THE UNIVERSITY OF HULL

Behavioural responses by marine fishes and macroinvertebrates to underwater noise

being a Dissertation submitted for the requirements for the Degree of

Doctor of Philosophy

in the University of Hull

by

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Soundwaves Consortium (2013) Evidence project final report ME5205, Newcastle University, Loughine Ltd., Subacoustech Environmental Ltd., Institute of Estuarine and Coastal studies. *Unpubl.*

Ethical note

The experiments of this thesis 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. Animals tested in the field were unrestrained. Laboratory animals were either kept for future experiments in the Hull University aquaria, or returned to the shore. Animals were handled as little as possible throughout the work.

Abstract

The aim of this thesis was to explore and evaluate the key behavioural responses of coastal UK marine fishes and macroinvertebrates to anthropogenic noise. Work focussed upon two key aspects, water-borne acoustics and the relatively unstudied substrate-borne vibration, with a combination of laboratory and field work using grouped and solitary individuals. A literature review on underwater vibroacoustics, detection abilities, anthropogenic noise sources and the effects of such stimuli was provided (Chapter 1).

Playbacks were undertaken in the field using a purpose-built underwater transducer array capable of accurately reproducing man-made signatures (Chapter 2 – 3). The behavioural responses of wild, unrestrained schooling pelagic fish to impulsive sound were observed using an acoustic observation system. Precise exposure levels were linked to specific responses, with dose response curves produced for two pelagic species of varied hearing abilities. Baited remote underwater video (BRUV) was used to observe the behavioural responses of free-ranging individual fish and crustaceans exposed to impulsive sound and shipping noise. In both cases responses varied according to the level of sound, the type of school and the species.

In the laboratory, animals were exposed to sinusoidal vibratory signals using a fully calibrated electromagnetic shaker system. The sensitivity of unconditioned invertebrates (crustaceans and molluscs) to substrate-borne vibration was quantified with controlled vibratory exposures, allowing the production of a sensory threshold curve for three species (Chapters 4 - 5). Response variation was described in terms of two behavioural indicators, and related to consistency within individuals (personality), morphological parameters and time in the laboratory prior to tests. Further work investigated the response of sessile invertebrates to vibration, with the observations fully described in terms of response occurrence, duration and variation for both grouped and solitary animals.

The responses described in each chapter were related to actual measurements of anthropogenic noise sources in terms of water-borne and substrate-borne energy, allowing behavioural responses to be translated to actual conditions. The data here provide evidence for the levels of playback sound to induce a behavioural response, and are fully reproducible to allow further testing of the responsiveness of fish to different sound levels and signatures. Furthermore, the data are a first step towards understanding the sensitivity of benthic invertebrates to substrate-borne vibration and indicate that the effects of substrate transmission should not be overlooked when investigating the effects of noise pollution on the marine environment. The results from the current work, along with the recommendations for future work, will be important to aid the filling of the ‘information gaps’ that exist within the underwater bioacoustics field.

Chapter 1 Introduction

In recent years there has been a growing concern that man-made noise is having an ecological impact and that the implications are far reaching, affecting for example, length and pitch of bird songs (Slabbekoorn and Peet, 2003; LeFrancois *et al.*, 2009), insect prey detection (Wu and Elias, 2014) and calling of other species such as frogs (Kaiser and Hammers, 2009; Parris *et al.*, 2009). Indeed in the case of vibration, it has been said that man produces so much 'bioseismic pollution' that seismic signalling in animals is now difficult to study (O'Connell-Rodwell *et al.*, 2001). With advances of technology enabling further exploration and usage of resources, man-made noise is also having an impact upon the marine environment. The ocean has a natural soundscape with wind, water currents, earthquakes, lightning, rainfall and marine organisms all contributing to the ambient noise (Chapman and Hawkins, 1973; NRC, 2003; 2010). However fishing, exploration for gas and oil, construction, shipping, sonar and recreational activities have added to this background level. Most recently the construction of wind farms has also contributed to this (Gill, 2005; Kikuchi, 2010), with hundreds of wind turbines now operating in coastal regions offshore, and thousands to be installed in deeper water in the future (Musial *et al.*, 2004; EWEA, 2011). Indeed levels of ocean ambient sound have shown a significant increase since the 1950s (Andrew *et al.*, 2002; NRC, 2003; McDonald *et al.*, 2006). The increase, estimated to be approximately 3 dB per decade is mainly in the low frequency range of 20 – 80 Hz and has been attributed to global economic activity and the approximate doubling of shipping activity during this period (McDonald *et al.*, 2006; Frisk, 2012).

In much the same way that humans use underwater acoustics to navigate, communicate and find food, many marine organisms are adapted to do the same (Hatch and Wright, 2007). Indeed it has been suggested that sound is more important to marine species than light (Boyd *et al.*, 2011). Research into the effects of increasing noise has largely focussed upon marine mammals. Observed effects of noise on these organisms have included physiological changes such as stress, physical damage, and acoustically-induced stranding (Nowacek *et al.*, 2007; Weilgart, 2007; Rolland *et al.*, 2012). In addition to this, behavioural changes, such as avoidance of certain areas, feeding, migration disruption and decreased surface time have been demonstrated (Kastelein *et al.*, 2013). These variations are not only exhibited in mammals, negative effects upon fishes range from physical damage and physiological responses (McCauley *et al.*, 2003; Smith *et al.*, 2004) to a suite of behavioural changes (Engås *et al.*, 1996; Picciulin *et al.*, 2010). Noise pollution may also have an effect on invertebrate species (Christian *et al.*, 2003; Wale *et al.*, 2013b;a; Hughes *et al.*, 2014; Morley *et al.*, 2014) although the detection capabilities of these are still under scrutiny.

Increasing concerns about marine noise have recently led to inclusion in the OSPAR convention (guiding international co-operation for protection of the North-East Atlantic) and inclusion of noise within the Marine Strategy Framework Directive (2010). This directive aims to achieve GES (Good Environmental Status) in European seas by 2020. GES is defined by eleven descriptors, the final one being "introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment" (Borja *et al.*, 2010; 2010). Such criteria requires the setting of sound exposure criteria for marine species, however this task is complex, for example due to the wide

range of hearing abilities, source types and propagation conditions in the marine environment, and a lack of data linking specific responses with particular levels. Indeed a recent attempt to set exposure levels for fishes and turtles found that for many anthropogenic sources there were simply insufficient data to define levels which would elicit behavioural reactions (Popper *et al.*, 2014). As such there has been a call to fill such 'information gaps' (Hawkins *et al.*, 2014a) and suggestions of different ways to estimate effects without sufficient data (Hawkins and Popper, 2014).

1.1 Underwater sound

Sound can be described as the oscillation of molecules (mechanical disturbance) in an elastic medium, such as air or water (Götz *et al.*, 2009). Sound waves travel five times faster in water than in air, approximately 1500 m s^{-1} in water versus 334 m s^{-1}. This velocity is the same for all frequencies, but in water is altered by environmental conditions which affect the density of the water such as salinity, temperature and depth (Hatch and Wright, 2007). Water is an efficient medium for sound propagation due to low absorption rates and thus low attenuation.

Sound energy propagates as a longitudinal (compressional) wave, alternately compressing and rarefying the particles across the medium (pressure change, Figure 1.1B), and causing a back and forth oscillation of molecules parallel to the direction of travel (particle motion), (Figure 1.1A). Particle motion is a vector quantity and may be measured in terms of acceleration, velocity or displacement (m s^{-2}, m s^{-1}, m) in a particular direction. In contrast to this, pressure is a scalar quantity acting in all axes, measured in terms of pascals.

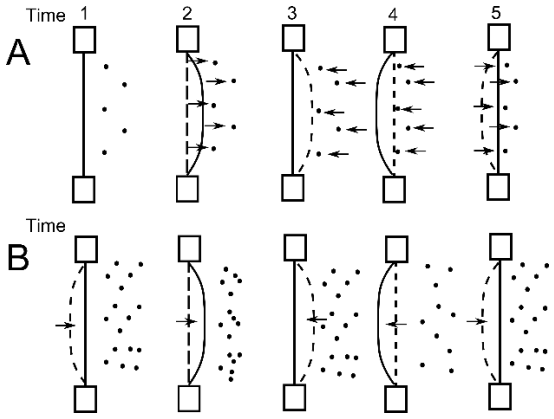

Figure 1.1 A speaker membrane producing sound. Particle velocity component, displacement of fluid particles (A), pressure, compression of nearby fluid (B), figure redrawn from Breithaupt (2002).

The frequency (pitch) of a sound wave is the number of cycles in a second measured in Hertz (Hz, one cycle per second) or Kilohertz (kHz, 1000 cycles per second). The wavelength is defined as the distance travelled in one cycle. Wavelength may be related to velocity and frequency using the following equation:

$$1.2\lambda = v/f \qquad [1]$$

Where λ= wavelength (m), v = velocity (m s^{-1}) and f = frequency (Hz), hence as frequency changes, so does wavelength, for example the wavelength of sound in water at 100 Hz is approximately 15 m. Some sounds, known as pure tones, have only one frequency, whilst others are broadband being made up of many different frequencies, known as harmonics. The frequency response of the human ear is approximately 20 Hz to 20 kHz (Richardson *et al.*, 1995), with the peak sensitivity (the ability to hear quiet sounds) between 1000 – 4000 Hz within which speech falls. In contrast, many fishes detect low frequency sounds (up to 500 – 1000 Hz, with best sensitivity between 100 - 400 Hz) (Popper, 2003), with some fishes being able to detect infrasound (< 20 Hz) (Sand and Karlsen, 1986; Sand *et al.*, 2000). The frequency of a sound can be displayed using a sound spectrum, this displays the amplitude as a function of frequency, plotted as a plot of pressure or intensity against frequency.

Measurement of sinusoidal waveforms such as sound may be given in terms of amplitude (Figure 1.2). In the case of sound, the amplitude is the change in pressure above and below the ambient pressure levels of the medium. Amplitude is measured typically as the difference between the maximum positive and minimum negative pressure of a waveform (peak-to-peak) the difference between equilibrium and maximum positive peak pressure (peak), or the root mean squared (RMS), defined as the square root of the mean of the squares of the amplitudes.

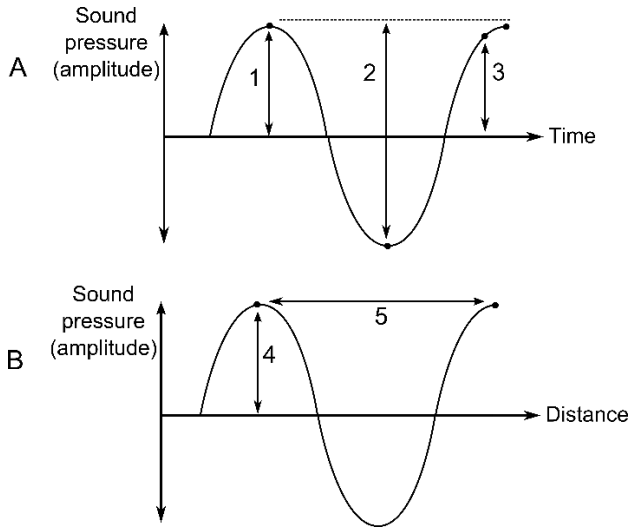

Figure 1.2 The pressure variation of an acoustic wave over time and distance (A), the waveform showing the commonly measured features of the wave (B). 1. Peak, 2. Peak-to-peak, 3. Root mean square (RMS), 4. peak amplitude, 5. wavelength (adapted from OSPAR, 2009).

In the acoustic free field where there are no physical boundaries to sound, particle motion and pressure are proportional and may be related by the plane wave equation:

$$P = pcV \qquad [2]$$

Where p is the density of water, c is the speed of the sound, V is the particle velocity (m s^{-1} first derivative of the particle acceleration m s^{-2}), P is the pressure of a sound field (Pa), pc is the acoustic impedance of the medium.

However in the area near to a source (the 'near field') or in shallow water and boundaries, the particle component is much greater than the pressure component and the sound field is variable and complex (Nedwell *et al.*, 2004; Hawkins *et al.*, 2012a). This has implications when considering experiments with sound in water, since the 'far field' is acoustically more predictable. In the near field the wave front may be described by the spherical wave equation:

$$d = \frac{P}{2\pi pcf}\sqrt{1 + (\frac{\lambda}{2\pi r}^2)} \qquad [3]$$

Where P is pressure (µbar), pc is the acoustic impedance of the medium (g cm^{-2} s^{-1},) λ is the wavelength of the sound (cm), and r is the distance from the sound source (cm). For a monopole source (the simplest type of source, radiating equally in all directions) the near field is described as being half the wavelength of the sound.

The intensity of a sound is the acoustic particle velocity and pressure component together and is the average amount of energy passing in a particular direction per unit of time, it is measured in watts/m^{-2}. The highest intensity humans can hear is greater than 10^{12} times as loud as the quietest intensity (ISO, 1961; MacLennan and Simmons, 1992). For this reason, it is expressed by a logarithmic scale in decibels (dB), called the sound pressure level scale. For example the doubling of a sound pressure would lead to a 6 dB increase in sound pressure level. The sound pressure level (SPL) of a sound is given by:

$$SPL = 20\log 10(P/_{Pref}) \qquad [4]$$

Where SPL is the sound pressure level (dB), P the measured pressure level, $Pref$ is the reference pressure level. For example 1 Pa would be 120 dB re 1µPa. To compare intensities to each other, a standard reference intensity or pressure is used. For underwater sounds this reference value is 1 µPa, whereas in air it is 20 µPa. Consequently intensity values between air and water are not directly comparable. The oscillation of particles in water (particle motion, in velocity) may also be expressed in decibel (dB):

$$PVL = 20log_{10}(\frac{u}{Pref/_{pc}}) \qquad [5]$$

Where u is particle velocity (m s^{-1}), pc is the acoustic impedance of water, and PVL is particle velocity level.

In addition to this, acoustic impedance of water is higher than air, hence sound does not traverse the air-water interface easily and is reflected back into the water column. This is an important consideration for acoustic experiments undertaken in small tanks, with numerous reflective boundaries, which make the acoustic field unpredictable and difficult to model (Parvulescu, 1964b;a; Rogers, 2015).

In the marine environment sound can travel in several ways, for example by reflecting from surface to seabed, passing sideways through rocks and by being trapped in sound channels (Nedwell *et al.*, 2004). As a result the acoustic power of the wave reduces over distance, known as

transmission loss. This occurs via refraction, reflection between the sea floor and the surface, and absorption (Harwood, 2002; Götz *et al.*, 2009). This has implications for the prediction of sound levels, and so measurements must be taken at distance from source. For single, well-defined sources, source level and transmission loss are commonly used to estimate how quickly sound levels reduce over distance (Nedwell and Edwards, 2004).

1.2 Measurement of pressure and particle motion

Underwater pressure is measured using receiving transducers known as hydrophones. These contain a piezoelectric material, such as a crystal or ceramic element, which produces an electrical voltage when compressed by the pressure of a sound wave (Götz *et al.*, 2009), which can then be amplified and measured. The electrical signal can then be characterised in terms of amplitude and frequency. Hydrophones measure the pressure of a sound wave, and from this the intensity can be calculated.

There are no current universal standards for measuring particle motion, although the ISO has recently proposed 1 pm, 1 nm s^{-1} and 1 µ ms^{-2} (ISO/DIS 1683). In the far field, water-borne particle motion may be calculated from pressure measurements using the plane wave equation, for example. However in the near field, or in complex acoustic fields with reflective boundaries, particle motion must be calculated using different models or measured. It is of note that measurements on particle motion often use the plane wave equation or the spherical wave equation which are not valid in tanks, shallow water or near the surface of the sea (SoundWaves *et al.*, 2012). There are no commercially available sensors to measure particle motion. Two solutions to the problem exist: to measure motion with the dual hydrophone method (Popper *et al.*, 2005; Zeddies *et al.*, 2010) or to use purpose-built sensors consisting of a motion sensor inside a neutrally buoyant casing (Kaifu *et al.*, 2008; Zeddies *et al.*, 2012). The implications of such sensor uncertainties are that data, where available, are stated in dissimilar units, and are taken with different methodologies making comparisons challenging.

1.2.1 Substrate-borne particle motion

In solids, such as the seabed, this energy can travel as longitudinal (compressional ‘P’ waves), transverse (shear, ‘S’ waves), or surface (Rayleigh, ‘ground roll’) waves (Markl, 1983; Aicher and Tautz, 1990; Hazelwood and Macey, 2015), with energy being transmitted in one or multiple waveforms depending on the substrate type, boundary layers, and connection to the substrate (Aicher and Tautz, 1990; Lowrie, 2010).

Longitudinal waves consist of particle oscillations in the direction of the wave propagation, these may occur in liquids and solids and comprise of compression and rarefactions of particles (Brownell, 1977; Markl, 1983; Hill, 2009a), (Figure 1.3A). Quasi-longitudinal waves (symmetric waves) are those produced by longitudinal waves at boundaries too narrow structures for example rods (Hill, 2009a). Shear waves consist of particle oscillations perpendicular to the direction of travel, and are only transmitted through solid materials since they depend on a resistance (shear force, Figure 1.3C), and travel at 60% the speed of compressional waves in a material (Markl, 1983; Aicher and Tautz, 1990; Hill, 2009a).

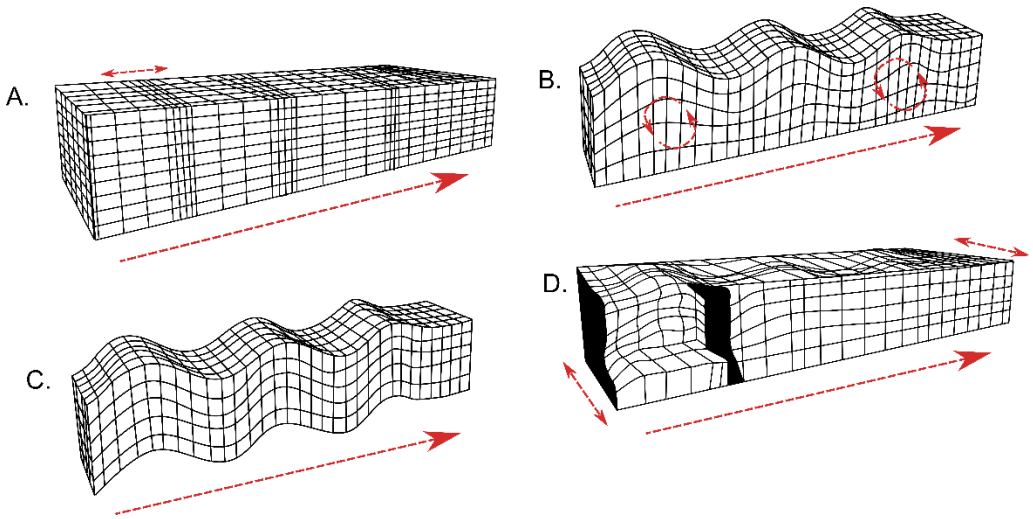

Figure 1.3 Block diagram of types of waves in a 3D medium with propagation direction marked with arrows, compressional (A), Rayleigh (B), shear (C) and Love waves (D), redrawn and adapted from Aicher and Tautz (1990) and Peterie *et al.* (2014).

Surface waves travel at the upper surface of boundaries, for example at a water-solid boundary (Hill, 2009a). There are two types of these, plate waves and Rayleigh waves (Lowrie, 2010) these are distinguished by the direction of motion and speed of propagation. Plate waves travel in materials only a few wavelengths thick; one type of these are Love waves, which travel perpendicular to the direction of travel (Figure 1.3D). Such waveforms may be found in plant stems for example (Hill, 2009a), and range from 1 – 1000 Hz with speeds of up to 19 000 kph (Garstand, 2009). Another type of surface wave, the Rayleigh wave (Figure 1.3B), travels through the surface of a thick solid substrate, penetrating down to one wavelength depth (Markl, 1983; Lowrie, 2010). The motion of Rayleigh waves is a combination of transverse and longitudinal motion, with the particles moving in an elliptical pathway, with most energy in the vertical direction perpendicular to the direction of motion (Brownell, 1977; Lowrie, 2010). Rayleigh waves are also described as 'ground roll' due to the elliptical motion, and travel 90% of the speed of shear waves, and can cause compressional waves in the water above (Hazelwood and Macey, 2015). For example, Brownell (1977) measured two types of waveform in dry sand - the first had a conduction velocity of 91 – 120 m s^{-1} greatest in the direction of travel, and the second of 40 m s^{-1} perpendicular to the surface. These were described as compressional and surface (Rayleigh) waves respectively. On land, depending upon the substrate, such waves are thought to travel up to 3800 kph (Garstand, 2009). The energy of Rayleigh waves is trapped within the surface of the transmitting substrate, with minimal absorption and cylindrical spreading losses, hence these surface waves are likely to propagate for large distances (over 1 km, possibly up to 2 km) from source, especially at low frequencies (Hazelwood and Macey, 2015). This is of particular relevance when considering anthropogenic activities such as piling and drilling into the seabed which are likely to produce large substrate-borne vibrations. It is also likely that, since this waveform may be the only wave to be detected at a distance from a source, it would be Rayleigh waves used by animals for the detection of disturbances (Brownell, 1977; Brownell and Farley, 1979; Brownell, 1984).

For a sinusoidal waveform conversions between displacement, velocity and acceleration in terms of amplitude may be undertaken using the following formulae:

$$D = (\frac{1}{2\pi f^2})A \quad [6]$$

$$v = (\frac{1}{2\pi f})A \quad [7]$$

$$A = 2\pi f V \quad [8]$$

Where D = displacement (mm) A = acceleration (m s^{-1}), f = frequency (Hz), v = velocity (m s^{-1}).

The level of substrate vibration produced by a source depends upon the solid type, the layering of the solid structure, distance from source and the type of wave propagation (Svinkin, 2004); The same applies to vibration within the marine environment. Attenuation of waveforms, being the reduction in amplitude with distance, occurs depending on the substrate type, with the extent of this occurrence varying according to the elasticity of the solid, frictional and spreading losses- because of this, the spectrum of a vibration is likely to change with distance (Athanasopoulos and Pelekis, 2000; Lowrie, 2010). For example a generalised attenuation factor for rock was calculated to be 0.39 x 10^{-3} s/m, compared to 2.05 x 10^{-3} s/m for sands (Athanasopoulos and Pelekis, 2000).

1.3 Measurement of substrate-borne particle motion

Substrate-borne vibration in the marine environment may be measured using waterproof geophone systems, which produce a voltage in response to a change in particle velocity (Lowrie, 2010). These sensors work on the principal of electromagnetic induction, with a spring-mounted inertial mass surrounded by a coiled wire, and a magnet. Movement of the magnet produces an electrical voltage proportional to the vibrational velocity of the ground. Since particle motion is a vector, these typically measure motion in one or all three axes, being vertical, the direction of the source-receiver (radial, R direction) and perpendicular to the radial (transverse, T direction) (Athanasopoulos and Pelekis, 2000). These measurements may be described separately, summed to produce a vector sum of vibration intensity, or described in the vertical plane only, depending upon the scenario (Athanasopoulos and Pelekis, 2000; Svinkin, 2004). Waterproof piezo-electric accelerometers may also be used, one for each axis of motion.

As discussed in the previous section, there are no standards for measuring underwater particle motion, either water or substrate-borne. There are standards for measuring vibration and shock regarding humans, and protection of buildings (ISO, 1997, 2010). The implications of such underwater measurement uncertainties are that data, where available, are stated in dissimilar units, making comparisons challenging.

1.4 Detection abilities of marine organisms

The detection ability of animals depends on the type of 'ear' receiving the stimuli, whether it is sensitive to pressure, particle motion, both or neither.

