larvosonic* were in accordance with the original wav files (Fig. 6), although some frequencies between 50 and 200 Hz and at 1 kHz were under expressed. During experiments, SPL levels (pp or RMS; ± standard error) were adjusted and the

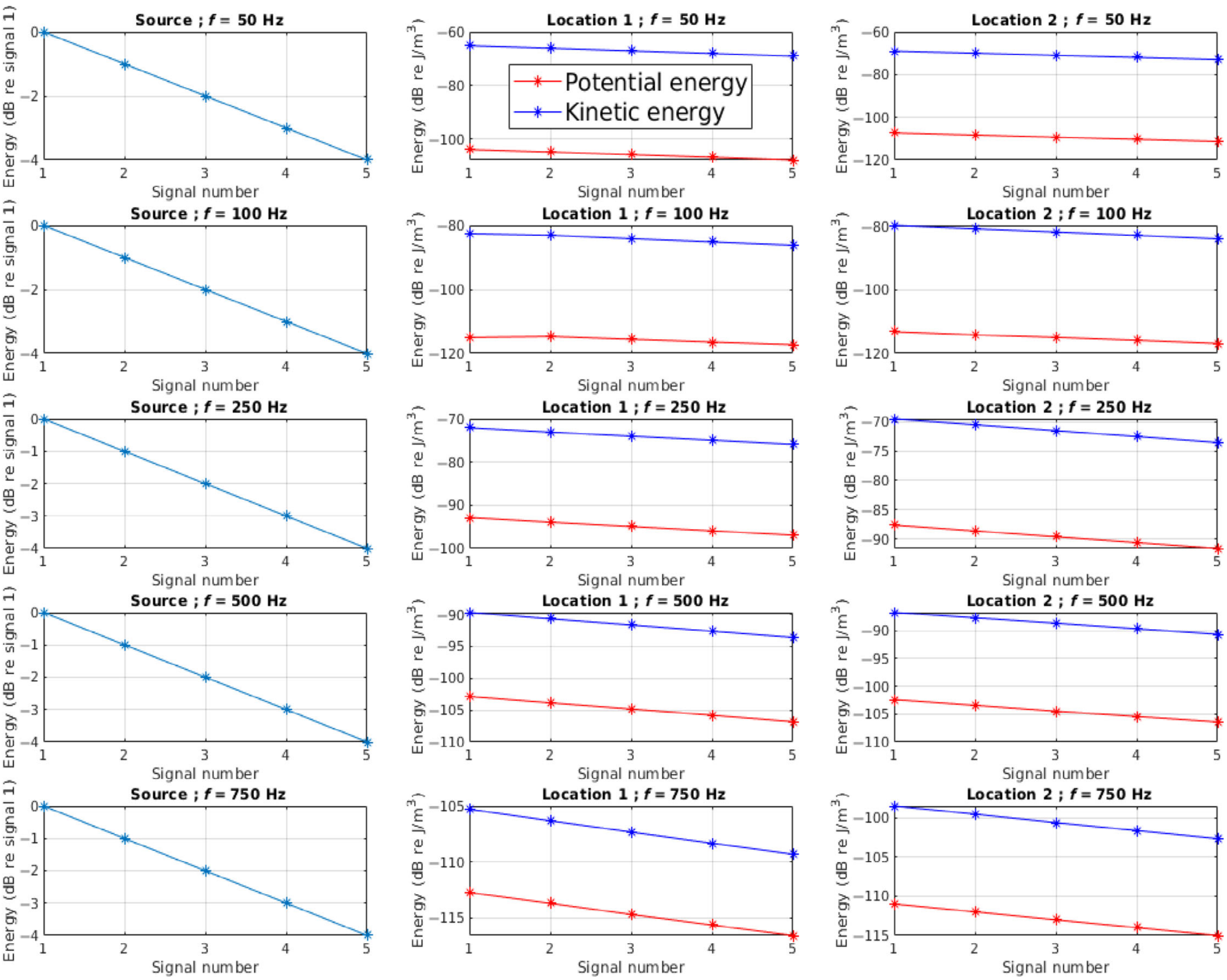


Fig. 4. Signal energy at the source (left graphs) and received at two different positions L1 (middle graphs) and L2 (right graphs) in the tank. Note that the energy of the source signal is given on a dB scale referenced to the energy of the first signal.

