Sensitivity of the mussel *Mytilus edulis* to substrate-borne vibration in relation to anthropogenically-generated noise

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Abstract

Many anthropogenic activities in the oceans involve direct contact with the seabed (for example pile driving), creating radiating particle motion waves. However, the consequences of these waveforms to marine organisms are largely unknown and there is little information on the ability of invertebrates to detect vibration, or indeed the acoustic component of the signal. Here sensitivity of the marine bivalve *Mytilus edulis* to substrate-borne vibration was quantified by exposure to vibration under controlled conditions. Sinusoidal excitation by tonal signals at frequencies within the range 5 – 410 Hz was applied during the tests, using the 'staircase' method of threshold determination. Thresholds were related to size and to seabed vibration data produced by anthropogenic activities. Clear behavioural changes were observed in response to the vibration stimulus. Thresholds ranged from 0.06 – 0.55 m s^{-2} (acceleration RMS, root mean squared), with valve closure used as the behavioural indicator of reception and response. Thresholds were shown to be within the vibrations measured in the vicinity of anthropogenic operations such as pile driving and blasting. The responses show that vibration is likely to impact the overall fitness of both individuals and mussel beds of *M. edulis* due to disruption of natural valve periodicity, which may have ecosystem and commercial implications. The data here provide a valuable first step to understanding the impacts of such vibration upon a key coastal and estuarine invertebrate which lives near industrial and construction activity, and illustrate that the role of seabed vibration should not be underestimated when assessing the impacts of noise pollution.

Key words: substrate-borne vibration, anthropogenic noise, seismic energy, mussels, sensitivity threshold, marine energy.

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Introduction

Sound energy travels as a longitudinal (compressional) wave, alternately compressing and rarefying the particles across the medium (pressure), and causes an oscillation of molecules parallel to the direction of travel (particle motion) (Van der Graaf et al. 2012). For an underwater sound source encountering a solid, the particle motion may disperse not only via the water column, but also by the substrate (Hazelwood 2012, Hazelwood & Macey 2015) hence causing ‘water-borne’ and ‘substrate-borne’ particle motion. Once in the seabed, the energy may be propagated as longitudinal (compressional ‘P’ waves), shear (transverse, ‘S’ waves), or surface (Rayleigh, ‘ground roll’) waves (Markl 1983, Aicher & Tautz 1990, Hazelwood & Macey 2015), with energy being transmitted in one or multiple waveforms depending on the substrate boundary layers, and connection to the substrate (for a review see Aicher and Tautz, 1990). For Rayleigh waves, the energy is confined to the surface of the seabed and the waves are likely to propagate for large distances from source (Hazelwood & Macey 2015). Energy in the substrate may also re-enter the water column at high levels, at large distances from the original source (Popper & Hastings 2009). Anthropogenic activities, especially those directly in contact with the seabed such pile driving and drilling, may produce such substrate-borne vibrations. Underwater noise has been identified as a major stressor in marine systems and is subject to recent governance initiatives, for example the European Marine Strategy Framework Directive, which includes underwater energy and noise as one of its 11 descriptors against which Good Environmental Status is measured (Borja et al. 2013). Seabed vibration is not specifically mentioned yet the consequences of these waveforms to marine life are largely unknown; indeed there is little information on the ability of invertebrates to detect these waves in general (Roberts 2015).

Detection of substrate-borne vibration (from now on referred to as vibration) has been described in various terrestrial organisms such as spiders, snakes, lizards, scorpions and insects, reviewed in Hill (2001). Semi-terrestrial fiddler crabs (*Uca* sp.) have also been shown to be receptive to, and indeed to communicate using such substrate vibrations (Salmon & Atsaides 1969, Salmon & Horch 1973, Popper et al. 2001). In the marine environment, other crustaceans have detection systems for particle motion, which may also be used for vibration (Tautz & Sandeman 1980, Breithaupt & Tautz 1988, 1990, Roberts & Breithaupt 2015). Indeed reception, and perhaps communication, seems likely in marine invertebrates since vibrations can propagate large distances through solids, making the seabed an ideal medium for transmission, yet this area is still relatively unstudied (Hill 2001).

There are few data regarding vibration detection in benthic invertebrates, indeed the research field has not progressed greatly since the review of Frings & Frings (1967). Of the data available, reactions to unquantified vibration stimuli < 500 Hz have been observed in decapods, coelenterates, and nudibranchs (Frings & Frings 1967), but focus has predominantly been upon semi-terrestrial crustaceans which use vibration during courtship (Salmon & Atsaides 1969, Horch 1971, Salmon & Horch 1973).