1.4.1 Fishes

Most fishes detect the particle motion element of sound using two sensory systems, the inner ear and the lateral line system (Fay and Popper, 2000; Popper and Lu, 2000). The fish ear varies between species but the basic components are shared. An accelerometer system is used, defined as a mass (otolith) that moves in relation to a receptor (sensory hair cell). The basic fish ear consists of semi-circular canals and sensory cristae, and three otolith organs (the saccule, lagena and utricle). The otoliths are situated next to a sensory epithelium covered in hair cells, and are overlain with a calcium carbonate mass. Each hair cell has a cilliary bundle made up of sensory hair cells that fill the area between the epithelium and the otolith. The body of the fish is of similar density to water, so that when water particles oscillate, the body itself moves. The density of the otolith is greater than the rest of the tissues which means that it moves with a different amplitude to the body, thus there is movement between the otolith and the epithelium causing flexion of the cilliary bundles. The movement of the cilliary bundles produces a nervous impulse (Popper and Fay, 2011). The sensory epithelia of the otoliths contains a variable amount of sensory hair cells, arranged in groups. It has been suggested that the total number of these groups is linked to the hearing ability of the fish for example fishes with sensitive hearing capabilities have highly specialised sensory epithelia around the head region, to detect water motion (Tasker *et al.*, 2010; Popper and Fay, 2011).

Unlike the inner ear, the lateral line system is more sensitive to low frequency sounds less than 100 Hz (Denton *et al.*, 1979), and has a low detection range. It is thought this system is most sensitive to small-scale hydrodynamic stimuli, within a few body lengths of the fish (Popper and Fay, 2011). The sensory structures in the lateral line are neuromasts, located in canals, consisting of hair cells innvervated by afferent nerve fibres (Dijkgraaf, 1963; Meyer *et al.*, 2012). There are two classes of lateral line receptor: superficial (innvervated by type I afferents) and canal neuromasts (type II afferents). These have different functions, the former being sensitive in still water, and the latter in fast water (Engelmann *et al.*, 2000). The two types help fishes to detect stimuli, for example predators, against background noise (canal neuromasts) and to achieve rheotaxis in fast water (superficial).

In some species the pressure component of a sound wave may be detected in addition to the particle motion, by using the swim bladder or an air-filled cavity. If there is a coupling (otophysic connection) between the ear and the swim bladder this signal can induce motion of the sensory epithelium and activation of the hair cells within the sacculus (Fay and Popper, 1974; Popper and Fay, 2011). The coupling may involve Weberian ossicles (specialised bones), air-filled bubbles by the inner ear, or anterior projections of the swim bladder (Popper and Lu, 2000). For example, gouramis (Osphronemidae and Helostomatidae) are likely to be sensitive to pressure due to a specialised air-breathing organ by the inner ear (Ladich and Yan, 1998; Yan, 1998).

1.4.2 Classification of fish hearing abilities

All fishes studied to date are able to detect acoustic stimuli in some capacity (Fay and Popper, 2000; Popper and Fay, 2011). However only 100 species of the known 32 000 fish species have been investigated (Hastings and Popper, 2009; Ladich and Fay, 2013), with most focus being upon the Sciaenidae (drums and croakers, known for widespread sound production).

In general, most fish have been shown to detect low frequency sounds up to 500 – 1000 Hz, with best hearing at 100 – 400 Hz (Popper, 2003), (Figure 1.4). However there is large variation between species, with some fishes such as Atlantic Cod (*Gadus morhua*) and European silver eel (*Anguilla Anguilla)* being able to detect infrasound (Sand and Karlsen, 1986; Sand *et al.*, 2000) and others sensitive to frequencies up to 5000 Hz such as the Pacific herring (*Clupea pallasii*) (Mann *et al.*, 2005). Most recently, for the purposes of defining sound exposure criteria, Popper *et al.* (2014) classified fishes into three categories in terms of hearing ability and detection mechanism. These are:

1. Fishes without a swim bladder or other gas chamber e.g. particle motion sensitive only.

2. Fishes with swim bladders which are not involved in hearing e.g. particle motion sensitive only.

3. Fishes in which hearing involves a swim bladder or gas volume e.g. pressure and particle motion sensitive.

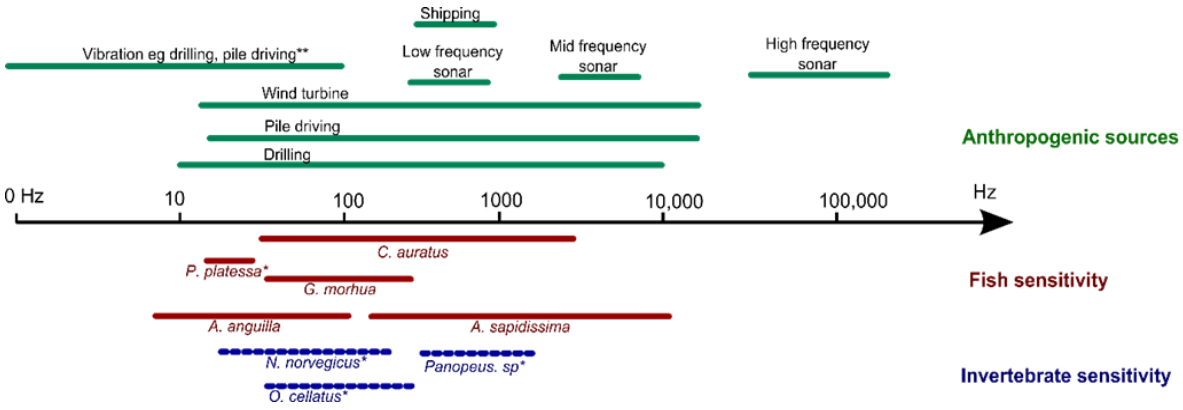

Figure 1.4 Fish and invertebrate sensitivity to sound in terms of frequency (Hz) (bottom) compared to the frequency range of typical anthropogenic sources (top). Figure inspired by and expanded from Slabbekoorn *et al.* (2010) with data of *C. auratus*, *P. platessa, G. morhua, A. anguilla, A. sapidissimia, N. norvegicus, Panopeus sp., O.cellatus* from Chapman and Sand (1974), Sand and Karlsen (1986), Goodall (1988), Karlsen (1992), Popper and Lu (2000), Sand *et al.* (2000), Kaifu *et al.* (2008), Ladich and Fay (2013), Hughes *et al.* (2014). Particle motion detection only is denoted with an asterisk (*).

Flatfish such as plaice (*Pleuronectes platessa*), dab (*Limanda limanda*), and Dover sole (*Solea solea*) are examples of category one fish. Lacking a swim bladder these species are sensitive to the particle motion of sound only (Chapman and Sand, 1974; Hawkins and MacLennan, 1975; Berghahn *et al.*, 1995; Nedwell *et al.*, 2004; Sigray and Andersson, 2011). For example sensitivity of *P. platessa* and *L. limanda* has been demonstrated in the range of 30 – 300 Hz, with peak

sensitivity at 110 – 160 Hz (Chapman and Sand, 1974; Karlsen, 1992). For fish within this category, particle acceleration thresholds are within the range of 30 – 70 dB re. 1 μm^{-2}, with best detection in the low frequency range (< 100 Hz) (Ladich and Fay, 2013).

Salmonids are an example of a category two fish, having a swim bladder thought not to be involved in sound detection (Hawkins and Johnstone, 1978). The cuckoo wrasse (*Labrus mixtus,* family Labridae), is another species with a gas bladder lacking a connection to the inner ear, with best sensitivity up to 1 300 Hz (Tavolga and Wodinsky, 1963; Schuijf *et al.*, 1971).

The pelagic fish sprat *Sprattus sprattus*, is thought to be sensitive to both pressure and particle motion due to a specialised auditory system involving an air-filled bulla next to the utricle (Enger, 1967; Blaxter *et al.*, 1981; Nedwell *et al.*, 2004). Indeed the sensitivity of clupeids has been shown to range up to 20 – 90 kHz (Ladich and Fay, 2013). These would be classed as category 3 species according to the criteria above. Gadiformes, such as Pollack *Pollachius pollachius,* Atlantic cod *Gadus morhua* and walleye Pollack *Theragra chalcogramma* are thought to have similar hearing abilities, sensitive to both particle motion and pressure due to the involvement of the swimbladder (Chapman and Hawkins, 1973; Nedwell *et al.*, 2004; Mann *et al.*, 2009). These would also be classed as category 3 species in the above list. In general, species in category 3 have a lower sound pressure threshold and respond to higher frequencies (best sensitivity at 200 – 3 kHz) than category 2 (best sensitivity at 100 Hz – 1 kHz) (Ladich and Fay, 2013).

The categories could be subdivided further for example category three could be divided into fish with a swim bladder and specialised connections to the inner ear (for example herring *Clupea harengus*, and Sciaenidae), and fish with a swim bladder without a connection (cod *Gadus morhua,* for example) (Popper *et al.*, 2014). A fifth category could be added to include fish with hearing above 20 kHz (ultrasonic) such as the American shad *Alosa sapidissima* (Mann *et al.*, 2001).

It is of note that the relationship between morphological adaptations and hearing sensitivity is not clearly defined- for example some Clupeids have a connection between the gas bladder and the inner ear, but have poor hearing sensitivity overall (Mann *et al.*, 2001). Furthermore, Ladich and Fay (2013) noted that several species had pressure sensitivity but lacked specialisations, for example the red sea bream (*Pagrus major*), European eel (Jerkø *et al.*, 1989) and the Atlantic cod (*Gadus morhua*) (Chapman and Hawkins, 1973). It was noted that for such species data were lacking, and that decisions were currently based upon opinion only (Ladich and Fay, 2013).

1.4.3 Invertebrate detection mechanisms

A number of semi-terrestrial crustacean species have been found to use vibration and acoustics (Salmon and Atsaides, 1969; Horch, 1971; Salmon, 1971; Salmon and Horch, 1973), but there is little information on the ability of marine crustaceans to detect such signals (see Popper *et al.* 2001, for a comprehensive review).

It has been conjectured that crustaceans are responsive to particle motion rather than sound pressure (Breithaupt and Tautz, 1990; Goodall *et al.*, 1990; Popper *et al.*, 2001), and thus are thought to be capable of vibration reception (Tautz and Sandeman, 1980; Plummer *et al.*, 1986; Breithaupt and Tautz, 1988, 1990; Goodall *et al.*, 1990; Monteclaro *et al.*, 2010; Roberts and

Breithaupt, 2015), Chapter 4. This is due to a lack of air filled spaces, and compressible tissue, in crustacea. These features are required to detect pressure in water, since they act as a pressure to particle motion transducer. Although evidence for this is largely still lacking, a few studies have indicated particle motion reception directly in crustaceans (Goodall, 1988; Hughes *et al.*, 2014). For example, Goodall *et al.* (1990) studied the response threshold of *N. norvegicus* by observing postural changes such as abdominal extension and claw waving. Distinct and reliable postures were exhibited in response to certain stimuli, for example abdominal extension occurred in response to frequencies of 20 – 80 Hz. The thresholds obtained were then re-tested in field conditions, and it was found that the source had to be less than a metre away (in the acoustic near field) from the subject to initiate a response. This suggests that the subjects were detecting particle motion, greater in the near field, rather than pressure. The sensitivity of the receptor systems in crustacean appear to be much less sensitive compared to fish - up to 10^5 times lower in terms of particle velocity (Fay and Simmons, 1998).

Particle motion detection in decapods is thought to involve mechanoreceptors which can be divided into three groups: superficial surface receptors, internal statocyst receptors and the chordotonal organs (Budelmann, 1992a), in the literature these have been described as hydrodynamic receptors, which includes the detection of water flow and turbulence (see Breithaupt 2002 for a review of mechanoreception) in addition to reception of vibratory stimuli that has been demonstrated in decapods (Tautz and Sandeman, 1980; Plummer *et al.*, 1986; Heinisch and Wiese, 1987; Breithaupt and Tautz, 1988; Goodall, 1988; Breithaupt and Tautz, 1990; Goodall *et al.*, 1990; Monteclaro *et al.*, 2010). The term 'superficial surface receptor' is used to describe a suite of cuticular mechanoreceptors consisting of sensory hairs, for example on the carapace, telson, chelipeds, antennal flagellae, and second antenna (Mellon, 1963; Wiese, 1976; Tautz and Sandeman, 1980; Heinisch and Wiese, 1987; Breithaupt and Tautz, 1988; Goodall, 1988). These receptors have been shown to function in a similar way to the fish lateral line (Wiese, 1976; Breithaupt and Tautz, 1988; Goodall, 1988). The principal of such hair cell receptors in similar between vertebrates and invertebrates, a mechanical displacement causes the hair to move which in turn stimulates a sensory receptor cell, such cuticular setae are found all over the body, on the antenna and second antenna and inside the statocyst (Breithaupt and Tautz, 1988; Goodall, 1988). For example movement of the mechanosensory setae in crayfish excites two neurons which respond according to the direction the hair is bent (Wiese, 1976). Receptor cells can be stimulated by solitary hairs (sensillum) or groups of hairs (hair pit organs), for example there are two types of hair cells on the second antenna of *Orconectes limosus* with varied thresholds (Tautz, 1987), and also on the chelipeds arranged in groups (Tautz and Sandeman, 1980). Similar hair cells can be found on the telson (Wiese, 1976). Such mechanoreceptors have been demonstrated to respond to water movement (Breithaupt and Tautz, 1990; Breithaupt, 2002).

Chordotonal organs in the joints of appendages, which signal joint extension and positional changes, also appear to be sensitive to vibration, perhaps incidentally (Burke, 1954; Horch, 1971; Salmon *et al.*, 1977; Barth, 1980; Aicher and Tautz, 1984; Budelmann, 1992a). For example in the propodite-dactylus joint (Burke, 1954). In semi-terrestrial crustaceans such as the *Uca sp.* this myochordotonal organ (Barths) is used in the detection of sound and vibration - this is a thin

membrane which vibrates with mechanical vibration eliciting an internal sensory receptor cell response (Horch, 1971; Salmon *et al.*, 1977). For example two genera of semi-terrestrial crabs, Ocypode and Uca, are thought to be able to detect particle motion up to 2 kHz and 700 Hz respectively using this organ (Horch, 1971, 1975). The Chordotonal organ is thought to be widespread (reviewed in Popper *et al.,* 2001), and it has been proposed that its role in substrate vibration reception varies with species for example in the ghost crabs *Ocypode sp.* it was demonstrated to be sensitive to air and substrate vibration, whereas in the fiddler crabs mainly to substratum motion (Salmon *et al.*, 1977).

Furthermore the statocyst, a fluid filled chamber with a mass loaded statolith inside, is used primarily for gravity detection (Cohen, 1955; Cohen and Dijkgraaf, 1961; Lovell *et al.*, 2006) but may also be involved in the detection of particle motion, in a similar way as in the cephalopods (Neumeister and Budelmann, 1997; Kaifu *et al.*, 2008; Hu *et al.*, 2009; Mooney *et al.*, 2010). The statocysts are widespread amongst the Malacostraca and are typically located in pairs within the basal segment of the antennule or in the abdomen (Cohen and Dijkgraaf, 1961; Popper *et al.*, 2001; Lovell *et al.*, 2005; Lovell *et al.*, 2006), and consist of a dense mass (statolith) inside a fluid-filled chamber lined with sensory hair cells (Cohen, 1955; Cohen and Dijkgraaf, 1961; Budelmann, 1992a; Lovell *et al.*, 2005). The statocyst is thought to act as a particle motion detector being sensitive to such motion in all three planes (Cohen, 1955; Breithaupt and Tautz, 1988; Nakagawa and Hisada, 1990). It has therefore been suggested that it may be involved with acoustic reception (discussed in Popper *et al.,* 2001). This statolith is made up of sand grains and other foreign materials in a mucus matrix, although in some cases this is entirely absent (Cohen and Dijkgraaf, 1961).The statolith is analogous to the otolith in the fish inner ear. Further details of the mechanism of hair cell stimulation are provided in Cohen and Dijkgraaf (1961). There are no air filled cavities in crustaceans and they are a similar density as water, therefore acoustic and vibratory stimuli travelling through the body encounter the statocyst and set it in motion. In this way the particle motion is detectable, for example movement of water currents created by predatory fish, or seismic waves.

Therefore there are a suite of receptors and organs that may be involved directly, or indirectly, with particle motion detection in crustaceans, which are particularly sensitive to low frequencies, but the extent of their involvement in the reception of higher frequencies and to sources at greater distances is currently unknown. The sensitivity of such receptors is greatly reduced compared to fishes (Chapter 4) and no structures have yet been found to indicate detection of pressure. It is likely though, that sensitivity of marine crustaceans to substrate-borne vibration would be similar to semi-terrestrial species, which are able to use substrate-borne vibration to detect conspecifics, environmental stimuli and predators (Popper *et al.*, 2001).

In other crustaceans, such as the cirripedia (Barnacles, Chapter 5), detection of vibration is likely to be similar to the postlarvae of decapod crabs, although they lack a specific statocyst-receptor. Both pelagic fish and crustacean larvae, being only a few millimetres long, are small in comparison to the wavelengths of sound in water and therefore move with sound waves, detecting motion only. In crab postlarvae otoliths and statoliths are present and act as differential density accelerometers (see Montgomery *et al.,* 2006 for a comprehensive review). The receptors used in barnacles may

be mechanoreceptors which detect differences in movement between the otolith/statoliths and their surroundings (Budelman, 1989), these may be present on the cirri for example. In addition to this, sensory structures have been found on the antennal and epidermal regions, for example in juvenile lobster (Denton and Gray, 1985; Wilkens *et al.*, 1996). These may be similar in function to the lateral line of fishes. It is likely then, that the larvae of cirripedes (as well as ascidians, braachyurans, bryozoans, hydroids and polychaetes) may use vibration as a cue (Rittschof *et al.*, 1998), and perhaps orientate and settle in response to ambient sound and vibration levels (Stanley *et al.*, 2014), although it is uncertain the frequency range that is important, demonstrated by conflicting results of (Branscomb and Rittschof, 1984; Guo *et al.*, 2011a; Guo *et al.*, 2011b). Such a phenomenon of sound as an attractant is now widely recognised for crab larvae (Radford *et al.*, 2007; Simpson *et al.*, 2008; Stanley *et al.*, 2011; Stanley *et al.*, 2014) and reef fishes (Simpson *et al.*, 2004; Montgomery *et al.*, 2006; Simpson *et al.*, 2008; Simpson *et al.*, 2010), and for bivalve larvae (Lillis *et al* 2014), see Slabbekoorn and Bouton (2008) for a comprehensive review. It is not yet known which component of the sound or vibration provides the information about the settlement site, although studies have begun to use passive acoustics to characterise such reef soundscapes (Lillis *et al.*, 2014).

The vibroacoustic reception ability of bivalvia is relatively unstudied, even less so in the lamellibranches (clams, mussels) (as in Chapter 5). It is largely understood that a range of sensory systems exists, consisting of chemoreceptors and mechanoreceptors (Olivo, 1970; Lacourse and Northrop, 1977). A statocyst, functioning principally as an equilibrium receptor (as in the crustacea), is present (Fraser, 1990; Zhadan, 2005) as found in other molluscs such as the cephalopods (Neumeister and Budelmann, 1997; Kaifu *et al.*, 2008; Mooney *et al.*, 2010), although the role of this in bivalve sound detection is relatively unstudied. Since there are no air filled spaces in such invertebrates, sound waves travelling through the body encounter the various structures, such as statocysts and cilia hairs, which then move in response. In this way these organisms detect the particle motion component of sound rather than the pressure (Zhadan, 2005). Since low frequency sound produces near field flow motion (and far field particle motion), it is somewhere in between an equilibrium stimuli and a sound. This motion is likely to be detectable by the statocyst which has been shown to develop in the late larval stage e.g. *Pecten maximus* (Cragg and Nott, 1977).

There is some evidence of such particle motion detection in bivalves, for example *Donax variabilis* was shown to respond to sounds within the near field but not the far field suggesting the detection of particle motion rather than pressure (Ellers, 1995). Although the acoustics in the laboratory environment should be viewed with caution, the authors suggest that the clams were responding to particle motion, or perhaps to compressional waves under or on the border of the sand as in scorpions (Brownell, 1984). The more active Pectinids are highly sensitive to water-borne vibrations (Zhadan, 2005), and the presence of the abdominal sense organ (ASO) is thought to be responsible, although after removal of the ASO cilia cells on the mantle and tentacle surfaces are able to detect stimuli to a lesser degree. The ASO has also been found in the subclasses Pteriomorpha and Palaerohetereodonta. In subclasses lacking the organ, similar analogies have been described (for example the cruciform sense organ in *Tellinidae* (Frenkiel and Moueza, 1980).

However, although mechanoreceptors in other bivalves such as *Mytilus edulis* have been described (Lacourse and Northrop, 1977) the extent to which these are used in reception of vibratory stimuli is unknown.

1.4.4 Classification of invertebrate detection

The lack of air filled spaces in crustaceans has led to a widespread belief that they are 'deaf' (Popper *et al.*, 2001) however whether or not this is true depends largely upon the definition of hearing used. For example, 'deaf' humans are still able to detect vibrations of sound in solids. If hearing is defined as a response to the pressure component of sound using a specialised receptor organ, then crustaceans cannot 'hear' (Breithaupt, 2002), but if hearing is defined as being able to locate a moving object then the opposite is true (Cohen, 1955). Hanlon and Budelmann (1987) define hearing as 'the ability of an animal to sense vibrations (either pressure oscillations or particle displacement) covering a wide frequency range and to integrate this information to produce an appropriate behavioural response'- they use this definition to argue that cephalopods cannot be considered deaf since they are able to detect particle motion, and to discourage theories which highlight pressure detection as the criteria for hearing. The same may apply to other invertebrates and to fish species that cannot detect pressure. It is also of note that a sound does not need to be 'heard' to cause damage to an organism.

In the crustacea and molluscs, it seems that many receptors that are not specifically designed for the purpose respond incidentally to vibration, but it is whether thresholds of these are ever reached (Burke, 1954). In any case, it could be argued that if sensitivity to an acoustic stimulus, in any form, causes a behavioural change then it is of relevance to the animal, regardless of the label assigned to the process. Perhaps more realistic then, is to agree then that these invertebrates have an 'acoustic sense' (Goodall *et al.*, 1990).

1.5 Methods of studying sensitivity

When studying sensitivity to vibroacoustic stimuli, experiments must be undertaken to determine the frequency range that an organism can detect. Behavioural bioassays are a valuable way to test for sensitivity since perception of a stimulus may often be associated with a behavioural response. The lowest level of sound for each frequency is measured is the threshold value. This threshold value is presented as an audiogram (sensitivity curve), measured in pressure or particle motion, or ideally both (Popper and Hastings, 2009). It is difficult to produce accurate audiograms, since experiments must be undertaken in a uniform and calibrated sound field equivalent to the free field; special tanks are required, and ideally would be used to separate out the particle motion and pressure components using transducers in and out of phase e.g. Hawkins and MacLennan (1975), for a comprehensive review see Nedwell *et al.* (2004). In addition to this, the work must be undertaken in conditions of low environmental acoustic and electrical interference (Nedwell *et al.*, 2004), for example in the natural environment (Chapman and Hawkins, 1973) since any increase in ambient noise level may cause an increase in auditory threshold (Hawkins, 1993; Nedwell *et al.*, 2004). Auditory thresholds may also vary with time the organism is held within the laboratory prior to tests, and with repeated exposure to stimuli, as demonstrated in fishes (Chapman and Hawkins,

1969; Knudsen *et al.*, 1992; Peña *et al.*, 2013). Threshold results may also be affected by 'personality', termed as a consistency in response within individuals, but a variation between individuals (Dingemanse and Réale, 2005; Briffa *et al.*, 2008; Dingemanse and Wolf, 2010; Briffa *et al.*, 2013).