mean values in the six associated cylinders as well as SEL calculated on 24 h are detailed in Table 3. Based on the original *in situ* sound recordings, we hypothesized of *in situ* emitting source levels of either 172 dB (Drilling D0) or 221 dB re 1 μPa (Pile-driving P0) and of losses of propagation of 15 * Log_{10} (distance in m) (intermediate model between spherical and cylindrical models of TL), the estimated distances from the source for drilling treatments corresponded to ~ 21,500, ~ 1000, and < 1 m for D1, D2, and D3 treatments, respectively. For pile driving treatments, distances corresponded to ~ 78,500, ~ 3900, or 169 m for P1, P2, and P3, respectively. Note that P3 and D3 levels correspond to the maximal attainable values that the AQ339 speaker can produce.

Survival and growth rates

Survival rates of scallop postlarvae were very high in both pile driving (mean 99.18% ± 0.13%) and drilling (mean 98.21% ± 0.19%) experiments. No significant difference in survival rate was detected between the four conditions of each sound ($p = 0.77$ and $p = 0.41$ in pile driving and drilling experiments, respectively), without any diet effect ($p = 0.22$ and $p = 0.72$ in pile driving and drilling experiments, respectively). Daily growth rate (GR) of postlarvae was significantly different ($p = 0.043$) between the two diet conditions in the pile driving experiment. GR was 7.2% higher in the DTCS+N group (mean 5.07% ± 0.21% d^{-1}) than the DTCS-N group (mean 4.73% ± 0.20% d^{-1}) (Fig. 7). In contrast, GR was not affected by diet ($p = 0.49$) in the drilling experiment. Significant differences ($p = 0.022$) were

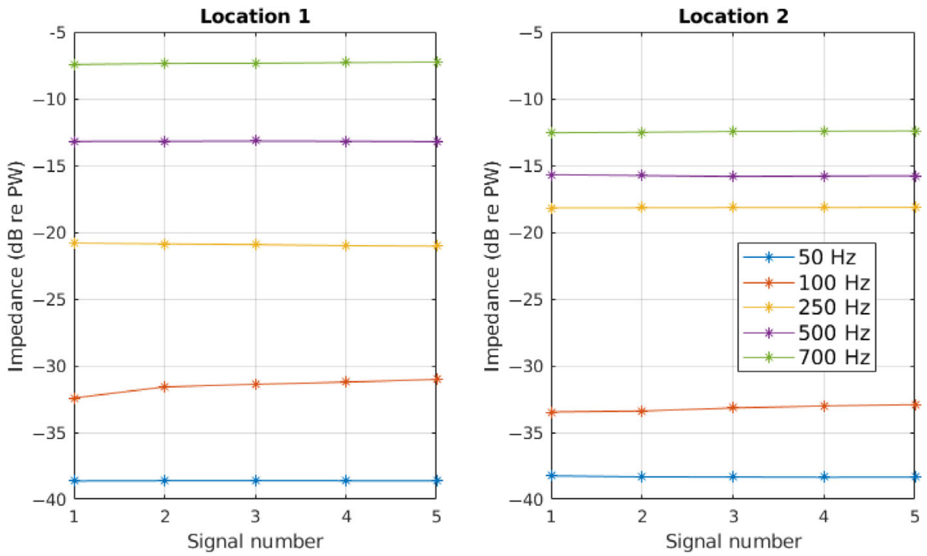
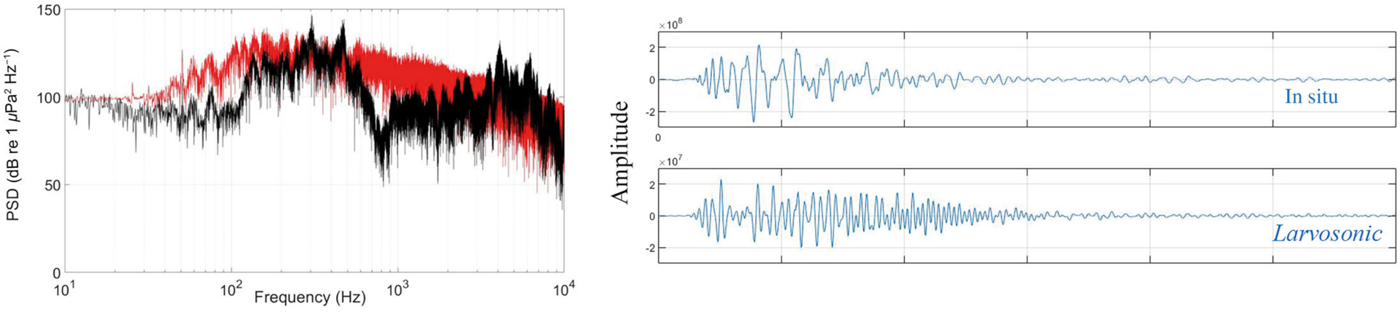


Fig. 5. Acoustic impedance as measured at two different positions in the tank. Note that the impedance is given in dB relative to Plane Wave propagation, that is, 0 dB means that PE = KE, as is the case for a plane wave.