There is relatively little information regarding the reception and use of vibration by molluscs, as commented upon by Markl (1983). Work has largely focussed upon the more active cephalopods, where reception to water borne particle motion has been described, with the epidermal lines on the head proposed as the analogue to the fish lateral line, reviewed in Budelmann (1988). More recently ink-jetting behaviour has been linked to sound exposure (Fewtrell & McCauley 2012). In bivalves, the specific receptors to detect acoustic and vibrational stimuli are relatively unstudied although there is some support for reception (Mosher 1972, Kastelein 2008), and of detecting particle motion rather than pressure (Ellers 1995). Responses described include siphonal retraction, closure of the valves and, in the more active Pectinids, jumping from the substrate (Mosher 1972, Ellers 1995, Kastelein 2008), although in many cases the precise levels of vibration are unspecified.

The auditory evoked potential technique (AEP) (Nedwell et al. 2007) to determine vibroacoustic sensitivity has been successfully used in the cephalopods and one crustacean species (Lovell et al. 2005, Mooney et al. 2010), although behavioural conditioning produces more accurate thresholds (Ladich & Fay 2013, Sisneros et al. 2015). Such behavioural conditioning is difficult in invertebrates, although has been successful in crustaceans (Offutt 1970). An alternative to this approach is to use small behavioural changes as markers for reception, for example postural changes, antenna movement and walking leg displacement are commonly used as response indicators in crustaceans (Heinisch & Wiese 1987, Goodall 1988, Breithaupt 2002), or monitoring of respiratory action as demonstrated in cephalopods (Kaifu et al. 2008).

The current study tested the hypothesis that a common intertidal bivalve, *Mytilus edulis* (L., Family Mytilidae), would be sensitive to a precise and repeatable, quantifiable source of vibration, and that the stimulus would affect behaviour. The species occurs on both sheltered and wave exposed shores (Seed & Suchanek 1992), creating biogenic reefs (Borthagaray & Carranza 2007) and is a common biofouling species, also of great commercial importance. The sensitivity of this species to vibration has not been previously determined but, due to a lack of any inner ear or ear-like structure, is likely to involve an array of mechanoreceptors across the body, or a statocyst, as found in other bivalves (Cragg & Nott 1977, Zhadan 2005). To our knowledge, this is undescribed for *M. edulis.* As an organism adapted to low, moderate and high energy shores, it is hypothesised that this species would be sensitive to vibrational changes.

Materials and Methodology

Experiments were undertaken in one session with mussels collected from the intertidal area of Filey Brigg shore, Filey (54° 13' 02.5"N 0° 16' 28.3"W). The animals were transported in seawater and placed directly in a glass holding tank (600 x 300 x 300 mm) with a partially sandy substrate, strewn with small rocks for attachment. Mussels were retained in natural groups until testing days and were not specifically fed for the duration of their time in the laboratory; however the seawater supply to the tank was unfiltered, therefore it is likely that some algae were present in the water, allowing some limited feeding. Valves were not cleared of external fouling organisms (e.g. barnacles), to avoid stressing the animal. One to two partial water changes were undertaken during the period in the laboratory. Subjects were given, at minimum, 72 hrs in the holding tanks prior to experiments.

Experimental setup

A shaker system was used to expose animals to primarily substrate-borne particle motion, with minimal pressure or water-borne particle motion elsewhere in the tank. External ground vibrations affecting the experimental tank (400 x 600 mm) were minimised using a purpose-built layered structure (Figure 1). A weighted steel frame, completely separate to the base, held an electromagnetic shaker (LDS v101, sine force 8.9 N, 5 – 12,000 Hz) above the tank, with a carbon fibre stinger rod descending to the substrate. The rod terminated in a plastic cap (35 mm) buried in the substrate to increase vibration propagation. At the other end of the tank an arena (100 x 50 mm) was positioned, consisting of a circular piece of plastic without a base. The arena was screened to eliminate visual disturbance. The experimental tank had a substrate of fine white aquarium sand (depth 30 mm) and a water depth of 150 mm. While mussels attach to hard substratum, this can be a shell or stone on sand. However, such an attachment was avoided here as it would have (a) required cutting the byssal attachment during the experiment, and (b) influenced the vibration received by the animal depending on the strength of byssal attachment.

Inside the arena each mussel was placed with the umbo (adjacent to the hinge margin) into the substrate and the exhalant siphon pointing upwards, and was not restrained in any way. A camera (Microsoft Lifecam web-camera in a subsea housing) was situated above the arena allowing behaviour to be monitored live, and the presentation signal to be modified accordingly. The external monitor for the camera eliminated disturbance by the experimenter.