The auditory threshold may be measured in two different ways (Nedwell *et al.*, 2007): either by behavioural conditioning technique (Chapman and Hawkins, 1973) or by using the Auditory Brainstem Response (ABR) (Scholik and Yan, 2002; Smith *et al.*, 2004). Behavioural conditioning involves training the animal to respond to a stimulus, with a mild electric shock being applied after the sound. This elicits a change in cardiac rhythm. The ABR directly measures the electrical potentials of the brain when it is stimulated by sounds (Smith *et al.*, 2004), this technique is also called AEP (auditory evoked potential) which uses electrodes upon the skin rather than in the brain (Nedwell *et al.*, 2004). The sensitivity curve presents the auditory threshold against frequency graphically and the auditory threshold is the softest sound that the animal can detect at a given frequency.

It is of note that the hearing capabilities of many fish species are reliant on audiograms produced in varied acoustic conditions and with differing methodologies, and, in many cases there is an absence of threshold data produced by behavioural conditioning, which is thought to produce more realistic thresholds (Ladich and Fay, 2013). As such, a single audiogram cannot be used as the definition of a species hearing, since it is highly context dependent. It is also now generally accepted that the two methods (AEP and behavioural) do not yield the same sensitivities and therefore cannot be treated as equivalent descriptions of auditory response (Ladich and Fay, 2013). For this reason there has been a call for measurements of hearing sensitivity, taken in terms of particle motion and pressure, within a fully characterised acoustic field. Laboratory tank studies when undertaken should be in appropriate standing wave tanks (Halvorsen *et al.*, 2011; Ladich and Fay, 2013; Hawkins *et al.*, 2014a).

Similar techniques may be applied to crustaceans, for example AEP in the prawn *Palaemon serratus* (Lovell *et al.*, 2006). Conditioning of crustaceans has also been undertaken using operant and classical conditioning (Offutt, 1970; Abramson and Fieinman, 1990; Feinman *et al.*, 1990; Burnovicz, 2010). For example Abramson and Fieinman (1990) used operant conditioning, where an organism learns to associate a stimuli with its own motor actions, to 'teach' crabs to press a lever to receive food. More appropriately, classical conditioning, where the animal associates two stimuli has been used to train *C. maenas* to associate carapace vibration with a mild air puff to the eye stalk (Feinman *et al.*, 1990). In this way the crab 'learned' to retract the eye stalks after a vibration occurred. Furthermore, Burnovicz (2010) showed that it was possible to classically condition an autonomic response (heart rate) in crabs (*Chasmagnathus granulurus*), by using a light pulse associated with a visual danger stimulus. However there is only one published attempt of a conditioned crustacean responding to sound (Offutt, 1970) due to the logistical difficulties of such attempts. Indeed, the heart rate of crustaceans is naturally irregular and may fluctuate over time, even cease, in relation to laboratory stimuli (Florey and Kriebelm, 1974). The use of behavioural changes such as postural and antennal modifications has been more successful to indicate reception of vibroacoustic stimuli (Heinisch and Wiese, 1987; Tautz, 1987; Goodall *et al.*,

1990; Berghahn *et al.*, 1995; Breithaupt, 2002). For example Goodall (1988) used postural changes such as abdominal extension in *Nephrops norvegicus* to demonstrate sensitivity to near field particle motion. In a similar way to fish, a sensitivity curve may be produced to demonstrate the capabilities of the invertebrates detection system.

1.6 Importance of sound and vibration to biological organisms

Sounds in the oceans are produced by a range of abiotic sources including waves, bubbles, wind, earthquakes, iceberg shift, spray and turbulence. In addition to this, biotic sources can include incidental feeding sounds of marine organisms, hydrodynamic sounds created by shoals, marine mammal and fish vocalisations, invertebrate and fish choruses (Richardson *et al.*, 1995; NRC, 2003). The ocean is therefore not a quiet environment (Urban, 1993), with this variety of sources producing sound from 1 – 100 000 Hz, dominated by waves at high frequencies (Wenz, 1962). It is of note that since background noise may be defined as 'background noise without distinguishable sources' (Knudsen *et al.*, 1948; Wenz, 1962) distant anthropogenic noise, such as shipping, is also included in ambient noise measurements (Blondel *et al.*, 2014).

In the animal kingdom, there are many cases where the use of vibration would be advantageous for communication, detection of environmental cues, predator-prey interactions and during courtship displays; for a comprehensive review see Hill (2001); Hill (2009b). This is due to the low attenuation rate of a solid compared to air (approximately 3 dB reduction with a doubling of distance compared to 6 dB), making it a more efficient form of energy transfer, particularly for waveforms such as Rayleigh (Hill, 2009a). This means that whilst in air, two animals would need to be within centimetres of each other to communicate seismically, in water they could be metres apart (Taylor and Patek, 2009). Animal vocalisations can be detected at considerable distance from source, for example lion roars (*Panthera leo*) with peak energy at 30 Hz have been measured seismically up to 300 m, and black rhinoceros (*Diceros bicornis*) up to 100 m (O'Connell-Rodwell *et al.*, 2001). Other environmental cues, such as thunder may also be detected in the substrate up to 1 km or further (O'Connell-Rodwell *et al.*, 2001), for example early detection of tsunamis (1 – 100 Hz) has been demonstrated in elephants (Garstand, 2009). Indeed, in times of high environmental background noise levels, for example in strong wind, use of vibration may be advantageous over acoustics. However, as wind, temperature and background noise affect acoustics transmission, so substrate type and substrate composition affect vibration transmission (O'Connell-Rodwell *et al.*, 2001).

It of note that many marine acoustic signals are also likely to elicit particle motion and pressure in the substrate as well as in the water, and therefore, whilst discussed separately here, there is considerable overlap between the two.

1.6.1 Marine acoustics as a biological stimulus

Many fish can produce sound, in addition to detecting it. For example when competing for food, attracting mates and to scare predators (Brawn, 1961; Colson *et al.*, 1998; Amorim and Hawkins, 2000; Hawkins and Amorim, 2000; Amorim *et al.*, 2004; Wilson *et al.*, 2004; Codarin *et al.*, 2009). There are three sound producing mechanisms in fishes: stridulation of body parts, use of the swim

bladder and hydrodynamic swimming sounds (Tavolga, 1971). Some of the more distinctive sounds produced by fishes involve the gas-filled swim bladder, which acts as a resonator of the sound. For example, haddock (*Melanogrammus aeglefinus*) produce a series of 'knocks' and 'hums' made of a number of pulses during spawning (Hawkins and Amorim, 2000) and cod (*G.morhua*) also produce 'grunts' using drumming muscles (Brawn, 1961). Sound may also be produced by expelling air from the gas bladder, for example in Atlantic herring (*C. harengus*) (Wilson *et al.*, 2004). Sound production in some species is so prominent that it may be used to passively locate spawning populations, for example the 'knocking' sounds to trace haddock *Melanogrammus aeglefinus* within Norwegian fjords (Casaretto *et al.*, 2014).

Sound is also produced by invertebrates such as crustaceans, from snapping shrimp (Johnson *et al.*, 1947; Knowlton and Moulton, 1963; Schmitz and Herberholz, 1998; Versluis *et al.*, 2000) to lobsters (Moulton, 1957; Patek, 2001; Henninger and Watson, 2005; Patek *et al.*, 2009), crabs (Horch, 1971, 1975; Aicher *et al.*, 1983; Field *et al.*, 1987) and even mantis shrimps (Patek and Caldwell, 2006; Staaterman *et al.*, 2011b). One relatively well-studied crustacean species that produces sound is the snapping shrimp, of the genera *Alpheus* and *Synalpheus.* These shrimp create a distinctive 'crackling' or 'frying' sound when in large numbers which is a large contribution to soundscapes in some areas (Johnson *et al.*, 1947; Hazlett and Winn, 1962). Each shrimp produces a 'snap' of only half to one millisecond in length caused by the collapse of a cavitation bubble produced by chela closure (Johnson *et al.* 1947; Versluis *et al.* 2000). Another well studied mechanism of sound production is that of the Carribean spiny lobster (*Panuliris argus*), family Pallinuridae (Moulton, 1957; Patek, 2001; Patek *et al.*, 2009). These lobsters produce a 'rasping' sound which is produced by stridulation using a 'stick and slip' mechanism (Patek, 2001). A similar stridulatory mechanism has been shown in the *Trizopagurus* genus of hermit crabs (Field *et al.*, 1987) and in the *Ocypode* (Horch, 1975).

It has been suggested that by using the range of abiotic and biotic sounds in the ocean, marine animals are able to perceive an 'auditory scene' in a similar way to our visual scene (Bregman, 1990), this has led to a newly emerging field of acoustic science, the analysis of 'soundscapes' (Slabbekoorn and Bouton, 2008; McWilliam and Hawkins, 2013). For example, sound appears to be important for larval orientation and navigation (Simpson *et al.*, 2005; Simpson *et al.*, 2008; Simpson *et al.*, 2010), and more generally for communication and foraging (Amorim *et al.*, 2004; Wisenden *et al.*, 2008). It is of note that the production of sound does not necessarily imply detection of sound (discussed in Popper *et al.,* 2001).

1.6.2 Substrate vibration as a biological stimulus

Sensitivity of terrestrial animals to substrate vibration has been widely described, with detection abilities documented, for example, in frogs (Lewis and Narins, 1985), large mammals such as elephants and rhinoceroses (O'Connell-Rodwell *et al.*, 2001), subterranean mammals (Rado *et al.*, 1987), and insects (Fabre *et al.*, 2012). Indeed, due to the lack of data on vibration use and detection in benthic marine invertebrates it is useful to consider the abilities of terrestrial species to detect and use vibration, recently reviewed in Morley *et al.* (2014).

Use of vibration for courtship is widespread in terrestrial invertebrates (Hill 2001, 2009). For example, the fruitfly *Drosophila melanogaster* creates a solely substrate-borne vibration using a 'quivering' of the abdomen during courtship (Fabre *et al.*, 2012). Similarly, the wolf spider *Schizocosa retrorsa* uses 'drumming' on the substrate (Hebets *et al.*, 2008). There is also evidence to suggest that small insects are able to localise vibration sources for the purposes of communication, for example the treehopper *Umbonia crassicornis (Cocroft et al., 2000).* Vibration may also be used for communication and co-ordination amongst large groups, for example in worker bees, thought to promote productivity (Lewis and Schneider, 2000). Seismic waveforms may also be important for prey-predator interactions, either to avoid or warn others of predators, or to locate prey (Brownell and Farley, 1979). Brownell (1977) also concluded that for animals dwelling on the surface the Rayleigh wave would become the predominant waveform at distance from a vibrating source.

Evidence for the use of substrate-borne vibration in benthic marine invertebrates such as crustaceans is lacking although, as discussed previously, there is evidence to suggest that detection of such signals is possible (Goodall, 1988; Popper *et al.*, 2001). However, in two families of semi-terrestrial crab, *Ocypode* and *Uca*, sound is produced during courtship, this has led to focus upon these species as detectors and producers of substrate-borne vibration (Salmon and Atsaides, 1969; Horch, 1971; Salmon, 1971; Horch and Salmon, 1972; Popper *et al.*, 2001), and interest in whether other crustaceans are able to use and detect such signals (Taylor and Patek, 2009). For example, species of *Uca* (fiddler crabs) produce sound by stridulation and 'rapping' of the major chela against the ground (Salmon and Horch, 1973; Aicher and Tautz, 1990; Popper *et al.*, 2001). Similarly ghost crabs *Ocypode* drum against the inside of the burrow to signal to conspecifics (Horch, 1971; Horch and Salmon, 1972; Horch, 1975). It is thought such signals are detected by Barths myochordotonal organ in the meral segment of the leg (Horch, 1971; Salmon *et al.*, 1977), which in *Uca* and *Ocypode* appears to be more sensitive (Popper *et al.*, 2001). Indeed Horch and Salmon (1972) suggested that, according to attenuation rates of approximately 6 dB per metre, *Ocypode ceratophthalmus* was likely to detect conspecifics 'rapping' upon the substrate up to 10 m. It is also likely that the crabs are able to detect the acoustic part of the signal. Due to similar receptor organs and mechanisms being present in other decapods species, it is likely that substrate-borne vibration would be detectable (Popper *et al.*, 2001), but the extent of sensitivity to such signals is largely unknown (sensitivities summarised in Roberts and Breithaupt (2015) see appendix, and Chapter 4). Other vibration producers are hermit crabs, for example terrestrial *Coaenobita sp.* and aquatic *Trizopagurus sp,* shown to stridulate (Field *et al.*, 1987; Taylor and Patek, 2009), although there is little evidence the vibration is for communication purposes.

In the molluscs, such as bivalves, there are few studies investigating the production of vibration let alone the detection of vibration (Mosher, 1972; Kowalewski *et al.*, 1992; Ellers, 1995; Zhadan, 2005; Kastelein, 2008). Indeed the lack of acoustics in mollusc life has recently been discussed (Vermeij *et al.*, 2010). There is however, some evidence to suggest behavioural responses to vibration such as retraction of siphons, digging and closure (Mosher, 1972; Ellers, 1995; Zhadan, 2005; Kastelein, 2008). In tidal areas, it would be advantageous to detect natural sources of

vibration such as predators and wave action, for example to enable closure of the valves when under threat.

The extent to which benthic and demersal fish may detect and use seabed vibration in the marine environment is relatively unknown, although particle motion detection capabilities have been described for flatfish (Chapman and Sand, 1974; Hawkins and MacLennan, 1975; Berghahn *et al.*, 1995; Nedwell *et al.*, 2004; Sigray and Andersson, 2011). It seems likely that such species would utilise vibrations to sense predators, for example, *P. platessa, Solea solea, Microstomus kitt* have been shown to hide under the sediment after exposure to sinusoidal vibration (Berghahn *et al.*, 1995).

1.7 Anthropogenic noise and vibration

Advances in technology have enabled increased use of the marine environment which has led to concern about the increase of anthropogenic noise in the ocean (NRC, 2003, 2005). For example at 25 – 50 Hz an increase of 16 dB has been demonstrated between 1950 – 2007 for the Northeast Pacific Ocean using a meta-analysis approach (Frisk, 2012). McDonald *et al.* (2006) and Andrew (2003) compared ambient noise in the deep ocean for the North east pacific west of California between the 1960s and 1994 – 200 and 2003 – 2004 respectively. It was found that ambient noise was 10 – 12 dB higher < 500 Hz in 2003, estimated to be 2 – 3 dB per decade (McDonald *et al.*, 2006), and there was a 3 dB increase at 100 Hz (Andrew, 2003). Both of these were attributed to increases in shipping in those time periods. These trends have since been related to global economic conditions and world gross product (Frisk, 2007). A linear increase of approximately 3 dB per decade was demonstrated enabling an overall equation to be suggested relating these parameters together, deemed 'noiseonomics' theory (Frisk, 2012). It is notable that there are few long-term ambient noise level data sets for other global regions. The levels of seabed vibration are relatively unknown, but are probably rising in a similar way to noise levels.

Ocean noise is produced, for example, by fishery activities, gas and oil exploration, dredging, seismic surveys, shipping, offshore renewable energy developments and recreational activities. Pile driving is typically undertaken during the building of oil and gas platforms, and construction of offshore wind farms. Foundations are constructed by driving thick piles, often made of steel, into the ground. These piles range in diameter from 3 – 12 m, with their introduction producing variable source levels (Thomsen *et al.*, 2006; Nedwell *et al.*, 2007; Götz *et al.*, 2009). There are various types of pile driving, from vibropiling (vertical vibratory force) to impact piling (double-acting hammers to impart downward force on the pile), and within these the sound levels vary with size of pile and construction material.

Seismic surveys are another common source of noise pollution and are undertaken during exploration for oil, mineral and gas resources or as part of general bathymetric surveys. Airgun arrays are commonly used for the surveys, the seismic signal produced by the airguns is reflected back by the seabed, providing details about features beneath the ocean floor (Götz *et al.*, 2009). Sound profiles of common anthropogenic noises and vibrations are indicated in Table 1.1, Table 1.2. It is of note that many sources contacting the substrate also elicit seabed vibration in addition to changes in the acoustic field.

Table 1.1 An overview of approximate source levels produced by key anthropogenic activities, as summarised from Götz *et al.* (2009).

Sound source	Source level (dB re 1µPa m)[a]		Bandwidth (Hz)	Major amplitude (Hz)	Duration (ms)	Directionality
TNT (1 – 100 lbs)	272 – 287	pk	2 –1000	6 – 21	1 – 10	
Pile driving	228	pk	20 – 20 0000	100 – 500	50	Omnidirectional
Dredging	168 – 186	RMS	34	100 – 500	-	
Drilling	145 – 190[b]	RMS	10 – 10 000	< 100	Continuous	
Wind turbine	142	RMS	16 – 20 000	30 – 200		
Small boats < 50 m	-180		20 > 10 000	> 1000		
Large vessels	180 –190		6 – 30 000	>200		
Low frequency sonar	215	pk	100 – 500	-	600 – 1000	Horizontal
Mid frequency sonar	223 – 235	pk	2800 – 8200	3500	500 – 2000	
Echo sounders	260 – 262	pk-pk	10 –100 000	10 – 120	30 – 60	Vertical

[a] nominal source

[b] higher source levels from drill ships.

Table 1.2. An overview of the seabed vibration produced by key anthropogenic activities, as summarised from S. Cheesman *Pers. Comm.* (2014)[1], Edwards and Kynoch (2008), East and Collett (2014).

Vibration source	Distance (m)	Vibration levels ($m\ s^{-1}$)		Frequency range (Hz)
Drilling	23	7.0E-3		< 100
Shell auger	70	3.7E-5		Unspecified
drilling	200	6E-4 – 1E-3		
Pile driving	17	4.1E-3	pk	5 – 50
	34	1.7E-3	pk	5 – 50
Dredging	5	3.8E-4	pk	1 – 30
	220	3.0E-6	RMS	1 – 30
Blasting	24	6.0E-2		Unspecified
	296	<1.0E-3		
Tunnel boring machine	< 5	1.6E-5		1 – 100
	80	3.5E-6		1 – 100

[1] Mr S. Cheesman, Acoustic consultant, Subacoustech Ltd., Southampton, UK.

Noises may be transient, short pulses of energy such as airguns, or may be continuous, such as shipping. Noise sources also vary with frequency content (i.e. a mix of frequencies, broadband or tonal, measured in Hz or kHz), duty cycle (occurrence pattern), movement (stationary or mobile) and amplitude. Similarly, energy may radiate predominantly through the water column (pressure and particle motion), may be originating predominantly from the contact with the seabed (substrate-borne vibration), or may involve both components. For example in the case of pile driving, energy propagates through the air and passes down into the water column, energy radiates out through the water column from the pile itself, and also causes seismic waves in the seabed (surface, shear and compressional waves propagating out from the tip of the pile and from the sides) (Athanasopoulos and Pelekis, 2000; Kim and Lee, 2000; Nedwell and Edwards, 2004; Svinkin, 2004; Thandavamoorthy, 2004) (Figure 1.5). For example, vertically polarised shear waves are generated, propagating outwards from the tip of the pile, and compression and shear waves radiate outwards from the pile spherically (Athanasopoulos and Pelekis, 2000). In addition to those three paths, energy may also re-enter the water column from the seabed at distance from the pile (Nedwell and Howell, 2004). The sound level produced depends largely upon the diameter and length of the pile, the pile material, power of the hammer, type of seabed and heterogeneity within it, and the duration of the operation (Svinkin, 2004). The acoustic field produced by such a source is complex and is highly dependent upon the scenario of the construction operation, making extrapolation between sources unreliable (Blondel *et al.*, 2014). Similarly, the energy produced by shipping, though originating from a water-borne source may also propagate indirectly through the seabed. Noise is produced from many parts of the ship for example the engine, propellers, wave slap and flow against the ship itself.

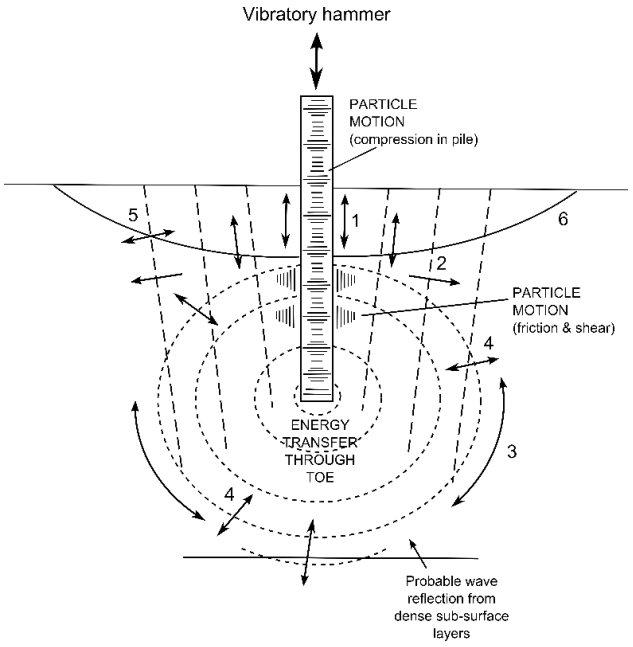

Figure 1.5 Mechanisms of seismic waves during pile driving (vibratory or impact) into soil 1. Particle motion within the pile (compressional), 2. Shear wave front, 3. Body wave front S-wave spherically expanding, 4. P-wave, 5. Interaction between reflected P wave and S wave causing surface motion, 6. P-wave reflection (minor surface reflection), adapted and expanded from Athanasopoulos and Pelekis (2000); Kim and Lee (2000).

A range of metrics can therefore be used to describe noise sources, and the relevance of each of these are still in debate (Hawkins *et al.*, 2012a), depending upon the source type described. For continuous sources where sound pressure varies over time, the sound pressure level, as described previously, is typically used, measured in terms of RMS. For such sources, the source level, expressed in terms of SPL at 1 m is used (dB re 1 µPa @ 1 m). The source level is the acoustic output of a source regardless of the propagation conditions and the acoustic field (Blondel *et al.*, 2014). Theoretically this would be measured with a hydrophone at one metre, however due to the near field effect the sound pressure level is highly variable close to the source, making such measurements inaccurate. Also such close-up readings are not possible for multiple or large sound sources such as whole airguns arrays or moving vessels. For this reason, the level is measured in the far field, and the 'apparent' source level at one metre is back-calculated to the standard distance of 1 m (Götz *et al.*, 2009; Blondel *et al.*, 2014). As this is an estimation, the true level may be slightly different from the source level.

Averaging of data in this way is not appropriate for shorter impulsive signals, since averaging across pulses is likely to misrepresent the source level. For these sources, the sound exposure level (SEL), defined as the integral of the square of the acoustic energy of a specific time period may be more appropriate:

$$SEL = 10log_{10}\frac{E}{E0} = SPL + 10log_{10}T \qquad [9]$$

Where SEL is the sound exposure level, E is the sound level, E_0 is the reference value (1 µPa^2•s), T is time. The SEL may also be used for continuous sources. When describing impulsive sources, the cumulative SEL ($SEL_{cum.}$) could also be calculated, defined as the sum of the energy across a series of pulses. If this measure is used, the number of pulses and the total time duration of the integration must be included (Blondel *et al.*, 2014). It is often the case that SEL is calculated, but details are not given about whether it is cumulative or single pulse (Hawkins *et al.*, 2012a). Furthermore the calculation assumes all pulses are equal in sound level, which may not also be true (Hawkins *et al.*, 2012a). Impulsive sources may also be described in terms of SEL per single pulse. Peak sound pressure level may also be used for impulsive sounds or peak-to-peak (previously defined).

The particle motion of anthropogenic sources measured comparatively less than pressure due to the difficulties of doing so, see previous section. In terms of substrate-borne particle motion, there are few publicly available measurements of such energy (Hazelwood, 2012; Hazelwood and Macey, 2015; Miller, 2015), indeed there are not yet international standards for measuring particle motion (although the ISO, (ISO) has recently been proposed 1pm, 1 nm/s and 1 µms^2). Due to the complexities of waterborne particle motion often measurements are given in terms of pressure only (Blondel *et al.*, 2014). Many anthropogenic activities specifically contact the seabed, such as drilling and piling, but without measurements the consequences of these to marine organisms cannot be fully understood.

