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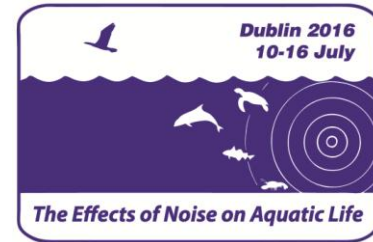
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Exposure of benthic invertebrates to sediment vibration: from laboratory experiments to outdoor simulated pile-driving

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Activities directly interacting with the seabed, such as pile-driving, can produce vibrations that have the potential to impact benthic invertebrates within their vicinity. This stimuli may interfere with crucial behaviors such as foraging and predator avoidance, and the sensitivity to vibration is largely unknown. Here, the responsiveness of benthic invertebrates to sediment vibration is discussed in relation to laboratory and semi-field trials with two marine species: the mussel (*Mytilus edulis*) and hermit crab (*Pagurus bernhardus*). Sensory threshold curves were produced for both species in controlled laboratory conditions, followed by small-scale pile-driving exposures in the field. The merits of behavioral indicators are discussed, in addition to using physiological measures, as a method of determining reception and measuring responses. The measurement and sensors required for sediment vibration quantification are also discussed. Response and threshold data were related to measurements taken in the vicinity of anthropogenic sources, allowing a link between responsiveness and actual operations. The impact of pile-driving on sediment-dwelling invertebrates has received relatively little research, yet the data here suggest that such activities are likely to impact key coastal species which play important roles within the marine environment.



1. INTRODUCTION

There is increasing evidence to suggest that anthropogenic substrate-borne energy is likely to adversely impact benthic invertebrates (Roberts *et al.*, 2015; Roberts *et al.*, 2016). One example of such an activity is pile-driving, which produces a strong vibration radiating from the tip and sides of the pile as the pile is driven into the sediment (Athanasopoulos and Pelekis, 2000). The potential impacts of this stimuli on benthic invertebrates have not been directly investigated (prior to the authors' work), although modeling (Miller *et al.*, 2016) and some preliminary playback experiments in sediment (Roberts, 2015) suggest that epifauna will be affected.

Vibration could be used in a similar way by marine species as by terrestrial animals (for a review of terrestrial vibration detection, see Hill, 2009). For example, vibration may be used by the deep sea scavenger shrimp *Pandalus borealis* to detect large falling prey items (Klages *et al.*, 2002), and it is also possible that the agonistic 'rumbles' produced by the stomatopod *Hemisquilla californiensis* are detected via the sediment (Patek and Caldwell, 2006). On the seashore, there is some evidence to indicate that bivalves and infaunal invertebrates (such as isopods and amphipods) are able to detect the footfalls and beak-probes of predatory birds (Hughes, 1970; Pienkowski, 1983). Despite this, the exact sensitivities to vibration are generally unknown for marine invertebrates (Frings, 1964; Frings and Frings, 1967). Therefore, a measure of sensitivity must first be obtained to understand the impact of high amplitude anthropogenic vibrations. Furthermore, because of the infancy of this research area, we also outline methodological considerations to investigate the impacts of sediment vibration on benthic species, from understanding their sensitivity, to investigating short-term responses. A case study is provided, using small-scale pile-driving as an example.

2. EXPERIMENTAL CONSIDERATIONS

The first consideration relates to species-specific sensitivity, in which the sensitivity threshold may be measured either by behavioral conditioning techniques (Chapman and Hawkins, 1973) or by using auditory evoked potential (Kenyon *et al.*, 1998). AEP (auditory evoked potential) has been used in the prawn *Palaemon serratus* (Lovell *et al.*, 2006), although there are now concerns over these methods compared to behavioral when measuring a whole animal response (Sisneros *et al.*, 2016). Conditioning of the animal may be used for threshold determination, for latest examples see other papers in this volume. Although crustacean conditioning has been undertaken before (Abramson and Feinman, 1990; Feinman *et al.*, 1990; Burnovicz, 2010), there is only one peer-reviewed attempt of a response to sound in this way (Offutt, 1970). The use of behavioral indicators has been more successful for vibroacoustic sensitivity determination, avoiding lengthy training procedures (Heinisch and Wiese, 1987; Tautz, 1987; Goodall *et al.*, 1990; Berghahn *et al.*, 1995; Breithaupt, 2002; Roberts *et al.*, 2016). In a similar way to fish, a sensitivity curve may then be produced to demonstrate the capabilities of the detection system across a range of frequencies (Hawkins and Chapman, 1975; Hawkins and Johnstone, 1978).

While behavioral indices are a valuable tool for quantifying sensitivity to vibration, physiological and physical observations are valuable to understand the consequences of

anthropogenic exposures, as seen in acoustic studies with fish (Knudsen *et al.*, 1992; Smith *et al.*, 2004). This allows a preliminary translation from short term to longer term, using measures of oxygen consumption, heart rate, or tissue damage, or better still an indication of fitness consequences, for example reproduction.