Each mussel was acclimated in the experimental tank for 1 hour without vibration prior to threshold determination. Preliminary tests indicated that a response of an individual to vibration could be classed as full or partial valve closure (a reduction in valve gape by approximately half), hereafter termed ‘valve closure’ as the reception indicator. Additional responses were observed such as retraction of the foot, a ‘twitch’ of the valves (minor movement of the valve as if to close, but remaining open) and digging in the sandy substrate.

Shell length (maximum anterior-posterior axis) and shell width (maximum lateral axis) were measured after testing (using Vernier callipers, ± 0.1 mm), and length-width ratio was derived.

Vibration stimuli and threshold determination

Sinusoidal signals (8 second duration) with a 1 s rise and decay time to prevent distortion, were presented at 11 amplitudes (incrementally 6 dB below the maximum amplitude the shaker could produce) (Figure A1, supplemental). Seven frequencies, equally spaced across a frequency range of 5 – 410 Hz were presented. Signals were played back through a Roland R-09HR MP3 recorder, after being created in AUDACITY (open source, version 2.0.5) and exported on an SD card. The recorder was connected to the electromagnetic shaker and a car amplifier (JL Audio XD 200/2 200 W 2 channel, full range 12 – 22 kHz).

[Figure 1]

Threshold determination was undertaken using the ‘standard staircase’ method (Cornsweet 1962), which involves exposing the subject to the stimulus and choosing the next signal according to the observed response. A negative response prompted an increase of the signal and vice versa, until two amplitudes were repeatedly presented, with positive and negative responses consistent i.e. the staircase had reached a plateau. An average of ten iterations was taken to be the threshold (Cornsweet 1962), (Figure A2, supplemental). Full and partial valve closure were used as the response indicator to calculate the threshold.

A threshold value was calculated at each frequency. At a random point across each test session animals were also exposed to a ‘blank’ clip (a zero amplitude file, no vibration) to investigate the effect of the equipment itself (hereafter termed a control trial). The presentation of frequencies was randomised and an interval of 10 – 15 minutes was given between frequencies to allow for recovery. Each individual was tested at seven different frequencies at eleven amplitude levels. Amplitudes were presented 2 – 5 minutes apart, depending on the duration of response. Two mussels were tested per day, one per session (morning and afternoon) respectively. There was no indication of habituation across the tests, which typically lasted 4 - 5 hours. As such no further tests for habituation were undertaken.

Stimulus measurements and signal analysis

Vibrations emitted to the substrate were measured in the vertical axis (m s^{-2},1 k/s sampling rate) using a waterproofed Brüel & Kjær piezo-electric accelerometer (Type 4333, sensitivity 20.60 mV/g) connected to a Brüel & Kjær Charge Amplifier type 2635. The accelerometer was placed next to the arena, on the outside, throughout the experiments, as the subjects were likely to interrupt the signal if they came into contact with the sensor (Figure 1).

Since particle motion is a vector quantity, a three-dimensional geophone sensor system was used to demonstrate the relative magnitude of velocity in all three planes (m s^{-1}) (Sensor Nederland, SM-7 370 ohm, IO, 28.8 V/m/s). The geophone was adjacent to the arena on the sandy substrate and was connected to an ADInstrument Powerlab data acquisition module and an IBM Laptop with CHART software (version 5.5.6). The positioning of the geophone was such that the x axis was between the shaker stinger rod and the arena, the y axis vertical and the z axis perpendicular, across the tank. The signal was of greatest amplitude in the vertical axis (Figure 2) although at 5 Hz the z axis was slightly greater perhaps due to interference.

[Figure 2]

Data from both sensors were recorded simultaneously (and continuously) using CHART 5.5 software. All measurements were made in terms of root mean square (RMS), defined as the square root of the sum of the squared amplitude of the points. All four sensor channels were selected simultaneously allowing RMS calculations for the accelerometer and the geophone signals (x, y, z axis). Exactly 6 seconds of each signal were used for the measurements, with the 1 s rise and fall part of the signal omitted. These values were then adjusted using a correction value (calculated as the difference in

RMS between inside and outside the arena) to calculate the vibration received inside the arena, and then were averaged to calculate the threshold value for each frequency.

Spectra of the excitation signals were calculated from all time periods using a 1024 FFTs, Blackman window (1 k/s). In each stimulus frequency there was a prominent peak at the desired frequency with slight variation of signal per experimental session. It is of note that in some cases at 40 Hz there were harmonic peaks due to resonance. At the maximum these peaks were 10 – 30% of the maximum peak amplitude, as such the 40 Hz results were viewed with some caution (Figure A3, supplemental).

A six second sample of background level for each day and frequency was used and averaged to calculate average background levels (RMS) across each experimental day, for the main experimental periods. There was no significant difference between background levels of the compared periods (Kruskal-wallis, $H = 0.68$, $df = 2$, $p = 0.71$), hence the average background level across all periods (0.0074 m s^{-2}, RMS) was compared to threshold values.