Despite various metrics being available, it is not generally agreed which is the most representative of each source, especially as the hearing abilities of most aquatic animals are not precisely known.

Furthermore often acoustic fields are not described in known metrics, or are incompletely described (Hawkins *et al.*, 2012a). This is a problem when studying the impacts of anthropogenic sound upon marine organisms.

1.8 Frequency weighting

In addition to the variation between sound signals, hearing sensitivities of marine species vary. This means that it is difficult to measure the effect of a signal upon a specific organism. To overcome this, metrics can be used to 'weight' sound measurements to the hearing ability. One proposed solution to this problem is to use the dB_{ht} (species) scale (Nedwell and Edwards, 2004). This is a value estimated by passing a sound through a filter mimicking a particular hearing ability of the species, and measuring the sound output. The calculated value then corresponds to the perception of sound by that species, with $90dB_{ht}$ (species) thought to cause significant avoidance reactions (Nedwell and Turnpenny, 1998; Nedwell *et al.*, 2007). This value varies with species and represents its particular hearing ability and the effects of sound upon the species (Nedwell and Edwards, 2004). However metrics such as these and others (Malme *et al.*, 1989) have not been widely adopted in peer-reviewed literature as it has been argued that perceived loudness does not relate just to the audiogram of that species (Thomsen *et al.*, 2009). In addition to this, as discussed earlier, there are few fish audiograms which are reliable due to the complexities of small tank acoustics and varying experimental methodologies between research groups (Ladich and Fay, 2013). There are few reliable audiograms available for invertebrates (Popper *et al.*, 2001). Furthermore the metric does not consider the duration of the impact, the effects of long-term exposure, recovery time required to return to pre-exposed state, or the life stage/health of the organism in question. On the other hand it is useful to scale a behavioural reaction to a species hearing capability of a species, and allow a non-specialist to use a single number to describe the effect of sound on that species and others.

1.9 Considerations for investigating the effects of noise

The effect of a sound or vibration upon a biological organism depends upon the hearing abilities of the receiving organism, the levels received, the background levels of sound and the properties (e.g. source level, duration, repetition, plane of motion, pressure level) (Chapman and Hawkins, 1973; Tasker *et al.*, 2010; Hawkins *et al.*, 2014a; Hawkins and Popper, 2014). As outlined above, since every sound has distinct characteristics varying in source level, frequency content, pattern of occurrence and movement (stationary or mobile source) (2010), it is difficult to measure the effects. The use of appropriate metrics is important to enable the effects of noise sources to be understood between scenarios (Hawkins *et al.*, 2012a).

There are several experimental considerations to be included when studying the effects of noise upon marine animals. Most of these will be addressed in the respective chapters, however more general considerations are provided here.

Table 1.3. Considerations and measurements to ensure a 'good' playback experiment (McGregor *et al.*, 1992; Popper, 2006; Popper *et al.*, 2006)

Feature	Considerations
Test tapes and sounds	Fully calibrated sound source (e.g. duration, intensity known, SPL, s/n ratio)
	Received sound quantified (RMS, peak pressure, SEL and particle velocity)
	Sources defined and decided by acousticians
	Distortion and transmission loss calculated
	Background sound levels calculated
Playback equipment	Speaker directionality
	Fidelity of equipment (e.g s/n ratio, frequency range).
	Video recording of responses, multiple cameras at angles
	Analysis of data (e.g. motion analysis)
Playback procedure	Quantitative design to ensure statistically valid results (pseudoreplication)
	Control trials, and control/base animals, blind analysis
	Loudspeaker, vessel and observer position (e.g. experimental area in far field)
	Response measures (e.g. single, multiple)
Test animals	Subjects location in relation to territorial boundaries (if applicable), predators and conspecifics
	Time of day
	Proximity to important resources (mates, food etc.)
	Health and acclimatisation of subjects if enclosed
	Audiogram for the species known (ideally)
	Standard procedures to determine damage and physiological changes
Environmental conditions	Time of year (influences ambient sound level, flora, behaviour of subjects and other species)
	Weather (same influence as above)
	Time of day (degradation effects)
	Temperature, salinity, visibility measurements
	Tidal period and moon phase

1.9.1 Playback experiments

Whilst a real noise source is the ideal noise exposure source, it is not always possible to undertake experiments near actual sources, particularly if they are mobile. Playbacks of actual recorded signatures are a commonly used method to overcome this problem. Playback allows the exposure source to be fully adjustable and controlled, allowing, for example, dose response curves to be produced associating the level of the stimulus to a particular response.

Several authors have mentioned the lack of continuity between marine playback studies (Popper, 2006; Popper *et al.*, 2006; André *et al.*, 2011), largely due to the methods of published literature lacking detail. In order to address this deficit, Popper (2006) lists the design and considerations for a 'good' playback experiment (McGregor, 2000), (Table 1.3).

Most notably the author details a fully calibrated sound source of known duration and intensity and a detailed analysis of the received sound in terms of exposure over time (SEL) rather than RMS and peak pressure. In addition to these factors, controlled exposure experiments (CEE) differ from normal playback experiments in that the acoustics of the source have to be measured more precisely, whereas typically a 'normal' playback involves modifying the stimulus until a response is seen (Tyack, 2009).

Whilst there have been various playback studies published, few have used complex sources of anthropogenic sound. This is largely due to the difficulties of a sound projector array being able to produce a complex signature in a way that accurately mimics the original signature in terms of waveform, frequency and sound field. This includes the spectrum (what the animal hears, determining whether the organism reacts) and spatial dependence (where it comes from, determining an organisms ability to react directionally). Furthermore, due to the effects of near and far field (discussed earlier), the projector array must be placed in such a way that the experimental area is in the far field to enable a stable sound level. This can be calculated by taking the size of the transducer array into consideration. Another consideration is the depth of the array to enable propagation in a similar manner to the original signature.

It is of note that sound projectors cannot mimic the vibrational component of many anthropogenic sources contacting the seabed, such as pile driving and drilling. Studies incorporating these, which include benthic animals, must produce additional vibration in the substrate.

One issue common within playback experiments is pseudoreplication (Hurlbert 1984), which arises when an insufficient number of recordings are used to test for a certain response (McGregor, 1992). For example to test the hypothesis 'birds singing with dialect X respond differently to dialect Z', to test this, the experimenter may play the bird one recording of dialect X (X_1) and one of dialect Z (Z_1). This is pseudoreplication, since the two dialect recordings cannot be representative of the whole of dialect X and Z- the recordings may have features (perhaps inaudible to the experimenter) which are not of interest. To test the hypothesis correctly, a number of dialect recordings must be used (X_1, X_2, X_3, Z_1, Z_2, Z_3) to ensure that there is sufficient 'n' to test (McGregor *et al.*, 1992). Kroodsma *et al.* (2001) noted that in some cases authors were presenting multiple stimuli (e.g. C_1, C_2, C_3) but then *combined* for statistical analysis into stimulus 'C'. The

justification for this is that a statistical test is used to show that C_1, C_2 and C_3 are not significantly different; however C_1, C_2, C_3 are still not identical and cannot be pooled together as stimuli under the heading of 'C' (Kroodsma *et al.*, 2001). Another common error is to combine C_1, C_2, and C3 into one composite recording to represent stimulus 'C'. To avoid pseudoreplication, Hurlbert (1984) and Kroodsma *et al.* (2001) recommend carefully thought out hypothesise with clearly defined variables.

Another consideration for playback experiments is that of external validity, that is, the ability to generalise from the results of an experiment. For example it is difficult to undertake two playbacks in two different places at the same time of day, which would be the ideal scenario (McGregor *et al.*, 1992).

1.9.2 Field versus laboratory studies

Many bioacoustic studies have been undertaken using captive or caged animals. In a tank, the sound field is affected by reverberation due to reflection upon the tank walls and resonance. Multiple reflected waves in the system create standing waves with frequencies differing from the original waves (Parvulescu, 1964b; Carr *et al.*, 2007; Rogers, 2015). In addition to this, particle movement of the water is further affected by the presence of boundaries. This means that it is difficult to replicate a noise or biological source accurately in the laboratory, and the received sound may not be representative of the original source (Parvulescu, 1964b;a; Carr *et al.*, 2007; Rogers, 2015). Unless experimental tanks are sufficiently large, experiments will be in the near field and all parameters within the tank should be measured to fully quantify the acoustic field. Some experimenters have tried to overcome these problems by using air-mounted transducers (Fay and Popper, 1975; Akamatsu *et al.*, 2002). Another approach is the creation of special tanks specifically to overcome the problem (Hawkins and MacLennan, 1975).The ideal situation would be to undertake experiments in the acoustic free field.

In terms of investigating substrate-borne motion, since there are few data on the subject, initial work on a small-scale is appropriate. With predominant motion in the substratum, the above concerns of an erratic pressure and particle motion sound field in small tanks are minimal, since the motion will be confined within the substrate.

1.10 Effects of noise and vibration upon fishes and invertebrates

1.10.1 Physical and physiological effects

There is substantial overlap in the range of anthropogenic and biological sounds, making marine species vulnerable to noise pollution, (Figure 1.4, Figure 1.6). The physical effects of noise pollution upon fishes can be divided into four categories: auditory changes (auditory threshold shift, temporary or permanent), physical damage (such as membrane and gas bladder ruptures), stress and mortality (indicated in levels of cortisol and glucose). Since the focus of this review is behavioural changes, physical effects will only be mentioned briefly here (for a comprehensive review see Popper and Hastings, 2009) .

Temporary elevation of auditory thresholds in response to noise has been shown in several fish species (Popper *et al.*, 2001; Scholik and Yan, 2001; Smith *et al.*, 2004; Wysocki and Ladich, 2005; Popper *et al.*, 2007; Vasconcelos *et al.*, 2007). For example elevated thresholds in *Oncorhynchus mykiss* (rainbow trout) and *Halobatrachus didactylus* (toadfish) in the presence of low frequency sonar and boat sound respectively (Popper *et al.*, 2007; Vasconcelos *et al.*, 2007).

In addition to auditory threshold changes, permanent and temporary damage has been exhibited in exposed fishes (McCauley *et al.*, 2003; Smith *et al.*, 2004). For example caged pink snapper exposed to an airgun source level of 203 dB re 1µPa had damaged sensory hair cells in the inner ear, which remained damaged up to 58 days after noise exposure (McCauley *et al.*, 2003). Similar damage has been seen in goldfish hair cells after exposure to 170 dB re 1µPa (Smith *et al.*, 2004). At the extremes, acoustic stimuli at high amplitudes can result in death as a result of barotrauma (a result of hydrostatic pressure differences). Noise exposure may also increase levels of cortisol, produced during the stress response (Smith *et al.*, 2004; Wysocki *et al.*, 2006; Popper *et al.*, 2007; Anderson *et al.*, 2011).

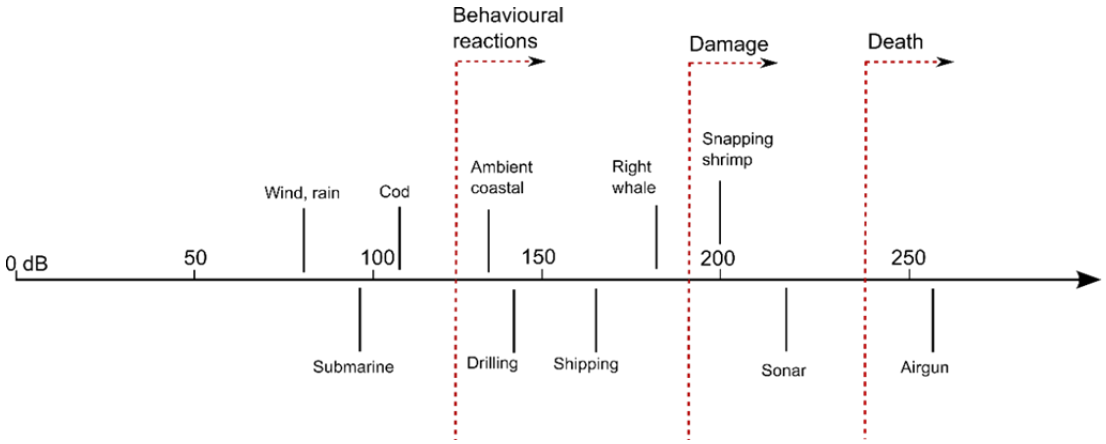

Figure 1.6 Approximate intensities of a selection of anthropogenic and biological sources of sound. The higher the intensity, dB, the more likely to cause damage, dotted lines represent thresholds above which behavioural responses and damage can be seen. Figure created from Nedwell and Howell (2004); Thomsen *et al.* (2006); Nedwell *et al.* (2007); Götz *et al.* (2009); Slabbekoorn *et al.* (2010).

In the invertebrates work has focussed upon the cephalopods, for example André *et al.* (2011) found that sounds (peak levels 175 dB re 1µPa) induced trauma in the cephalopods *Loligo vulgaris*, *Sepia officinalis*, *Octopus vulgaris* and *Illex coindetti* when exposed to low frequency sounds. Lesions, ruptured membranes, mitochondrial damage and swollen nerve fibres were found in exposed animals, in addition to hair cell damage. Similar damage was observed in multiple giant squid (*Architeuthis dux*) (Guerra, 2004). Anthropogenic sound may have an effect upon crustaceans, affecting, for example, metabolism and survival rates, pathogen resistance, growth and feeding in *Crangon crangon* (brown shrimp) and *Homarus americanus* (American lobster) (Lagardere, 1982; Regnault and Lagardere, 1983). Changes in oxygen consumption and foraging have also been demonstrated in *Carcinus maenas* (Wale *et al.*, 2013b). However, investigations in the field have failed to show differences in physiological parameters, for example unchanged

haemolymph solutes, serum proteins and enzymes in snow crabs exposed to airgun discharges (Christian *et al.*, 2003; DFO, 2004), although turnover rate was faster in seismic exposed animals (Chadwick, 2004).

1.10.2 Behavioural effects

The behavioural effects of noise and vibration upon fishes and invertebrates will only be described briefly here, since Chapters 2 – 6 focus upon these aspects (Table 1.4).

Investigating the effects of noise upon free-living fishes is logistically difficult without influencing behaviour. The logistics become further compounded when studying schools which are often large and highly complex structures (Misund *et al.*, 1998). To overcome difficulties, previous work has monitored fish behaviour in the laboratory (Blaxter *et al.*, 1981; Blaxter and Hoss, 1981; Engås *et al.*, 1998; Kastelein *et al.*, 2007; Kastelein *et al.*, 2008) however as discussed previously the acoustics within tanks is unpredictable (Rogers, 2015). Results from such studies have indicated involuntary flexion of the body, the c-start response, and an increased swimming speed and velocity in response to noise (Blaxter *et al.*, 1981; Blaxter and Hoss, 1981). Captive fish also showed a startle and increased swimming speed in larger tanks (Kastelein *et al.*, 2008). There is also evidence that anthropogenic noise 'masks' vocalisations of fishes (Chapman and Hawkins, 1973).

In the field large pens and cages may be used to monitor fishes behaviour throughout exposures (Schwarz and Greer, 1984; Engås *et al.*, 1995; Engås *et al.*, 1998; Fernandes *et al.*, 2000; Sara *et al.*, 2007; Picciulin *et al.*, 2010; Fewtrell and McCauley, 2012a). Whilst results are difficult to compare due to different experimental methodologies and exposure levels, typical behavioural responses include directional avoidance, increased density of schools and increased vertical avoidance (Schwarz and Greer, 1984; Engås *et al.*, 1995; Fewtrell and McCauley, 2012a).

Three approaches have been successful to monitor free-living fishes: underwater video, tagging and acoustic observations (Engås *et al.*, 1998; Mueller-Blenkle *et al.*, 2010; Hawkins *et al.*, 2014b). Video studies of smaller groups of free-ranging fish, or individuals, have indicated changes in time budgets after noise exposure (Picciulin *et al.*, 2010), and changes to the behaviour of reef fish, for example involuntary c-start behaviour, have been observed (Wardle *et al.*, 2001). Sonar studies on free-living fishes have indicated displacement from areas of noise (e.g. Løkkeborg and Bjordal, 1992, Engås *et al.*, 1996), vertical displacment, increased swimming speed, and changes in density of fish schools (Misund *et al.*, 1996; Pitcher *et al.*, 1996; Vabø *et al.*, 2002; Gerlotto *et al.*, 2004; Hawkins *et al.*, 2014b). However, the key exposure levels of these responses are not always fully understood and often the sound fields are not fully quantified, which makes the results difficult to interpret in a wider context.

There are few data on the effects of acoustic noise on invertebrates. In crustaceans exposed to high particle motion conditions, behavioural responses such as startle response, changes in foraging and anti-predator activities may be exhibited, but in general there is a lack of data obtained under suitable acoustic conditions (Chan *et al.*, 2010a; Staaterman *et al.*, 2011a; Staaterman *et al.*, 2011b; Wale *et al.*, 2013b;a).

Table 1.4 Behavioural, physical and population level effects of anthropogenic noise exposure to marine organisms, produced as part of a consortium review (SoundWaves *et al.*, 2012)

Impact	Effects on individuals	Expected effects on populations
Immediate or delayed death, injury	Trauma to tissues and organs e.g. gas filled organs	Mortalities, decreased abundance, reduced growth and reproductive outputs, changes in size and age structure of populations
Damage to the hair cells of the auditory system (PTS, TTS)	Hearing loss of damage temporary or permanent	Increased mortality risk, leading to decreased abundance, reduced growth and reproductive outputs.
Masking of biologically important sounds	Disruption of behaviour, increased vulnerability to predators, reduced access to prey, reduced communication, changes in vocal behaviour, disruption of spawning activities, stress.	Increased risk of mortalities Decreased abundance Reduced growth and reproductive outputs
Changes in behaviour	Direct modification of behaviour, avoidance reactions, stress responses	Displacement away from preferred habitats. Disruption of migrations/spawning Reduced growth and reproductive output
Changes in physiology	Changes in levels of stress hormones, changes to metabolism, decreased feeding, slower growth, changes to energy budgets	Reduced growth and reproductive output

Work in the field has focussed upon fisheries catch rates (Christian *et al.*, 2003; Andriguetto-Filho *et al.*, 2005; Parry and Gason, 2006) or early developmental stages (Pearson *et al.*, 1994; Radford *et al.*, 2008) with only the former indicating changes (in metamorphosis of postlarvae). There is also recent evidence to suggest that snapping shrimp (*Alpheus sp.*) have altered acoustic behaviour during impulsive sound playback (Spiga, 2013). In the molluscs, most work focussed upon cephalopods where exposure to noise has been linked to ink jetting behaviour (Fewtrell and McCauley, 2012a).

There are few data on the effects of substrate-borne vibration upon invertebrates, indeed there are also few commercially available data upon vibration levels produced by anthropogenic activities. In the bivalves, only a small number of studies have indicated reception of vibration and behavioural responses, which include closure of the siphons and, in more active molluscs, 'jumping' from the substrate (Mosher, 1972; Ellers, 1995; Kastelein, 2008).

Sensitivity of marine crustaceans to substrate vibration is also relatively unstudied, with all information on the subject being derived from semi-terrestrial species known to use vibration in mating behaviour (Aicher and Tautz, 1990). There are no data available on the responses and sensitivities of bivalves and marine crustaceans to substrate-borne stimuli. Despite calls to investigate sensitivities of benthic organisms (Hazelwood and Macey, 2015), the field is relatively unstudied. This lack of data also extends to terrestrial organisms where the role of anthropogenic vibration is also neglected (Wu and Elias, 2014). Indeed O'Connell-Rodwell *et al.* (2001) discuss the difficulties of studying terrestrial seismic signals in animals due to the levels of man-made signals present in the background, similar concerns have been expressed by Lewis *et al.* (2001). It is clear however, that as for marine species, the effects of anthropogenic vibration should not be underestimated since they may have population level effects.

1.10.3 Population level implications

Behavioural changes may have population level implications (Blickley and Patricelli, 2010), although extrapolating behavioural responses up to a wider context is problematic. Various methods have been proposed, involving conceptual models and individual based ecological models (NRC, 2005; Tasker *et al.*, 2010; Willis, 2011; Rossington *et al.*, 2013; Harwood and King, 2014), Section 6.5. However such approaches require empirical data, such as dose response data, to be implemented. At the moment such data are largely lacking for fishes as demonstrated recently with attempts to set sound exposure criteria for fishes (Popper *et al.*, 2014). For invertebrates, no such data exists.

There is a need for behavioural observations from empirical studies which provide data upon significant behaviours which may affect individual fitness, for example changes in anti-predator behaviour or time budget changes (Chan *et al.*, 2010a; Picciulin *et al.*, 2010; Wale *et al.*, 2013a). Such information would also be valuable to inform individual based ecological modelling approaches which aim to link sound exposures to behavioural responses (Rossington *et al.*, 2013). In addition to biological information, acoustic propagation data are required for modelling approaches, such as the INSPIRE model (Subacoustech Ltd.) (Hawkins *et al.*, 2012a)- but propagation is complex, for example, varying with bathymetry, boundaries and source types

(Harwood, 2002). In the light of the data deficit, other methods of scaling impacts to the population level have been suggested such as risk assessment frameworks (Tasker *et al.*, 2010; Hawkins and Popper, 2014).

It is important to be able to fully understand the effects of noise pollution on marine life to allow adequate mitigation measures to be created which would be economically viable and successful (Ducrotoy and Elliott, 2008; Normandeau Associates, 2012), (see Chapter 6 for a full discussion).

1.11 Definitions

The term 'noise' refers typically to any sound that is not the signal of interest (Hatch and Wright, 2007), but in the current work will be used to refer to anthropogenic signatures. When referring to playback of a sound or vibratory stimuli, the term 'sound playback' or 'vibration playback' will be used rather than, for example 'pile driving noise' since whilst the playback signals were similar to the original sources they cannot be deemed identical. The term 'vibration' will be used for substrate-borne motion, unless otherwise stated.

In the terrestrial environment, sound may be distinguished from vibration in that it travels through the air, whereas vibration through the ground (Goodall, 1988; Goodall *et al.*, 1990). This definition is not so clear within the marine environment, where sound and water-borne vibration may be used to describe the same energy. As such there is some ambiguity within the literature, with different terms used for particle motion and vibration. For example the term 'vibration' may be used to describe all types of particle motion, the acoustic field as a whole or just seabed motion. Similarly the term 'sound' may be used to discuss solely compressional waves (pressure), or to include particle motion additionally, or just to refer to the vibration an organism can perceive. However more commonly, 'sound' is used to describe pressure changes that may be detected by a specialised organ, which may result in an internal or external response (Hill, 2009a). Since the particle motion component of an underwater sound may propagate not only via the water column, but also by the substrate (Hazelwood, 2012; Hazelwood and Macey, 2015; Miller, 2015), it is common in the underwater bioacoustics literature to refer to 'water-borne' and 'substrate-borne' particle motion, although in reality a substrate may have increased fluidity and not be a clearly defined solid. The term 'substrate-borne' vibration is used predominantly in the terrestrial literature to refer to energy in the substrate, travelling perpendicular to the direction of travel (Hill, 2009a), the terms 'seismic' and 'substratum borne vibration' may be used synonymously. These terms will be used within the current work.