To understand vibration exposure levels fully, calibrated sensors (tri-axial) must be used to quantify: (1) particle motion levels within the sediment, and (2) particle motion in the water. A waterproofed geophone may be used for the former as this instrument is sensitive to low frequency vibrations. For the latter, there are many complexities of measuring particle motion in the water column, not outlined here, but considered elsewhere in this volume by other authors, and the previous proceedings volume (e.g. Martin *et al.*, 2016). It is probable that our stimulus was greatest within the sediment, but it is also valuable to understand the motion within the water column above the sediment since these animals are also sensitive to this additional particle motion. For completeness (although this may not be of importance to invertebrates; for a review see Popper *et al.*, 2001), it would also be valuable to measure water pressure, particularly in the case of measuring an anthropogenic source. This allows a link between the vibration of the signal, and the water-borne motion and pressure and so an ideal study would incorporate all three measurements.

The experimental setup must be carefully considered, both in terms of the practicalities of producing a stimulus within the sediment (e.g. real source, electromagnetic shaker, low frequency transducer for playback) and in terms of vibroacoustic propagation. The challenges of aquaria studies for bioacoustics will not be discussed here as they are outlined in detail by other authors in this volume and in previous volumes (e.g. Rogers *et al.*, 2016). Of most importance here is how these challenges relate to strong vibrations in aquaria. Low-frequency vibrational energy is likely to be confined to the sediment (e.g. Rayleigh waves; Markl, 1983, Aicher and Tautz, 1990), although motion may affect the water above both in terms of pressure and particle motion close to the sediment (Hazelwood and Macey, 2016). Reflective walls of the tank, beneath (and surrounding) the sediment, may also affect propagation of the various vibratory waves within the sediment itself, although the extent of this is not known. It seems therefore that while small-scale tank work with controlled vibration is valuable (and most practical), it is unlikely to totally represent the real field situation and hence field experiments with actual vibroacoustic sources are even more relevant and valuable where substrate-borne (and water-borne) stimuli can propagate freely.

A dual laboratory semi-field approach is outlined below for addressing the above considerations. This involved measurement of all stimuli, quantification of sensitivity in controllable laboratory conditions and exposures involving ‘actual’ sources in open water.

3. CASE STUDY

Experiments were undertaken using two intertidal marine invertebrates: the hermit crab *Pagurus bernhardus*, and the blue mussel *Mytilus edulis* (see Roberts *et al.*, 2015, 2016). These were chosen due to their coastal and ubiquitous distribution which is likely to bring them in contact with human activities, and the ease of quantification of clearly observable behaviors (e.g. valve closure, withdrawal into the shell; e.g. Elwood and Briffa, 2001). In addition, *M. edulis* is a commercial and a biofouling species, and *P.*

bernhardus a common species on the seashore. The sensitivities and responses of these species to substrate-borne vibration were previously unknown and unquantified.

Laboratory approach to determine spectral sensitivity: the sensitivity of *P. bernhardus* and *M. edulis* to vibration (5 – 410 Hz) was determined in the laboratory under carefully controlled conditions; the full methodology is outlined in Roberts *et al.* (2015); Roberts *et al.* (2016), and in more detail in Roberts (2015). At each frequency, animals were presented pure tone signals, of 11 amplitudes using the ‘staircase method’ (Cornsweet, 1962) to enable the calculation of sensory thresholds. Vibration was created using an electromagnetic shaker (LDS v101), and measured using waterproofed sensors (Bruel and Kjaer piezo-electric accelerometer Type 4333, sensitivity 20.6 mV/g; Sensor Nederland Geophone system SM-7 370 ohm, IO, sensitivity 28.8 V/m/s) allowing quantification in all three axes. Behavioral indicators were used as a measure of reception. These were defined by extensive observations during preliminary tests. Thresholds were compared to previously collected measurements of vibration measured adjacent to anthropogenic operations.

Behavioral indicators (e.g. antennal changes, movement, valve closures) allowed the calculation of sensory thresholds. On several occasions, *P. bernhardus* were seen lifting the shell from the sediment during vibration. It is particularly of note that at high amplitudes a number of individuals left their shell entirely, examined it and then returned. It is possible that this behavior was a result of misinterpreting the vibration as the shell-rapping behavior of another crab (Briffa and Elwood, 2000). Because of this, an investigation of these higher amplitudes would be valuable. Sensitivities at the detection range varied between 0.06 to 0.55 m s⁻² (root mean square, RMS, vertical plane). Thresholds were shown to be within the levels measured near anthropogenic operations such as pile-driving and up to 300 m from explosives testing (blasting). The sensitivity values were also valuable to check that field exposure levels here were well above the lowest thresholds.