(A) Pile driving



(B) Drilling

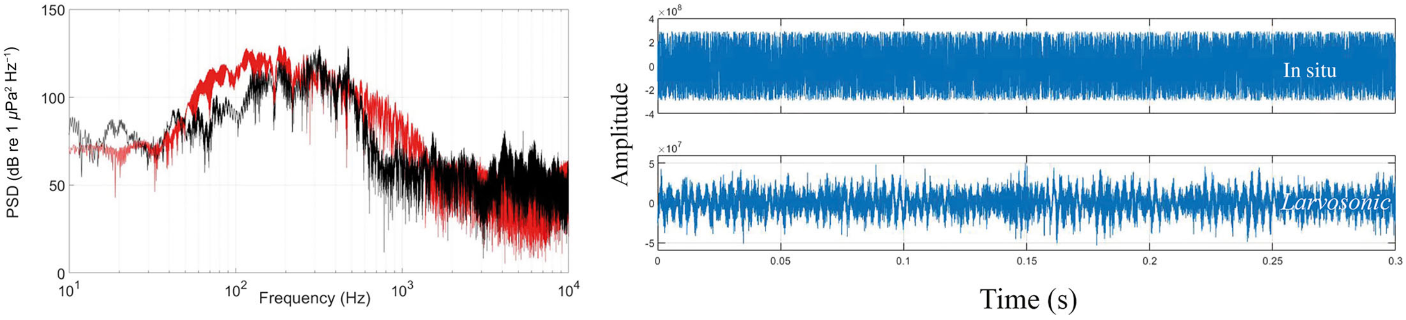


Fig. 6. PSDs of *in situ* recordings (red) and recorded at the center of the *Larvosonic* system (black) as well as time course during (**A**) pile-driving or (**B**) drilling sound emissions.

detected between sound treatments in the drilling experiment. D3 GR (mean 3.94% ± 0.26% d^{-1}) was 30.6% higher than C and D1 conditions (mean 3.01% ± 0.18% d^{-1}) with an intermediate value for the D2 treatment (mean 3.61% ± 0.26% d^{-1}) (Fig. 7). In contrast, no sound effect was found for the pile driving experiment ($p = 0.158$).

Total content and relative contributions of fatty acids in larvae

A total of 47 fatty acids were detected in our samples, with 18 of them reaching more than 1%. Collectively, these fatty acids contribute to 91.95% of the total fatty acids (Supplementary Material S5). Dominant fatty acids were 16:0 (14.50% ± 0.44%), 22:6ω3 (12.67% ± 0.69%), and 20:5ω3 (11.90% ± 0.40%). We also detected elevated proportions of 16:ω7 (8.39% ± 0.19%), 18:1ω7 (7.69% ± 0.17%), 18:0 (6.19% ± 0.15%), 18:1ω9 (5.52% ± 0.09%), and 14:0 (5.24% ± 0.16%). The Permutational multivariate analysis of variance (PERMANOVA) did not show any significant difference between fatty acids profiles for sound ($p = 0.9777$) or diet ($p = 0.5475$), but indicated strong differences ($p = 0.0021$) between the reference group collected the first day of the experimentation and the tested group collected 4 d after. A Similarity percentage procedure (SIMPER) analysis revealed that the fatty acids responsible for 50% of the difference between the two groups are 22:6ω3, 18:4ω3, 20:5ω3, 16:0, and 18:0 (*see* Supplementary Material S5). When considering the total fatty acid concentrations, pile driving sonorous and control groups do not differ ($p = 0.08731$; mean $= 274 \pm 7$ mg g^{-1}). However, drilling had a negative impact ($p = 0.015$) on total fatty acid concentration with mean value for control (317 ± 10 mg g^{-1}) significantly larger than the mean value for drilling (280 ± 5.6 mg g^{-1}). The PERMANOVA analysis did not show any significant impact of the diet on total fatty acid concentration ($p = 0.6283$; mean $= 286 \pm 6$ mg g^{-1}).