1.12 Aim, Objectives, hypotheses and structure of the thesis

Noise pollution is prominent in the marine environment and is steadily increasing. However, data and understanding on the potential effects of this pollution upon fishes and invertebrates are lacking. Such data are important to set even general sound exposure guidelines for many anthropogenic sources and marine species (Popper *et al.*, 2014). In particular, the behavioural responses to such stimuli are not well studied, due to the wide range of hearing abilities in marine

organisms and logistical difficulties of working in a natural habitat. In addition, the measurement of the level of seabed vibration produced by anthropogenic activities is lacking and the effects of such vibration upon benthic fishes and invertebrates are almost completely undocumented, despite the majority of marine construction techniques involving contact with the seabed. Indeed, the sensitivities of these organisms to vibration are largely unknown.

The aim of this project is therefore to explore and evaluate the key behavioural responses of coastal UK marine fishes and invertebrates to anthropogenic noise. To do this, the project aims to focus on the different components of anthropogenic noise, acoustic and vibratory stimuli, in addition to measuring responses on a group and individual level, in ocean and laboratory conditions.

In order to address this aim, controlled exposure experiments (playbacks) will be undertaken in a fully described acoustic free field (in the natural habitat) using a specifically designed underwater transducer array capable of reproducing man-made noise signatures. Exposure levels will be documented in the appropriate metrics to the source as advised by the current literature. To determine the sensitivity of invertebrates to vibratory signals, threshold determination experiments will be undertaken in the laboratory under fully described, controlled vibration conditions, using an electromagnetic shaker system to expose the animals to sinusoidal signals. The above will be achieved with the objectives below:

1. The behavioural responses of free-ranging fish schools to acoustic playback will be measured and linked with specific exposure levels to predict the exposure levels that will elicit responses.

2. The responses of individual crustaceans and fish to acoustic playback will be measured and linked with exposure levels to predict the exposure levels that will elicit behavioural responses.

3. The behavioural responses of benthic marine invertebrates to substrate-borne vibration will be measured and interpreted with vibration level data to predict the levels of response, and to calculate threshold sensitivity.

4. The data from all objectives will be synthesised to discuss the overall impact of anthropogenic noise upon the behaviour of marine species, from individual to population level.

A brief outline of each chapter is as follows:

Chapter 2 examines the behavioural responses of free-living fish schools to playback exposures undertaken in the acoustic free field, using an active acoustic observation system (SONAR).

Chapter 3 describes preliminary behavioural responses of individual free-living fish and crustaceans to playback exposures undertaken in the acoustic free field, by the use of underwater video (BRUV).

Chapter 4 investigates the threshold sensitivity and behavioural responses of a crustacean species to substrate-borne vibration exposures undertaken in controlled laboratory conditions.

Chapter 5 examines the threshold sensitivity and behavioural responses of a mollusc species in controlled laboratory conditions to substrate-borne vibration exposures.

Chapter 6 links the data from chapters 2 – 5 together to provide a synthesis of the effects of anthropogenic noise (vibroacoustic) upon marine species and to provide discussion of methods to scale such data up to the population level.

Chapter 2 Acoustic imaging to investigate responses of free-living coastal pelagic fish to impulsive sounds

Note

The work of this chapter was undertaken as part of the SoundWaves consortium (SoundWaves, 2013). The results of this work were published as a collaborative paper:

Hawkins, A.D., **Roberts, L.**, Cheesman, S. (2014) *Responses of free-living coastal pelagic fish to impulsive sounds.* Journal of the Acoustical Society of America 135 (5): 3101-16 (*see appendix*).

The author of this chapter was involved at all stages of the work- including initial experimental planning, data collection, statistical data analysis and production of the paper.

Abstract

Wild unrestrained pelagic fish were observed using acoustic observation techniques during sound playback experiments. Fish aggregations, including sprat *Sprattus sprattus* and Atlantic mackerel *Scomber scombrus* were exposed to synthetic signatures created to mimic the short impulsive sounds produced by a pile driver. An underwater sound projector system, built for purpose, was used to play the clips at a variety of levels. As exposure level increased the incidence of responses increased. The 50% incidence rate of response was 163.2 dB re 1 µPa peak-to-peak (135.0 dB re 1 μPa^2•s SEL) for sprat and 163.3 dB re 1 µPa peak-to-peak (140.0 dB re 1 μPa^2•s SEL) for mackerel. Layers of zooplankton appeared to show changes in response to levels of 155.8 - 158.8 dB re 1 µPa peak-to-peak, and received single strike SEL of 131.1 – 140.9 re 1 μPa^2•s SEL. Responses varied according to the level of sound, the type of school and the species. The methods given here have proved successful, and are unique in examining the behaviour of unrestrained fish aggregations to playback noise. Methods such as those described should allow further testing of the responsiveness of fish to different sound levels and signatures.

2.1 Introduction

To assess the impact of man-made sounds there is a need to fully describe the behavioural responses of wild fishes, both on an individual and a group level. Responses of a school to a stimulus may be of importance since small changes in behaviour could have implications for migration, reproduction, feeding and predator-prey interactions. Disruption of a school as a whole may be important since schooling is thought to aid foraging and to reduce successful predatory attacks (Grunbaum, 1998). Here the term school is defined as “groups of fish characterised by polarised, equally spaced individuals swimming synchronously” (Pedersen, 1996) although actually the current work will deal with ‘acoustic’ schools (Section 2.3.5).

There are many logistical difficulties involved when designing a playback experiment on a school level, since these are often large, dynamic structures changing status regularly making observations a challenge (Misund *et al.*, 1998). Many experiments of this nature have been undertaken within the confines of the laboratory where behavioural responses are easily monitored (Blaxter *et al.*, 1981; Blaxter and Hoss, 1981; Kastelein *et al.*, 2007; Kastelein *et al.*, 2008). For example, North Sea fish kept in large outdoor tanks exhibited a startle response (increased speed and increased turning) after exposure to pure tones (0.1– 64 kHz) (Kastelein *et al.*, 2008), and were also shown to increase swimming speed and drop in the water column when exposed to acoustic alarms (used to deter cetaceans) (Kastelein *et al.*, 2007).

However, acoustic conditions within laboratory tanks are not comparable to the acoustic free field of the ocean (Section 1.11.2). Sound waves inside a tank propagate differently, for example due to the reflective walls, and the air-water boundary (Parvulescu, 1964b;a; Rogers, 2015). Unfortunately, due to the more controllable nature of laboratory playback experiments, this is often overlooked. Furthermore, since fish aggregations can be large and changeable, behaviour may change within the confines of laboratory tanks.

A more realistic approach would be to undertake such experiments in the field. A number of field-based playback studies have been undertaken in this manner, typically using large net pens (Schwarz and Greer, 1984; Pearson *et al.*, 1992; Engås *et al.*, 1995; Sara *et al.*, 2007; Mueller-Blenkle *et al.*, 2010; Doksaeter *et al.*, 2012) and cages/tanks in the sea so that the fish can be observed easily (Nedwell *et al.*, 2003b; Hassel *et al.*, 2004; Boeger *et al.*, 2006; Popper *et al.*, 2007; Fewtrell and McCauley, 2012a). However this confinement may still be disruptive to the behaviour of the fish, for example behavioural changes have been described for pelagic fishes in tanks (Kastelein *et al.*, 2008), especially if they have been damaged during handling or bred in captivity (Balaa and Blouin-Demers, 2011), and many fishes are highly fragile (such as sprats, for example) and suffer in confinement making captive experiments with these impossible.

In an attempt to overcome such difficulties, some studies have observed fishes in the wild. For example the use of tags to track the movement of fish (Engås *et al.*, 1998; Mueller-Blenkle *et al.*, 2010). However tagging studies are more suited to the study of individual fish rather than whole schools due to the expense of the tags and the invasive nature of the approach. One way around this problem is a combination approach, using net pens and tags. For example Mueller-Blenkle *et al.* (2010) tagged *Gadus morhua* and *Solea solea* in net mesocosms to investigate behavioural

thresholds in response to playback of pile driving. This approach may have been advantageous however the stress of captivity, in addition to being tagged, may have changed the natural responses of the fish.

To observe whole schools of fish, observations of schools would preferably be passive, without influencing behaviour. Single and multibeam sonar systems have proved useful for this purpose, determining the dynamics, density and internal structure of free-ranging schools (Misund, 1993a; Gerlotto and Paramo, 2003; Brehmer *et al.*, 2007; Paramo *et al.*, 2007; Knudsen *et al.*, 2009; Paramo *et al.*, 2010; Hawkins *et al.*, 2012b). There have been two approaches to using sonar to investigate behavioural responses of fish schools to anthropogenic noise. The first is to take advantage of fisheries data and study abundances of fishes pre- and post-noise exposure (Engås *et al.*, 1996; Hassel *et al.*, 2004). Such studies have shown decreased catches in areas near seismic surveys (Engås *et al.*, 1996), in some areas being up to 80% decline. The problem this approach is that in some cases it is only possible to work with the available data. Methodology varies between data sets which imposes limitations upon experimental design. Furthermore, often the sound levels are not specified or are not measured in an appropriate way since the experimenter is not necessarily in control of this aspect.

The second approach is to specifically design experiments for the purpose of investigating the effects of noise. Studies such as these have monitored the responses of fish schools to trawls and vessel noise (Misund and Aglen, 1992; Misund, 1993b; Misund *et al.*, 1996; Pitcher *et al.*, 1996), and to seismic surveys (Slotte *et al.*, 2004; Peña *et al.*, 2013), and sonar (Sivle *et al.*, 2012). Reactions of schools in these situations appear to include vertical and horizontal displacement, angular changes, density changes and increased swimming speed (Misund *et al.*, 1996; Pitcher *et al.*, 1996; Vabø *et al.*, 2002; Gerlotto *et al.*, 2004). These responses are similar to that exhibited in the presence of a predator (Misund *et al.*, 1996; Pitcher *et al.*, 1996; Wilson and Dill, 2002; Gerlotto *et al.*, 2004). In other cases schools have been unresponsive, for example in response to airgun arrays of SEL 180 re1 μPa^2•s and low/mid-frequency sonar of SEL 181 dB re 1 μPa^2•s (Jorgenson and Gyselman, 2009; Sivle *et al.*, 2012; Peña *et al.*, 2013). However exposure levels to prompt such responses are not fully understood, and are often unquantified and are not reproducible, especially in the case of vessel avoidance studies (see De Robertis and Handegard (2013) for a comprehensive review).

Hence in order to understand the reaction of fish schools to anthropogenic sound, experiments should ideally be based in the field, using unrestrained schools, passively observed. Moreover, the sound exposure must be reproducible and fully quantifiable. The merits of playback experiments, as opposed to real-life sound sources, will not be discussed again here since they were fully covered in Section 1.11.

2.2 Aim, Objectives and Null hypotheses

The aim of the current work was to investigate the behaviour of unrestrained pelagic fish in response to playback of sound stimuli. To do this, experiments were undertaken in a quiet, undisturbed area with minimal human influence, using a novel small-scale experimental setup, with a sonar system to monitor school behaviour. A fully calibrated purpose-built underwater sound

projector array was used to accurately reproduce low frequency pulses similar to that produced by pile driving and airguns.

It is of note that the current work deals with schools as represented by the sonar system- since detailed structure and behaviour within the schools cannot be resolved from the echograms, these schools are essentially 'acoustic' schools, being "acoustically unresolved, multiple fish aggregations" (Kieser *et al.*, 1993). That is, the image seen is a two-dimensional slice of the school only and does not represent the whole school itself, since the vertical cross section may or may not be at the centre of the school, hence maximum dimensions may vary.

The main species of fish investigated were Atlantic mackerel *Scomber scombrus* (L., family Scombridae) and sprat *Sprattus sprattus* (L., family Clupeidae). The sprat, like its close relative the herring *Clupea harengus* is thought to be sensitive to sounds with a specialised auditory system (Enger, 1967; Blaxter *et al.*, 1981; Mann *et al.*, 2001; Mann *et al.*, 2005) having sensitivity up to 5 kHz, although there are few data available due to difficulties of maintaining them in captivity (*Pers. Comm.*[2]). The hearing abilities of mackerel are not well understood, however since they lack a swim bladder it is thought that they are only capable of detecting particle motion and would have a restricted hearing range (Popper and Fay, 2011; Ladich and Fay, 2013). The closely related mackerel tuna *Euthynnus affinis* has been found to be less sensitive than other scombrids (Iversen, 1969). It was expected that the sound levels required to elicit a response between the two species would differ.

The null hypotheses were:

1. Response presence or absence will have no relation to sound exposure.

2. Responses will not vary with species, in terms of the manifestation of the response type and the sound level to elicit such response.

3. Environmental parameters will have no relationship with response presence.

2.3 Methods

2.3.1 Location

Experiments were undertaken at Lough Hyne, County Cork on the South West coast of Ireland (51° 30' N, 9° 18'W). This site has been a marine nature reserve since 1981 (Anonymous, 1981) and is a well-known location for scientific study owing to its reserve status, rich biodiversity, unique set of environmental conditions and to the presence of the Renouf Laboratory, University College Cork (UCC). Due to the nature reserve status of the lough, human influence is minimal enabling ideal quiet conditions for sound experiments. As such, a permit was required from the Irish Government in order to work.

The lough itself is approximately 1 km long and 750 m wide, connected to the sea via a narrow gap known as the rapids (Figure 2.2A). The tidal range within the lough is approximately 1 m compared with 4 m in Barloge creek outside the lough. The lough has a maximum depth of 49 m, and a

[2] Prof. A. Hawkins, Loughine Ltd, Aberdeen, UK.

thermocline forms each year in April between 20 – 30 m and lasts until November (Kitching *et al.*, 1976).

The playback experiments were completed in two trips (20th – 27th October 2012 and March 17th – 23rd 2013), with four previous trips that defined the methodology for the experiments and identified suitable sites for the work within the lough (27th May – 12th June 2011, 19th – 27th August 2011, 17 – 24th February 2012, 8 – 22nd May 2012). The work was also underpinned and aided by annual fish school observations undertaken by A. Hawkins, (Loughine Ltd.) which span a number of years. These observations assisted the development of the methodology considerably.

2.3.2 Observation method

An outboard powered rigid inflatable boat (RIB, 4 m) and a rowing boat (4 – 5 m) were tethered together for the experiments (Figure 2.1 A, C–D). The rowing boat was equipped with a Humminbird 998c SI sonar/echo sounder system, consisting of a downward pointing echo sounder (200 kHz, 20°beam) and a side-scan sonar (800 kHz), this will be termed 'sonar' from here onwards. The system operated at frequencies well above the hearing ranges of the species tested (ultrasonic). The transducer was mounted onto a wooden beam that was G-clamped to the side of the boat, held vertically in the water at approximately 0.5 m depth. The echo sounder and the sidescan data were recorded onto an SD card, with GPS positional data automatically recorded.

2.3.3 Sound projector array and playback signatures

A purpose-built sound projector array (Subacoustech Ltd.) was used in the experiments, consisting of four speakers bolted together as a unit (Figure 2.1B). The array was hung from the opposite side of the two boats, as far away from the sonar beam as possible to prevent it appearing as a target. The array was suspended horizontally in the water by ropes and buoys at a depth of 3 – 5 m (to the top of the projectors), with the cable running to the surface. The projectors were connected to an InPhase IPX2400 car amplifier powered by a car battery, into which a signal was fed from either a Tascam model DR05 sound recorder, or an IBM thinkpad laptop computer.

A synthetic playback signature of pulsed sound 20 s duration was used. The signal was constructed by filtering white noise to mimic the spectral and temporal characteristics of impact piling sound, with a range of 50 to 600 Hz. To produce a signal similar to a single pile driver strike, a rapid onset followed by an exponential decay was set (decay constant of 0.15 s). One playback consisted of ten sharp-onset, low frequency pulses, with a gap of two seconds in between each.

In order to avoid pseudoreplication (Hurlbert, 1984), six versions of the signature were produced by using a different random seed in the production of the original white noise, but using the same onset time and filtered frequency range. The signal was presented at six different levels, incrementally 6 dB below the maximum volume produced by the array. The maximum source level was typically 185.0 dB re 1 µPa peak-to-peak @ 1 m, although this was dependent on the depth of the projectors and of the surrounding water. The order of playback clips was fully randomised. 'Blank' clips were randomly interspersed to ensure that equipment alone did not influence subjects, referred to as control trials.

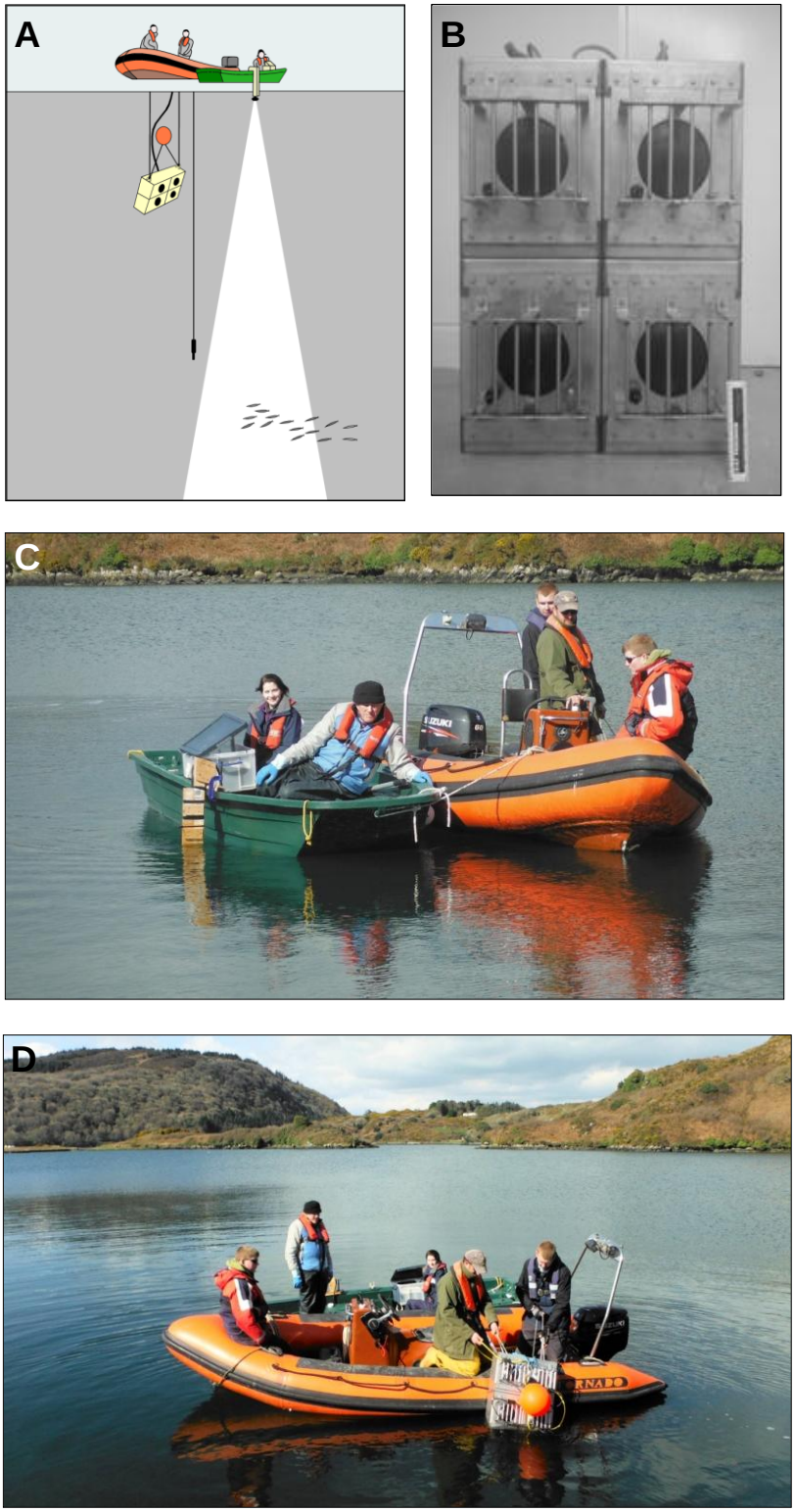

Figure 2.1 A pictorial representation of the experimental setup. A RIB (rigid inflatable boat) and a small rowing boat were tethered together and allowed to drift during the experiments. Suspended from the smaller boat was a sonar system. A sound projector array was suspended from the RIB. At the end of the experiments, sound levels were measured with a hydrophone at set depths where the sonar beam would have been. Diagram courtesy of A. Hawkins (Hawkins *et al.*, 2014b), B. An underwater sound projector array was used for the playback of synthetic piling noise, 150 mm rule for scale, C &D. Photographs of the experimental setup, courtesy of J. McWilliam, the array is shown partially out of the water in (D).

A caged, fully calibrated hydrophone (Reson model TC4014, sensitivity -186 dB re 1V/ µPa, frequency range 0.1 Hz – 400 kHz, measurement bandwidth 15 Hz – 100 kHz) was used to measure the received sound levels on each day of the experiments. The measurements were made directly beneath the sonar (when offline). The hydrophone was calibrated before each trip and was fully calibrated in February 2013. A purpose-built battery powered amplifier was used to amplify the hydrophone signal between 0 – 40 dB, a National instruments type 6062E data acquisition device was used to digitise the signal (sample rate 350 kHz) prior to storage on a laptop computer.

The sound pressure levels (SPLs) were measured daily at different distances (depths) from the projectors, from 4 – 19 m. The measurements were initially recorded as peak-to-peak SPLs, but using the assumption of equal magnitudes of positive and negative peaks, were converted to peak values (subtraction of 6 dB). To allow comparison of SPLs between different studies (Section 2.5.2) the same assumptions were used allowing conversion between peak-to-peak and RMS values (subtraction of 9 dB). Where this has been undertaken the new values are italicised in brackets.

Where appropriate the sound exposure levels (SELs) are presented, calculated as the time integral of the sound pressure squared for a single pulse and for ten pulses (dB re 1 $\mu Pa^2 \bullet s$). Particle velocity was converted from the sound pressure measurements (dB re 1 m s^{-1}) since it is likely that this was proportional to the sound pressure at the position of the measurements, but these are to be viewed as conservative estimates.

2.3.4 Experimental procedure and recording responses

The boat setup was allowed to drift across the lough without the engine on. Fish aggregations were clearly visible as strong acoustic targets. Noise signatures were then immediately projected and the time of playback was recorded. The echograms from the sonar were recorded throughout. A new recording was created every time the boat was driven to a different area, each linked to a GPS location that was exportable to Google Earth during analysis. The coupled boats drifted across the lough under the action of the wind and tide, with sound playback being undertaken when targets were encountered. Multiple schools were encountered on each track; intervals of 5 to 10 minutes were left on these occasions to avoid exposing the same school to multiple playbacks. Playback of 'blank' sound files (controls) was carried out at random intervals interspersed between full experimental sound presentations.

Playbacks were typically undertaken in less than 25 m of water since in deeper areas targets were less apparent due to the thermocline at the time, which was at approximately 30 m (Kitching *et al.* 1976). In the March experiment (17^{th} – 23^{rd}) the thermocline was absent, as expected. Sampling was generally undertaken in calm sea conditions (Beaufort Sea state two and below) to ensure that the vessel drifted at a suitably slow speed ($\bar{x}$ speed = 0.16 m s^{-1}).

Aggregations (schools) were found in similar regions of the lough for both sampling trips, being two main areas in the North and South basin (Figure 2.2A). On five of the sampling days, schools were found in the South basin during the morning and the North Basin during the afternoon. In addition to these two areas, the Whirlpool area was found to have diffuse layers of small targets (Figure

2.2D). In March, additional observations and experiments were made during the night time, to investigate variation in response with time of day. To determine the species responsible for the most common acoustic targets, sampling was undertaken at the end of each experimental period using a small plankton sampling net and by rod and line fishing.