At the end of the experiments, the 4333 accelerometer and the geophone data were calibrated against a type 4370 accelerometer (Brüel & Kjær, sensitivity 80 mV/g) which was used for the sole purpose of calibration, for method see Roberts (2015).

Statistical analysis

All data sets were tested for normality (Shapiro-Wilk) and log transformed as appropriate to fulfil the assumption of parametric tests. Where this was not possible non-parametric tests were used. Mussel thresholds were averaged across individuals at each frequency. Shell width (mm), length (mm) and shell length/width ratio were correlated with average threshold values (m s^{-2}) using Pearsons R correlation (m s^{-2}, RMS) (data separated according to frequency) or Spearmans Rho correlation when assumptions for parametric test were not fulfilled.

Thresholds were related to literature-derived values of vibration produced by anthropogenic vibration (measured as velocity, m s^{-1}) (Roberts, 2015; Roberts et al. *accepted*). Therefore sine wave equations were used to convert the thresholds from the current work into velocity (m s^{-1}) using the sinusoidal wave equation for amplitude:

$$A = 2\pi f V \qquad [1]$$

where A = acceleration (m s^{-2}, RMS), f = frequency (Hz) and V = velocity (m s^{-1}, RMS).

Anthropogenic vibration levels used here are given in terms of maximum peak amplitude across all axes - the axis of the maximum was not provided in the source literature and therefore it is not known which axis was predominant in the given signals.

Results

Threshold determination in M. edulis

Fifteen adult mussels, shell length 35.7 – 43.8 mm, were tested for sensitivity to sinusoidal waves at seven frequencies 5 – 410 Hz. The mussels were deemed healthy as there was no mortality and valve gape was frequent, gills and siphons were visible, and the foot explored the area sometimes leading to partial digging behaviour. Clear valve gape changes were observed in all mussels in response to the vibration stimulus, which were distinct from the valve movements during natural rhythms of feeding. No reactions were observed during control trials. Full and partial valve closure responses were frequent and clearly visible throughout the experiment. On average each mussel reacted to five out of the seven frequencies tested (n = 15), regardless of individual and the day tested. Response was similar across all frequencies with an average of 12 reactions per frequency out of 15 ($\bar{x}$= 11.57, SD = 2.15) (Table 1). With regard to observer bias, given the well-defined criteria for open, closed and partially closed, there was little ambiguity in response and so an independent verification was not needed. However, all experiments were filmed for later verification if required. Furthermore, real-time determination of response was necessary given that the observer also had to control the equipment settings. Finally, there was no incentive to bias a yes or a no response, since there were no previously known threshold values to aim towards, or other results to disprove.

The greatest sensitivity to vibration was measured at 10 Hz, with an average threshold of 0.06 m s^{-2} (RMS, n = 15) in the vertical direction. Thresholds ranged from 0.06 – 0.55 m s^{-2}, with an approximately consistent level but a prominent peak (reduction in sensitivity) at 210 Hz of 0.55 m s^{-2} (RMS), (Figure 3A).

There was a significant correlation between length of mussel (mm) and average threshold value (m s^{-2}) (Pearsons r = 0.59, n = 13, $p < 0.05$, log transformed), (Figure 3B) but not between width (mm) or length/width ratio and average threshold values (all frequencies together, Pearson r = 0.50, n = 13, p = 0.08 and r = -0.002, n = 13, p = 0.10 respectively, log transformed). When the data were subdivided according to frequency (Hz), there were no significant correlations between the threshold and the morphological variables (Table 2).

[Table 1]

[Figure 3]

[Table 2]

Discussion

Sensitivity of mussels to vibrations

Sounds in the oceans are produced naturally by various abiotic sources including waves, bubbles, wind, and turbulence. In addition to this, biotic sources can include incidental feeding and communication sounds of marine organisms, hydrodynamic sounds created by shoals, and byssal thread movement of bivalves (Di Iorio et al. 2012). Detection of ambient levels of substrate-borne vibration (and water-borne in some cases) may be advantageous, for example for detection of waves (e.g. Ellers, 1995) or predators.

Here, mussels responded to sinusoidal vibratory signals in the frequency range of 5 – 410 Hz. Responses were relatively constant across all frequencies, with a prominent decrease in sensitivity at 210 Hz (0.55 m s^{-2}, RMS). A reduction in sensitivity with increasing frequency such as this has been demonstrated in crustaceans, fish and cephalopods (Salmon & Atsaides 1969, Packard et al. 1990, Hughes et al. 2014). In our study the sensitivity increased again at 410 Hz. Spectral analysis indicated that the 410 Hz was relatively 'pure' in terms of frequency composition; therefore the reason for the anomaly is not known. Alternatively, it is possible that the 210 Hz value was over-estimated by the accelerometer, possibly affected by resonance. Use of a non-contact transducer such as a laser Doppler vibrometer (e.g. Breithaupt, 2002) to measure the vibration on the mussel valve itself would have been valuable to further understand these results.