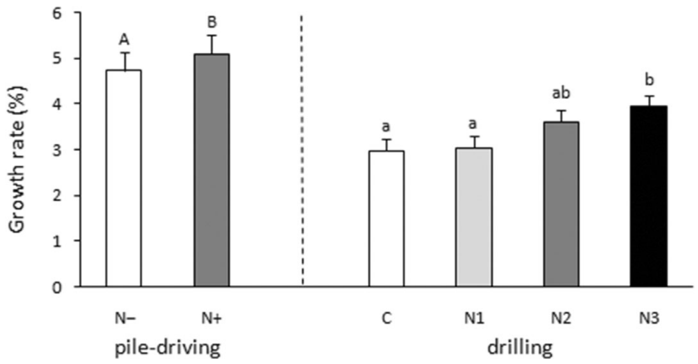


Fig. 7. Mean GR (% d^{-1}) of postlarvae exposed to two diet (with + or without – *Nannochloropsis*) or four intensities of drilling (C is the control without sound emission, N1 received a sound level like heard at 2500 m of drilling, N2 correspond to 250 m and N3 to 25 m). Groups annotated with the same letter do not differ significantly at p-value <0.05.

Discussion

We here propose a new laboratory tool, the *Larvosonic* system, to quantify the effects of anthropogenic noise on larval and peri-metamorphic stages of marine invertebrates subjected to accurate acoustic conditions. The development of such a standardized experimental mesocosm is necessary for comparison and translation of results arising from different marine laboratories worldwide. To decipher the complex processes involved in the recruitment of benthic invertebrates, ecologists in the early 1960s developed experimental approaches in the field, studying settlement of barnacle larvae through the use of panels deployed on rocky shores (Crisp and Barnes 1954), and in the laboratory, and studying settling larvae of bivalve in tanks (Bayne 1969). For more than half a century, a plethora of scientific articles has been produced on the various environmental abiotic and biotic cues involved in pre-settlement, settlement and postsettlement processes (but *see* Olivier et al. 2000 for references on *Amphibalanus amphitrite*).

Previous experimental work to assess noise impact on larval stages of invertebrates

With the exception of the sole work of Branscomb and Rittschof (1984) who tested the influence of narrow-band low-frequency sound on cyprid settlement, research conducted in the laboratory that focuses on the impact of natural or anthropogenic sounds on recruitment has been published only recently, beginning in the 2010s (but *see* details in Supplementary Material S1). Our analysis of the listed studies

Table 3. Summary of the resonant frequencies observed on the chirp (Fig. 2) recorded in the *Larvosonic* system, and associated theoretical resonant frequencies obtained from Novak et al. (2018) theoretical model.

Notation	Mode	Observed frequency (Hz)	Theoretical frequency by Novak (Hz)
f_1	(1,1,1)	1435	1436
f_2	(2,1,1)	1679	1713
f_3	(1,2,1)	1920	1970
f_4	(3,1,1)	2050	2095
f_5	(2,2,1)	2123	2181
f_6	(1,1,2)	2343	2357
f_7	(3,2,1)	2455	2492
f_8	(1,3,1)	2628	2630
f_9	(2,3,1)	2790	2790
f_{10}	(3,3,1)	3021	3040