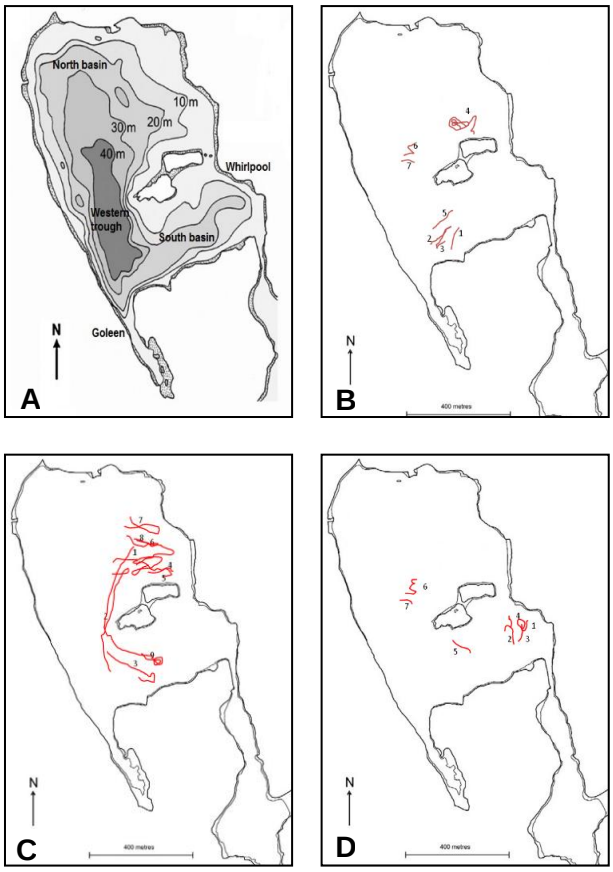

Figure 2.2 A: Bathymetric map of Lough Hyne, County Cork, the sampling site (51◦ 30′ N, 9◦ 18′ W). B & C: Boat tracks as an example of two days sampling (21st March 2013 and 23rd October 2012 respectively). Boat tracks are numbered, and were marked precisely using the GPS system of the sonar. D: Diffuse layers of targets were found in two regions of the lough, at 17 m depth (tracks 1 – 5) and 6 – 7 (30 m).

2.3.5 Classification of acoustic targets

Echograms were viewed using HUMVIEWER software (version 67). The sonar displayed the depth of acoustic reflectors in the water column, in addition to the sea surface, seabed, and time (horizontal axis). The colour of the echogram represented the strength of the signal, being blue to yellow and green to white as the echo increased for the 200 kHz beam and the 800 kHz side-scan beam respectively.

Two observers classified each target into groups according to density, overall size and their appearance. Targets were categorised into four main groups according to individual target size within the aggregation and overall morphology. Categories were further subdivided according to overall size, estimated by eye and defined as small, medium or large (Table 2.1).

Table 2.1 A summary of the four main types of targets observed, subdivided according to the size and disposition of the reflected echoes and whether aggregated or solitary.

Target	Description	Size classification
A	Diffuse layers of small reflectors identified as zooplankton	n/a
B	Aggregations of small reflectors identified as sprat	Small-Medium-Large
C	Aggregations of large reflectors, identified as mackerel	Small-Medium-Large
D	Individual reflectors, identified as zooplankters and fish from the breakdown of fish schools, identified as sprat	n/a

Targets classed as 'A' were observed as diffuse layers in the water column (Figure 2.3A). Although these reflectors were not sampled directly for identification, Hawkins *et al.* (2012) identified similar layers in the lough as zooplankton including bivalve, decapod and gastropod larvae. Targets classed as 'B' were described as small targets, identified as sprats (*S. sprattus*) seen in schools of variable size (classified as small, medium, large), (Figure 2.4A, C). Those assigned to type 'C' were aggregations comprised of larger targets, shown by spaghetti-type edges to the group rather than the dotted edges seen in type 'B' (Figure 2.4B, D). These were identified to be predominantly Atlantic mackerel *Scomber scombrus* with perhaps sporadically other predatory species such as pollack *Pollachius pollachius*. Rod and line fishing confirmed the identification of categories B and C to be sprat and mackerel, and the results are discussed in these terms. Targets classed as type 'D' were not definitively identified but were thought to be individual fish (Figure 2.3B). The individual targets seen more frequently during the night surveys were likely to have been sprats having broken up from their schools to feed. Ctenophores (comb jellies) may also have been responsible for some of the individual reflectors observed during both day and night.

2.3.6 Scoring responses

Times of playbacks were marked onto echograms using the Waypoint tool in HUMVIEWER. In the field not every fish aggregation was exposed to playback- since often schools were found close together, within a short space of time. Therefore in the echograms numerous schools had been recorded, but not exposed. These were later recorded in the data set as 'false' playbacks (false trials) - that is, where a target had been observed but a playback had not been undertaken. This was to allow comparison to control trials, where 'blanks' had been played back in the field. The position of the false trials was determined as if in the field, a waypoint was placed when a suitable amount of the school was sufficiently visible that a playback would have been undertaken. It is of note that a playback trial here is defined as an actual playback of sound, rather than simply a transect of data.

Both the echo sounder (200 kHz) and the side-scan sonar (800 kHz) images were inspected by two experienced observers, who scored possible responses (including false and control trials) both separately and then together. Data were scored depending on response presence (binary data). A change in density, depth or movement out of the sonar beam (cut-off) was deemed indicative of a

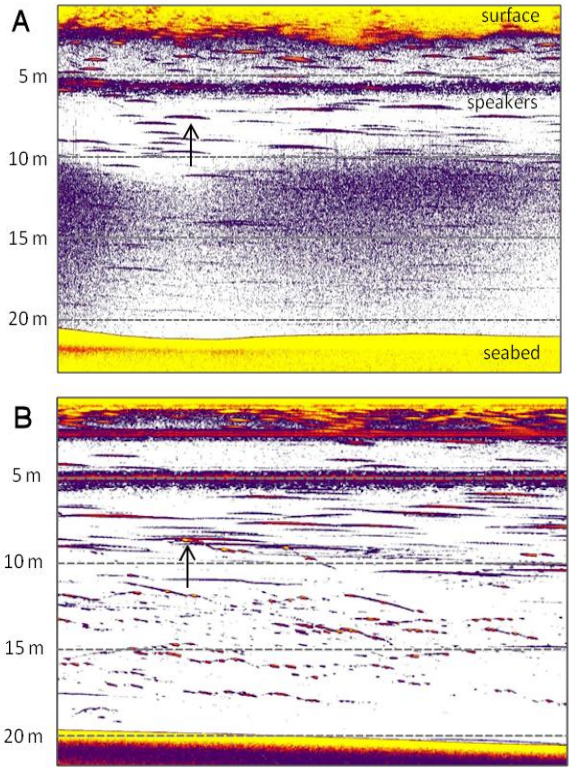

Figure 2.3 An example of a type A acoustic target, characterised as a diffuse layer of small acoustic reflectors, identified as zooplankton, echogram from 22nd October 2012, arrows mark individual larger targets classified as type D (A). An example of type D targets (individual reflectors) found in day and night time. Echogram from March 23rd. The small targets here appeared at dusk, thought to be sprat (B)

positive response. Responses were classified into three groups: a depth change was defined as the top of the school clearly dropping in depth in the water column; if the aggregation completely cut-off (left the beam) then appeared at another depth, this was classed as a depth change with re-emergence; and a density change was seen as a change in the echogram coloration, for example from deep orange to yellow- denoting a variation in the strength of echo.

Most responses were assigned to one of these categories without difficulty. If multiple response types were noted, the observers decided on the most predominant reaction, although this occurred rarely. Where possible, sidescan and downview (800 kHz) echograms were also used to determine if responses were present. Depths of targets were measured using the measurement tool in HUMVIEWER. This depth could then be linked to the corresponding sound level calculated in the daily sound level measurements. Experiments were undertaken in various water depths, and an aggregation at a similar depth from the surface could be closer to the seabed in a shallower area; therefore the depth of the school from the seabed was also measured from the echograms (taken to the top of the school) and was used in the analysis. The way an aggregation might respond may be affected by the space available to move, consequently depth from the bottom was taken as an indicator of depth for statistical analysis. The data set consisted of the parameters listed in Table 2.2.

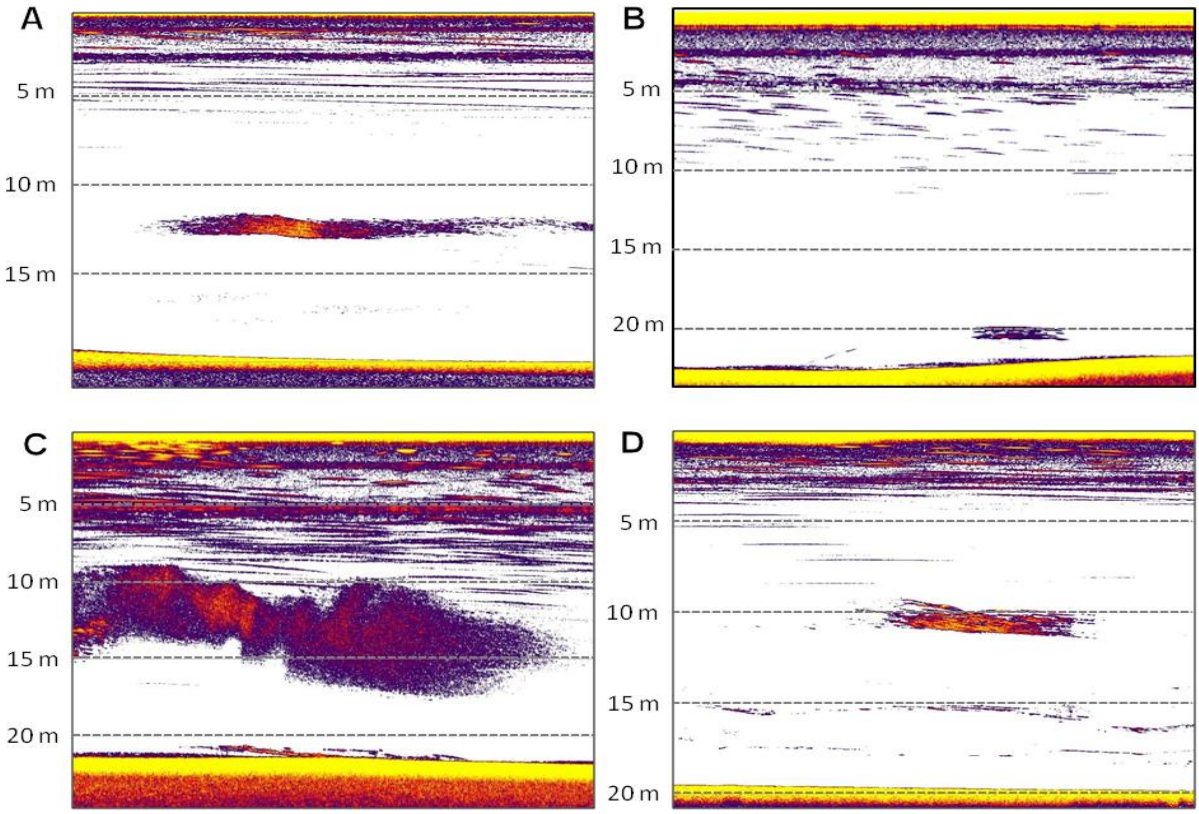

Figure 2.4 A small acoustic target, type 'B' identified as sprat, echogram from 25th October (A). A small type 'C' target, echogram from 19th March 2013 (B). A large type 'B' aggregation, echogram 25th October 2012 (C), A medium-sized 'C' target, echogram from 24th October (D). In B and C, individual reflectors are also present, identified as mackerel.

2.3.7 Statistical analysis

Data were grouped together as one data set for the purpose of analysis, since experimental setup and procedure was the same in both March and October, and the enclosed nature of the lough increasing the chances that the species seen in each trip were the same. The versions of recordings were also grouped for the analysis, since they were artificially produced (Section 2.3.3).

Data were split by aggregation type (four types, with further size subdivision in the largest two categories) for analysis, since there were varied school types likely to be different species, and hence grouping of the data set as a whole did not seem appropriate. Only the largest two aggregation types could be analysed statistically due to lack of responses for the first class and fourth class respectively- there were insufficient replicates to subdivide these further so the data were combined for analysis.

Descriptive statistics were calculated in EXCEL software (version 2007). Initially, Fishers exact test was used to compare control and false trials, to investigate whether the presence of the equipment itself had an influence upon subjects. The equipment was found to have no effect on the responsiveness of subjects (Fishers exact 1.00, df = 1, $p > 0.05$, n = 99, comparison of control and 'false' trial data). Control trials were therefore kept in the data set for comparison with the exposure data.

Table 2.2. Variables from the data collected during the experiments.

Factor	Variable type	Details
Sound level	Continuous	Measured as unweighted SPL, SEL (1 & 10 strike)
Response	Categorical	0 Presence
		1 Absence
Type of response	Categorical	0 None
		1 Density change
		2 Dispersal and re-emergence
		3 Depth change
Depth from the sea bed (m)	Continuous (m)	Used for regression analysis
		Top of the aggregation to the seabed
Depth (m)	Continuous (m)	Used to determine received sound level at the aggregation
		Measured to the top of the aggregation
Time of day	Continuous (hh:mm:ss)	Converted to decimal time
Type of aggregation	Categorical	1 Zooplanktors
		2 Small fish, sprat
		3 Large fish, mackerel
		4 Individual reflectors
Type of trial	Categorical	1 Playback
		2 False trial
		3 Control trial

The data were analysed in more detail using SPSS (version 19). Binary logistic regression was performed on response (presence or absence) data using sound level, depth from the seabed (m) and time of day (decimal) as predictors, to investigate which of these factors may have governed overall responsiveness. Sound level data were unweighted, that is, not weighted according to the species hearing ability (see Section 1.10).The data were then analysed by type of response (density change, dispersal with re-emergence and depth change). Multinomial logistic regression was performed on type of response (four categories, including 'no response' data), with sound level (unweighted), depth from the seabed (m) and time of day (decimal) as predictors. The reference category for the analysis was 'no response'. In one of the aggregation types, two response types were grouped together to allow analysis, due to insufficient replicates.

Although the data were analysed using linear logistic regression, dose response curves were plotted by fitting a cumulative normal distribution with non-linear regression (ORIGINLAB 9.1) (data analysis in collaboration with Subacoustech Ltd.). This was to enable comparability with published dose response curves for marine mammals, the standard method for these being non-linear. The curves allowed calculation of the sound level at which 50% of the fish would react. These curves

used the unweighted peak-to-peak sound level (dB re 1 µPa), single strike SEL (dB re 1 μPa^2•s), and 10 strike SEL (dB re 1 μPa^2•s). The 95% upper confidence limits could not be calculated for the higher sound levels since there were too few replicates. Although all reactions occurred immediately on the first piling strike, 10 strike SEL was calculated for completeness. In addition to this, peak-to-peak particle velocity (dB re 1 m s^{-1}) and single strike particle velocity exposure level (dB re 1 m s^{-1}) were included. These were calculated from the sound pressure measurements, assuming that at the point of measurement the particle velocity was proportional to the sound pressure.

2.4 Results

2.4.1 Hydroacoustic measurements

In the 13 days of fieldwork, a total of 321 targets (individual or aggregations of fish or zooplankton) were recorded, with 222 of these being exposed to sound playback. Slightly more aggregations were encountered in March ($\bar{x}$ = 35, SD = 36.4, n = 106) than October ($\bar{x}$ = 31, SD = 12.5, n = 215). Night time surveys in March increased the numbers of targets recorded, since individuals were more common at dusk.

Targets were found at varying depths, 2.0 – 35.0 m, and on average were 18.0 m (SD = 5.9, n = 215) deep, spanning 2.6 m of the water column (SD = 2.49, n = 215) during the October sampling. In March, schools were found in a depth range 3.8 m to 31.0 m, on average were 14.8 m deep (SD = 5.6, n = 106) spanning 1.08 m vertical distance (SD = 1.8, n = 106) of the water column. Transects varied in length, with the boat typically drifting at 0.14 m s^{-1} (SD = 0.2, n = 215) and 0.2 m s^{-1} (SD = 0.16, n = 106) in October and March respectively.

2.4.2 Sound exposures

The playbacks of the synthetic impulsive sound replicated the sound pressure from a typical piling strike at a distance of several kilometres (far field), having the characteristic sharp onset and decay, with predominant energy in the 50 – 600 Hz band (Figure 2.5A, B). However it is of note that the acoustic characteristics would be different close to a pile driver (in the near field). The stimulus had a rise time of 0.02 s, similar to that of a pile strike or other impulsive source (Nedwell *et al.*, 2007). The main difference between the recording and an actual piling strike was a longer strike duration (0.16 s longer than a real strike, 0.52 s longer than an airgun pulse). The sound levels of the impulses were measured over a bandwidth of 10 Hz to 20 kHz, a bandwidth at much higher frequencies than fishes can detect (relatively few detecting above 5 kHz). However there was comparatively little energy at the highest frequencies, and this bandwidth was selected due to standard measurement procedures (Subacoustech Ltd.), (Figure 2.5, Figure 2.6). For the example data (Figure 2.6A, B) the peak-to-peak sound level was 170.6 dB re 1 µPa, or single strike SEL of 147.9 dB re 1 μPa^2•s.

The playback sound and the original piling recording both had energy in the 50 to 600 Hz band range, with an upper range of 900 Hz (Figure 2.6B). The airgun signature was similar in the upper frequency range but had a strong low frequency component (Table 2.3).

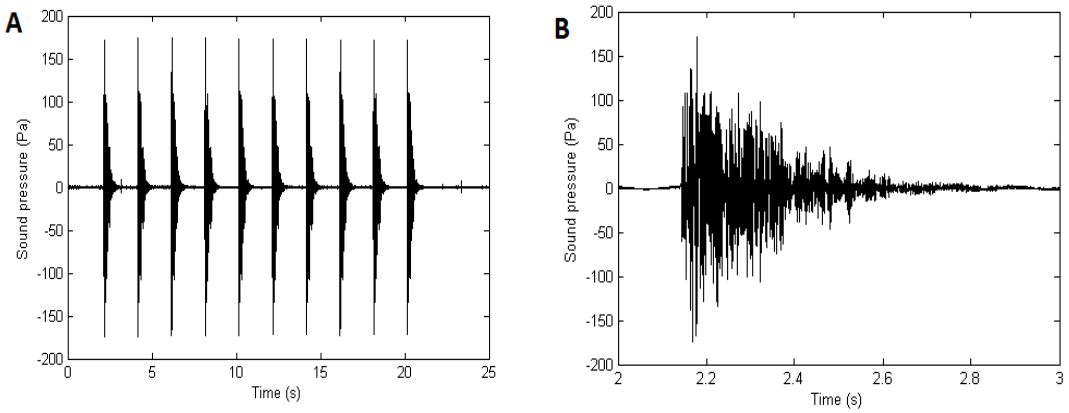

Figure 2.5 Sound measurements taken at 4 m (5 m from the sound projectors) on 19th March 2013. Time history of sound pressure measurements of one 20 s playback of synthetic piling (A); Expanded view of a single playback strike (B). Data courtesy of Subacoustech Ltd.

Table 2.3. Characteristics of the synthetic impulsive sound compared to recorded piling and airgun signatures, shown in Figure 7. Data reanalysed from Nedwell *et al.* (2007) and Parvin and Nedwell (2006), analysis courtesy of Subacoustech Ltd.

Source	Measurement range (m)	Specification	Water depth (m)	Rise time (s)	Strike Duration (s)	Peak-to-peak level (dB re 1 µPa)
Simulated piling	5	4 projectors	10 – 35	0.02	0.7	170.6
Seismic airgun	3700	Bolt Model 1900LL-X[a]	10	0.02	0.2	164.2
Piling	5000	4.7 m diameter[b]	7 – 24	0.03	0.5	167.6

[a] Note the airgun listed was charged to 60 bar, data reanalysed from Parvin and Nedwell (2006)

[b] Data reanalysed from Nedwell *et al.* (2007).

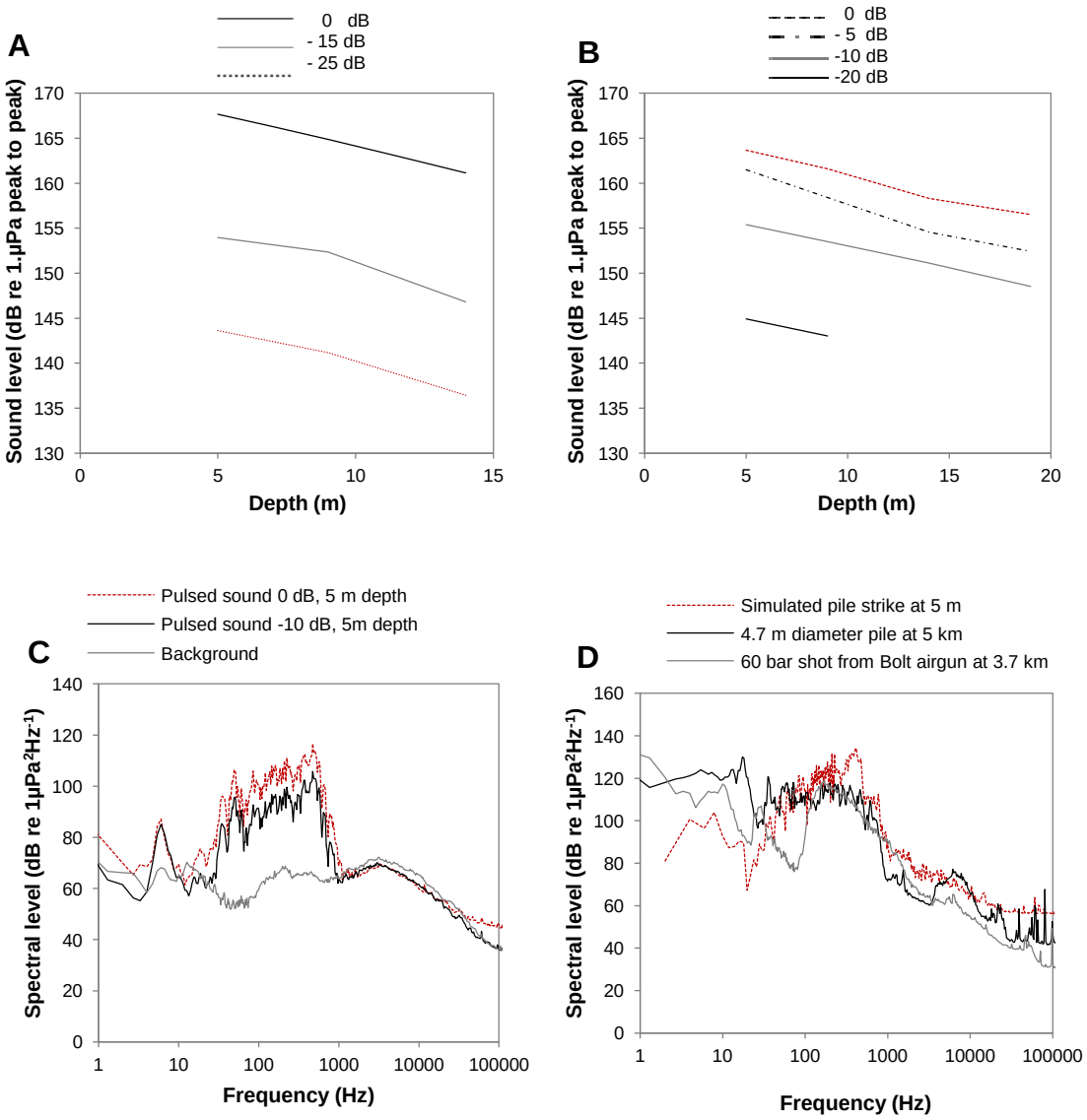

Figure 2.6 Sound pressure levels at different depths from the sound projector, 23rd October (A), 27th October (B). The sound at -20 dB on 27th was not distinguishable above background; The spectra of playbacks on 27th October (C); A comparison of playback to recordings of piling and airguns (D). Piling data reanalysed from (Nedwell *et al.*, 2007). Data courtesy of Subacoustech Ltd.