Sensitivity to vibration decreased with size (and hence weight) of the mussel. This may be caused by the higher inertia of larger mussels. Rayleigh waves will accelerate a mussel resting on the ground in the vertical axis. At a given force a heavier mussel needs more acceleration to be moved in the vertical plane than a lighter mussel. This dependence on size (or weight) is a consequence of the current experimental set up and may not be of biological significance. Naturally, mussels are attached to solid objects and depend on transmission characteristics of the solid.

There have been few studies investigating sensitivity of bivalves to vibration (Frings 1964, Mosher 1972, Kowalewski et al. 1992, Ellers 1995, Zhadan 2005, Kastelein 2008). Of these, only one provides detailed measurements of the exposure stimulus (Kowalewski et al. 1992) but focussed upon mortality of larval forms rather than responses of adults. Incomplete or undisclosed descriptions of exposures in other studies make it difficult to fully interpret the results, for example when references to sensitivity are given without data to confirm these observations. Therefore there are insufficient data with which to compare the current sensitivity results. Other studies do not provide details of the vibration stimulus in terms of amplitude, but do indicate the frequency range of reception. For example vibration sensitivity of *Cerastoderma edule* (Cardiidae) and swift scallop *Chlamys swifti* (Pectinidae) has been demonstrated in the region of 20 – 64 kHz (Zhadan 2005, Kastelein 2008); and sensitivity < 1000 Hz was shown for *Macoma balthica* (Tellinidae), *Mizuhopecten yessoensis* (Pectinidae) and *Donax variabilis* (Donacidae) (Mosher 1972, Ellers 1995, Zhadan 2005).

Whilst threshold data for bivalves are sparse, they are available for other more active molluscs, for example cephalopods (Packard et al. 1990, Kaifu et al. 2008), although these have a more complex nervous system. These studies indicate a greater sensitivity to particle motion than *M. edulis*, with threshold amplitudes ranging from 0.0003 – 1.1 m s^{-2} (water-borne particle motion,1 – 300 Hz) (Kaifu et al. 2008, Mooney et al. 2010). It is of note that the cephalopod studies use different methodologies, and water-borne stimuli rather than the vibration stimuli used here. However although cephalopods are more mobile than sessile bivalves, they are still in contact with the substrate. In general, most research focus has been upon crustaceans where threshold sensitivities are reported to be in the range of 0.002 – 0.81 m s^{-2} (20 – 1600 Hz, RMS acceleration) (Salmon & Atsaides 1969, Horch 1971, Salmon & Horch 1973, Breithaupt & Tautz 1988, Breithaupt 2002, Hughes et al. 2014), within the range demonstrated in the current work.

Due to a lack of any specific ear-like structure, two receptor systems are likely to be involved in the detection of vibration in non-cephalopod molluscs- the internal system (statocyst) and external system (superficial receptors such as mechanoreceptors on the epidermal layer) (Lacourse & Northrop 1977, Budelmann 1992), see supplemental text. Epidermal sensory cells may be stimulated by hydrodynamic and vibrational changes causing deflection of cilia cells on the body surface (Cragg & Nott 1977, Zhadan 2005) and in some cases may involve specialised abdominal sense organs (Budelmann 1988, Zhadan 2005). It is also likely that vibration travelling through the body may stimulate movement of the statocyst system (Lacourse & Northrop 1977, Ellers 1995, Kaifu et al. 2008, Mooney et al. 2010), as in other invertebrates (Budelmann 1988). However there are few data available on these systems in bivalves, (Budelmann 1992), although responses to water movements have been observed (Frings & Frings 1967), and there are statocyst descriptions for other bivalves (Cragg & Nott 1977, Zhadan 2005).

Behavioural responses and implications

In the current work, responses were clear and occurred at onset of the stimulus. The response of *M. edulis* to fully calibrated vibration sources has not been recorded previously, although responses may be similar across bivalves, where valve closure, siphon retraction and burrowing have been documented (Mosher 1972, Kádár et al. 2005, Kastelein 2008).

With all behavioural experiments involving presentation of stimuli, there is a risk of habituation. As with *Macoma balthica* (Mosher 1972), there was no evidence of habituation in the current work. Nevertheless it would be valuable to explore this further, in particular to assess the repercussions of the response, especially as habituation would not protect them from predators. Furthermore, an investigation into the response variability of the same animal *between* days would also be valuable, an aspect already explored with other invertebrates (Roberts 2015).