Semi-field experiments to determine responses: Experiments were undertaken with the same two species in a large enclosed dock (~90 m long, 18 m wide; Fig. 1), with water depth 2 – 3 m and sediment depth 3 – 4 m (Bruitjes *et al.*, in review). At one end of the dock was a small-scale pile driver consisting of a tractor, pile and hydraulic hammer, operated every 6 s for 2 hr periods creating sound and sediment vibration. *P. bernhardus* and *M. edulis* were deployed for 30 min in subdivided cages on the sediment within the dock, with cameras mounted above for recording behavior for later analysis. Before and after deployment within the dock, *P. bernhardus* were tested for turnover time (s), defined as the time taken for the crab to replace all appendages onto the substrate after inversion (turning over the animal) (Briffa *et al.*, 2008), used as an indication of recovery after stress. Another group of *P. bernhardus* were observed for behavioral changes, such as movement, ‘flinching’ of appendages, and shell retraction during deployment. *M. edulis* were deployed within enclosed vessels allowing oxygen consumption measurements (ppm/%, HANNA instruments H19146) to be carried out pre- and post-deployment. Another group of *M. edulis* was observed for behavioral changes, such as valve closure and foot movement during deployment. Control experiments were undertaken with animals deployed within the dock in ambient conditions (no pile-driving). The pile-driving stimulus was quantified using a 3D geophone system (Sensor Nederland, SM-7 375 ohm, IO, sensitivity 28.8 V/m/s, x axis between the piles, y axis vertically and z perpendicular), and a data-acquisition system

(ADInstrument Powerlab module) with associated software (CHART 5.5). Water-borne particle motion and pressure were also measured simultaneously (HiTech HTI-96-MIN hydrophone, sensitivity -164.3 dB re 1V/mPa; tri-axial accelerometer, M20L Geospectrum Technologies; Boss recorder BR-800). The sound and vibration data were used to calculate RMS of ambient levels (RMS, m s^{-1}) and peak amplitude of the pile strikes (10 strikes, m s^{-1}), in addition to spectra (Blackman, FFTs 1024).



Figure 1. Left- Semi-field experiments were undertaken in a large enclosed dock at OREC Catapult field site, Blyth; Right- the drained dock showing the small-scale pile-driving operation at one end. Photos taken by Roberts.

In semi-field conditions, animals exhibited behavioral and physiological changes compared to control animals, such as variation in valve gape and oxygen demand in *M. edulis*. There appeared to be some behavioral variation in *P. bernhardus*, but this was not significant, and may be due to stress during deployment. Propagation of the vibration from the pile driver varied with the position of the pile; vibration propagated further (up to 30 m) in shallower water compared to deeper water (up to 15 m). The signal in the sediment was predominantly low frequency, concentrated < 100 Hz, with core energy in the region of 25 – 35 Hz. The vertical axis was the strongest component of the signal nearest the pile.

4. CONCLUSIONS

In the aquatic environment, management and consenting procedures concerning anthropogenic sources should not only consider water-borne acoustic energy but should also include the potential impact of sediment vibration. Particular operations involve strong vibration (e.g. to allow the efficient driving of a pile into the sediment) whilst also generating acoustic signals. While all forms of introduced substances and their adverse effects are covered under the Environmental Impact legislation, such as the EU EIA Directive, and under licenses to operate, this usually relates to noise and heat energy than vibration energy. For example, the EU Marine Strategy Framework Directive, requires European Member States to determine if an area is in Good Environmental status according to a set of 11 Descriptors (Borja *et al.*, 2013). However, the Descriptor 11 termed introduction of energy has mostly been interpreted as noise (Tasker *et al.*, 2010) and the fact that current legislation only covers acoustic noise should be no excuse to

avoid mitigation of vibrational stimuli. Biological impacts of such operations can still be mitigated, such as avoiding sensitive benthic areas, or particular time periods, or by addressing the source characteristics such as using ramp up.

Despite the wide diversity of marine invertebrates, vibroacoustics work to date has focused on epibenthic or pelagic species (Aguilar de Soto, 2016), and the few vibration studies have focused on crustaceans and bivalves. Of all aquatic invertebrates examined in underwater noise assessments, *M. edulis* currently appears to be receiving most attention, presumably due to its commercial relevance and its long-held role as a sentinel organism in environmental assessment, but there are many other benthic invertebrates for which responses to vibroacoustic stimuli (natural or non-natural) are undescribed (Frings, 1964; Frings and Frings, 1967; Budelmann, 1992). For example, research should encompass infaunal species such as polychaetes which have key roles within benthic ecosystems (Gray and Elliott, 2009) and are likely to be affected by vibration. It is also worth considering that damage and displacement of macro- and meio-fauna may be of great relevance for prey availability, particularly with regards to the high amplitudes of pile-driving.

The case study outlined here is an example of the process required to understand the effects of substrate-borne stimuli at present, given the lack of data in this area. The results indicate that animals are sensitive to, and respond directly to, anthropogenic stimuli propagating within the sediment. However, given the intimate links between the infauna and the sediment and the role of species in structuring the sediments (Gray and Elliott, 2009), we must also consider indirect effects on benthos in terms of habitat destruction and sediment re-sorting, for example, when assessing the impacts of these sources. The sensitivities and responses to vibration must be considered within the context of coastal marine developments and offshore activities, and also in reference to natural sources of vibration (e.g. intertidal and sub-tidal).

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