2.4.3 Response descriptions

Type 'A' aggregations were seen responding to playback in a similar way each time- a small displacement at the onset of each playback clip (Figure 2.7A,B), which was shorter than the duration of the sound sequence. The same response occurred even when the layer was thinner (Figure 2.7A) for example, on seven out of nine presentations (Table 2.4). The sound level calculated to produce this response was 155.8 – 158.8 re dB 1 µPa (Single SEL 131.3 – 140.9 re 1 $\mu Pa^2 \bullet s$). Responses of B aggregations were most commonly seen as a complete 'cut off' of the aggregation, and then re-emergence at a lower depth (Figure 2.8A – C). This response was common with all sizes of aggregation. In other cases aggregations appeared to change density

immediately after playback (Figure 2.8D). Common responses of C aggregations were a complete 'cut off' of the group, a density change (Figure 2.9B) or a sharp depth change (Figure 2.9A). Since depth changes were rapid, it was thought that these aggregations consisted of fast moving predatory fish, most likely mackerel. None of the individual targets, classed as 'D' appeared to respond. Side-scan data helped, in some cases, to gain additional information on the responses of the aggregations (Figure 2.10).

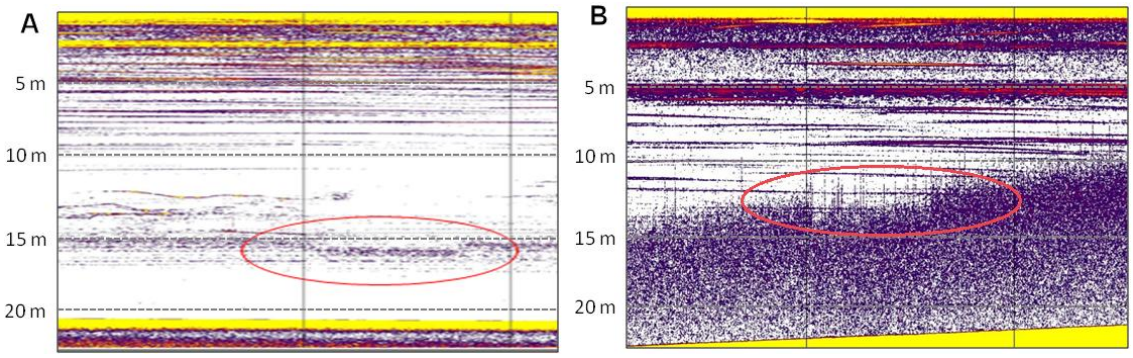

Figure 2.7 Example echograms indicating a layer of diffuse targets, thought to be zooplankton, responding to sound exposure, shown by a 'dent' in the layer. The response is encircled in red. Black vertical lines indicate the onset and offset of the sound sequence (20s duration) (A & B).

Table 2.4. The frequency of occurrence of the three main response types per target category, using exposed schools only.

Target[a]	Total response[b]	Density change	Dispersal and re-emergence	Depth change
A	7 (9)	0	0	7
B	49 (120)	21	18	10
C	16 (47)	5	2	12
D	n/a	0	0	0

[a] Abbreviations as defined in Table 2.1.

[b] Figures in brackets denote the number of presentations.

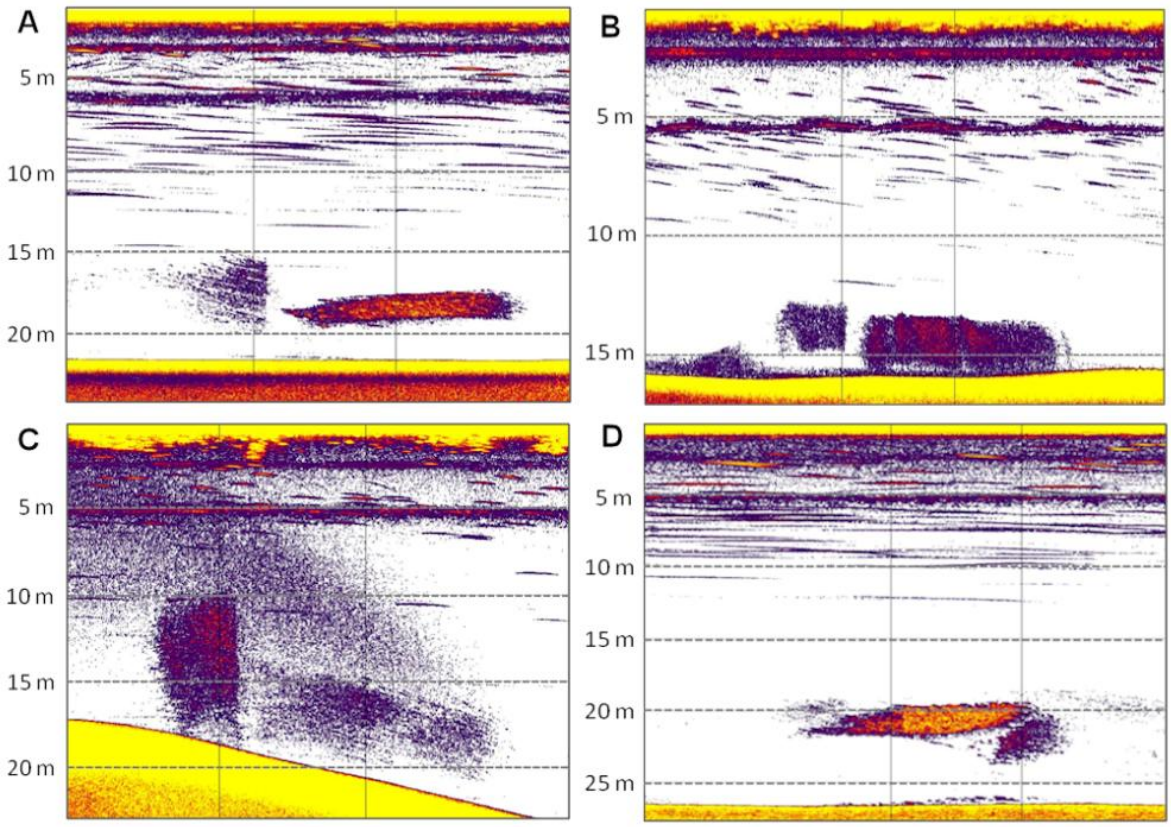

Figure 2.8 Example echograms of sprat school (category B) responses. A school appears to cut off at the beginning of the playback and reappeared a few seconds later, classified as dispersal with re-emergence, 25th October (A); A medium-sized school cut off and then reappeared lower down, 21st march (B); A school cut off at the onset of the sound and reappeared at a lower depth and density (C). A school appears to have compacted in response to the playback, shown by the change from red to yellow, an indication of increasing density, 24th October (D). Black vertical lines indicate the onset and offset of the sound sequence (20s duration).

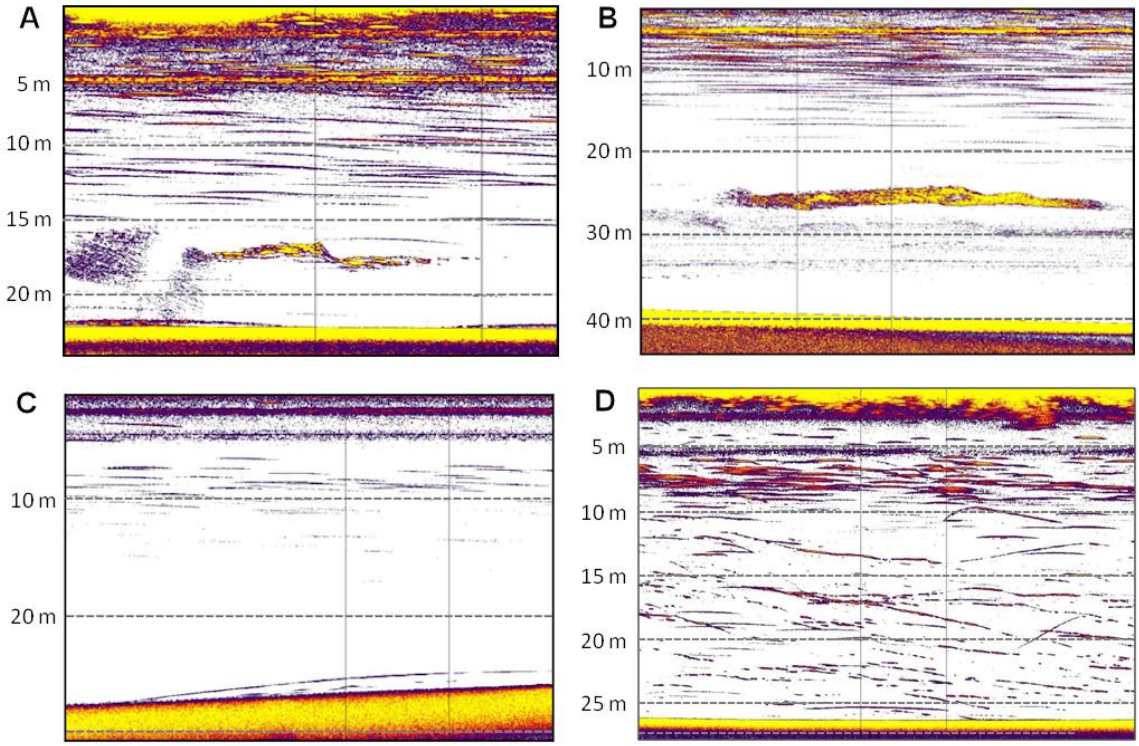

Figure 2.9 An aggregation of fast moving fish, most likely mackerel appear to dive and thin in response to playback, 20th October (A); A change in density is exhibited in response to the sound, 23rd October (B); Targets identified as zooplanktors and individual sprat did not respond to playback, 20th March 2013 (C & D). Black vertical lines indicate the onset and offset of the sound sequence (20s duration).

2.4.4 Responses and exposures

As sound level increased, likelihood of response increased significantly for both aggregation types ($p < 0.05$, n = 321 for all data, n = 150 for sprat and n = 63 for mackerel respectively) (Table 2.5). The type of response (e.g. density change, depth change) exhibited by both aggregations was significantly related to the sound level (Table 2.6). The likelihood of a density change and dispersal increased as sound level increased for sprat ($\chi^2 = 20.23$, df = 3, $p < 0.05$) and likelihood of a depth change increasing with sound level for mackerel ($\chi^2 = 12.28$, df = 3, $p < 0.05$).

The type of response was not correlated with depth of the school from the bottom, or time of day (Table A.1, A.2 appendix), hence there was no relationship between environmental parameters and response. There were only 8 responses recorded from the 99 control and false trials. Five of these responses were shown by mackerel (type C aggregations). These targets were fast moving even during non-exposures.

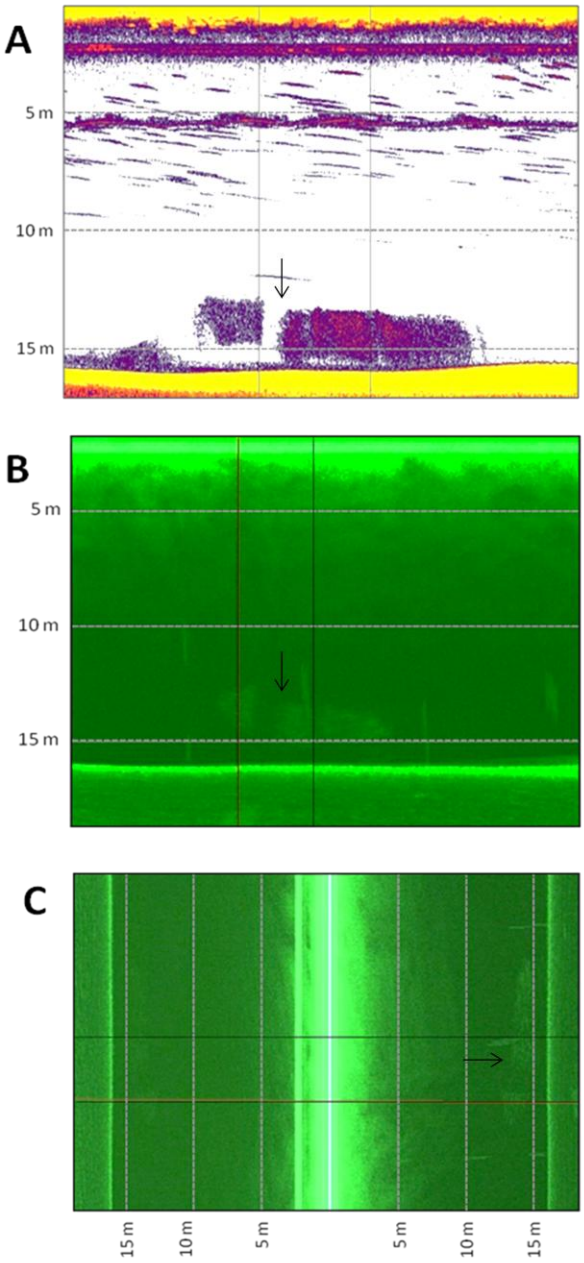

Figure 2.10 200 kHz echogram showing a large school responding to playback, 19th March, in all cases the school is marked in each case with an arrow after a cut-off (A); 800 kHz down view image of the same school (B); 800 kHz Sidescan image of the same school (C). In this echogram depth is on the horizontal axis and time is on the vertical, the centre line is the position of the boat. The school is shown to be on the starboard side of the boat/beam. Black vertical lines indicate the onset and offset of the sound sequence (20s duration).

Table 2.5. Binary logistic regression on response data using sound level, depth and time of day as predictors. Significance is denoted by asterisks (* p<0.05, ** p<0.01), B is the regression coefficient.

Category[a]	n	Predictor	B (SE)		95 % CI for odds ratio		
					Lower	Odds ratio	Upper
B (sprat)	164	Intercept	-18.57 **	(0.11)			
		Sound level	0.11 **	(0.03)	1.06	1.12	1.18
		Depth	0.95	(0.03)	0.89	0.95	1.01
		Time of day	0.04	(0.11)	0.85	1.04	1.28
C (mackerel)	74	Intercept	-25.33**	(9.11)			
		Sound level	0.16**	(0.06)	1.05	1.18	1.32
		Depth	-0.07	(0.07)	0.83	0.93	1.04
		Time of day	-0.05	(0.11)	0.77	0.95	1.18

[a] Abbreviations as defined in Table 1.

Table 2.6. A. Multinomial logistic regression on response data (type of response) using sound level as the predictor. Density and dispersal data were grouped for mackerel due to lack of replicates for this analysis. Significance is denoted by asterisks (* p<0.05, ** p<0.01).

Response type	Category[a]	n	Predictor	B (SE)		95 % CI for odds ratio		
						Lower	Odds ratio	Upper
Density	B (sprat)	164	Intercept	-17.46	(6.50)			
			Sound level	0.09*	(0.04)	1.01	1.09	1.18
Dispersal	B		Intercept	-25.88	(7.59)			
			Sound level	0.16**	(0.05)	1.08	1.18	1.29
Density with dispersal	C (mackerel)	74	Intercept	-25.14	(13.56)			
			Sound level	0.14	(0.09)	0.96	1.15	1.37
Depth change	B	164	Intercept	-14.16	(7.85)			
			Sound level	0.08	(0.05)	0.99	1.09	1.19
	C	74	Intercept	-27.29	(11.58)			
			Sound level	0.18**	(0.08)	1.04	1.20	1.39

[a] Abbreviations as defined in Table 1.

2.4.5 Dose response curves

Dose response curves (Figure 2.11) indicated that the level of sound responded to by 50% of sprats was 163.2 dB re 1 µPa peak-to-peak (135.0 dB re 1 µPa^2•s SEL, Figure 2.11A, B), and for mackerel was 163.3 dB re 1 µPa peak-to-peak (142.0 dB re 1 µPa^2•s SEL, Figure 2.11C, D). Therefore in terms of peak-to-peak SPL the two species were similar in response. Table 2.7 provides the 50% response levels in terms of a variety of sound metrics. The particle velocity level that 50% of mackerel responded to was 80.4 dB re 1 m s$^{-1,}$ single strike velocity SEL of 101.7 dB re 1 m^2 s^{-1}. Mackerel are more likely to respond to particle motion rather than sound pressure, since they lack a swim bladder.

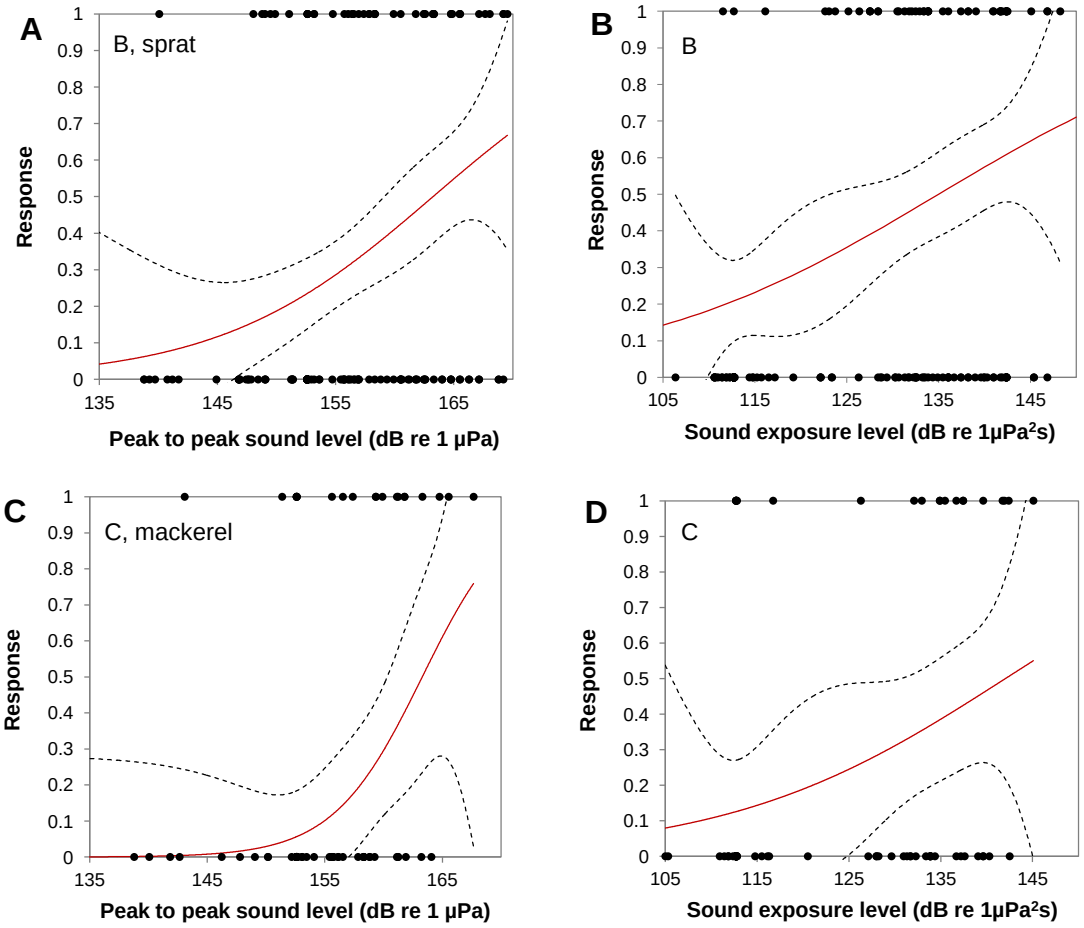

Figure 2.11 Responses of data groups to sound exposures. In each figure the solid line represents the non-linear regression fit to the data, 95% confidence intervals are represented by dashed lines. Response of sprat to sound measured in peak-to-peak levels, (A); Response of sprat to sound measured with single strike SEL, (B); Response of mackerel to sound levels measured in peak-to-peak levels, (C); Response of mackerel to sound levels measured in single strike SELs (D), (dose response analysis Subacoustech Ltd.).

Table 2.7. ***50% response levels for sprat and mackerel, as taken from*** **Figure 2.11.** ***Particle velocity is*** **provided for particle velocity sensitive species only (mackerel), data courtesy of Subacoustech Ltd.**

Category	Measure	95% CI lower	50% response level
B (sprat)	Peak-to-peak SPL [a]	159.1	163.2
	Single strike SEL[b]	123.2	135.0
	Cumulative SEL[b]	133.2	145.0
C (mackerel)	Peak-to-peak SPL	160.3	163.3
	Single strike SEL	130.7	142.0
	Cumulative SEL	140.7	152.0
	Peak-to-peak particle velocity[c]	- 83.0	- 80.0
	Single strike particle velocity[d]	- 112.6	- 101.3

[a] dB re 1 µPa

[b] dB re 1 μPa^2•s

[c] dB re 1 m s^{-1}

[d] dB re 1 m^2 s^{-1}

2.5 Discussion

2.5.1 Reception ability

A key factor affecting the results of the current work is the extent to which the fish can detect the sound, i.e. the hearing ability of the receiver. In the case of sprat, little is known of their hearing ability, largely due to their fragility in captivity making hearing studies a challenge. Lacking a swim bladder, it is likely that mackerel have limited hearing abilities with a restricted range, and are sensitive to particle motion only. In the current work sound levels have also been described in terms of particle motion (peak-to-peak particle velocity and single strike particle velocity) to account for the probable hearing ability of the mackerel. Sprat, as Clupeidae, are likely to have a good hearing sensitivity. This is due to a gas filled bulla in the head, which enables the detection of the pressure component of sound (Enger, 1967; Blaxter *et al.*, 1981; Blaxter and Hoss, 1981). Data from other Clupeids has shown a hearing range up to 5 kHz (Mann *et al.*, 2001). Therefore sprat and mackerel are at opposite ends of the spectrum in terms of hearing abilities; this is likely to affect the way they perceived the playback sound in this study. However despite this, the 50% response levels for both species are similar here, which may demonstrate the effect of individual and contextual differences in responses. It is of note that the results of this study should not be extended to other fish species, since hearing abilities vary widely between species, and the hearing abilities of many species are still unknown. Only approximately 100 of the 32 000 fish species have documented sensitivity and many of the existing AEP audiograms lack validity due to differing methodologies and acoustic conditions (Hastings and Popper, 2009; Ladich and Fay, 2013).

2.5.2 Sensitivity of fishes to impulsive sound

Investigating the effects of sound upon free-ranging schools is logistically difficult, due to the problems of reproducing the sound source accurately, and using a suitably passive method of observing behaviour whilst keeping the fish within range throughout the experiment. The current work was successful in using a small-scale setup, easily reproducible and with behavioural changes easy to monitor throughout the duration of the experiment. The use of control and false trials indicated that the setup of the two boats, drifting without power did not have an effect upon the behaviour of the fish. Furthermore, the sound projector array was able to accurately reproduce a pile driver signature.

The important aspects of this experiment are the choice of species (hearing ability varying between species), the use of a synthetic impulsive signature as a source, the use of free-ranging fish and a sonar system to observe their behaviour passively. Most important is the calculation of the sound level to which a specified proportion of the fish responded.

Due to the described difficulties of such an experiment, comparisons to other studies must be viewed with caution since there are few similar to the current work. Metrics used to describe the sound level vary between research groups (for example the received sound levels may be unrecorded, and the level that causes a response may not be calculated), as does the experimental methodology, enabling only tentative comparisons. To allow comparisons between studies giving sound levels in terms of SPL peak, peak-to-peak and RMS, positive and negative peaks of given signals were assumed to be equal magnitude allowing conversion between the three units (for example, subtracting 6 dB from peak-to-peak measures to convert to peak). Where this conversion was undertaken the newly calculated values are given italicized in brackets, to be viewed as more approximate than the original data given. By doing this it is possible to compare sound levels between studies.