The closure of the valves in response to a stressor, as seen here, is a costly behaviour in terms of energy, respiratory and heart rate disruption, and an impaired excretion ability, for example, a 3-hour valve closure has been demonstrated to halve oxygen within the shell and double carbon dioxide levels (Akberali & Trueman 1979). Since energy balance changes with feeding, respiration and excretion, scope for growth (energy balance) and body condition index (longer nutritional and energetic status) are also likely to be affected by valve movement changes; such changes have been demonstrated in response to other pollutants (Widdows et al. 1984, Widdows et al. 2002, Mazik et al. 2013). It is therefore possible that the valve closures exhibited here could affect the overall fitness of the individuals eventually leading to population effects (Widdows et al. 1984) although these aspects, and those in relation to synergistic effects of stressors (Mazik et al 2013) require further study.

Relation to anthropogenic vibration levels

The frequency range tested in the current work (5 – 500 Hz) was chosen since energy of key anthropogenic acoustic signatures is concentrated at low frequencies (Nedwell et al. 2003) as are many natural sounds (NRC 2005). The frequency range of such energy within the substrate is also likely to be < 100 Hz (Subacoustech Ltd., *unpubl.,* Roberts et al. - *accepted*). In terms of vibrations, the longer wavelengths of low frequencies are likely to propagate further and therefore are perhaps more likely to be present close to and at greater distances from anthropogenic operations. However, the lack of published field information makes it difficult to relate thresholds to actual values of anthropogenic signals (Hazelwood & Macey 2015). Due to the complexities of underwater sound measurement, many studies only measure sound pressure, without considering water-borne particle motion, or indeed the energy in the seabed. Anthropogenic activities that specifically contact the seabed are of most relevance to the current work - for example pile driving or those which intentionally produce high levels of substrate vibration, which produce vibrations as compressional, Rayleigh and shear waves (Athanasopoulos & Pelekis 2000, Thandavamoorthy 2004, Hazelwood 2012, Hazelwood & Macey 2015).

The threshold of sensitivity determined here ranged from 0.00005 – 0.002 m s^{-1} (RMS) after conversion to velocity. The sensitivity data here fall within levels measured near to anthropogenic operations, indicating that the mussels are able to detect such stimuli and show behavioural responses. For example, vibrations measured at 296 m from blasting are greater than the threshold range (6.25 kg charge weight) (Edwards & Kynoch 2008), indicating that the energy would be detectable and would be likely to elicit a behavioural change. Similarly, vibration levels at 35 m from pile driving (0.9m diameter pile, muddy substrate, 5 – 50 Hz, Subacoustech *unpubl.*) are several orders of magnitude higher than the threshold levels, as are those at 22 m from impact drilling (Parvin & Brooker 2008), indicating they would be detectable by mussels at considerable distances from the source. Vibrations measured at 45 m from auger piling (0.75 m diameter auger, 30 m deep) (Parvin et al. 2007) also fall well within the threshold range. Details of the measurement procedures for the above data are described in Roberts *et al.– accepted,* and summarised in Roberts (2015), alongside additional measurements. Close to source, strong vibrations probably elicit stronger behavioural changes, and perhaps injury, although the threshold for damage was not investigated here and that there is a lack of published vibration data for comparison. It is of note that a stimulus does not need to be ‘detected’ to cause damage to an organism, nor does detection necessarily always elicit a response.

Levels of vibration produced by man-made operations will vary significantly according to, for example, the sea bed composition, type of source and environmental parameters (Thandavamoorthy 2004). Therefore whilst the data here indicate potential detection at specific distances from various source types, actual detection would be scenario-specific. Impulsive signals such as pile driving and seismic surveys additionally produce a water-borne particle motion and a sound pressure component which were deliberately not replicated in the current work as they would confound the observed responses due to interacting factors. Furthermore, the noise from some activities which do not have specific contact with the seabed (such as shipping) may also produce seismic waves in the seabed after propagation through the water (Hazelwood 2012) and therefore be relevant, although levels of these are relatively unknown. The current methodology could be adjusted to incorporate other vibration stimuli, including different anthropogenic recordings, and variation in pure tones such as the duration of the signal. Repeating the tests in the natural environment would also be valuable.

The lack of information is important given the inclusion of underwater noise and energy as an environmental pressure in the OSPAR convention (guiding international co-operation for protection of the North-East Atlantic) and within the European Marine Strategy Framework Directive (Van der Graaf et al. 2012). These aim to set levels of sound exposure to protect marine species and Good Environmental Status (Borja et al 2013) even though seabed vibration is not mentioned specifically. By collating sensitivities of a key invertebrate and actual vibration data, the current work emphasises that substrate-borne vibration has a role within noise assessments, and therefore the setting of criteria for both substrate-borne and water-borne energy.