revealed several similarities in the experimental designs adopted that could induce potential bias and thus restrict any generalization of the results to natural populations in the field. One obvious problem is that replicates of larval samples consist of small-volume (< 100 mL) containers—flasks, petri dishes, or even tissue culture plates filled with filtered seawater arranged in water baths systems of various dimensions. As a result, larval samples used for experiments do not exceed 100 individuals and often are limited to less than 10 (sometimes limited to one), clearly restricting the possibility of fatty acid analyses for most invertebrate species requiring a minimum of biological material. For hatching larval stages of cephalopods, however, the size is much larger during the pelagic phase (mantle length up to 9 mm for *Loligo forbesii*; Villanueva et al. 2016). In such case, the *Larvosonic* system would be appropriate to test individual responses to sound, including behavioral and lipid/physiological analyses. Few works include acoustic measurements where the hydrophone is placed within the experimental container, except Guo et al. (2012), who measured the pressure 0.5 mm above the bottom of a 20-mm diameter glass vial through a 1-mm diameter needle hydrophone (Precision Acoustics). However, the considered frequencies (23, 63, and 102 kHz) were such that the wavelengths (about 65, 23, and 15 mm) were smaller than the water vial. In this context, assuming an acoustic point source, the sound field is known to decay exponentially from the source (Rogers et al. 2016), so that the acoustic dose received by an animal depends heavily on his location within the vial. For a more complex sound system, as seems to have been used by Guo et al. (2012), the variability of the sound field within the vial remains an open question. To avoid density-dependent effects that express a threshold above 1 larva mL^{-1}, some authors have increased the volume of experimental containers to 250–750 mL, a compromise between the number of replicates and the space available in the water bath (Pine et al. 2012, 2016; Stanley et al. 2012, 2015) and also a method to investigate responses of large larval populations to sound (Jolivet et al. 2016). Again, recording sound in the experimental containers is not common and most studies provide only sound characteristics of the main water bath tanks without any detail on the spatial heterogeneity that could occur in the containers (*see* Supplementary Material S1 for details). At the extreme range of volume of experimental containers, the studies of Solé et al. (2018), which are based on the protocol detailed in Solé et al. (2013), involve three tanks of 2 m^3. One tank maintains a batch of test organisms (adult stages of either cephalopods or Scyphozoan medusae); one tank is used for exposure of the same whole batch to sound; the last tank allows sequential sampling of the exposed individuals to assess latent impacts of sound on statocyst sensory epithelium. We note that such an experimental design based on a limited number of large tanks generates pseudo-replication (Hulbert 1984) because independence between replicates is not satisfied (here sequentially samples of several adults located in the last tank). Using a water bath experimental design, the *Larvosonic* system satisfies a true independence between replicates and the much larger volume of the containers (5-L cylinders) allows the use of large batches of larvae (up to 50,000 individuals) as well as measurements within the experimental containers, while limiting reverberation and spatial heterogeneity of sound emission between them. Care should, however, be taken during experiment to compare noise treatments with a level difference higher than 3 dB to avoid bias related to intercylinder (internal *vs.* external) sound-level heterogeneity.

Larvosonic mesocosms: Less reverberation and homogeneous sound diffusion

Understanding the whole frequency performance of the *Larvosonic* system is crucial because few previous studies have claimed that acoustic impacts on fauna, including invertebrates, are nearly independent of SPLs, but rely more on the frequency content (Pine et al. 2016). Within this context, research focusing on anthropogenic noise in marine organisms requires accurate reproduction of the frequency spectra of the sound(s) from *in situ* recordings. Doing so in a tank requires an accurate model of the sound field in the tank, and/or a complete experimental characterization of the sound field. In this research, we assess the sound speed variability inside the *Larvosonic*, and demonstrate that the main features of this variability (e.g., resonant frequencies) can be predicted accurately by an acoustic model.

Whereas most bioacoustic studies have included only one tested signal (Jones et al. 2019), we used a diversity of sounds to assess acoustic responses of the *Larvosonic* system. Using a chirp sound, we identified the resonant frequencies, which were confirmed by theoretical models. Spiny lobster sound was useful to assess the temporal effect of reverberation on broadband and transient sound. In addition, both sounds were useful to highlight reverberation and the efficiency of diffusers to reduce it. Different absorptive materials have been tested for this purpose, including "horse hair" and sand or air-filled bubbles (in Popper and Hawkins 2018), but their effectiveness was largely insufficient (Rogers et al. 2016). Built-in expanded polystyrene, designed to provide absorption and multireflection of sound in air, the vicoustic diffusers and bass trap components constitute effective underwater sound absorbents that reduce the reflection of the whole frequency bandwidth and thus should be prioritized in future bioacoustic systems.