Stimulus presentation

In the current work, *M. edulis* was exposed to sinusoidal waves which were greatest in the vertical plane (horizontal waves were also present to a much smaller degree), although it is difficult to determine the wave type present without further investigation. Rayleigh waves, whilst involving circular motion of particles, excite the substrate in the horizontal and the vertical plane hence these may be most relevant to the current work (Hazelwood 2012, Hazelwood & Macey 2015). These are detectable by semi-terrestrial crustaceans such as *Uca pugilator* (Aicher & Tautz 1990), by using receptors in the walking appendages, but data for bivalves are unavailable.

Whilst the vibratory signal here was predominantly substrate-borne, it may also have had included water-borne particle motion and perhaps even sound pressure in the experimental tank. However by using a shaker directly contacting the substrate, the sound pressure and interference phenomena found in small tanks (Rogers 2015) are likely to be minimal compared to the substrate signal. The energy of the signal was predominantly in the vertical axis, but energy was also present in the other two planes, and hence the animals may have been responding to this additionally. The present experimental set up was a trade-off between allowing natural behavioural responses and creating a relevant stimulus. Further tests could involve a tank where water-borne particle motion and pressure could also be controlled allowing an investigation of acoustics as well as substrate vibration.

Conclusions

As with all vibrational and acoustical studies, the results here should be taken within the experimental context, involving a particular exposure duration, frequency range, substrate, vibration stimulus, and species. Extrapolating the results is not possible since propagation of vibration energy varies according to, for example, substrate, environment, and propagation conditions (Kim & Lee 2000). Furthermore, behavioural responses of an individual may be affected by other individual-specific cues such as energy availability, size, respiratory requirements, interactions with conspecifics and perhaps even consistent individual behaviours, reviewed in Roberts (2015). It is not known how energetically costly the behaviours exhibited in the current work were, or to what extent they would affect the long-term fitness of the animals.

The present work has provided a valuable first indication of the sensitivity of a common intertidal species which is important on an ecological and a commercial scale. The methods are fully reproducible and the vibration stimulus was described in three axes; this allows comparisons with future studies. Vibration sensitivity is important within the context of marine noise pollution due to the prevalence of activities contacting the seabed. By comparing sensitivities to field measurements, the data here demonstrate that *M. edulis* is likely to detect such vibrations and is likely to exhibit behavioural changes at levels actually produced by operations. This is highly relevant since the shallow, coastal areas occupied by *M. edulis* are also those frequently used for man-made activities, the productivity of mussel beds may be therefore affected by exposure which could have both ecosystem and commercial implications. Hence this valuable first step towards demonstrating the sensitivity of a common bivalve species to substrate-borne energy clearly illustrates that the effect of seismic waves cannot be underestimated when considering the impact of anthropogenic noise in the marine environment.

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Ethical note

The experiments of this chapter were approved by Hull University Ethics Committee (university ethics reference no. U034). There were no obvious adverse effects on the animals before, during or after experiments. After use, animals were either kept for future experiments in the Hull University aquaria, or returned to the shore. Mussels were handled as little as possible throughout the work.

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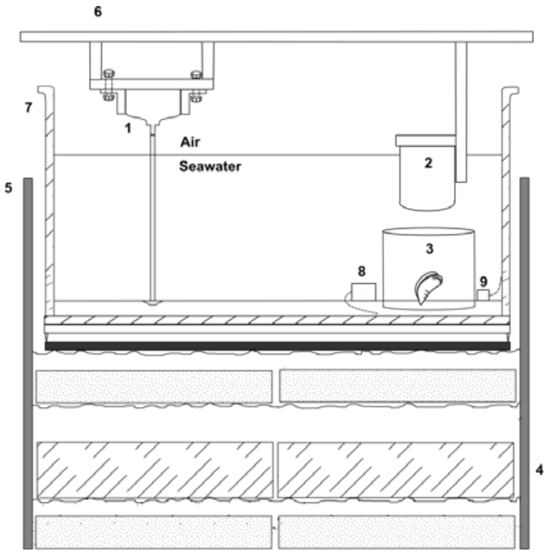

Figure 1 **Experimental setup (not to scale), consisting of electromagnetic shaker and stinger rod (1), underwater camera (2), experimental arena (3), layered base made up of mixed hard and soft insulation, acoustic dampening and concrete (4), wooden support structure (5), steel frame separate from the base (6), experimental tank with needlepoint legs and sandy substrate (7), position of geophone system (8), position of accelerometer (9).**

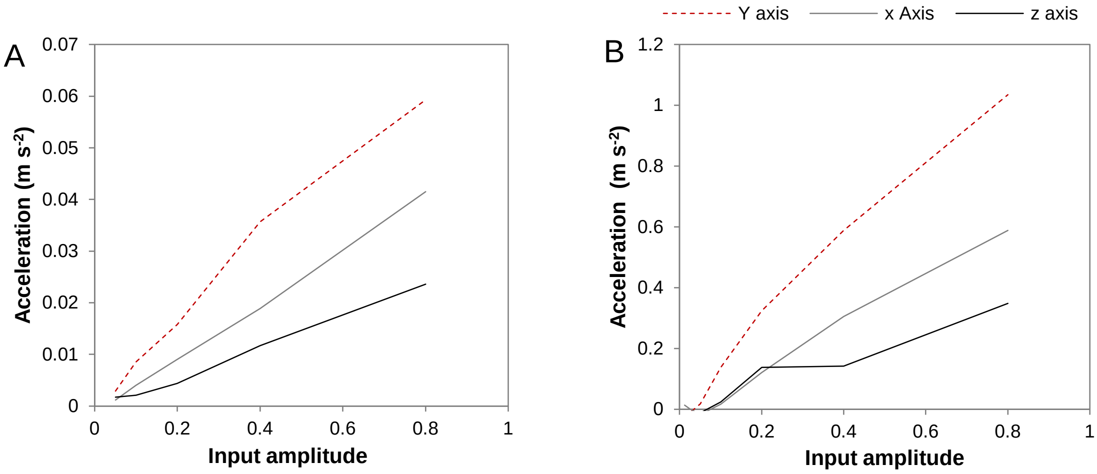

Figure 2 **The relative proportion in each axis (x, y, z; RMS) of the sinusoidal signal, for example at 40 Hz (A) and at 210 Hz (B). Maximum shaker input amplitude (x axis) is 0 dB, denoted as 1, reducing in -6 dB steps.**

Table 1 Descriptive statistics for the mussel *M. edulis* threshold experiments, with closure and partial closure used as the indicator of response (n = 15).

Frequency (Hz)	x̄ threshold (m s^{-2})	SD	Number of individuals responding
5	0.07	0.008	9
10	0.06	0.002	11
20	0.08	0.010	15
40	0.10	0.012	12
90	0.09	0.041	13
210	0.55	0.092	12
410	0.12	0.014	9

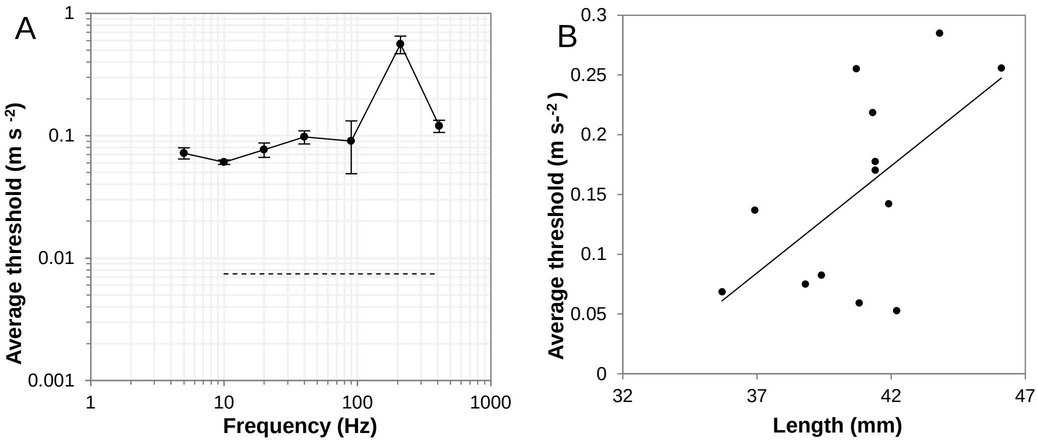

Figure 3 Sensitivity threshold (m s^{-2}, RMS, vertical plane) of *M. edulis* (n = 15 +/- SE) to substrate-borne sinusoidal vibration. Average background levels are denoted by the dotted line (A) Correlation of shell length (mm) and average threshold (m s^{-2}), (B).

Table 2. Correlation coefficients (Spearmans, ρ) between shell morphology (mm) and average thresholds per frequency (Hz) for *M. edulis.* Statistical significance is denoted by asterisks (* p < 0.05, ** p < 0.01).

Frequency (Hz)	**Length (ρ , mm)**	**Width (ρ, mm)**	**Length*width ratio (ρ)**
5	0.17	0.34	-0.14
10	0.24	0.30	-0.90
20	0.07	-0.07	0.17
40	0.03	-0.06	-.30
90	0.07	0.24	-0.20
210	-0.12	-0.57	0.08
410	0.25	0.46	-0.15