Anthropogenic impacts on P. maximus *recruits*

Survival rates of postlarvae of the bivalve *P. maximus* were very high and not influenced by any of the noise treatments during 4 d in the *Larvosonic* system. By contrast, both type and level of the emitted pile driving and drilling noises induced complex effects on postlarval growth, with interactive impacts between the trophic environment and noise level/spectra. This result is consistent with Jolivet et al. (2016), who also emphasized interactive impacts of boat noise and diet (with or without *N. oculata*) on the settlement rates of the blue mussel *M. edulis*. Interestingly, our study demonstrates that the most significant responses (daily GRs) of young invertebrate stages do not correspond to the most elevated noise levels and they do not follow a linear relationship among the three noise treatments. As suggested by Pine et al. (2012), who studied the time for megalopae of crabs *Austrohelice crassa* to reach metamorphosis, the frequency composition of anthropogenic noise was more relevant than noise levels. Our data provide evidence that (1) pile driving noise had no effect on daily rate of shell growth or total fatty acid concentrations, (2) the highest level of drilling noise (D3) significantly increased the daily rate of shell growth and diminished the total fatty acid concentration. These results demonstrate the potential contrasting impact of impulsive vs. continuous noise on benthic organisms, as shown by Solan et al. (2013) on the bio-irrigating and bioturbation behaviors of adults of the warty clam *Ruditapes philippinarum*. Further interpretation of the influence on growth and lipid content is speculative because physiological parameters and clearance rates were not monitored during the experiments.

The Larvosonic system, an efficient tool that can be improved

Characterization of the acoustics of the *Larvosonic* system has raised some limitations and overexpression of particular frequency bands that could be solved in the future. We have detected one 500-Hz band persisting for almost 0.7 s in the frequency spectrum only when all cylinders were settled in the *Larvosonic* system. This illustrates how the fine-scale geometrical shape within a tank may produce resonant frequencies lasting over a long period. By contrast, the 300-Hz band observed in the *Larvosonic* system disappeared when we made recordings in the pool. We do not know the origin of this band, but we hypothesize it could be linked to an unknown vibration of the *Larvosonic*'s aluminum frame. Overexpression of the resonant frequencies could be compensated by reducing their level at the source. Also, other diffusers with various textures, materials, and thickness, as reviewed in Fu et al. (2021), could be used to further mitigate the reverberation. Our study considers a single speaker whose broadband frequency response is not flat. In the future, one speaker could be replaced by several speakers dedicated to specific frequency bands, in order to create a source emission system with a frequency response as flat as possible (as is done commonly for music speakers). Sound detection abilities in marine invertebrate larvae are poorly known compared to fish larvae (Simpson et al. 2005). Because these organisms do not possess any gas-filled organs (such as the swim bladder in fish), they likely respond to particle motion rather than sound pressure (Popper and Hawkins 2018). Given that pressure and particle motion are usually not related linearly, a careful calibration of particle motion of the *Larvasonic* system would be required to assess absolute impact threshold. With a calibrated vector sensor, we have illustrated here that a single measure of impedance is useless and can be misleading because it has to be measured at every location occupied by animals under study (here postlarvae <1 mm). The negative impedance always observed in the *Larvosonic* is a known property of the acoustic field for measurements performed in a tank when considering frequencies that are lower than the first cutoff frequency of the tank (Duncan et al. 2016; Rogers et al. 2016). Alternatively, a small number of impedance measurements can be used to calibrate a numerical model, which in turn can be used to forecast impedance in the whole tank. When not done, it is better to stay away from absolute impact threshold and to work on relative trends. Whether using pressure or particle motion, the system demonstrates how impact evolves when source level (and thus both received pressure or motion) increases by a certain amount of dB: both pressure and particle velocity decrease by exactly the same amount. As a result, pressure-only measurement can be used to assess impact trends—even for animals that exclusively sense particle motion. As pointed out by Hawkins et al. (2020), there is actually no standardization in the bioacoustics community "both from the perspective of acoustic setup and behavioral methodology." We believe that future research focusing on the impact of noise pollution on marine invertebrates can be enhanced by improving the experimental designs (using dedicated mesocosms, avoiding pseudoreplication) so that acoustic conditions experienced in nature by young stages can be replicated accurately. The careful determination of impact threshold in terms of particle motion requires (1) the use of calibrated vector sensor (which are difficult to procure), (2) a full acoustic characterization of the mesocosm under study (which can be done using a combination of empirical measures and numerical models), and (3) a method to assess the variability of larval responses to sound, for which the *Larvosonic* system could be used. A set of four *Larvosonic* system allowing multiple experimental designs can be built in less than 2 months by companies using polymethyl methacrylate (PMMA) material for construction applications. Its costs of ~ 20 k€ make it constitute a quite affordable solution for multiple experimental projects conducted on several years. When possible, we also recommend to develop in parallel to *Larvosonic* studies additional *in situ* experiments with 'natural' anthropogenic sources as the time course of some very fast impulsive noises may be poorly simulated by the actual characteristics of underwater speakers.

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Conflict of Interest

None declared.

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