In the current work sprat and mackerel (categories B and C) responded to sound pressure levels as low as 140 dB re 1 µPa and 143 dB re 1 µPa (peak-to-peak) respectively. Individual targets did not respond to even the highest sound levels presented (170.6 dB re 1 µPa peak-to-peak). Similar response levels have been found for other studies using clupeids. For example herring responded to vessel noise at a received level of 130 dB re 1 µPa (RMS) (peak-to-peak 139 dB re 1 µPa) by packing closer together and dropping in the water column (Engås *et al.*, 1995) and startle responses (acceleration burst, increased velocity, c-start and directional changes) at sound pressure levels of 122 – 138 re 1 µPa have been demonstrated aquarium tanks (Blaxter and Hoss, 1981), with acceleration changes most prevalent (Blaxter *et al.*, 1981) when exposed to vibration from a sine-wave oscillator (70 – 200 Hz). However Clupeids such as these do not do well in captivity, therefore the results of these studies should be viewed with caution. Nonetheless these reactions levels are close to those observed in the current work.

Nedwell *et al.* (2003a) exposed caged brown trout (*Salmo trutta*) to vibro-pile driver signals of source level 194 dB re 1 µPa @ 1 m, with a received level of 134 dB re 1 µPa (peak-to-peak) at 400 m. There was no variation in behaviour which is in agreement with the current work, where

responses were seen at a slightly higher exposure level, bearing in mind the species and methodology difference between the two studies.

Thomsen *et al.* (2010) found that captive cod and sole responded to pure tones of sound pressure levels 140 – 160 dB re 1 µPa peak (146 – 166 dB re 1 µPa peak-to-peak). Conversion of the current results to peak (subtracting 6 dB) allows comparison, with SPL peak of 134 dB re 1 µPa (sprat) and 137 dB re 1 µPa (mackerel). Responses of rockfish in pens to impulsive sounds such as airguns include behavioural changes such as diving and startle responses, and changes in swimming movements at sound pressure levels of 154 dB re 1 µPa (peak), with the threshold for a startle response at 200 dB re 1 µPa (peak) (Pearson *et al.*, 1992). However caged freshwater fish exposed to seismic airguns of a peak SPL 205 – 209 dB re 1 µPa (211 – 215 dB re 1 µPa peak-to-peak) did not exhibit behavioural changes (Popper *et al.*, 2005) although the species investigated were different and the fish were also captive and enclosed in these studies. At higher levels than the current work, exposure of captive rainbow trout (*Oncorhynchus mykiss*) to low frequency sonar at a sound pressure level (RMS) of 193 dB re 1 µPa (202 dB re 1 µPa peak-to-peak) elicited a sudden burst of swimming immediately after sound onset (Popper *et al.*, 2007).

There have been few studies using sonar to observe responses to quantified playback sources, (Table 2.8). Results from these studies are varied, for example, freshwater arctic fish exposed to airguns of 180 dB re 1 $\mu Pa^2 \cdot s$ (SEL) did not exhibit behavioural changes (Jorgenson and Gyselman, 2009). Sivle *et al.* (2012) exposed Atlantic herring in pens to naval sonar 1 – 7 kHz at received sound pressure levels (RMS) of up to 176 and 157 dB re 1 µPa (184 – 166 peak-to-peak), no behavioural changes were observed. Similarly, Doksaeter *et al.* (2012) exposed captive herring to naval sonar of 1 – 1.6 kHz of sound pressure level 215 dB re 1 µPa at 1m (RMS) (224 re 1 µPa peak-to-peak) and simulated sonar at 190 dB re 1 µPa at 1m at 1.6 kHz and less. No changes in behaviour were observed although startle responses, density changes and horizontal avoidance were shown in response to engine noise. However these studies used pens or cages to enclose the fish.

A number of studies have examined the behaviour of free-living fishes using sonar observation. Of these, catch rates of free-ranging cod and haddock have been shown to decrease when exposed to sound pressure levels of 249 dB re 1 µPa (peak) (254 dB re 1 µPa peak-to-peak) (Engås *et al.*, 1996) and blue whiting have been shown to drop in the water column in response to airguns (2000 psi, 3090 in^3 chamber volume, no levels given) (Slotte *et al.*, 2004). However behavioural changes were not observed for free-ranging herring schools, exposed to airgun signals of sound exposure level 125 – 155 dB re 1 $\mu Pa^2 \cdot s$ (Peña *et al.*, 2013). In the current work, the 50% response threshold for sprat was 163.2 dB re 1 µPa peak-to-peak (135 re 1 $\mu Pa^2 \cdot s$ SEL), and for mackerel was 163.3 dB re 1 µPa peak-to-peak (140 dB re 1 $\mu Pa^2 \cdot s$ SEL). Kastelein *et al.* (2008) found that the 50% reaction threshold for herring was 30 dB above the estimated sound pressure level threshold for herring (Enger, 1967). It has been suggested that sounds 90 dB above the hearing threshold cause behavioural avoidance reactions (Nedwell and Howell, 2004; Nedwell *et al.*, 2007); that is, $90dB_{ht}$(species) (Section 2.3.3).

Here, layers, thought to be zooplankton, responded to a received sound pressure level of 152.6 dB re 1 µPa peak-to-peak.

Table 2.8. Summary of the literature most relevant to the current work. These studies were designed for purpose and used a fully quantified sound source accompanied by a sonar system to observe behavioural response. For comparative purposes the results of the current work are provided, shaded grey.

Reference	Species	Location	Condition (free or captive)	Observation method	Source type	Sound level	Results	Notes
Peña *et al.* (2013)	herring	Norwegian sea		Simrad SH80 omnidirectional fisheries sonar (multibeam)	approaching seismic vessel, airgun arrays	SEL 125 – 155 dB re,1 μPa^2•s, remained above 145.	No velocity changes, speed or directional response.	Vessel drifted during experiments. Few control replicates.
Misund *et al.* (1996)	herring	Norwegian sea		Simrad SR240 (multibeam)	vessel	125 – 500 up to 2000 Hz 146 dB re 1 µPa at 250 Hz.	Moved towards, not faster or deeper.	
Sivle *et al.* (2012)	herring	Norwegian sea	Free	Simrad sh80 omnidirectional sonar	sonar (1–2 kHz) mid freq (6–7 kHz) killer whale sounds	176 dB re 1 µ Pa (SEL 181 dB re 1 μPa^2•s). 157 dB re 1 µ Pa (SEL 162 dB re 1 μPa^2•s). 150 – 160 dB re 1 µPa.	No difference between sonar types and control. Moved deeper on all treatments as the boat passed.	Summer feeding. Behaviour and distribution varied night vs. day.
Doksaeter *et al.* (2012)		Bergen, Norway	Captive	Echosounder and video	sonar (1–1.5khz)	168 dB re 1 µPa (RMS, SPL).	No reaction to sonar. Dive reaction in response to engine.	
Jorgenson and Gyselman (2009)	arctic fish	Mackenzie river, Canada		Simrad split beam	airguns	180 dB re 1 μPa^2•s SEL	No clear behavioural changes seen.	
The present work (Hawkins *et al.*, 2014)	sprat and mackerel	Lough Hyne, Ireland	Free	Humminbird 998c SI	impulsive sound playback	163.2 –163.3 dB re 1 µPa (peak peak). (SEL 135 – 140 dB re 1 μPa^2•s).	Dispersal and depth changes.	Vessel drifted during experiments. Control exposures undertaken.

Directional swimming behaviour has been observed in postlarvae of five crab species, with orientation towards reef sounds (Radford *et al.*, 2007). It is possible that the zooplankton layers here, which include bivalve, decapod and gastropod larvae (Hawkins *et al.*, 2012b) may also be able to orientate to sounds, in this case away from the sound. Detection of water oscillations has, for example, been demonstrated in copepods (Heuch and Karlsen, 1997). The zooplankton communities in Lough Hyne have been shown to undergo complicated vertical migrations and have been extensively studied (Rawlinson *et al.*, 2004) and therefore are clearly sufficiently mobile to use avoidance as an anti-predator response. Further investigation of these zooplankton layers would be valuable in order to further understand the significance of the current observations.

2.5.3 Types of Response

Responses in this work were classified into three main types- a density change, a dispersal with re-emergence and a full depth change. For sprat, as sound level increased they were significantly more likely to exhibit a density change or dispersal, whereas mackerel were more likely to exhibit a depth change. This may be because mackerel are more predatory, mobile fish, and are able to change depth rapidly, whereas for sprat there may be a larger energetic 'cost' of such behaviour, since they need to expel air from the swim bladder to change depth (Knudsen *et al.*, 2009). Aggregations of zooplankton all responded with a clear depth change, but it was unclear whether this was a passive or active process. However due to a small body size they lacked the acoustic 'strength' to show sufficient density changes on the echograms. Individual reflectors did not respond to playback.

Similar responses have been noted in other work, for example horizontal avoidance of vessels has been described (Misund and Aglen, 1992; Misund, 1993b; Vabø *et al.*, 2002; Sara *et al.*, 2007), and avoidance has been indicated by reductions in catch rates after exposure to noise (Løkkeborg and Bjordal, 1992; Engås *et al.*, 1996; Engås and Løkkeborg, 2002). Increased packing density has been described for shoals of cod (Rosen *et al.*, 2012), herring and cod in captivity (Engås *et al.*, 1995; Doksaeter *et al.*, 2012) and freshwater species in cages (Jorgenson and Gyselman, 2009). Further, rapid diving and density changes have been described in response to airguns and engine noise (Schwarz and Greer, 1984; Engås *et al.*, 1995; Pitcher *et al.*, 1996; Slotte *et al.*, 2004; Fewtrell and McCauley, 2012a; Rosen *et al.*, 2012). These types of responses are similar to those described in response to a predator (Pitcher *et al.*, 1996; Misund *et al.*, 1998; Wilson and Dill, 2002; Southall *et al.*, 2009). Indeed many species appear to react to anthropogenic influences in this way (Frid and Dill, 2002). Staying together as a school is advantageous when responding to a predator, although the conditions within a school itself are not generally favourable, including that of reduced oxygen, disease, depleted food and waste (Hamner and Hamner, 2000; Hawkins *et al.*, 2012b). Pitcher *et al.* (1996) described herring schools reacting in varied ways according to the predator encountered (either cod *Gadus morhua,* haddock *Melanogrammus aeglefinus* or the faster saithe *Pollachius virens*). The results from the present work indicate that response to anthropogenic signatures may also vary depending on the perceived nature of the threat.

The decision of a school to respond to a stimuli of any sort depends on a variety of factors, including physiological state, parasite load, environmental parameters and the motivational state of

the individual and the whole group (reviewed in Lima and Dill (1990). The 'decision' of a school to react to a stimulus or not, can have detrimental impact on each individual, for example not reacting to a predator might have a high cost whereas a 'false' alarm 'only' causes reduced feeding or mating (Bouskila and Blumstein, 1992). Indeed, if a sound stimulus 'distracts' individuals within a school, a predator may be able to approach a greater distance without being noticed, a hypothesis proposed by (Chan *et al.*, 2010a). The time of year and motivation state may also affect the response to stimuli, for example herring are less reactive during feeding times than over wintering times (Fernandes *et al.*, 2000; Vabø *et al.*, 2002). Responsiveness of the fish studied here appeared to change throughout the day. Individual targets did not respond during the night time. The motivational state of schools may change across a day since many schools break up at dusk as light levels fall (Knudsen *et al.*, 2009; Hawkins *et al.*, 2012b; Solberg and Kaartvedt, 2014). The individual targets at night were likely to be sprat, which spend the day time schooling and disperse at night to feed, this is important since it has been shown that sprat schools deplete the zooplankton plumes significantly around themselves during the daytime and so are deficient in resources (Knudsen *et al.*, 2009; Hawkins *et al.*, 2012b). As is the case of any behavioural response to a sound, responses are likely to vary according to sound properties, ambient levels, species, environmental factors, as well as the school and individual condition. Furthermore, repeated exposure may lead to habituation to the source, as found in other playback studies (Chapman and Hawkins, 1969; Knudsen *et al.*, 1992; Peña *et al.*, 2013). This is of importance since the fish may show a reduced response to all sounds, including biological stimuli such as prey and predators (Hawkins *et al.*, 2014b).

2.5.4 Replication of the stimulus

The sounds played back here were an accurate representation of those produced by a real pile driver, this was shown by a similar spectral range and energy peaks. The difficulties of reproducing piling in this way can be illustrated by other studies, for example playback of recorded piling via an underwater transducer increased predominant energy from the 100 – 200 Hz region to 1000 Hz (Kastelein *et al.*, 2013). Furthermore, in the original signal of Kastelein (2013), 90% of the energy was at 63 – 400 Hz, whereas the transducer output had most energy at 630 Hz. In the current work the playback signature is compared to actual pile driving and air gun sounds, and is shown to be similar.

However it is important to note that whilst the current work was able to accurately reproduce the water-borne component of the sound in the far field, the array did not seek to represent the acoustic field closer to source (the near field). Furthermore, in the case of pile driving, there is also a strong substrate-borne vibration produced when a pile is driven into the seabed, which a projector cannot mimic. This vibration radiates outwards along the seabed, in the form of longitudinal (compressional 'P' waves), shear (transverse, 'S' waves), or surface (Rayleigh, 'ground roll') (Hazelwood, 2012; Hazelwood and Macey, 2015) in addition to entering the water and is likely to affect benthic animals, although the sensitivities of these animals to such stimuli are unknown (Roberts and Breithaupt, 2015), (Chapters 4, 5). Although this could be thought of as a large omission for this work, the fishes investigated here were pelagic and hence are unlikely to be in contact with these large seabed vibrations directly. As such the projector was adequate to

reproduce the sound field that these species were likely to detect. Further, the sound level produced in the current work is representative of the level at 1 – 10 km from a pile driver, seabed vibrations at this distance are likely to be low, although there is little data to conclude this for definite.

According to (Nedwell *et al.*, 2003a) the water-borne SPL of individual pile strikes may be over 210 dB re 1 µPa peak-to-peak at 100 m from the operation, and may still be over 140 re 1 µPa peak-to-peak at 10 km. In the current work zooplankton, sprats and mackerel were shown to respond to 155.75 – 163.2 re 1 µPa peak-to-peak indicating that they are likely to show a behavioural response in the vicinity of piling. In addition to this, the impulsive nature of the synthetic piling in the current work was similar to that of airguns, therefore these threshold levels give an indicator of responses to these. It is of note that the actual sound level of piling depends upon the pile properties (diameter, shape, thickness, depth into the seabed), the seabed composition and the propagation conditions at the time (Athanasopoulos and Pelekis, 2000; Harwood, 2002; Nedwell and Edwards, 2004; Thandavamoorthy, 2004). These factors all affect the level of the sound produced, and the frequency spectrum of the signal.

In the current work, sound levels were given in terms of peak-to-peak SPL and single/ten strike SEL. However it is still not known which features of an impulsive sound are important to marine organisms- the total energy of a pulse or the rise time may play a role not accounted for. For example the cumulative SEL is commonly used (Southall *et al.*, 2007), but in the current work fish responded immediately to the first strike, hence a measure of cumulative sound does not seem appropriate, especially since after reacting the distance from source to receiver may also differ. For this reason the SEL was calculated for one strike and for ten strikes. However the single strike SEL has been suggested as a suitable metric to use for these types of experiments, since it is a measure of the total energy in one second of the signal, and therefore would enable comparison between different anthropogenic sources (Popper *et al.*, 2007; Hawkins *et al.*, 2012a).

2.5.5 Critique

There are some caveats to the analytical approach taken here. One of these is the issue of observer bias within the data scoring procedure. To overcome this, observers were unaware of whether the echograms were exposures or not, until after a decision had been reached. Furthermore, the two observers worked independently initially so as not to influence decisions. It could be argued that responses in the echograms could have been quantified by measuring precise morphological and positional changes of the school. During the data exploration stage this approach was investigated, for example measuring the depth to the top of a school before and after playback, and thickness changes of the school. However in some respects this approach would be misleading since the numbers obtained would not be representative of the school morphology- to do this accurately requires a correction factor, taking into the account the beam shape and pulse length of the transducer (Kieser *et al.*, 1993; Misund, 1993a; Diner, 2001). Such detail is unnecessary since it is merely the change pre- and post-stimulus which is important rather than precise measurements of school dimensions. Additionally, when a subsample was analysed in this way, the outcomes were similar to the observer scored data. Had scoring procedures been

problematic then these procedures could have been implemented, for example quantification of density change may have been undertaken using IMAGEJ to calculate pixel colour changes.

It is of note that the data presented in the echograms are not representative of the 'true' school- it is an 'acoustic school', a 2D representation or slice through the aggregation (Paramo *et al.*, 2010). Although using the sidescan beam of the sonar system in the current work aided interpretation of the downview echograms, to understand the acoustic targets further a multibeam sonar system could be used (Pedersen, 1996; Paramo *et al.*, 2010). Multibeam systems use multiple transducers to cover a larger area under the vessel, enabling a three-dimensional image, for example, of a fish school (Gerlotto *et al.*, 2004; Brehmer *et al.*, 2007; Jorgenson and Gyselman, 2009; Paramo *et al.*, 2010). If used for the current work the additional third dimension could indicate the internal structure of the school, allowing more detailed changes to be seen. However this level of detail may also be a hindrance, since internal structure is thought to vary naturally, with many patches of high and low densities present within a school (Paramo *et al.*, 2010). In fact the internal structure of herring schools has been found to change approximately every five minutes with, for example, changing food opportunities and presence of predators (Pitcher *et al.*, 1996). This may make responses more difficult to distinguish from normal school behaviour.

The design of the experiment itself meant that it was not possible to know whether the same school was being exposed several times throughout the experiments. However to minimise this problem, there were large intervals between exposures; the boats drifted throughout the experiments; and the boats were periodically moved to a completely different area of the lough. It is also possible that each school type, rather than being the species we deduced, was in fact another species. However this is unlikely since previous work has described the schools in the lough in detail (Knudsen *et al.*, 2009; Hawkins *et al.*, 2012b), indeed there were estimated to be 8900 kg of sprats in the lough in 2008 (Knudsen *et al.*, 2009). Finally, there may be concerns that a positive response was merely a school moving rapidly in and out of the sonar beam as a matter of chance, however the large number of replicates in this work, with additional control and false trials, have greatly reduced the likelihood of this occurrence. There were only a handful of non-exposures where a response was seen.

2.6 Conclusions and recommendations

The consequences of the behavioural changes seen in the current results are difficult to ascertain. The energetic cost of diving in the water column or packing closer together is unknown, for example, and therefore the short or long-term consequences are unknown. It may be that such behaviour does not have repercussions within the lifetime of the fish, or, alternatively, such small changes, if occurring regularly may affect feeding and reproduction. It is a challenge to scale-up the responses exhibited in the current study to population affects, indeed this is an issue for the research area in general- there is a need for links between individual responses to fitness implications.

There are several ways that the current work could be expanded to investigate fitness implications of the responses described here. For example, a multibeam sonar system could be used to ascertain the specific 3D shapes of schools pre- and post-exposure, with the shapes then related

to the overall energetics of the school. A more streamlined school may allow each fish to expend less energy swimming, whereas a partially broken-up school may cause the opposite. Exposure to a continuous sound source may also indicate whether schools would break up permanently- since schools provide a degree of protection from predatory attacks, the results could then be linked to overall fitness or population effects. By using a longer exposure duration it would be possible to investigate whether habituation to the sound playback would occur (Chapman and Hawkins, 1969; Knudsen *et al.*, 1992; Peña *et al.*, 2013).

Similarly, by scaling up the experiment to oceanic conditions it may be possible to show displacement of reproductively actively fish away from key spawning grounds which would have implications on a population level. For example, whole population avoidance responses may be studied by using the results of acoustic surveys (Fernandes *et al.*, 2000). Fish densities and biomass can be calculated using the intensities of returning echoes during transects across an area and thus provide a comparison of densities and biomass from different survey vessels, generating different noise levels. Fernandes et al (2000) concluded that herring schools did not avoid a particular type of research vessel by comparing fish densities obtained with that vessel with those obtained using a much quieter autonomous vehicle. The extrapolation of the results to all vessels, however, has been criticised as the research vessel employed was an especially quiet vessel compared to many other similar vessels. Another approach would be to study commercial fishery data, to investigate whether nearby anthropogenic sources cause catch rate declines. Avoidance responses have been seen in studies undertaken in such a way (Skalski *et al.*, 1992; Engås *et al.*, 1996; Hassel *et al.*, 2004). Decreases in catches of cod and haddock have been exhibited in areas near seismic surveys (Løkkeborg and Bjordal, 1992; Engås *et al.*, 1996) in some cases catch rates were shown to have decreased by 80%. However the problem of using fisheries data is that it is only possible to work with the data available, so there are limitations on the experimental design, and often sound levels are unspecified or not measured in a useable way. The present work did not encounter such problems.

Alternatively, invasive techniques could also be used, for example with the use of energetic tags, which track fish movements and allow calculation of swimming energetics (Cooke *et al.*, 2014). A select number of large schooling fish could be tagged, for example mackerel or pollack, with the schools kept in sizeable net pens in the natural environment. Observation of the schools with a fixed sonar system (Jorgenson and Gyselman, 2009) would allow energetic parameters to be linked to specific school responses such as density change , found in the current work.

Furthermore the sound signature could also be varied to represent other anthropogenic source types, or actual recordings such as shipping could be used (as Chapter 3). Indeed the method outlined here can be expanded to investigate any number of sound stimuli. For example, to investigate the efficacy of key mitigation measures such as ramp-up and acoustic deterrents, or even to incorporate the use of an actual pile driving rig or an airgun. Few studies have investigated variability of response with ramp-up or soft-start, of anthropogenic sources, although there is evidence that, for example, alarm responses in squid are reduced after such stimuli (Fewtrell and McCauley, 2012a)

The current work was successful in enabling the behavioural responses of free-ranging fish in the wild to be observed in response to playback of impulsive sound. The responses were easily observed throughout the experiments, and the results indicate that the type of response exhibited varies with sound level and species exposed. More work of this kind with other species of fish is necessary to inform the research community of the behavioural changes that man-made sources can elicit. There was a significant correlation between sound level and response, and response type varied between the two species investigated, hence the first two null hypotheses were rejected here. The third null hypothesis was accepted- there was no relationship between external parameters (for example depth of the water, time of the day) and response. However, during initial data exploration stage when data were analysed as a whole (without subdivision by species type), there was a highly significant trend between depth and response, indicating that more replicates of each species type may have led to rejection of the hypothesis. A low response to control trials indicated that the equipment itself was not having an effect on the behaviour of the animals and therefore the responses seen can be considered reliable.

As with all playback studies, the results here must be viewed with caution since it is a specific circumstance with a particular sound exposure, environment and species. To extrapolate the results here, in particular the use of the 50% response levels to inform policy, for example, would be incorrect. Whilst sound exposure criteria are still largely undefined in terms of behavioural responses (Popper *et al.*, 2014), the response of a fish school and an individual fish may be a result of previous experience, the time of year, the motivation of the fish and the hearing abilities of fish. The two species here give an indication of how that species responds in this particular scenario, however this response may vary according to, for example, motivational state, propagation of the sound, environmental parameters, length of the exposure time, type of piling method. Furthermore, the experiments here were undertaken in a quiet area with low amounts of human influence. Other coastal areas are likely to have higher ambient sound levels for example, and this may have implications on the response of sprat and mackerel in those areas.

With the paucity of information in this area, the current work has provided a reproducible method of observing the response of unrestrained fish schools to a fully calibrated sound source. Details of the sound levels in the current work are well documented and informative, enabling comparisons with other studies to be made. Popper *et al.* (2007) suggest that new contributions to this field of research include fully calibrated sources, fully described acoustic conditions, measures of sound pressure and particle velocity, in addition to good experimental designs including numerous control trials and known hearing information for the species involved. The methods here complete these requests and may allow more testing on other species, to investigate the link between response and type of response with sound level.

Chapter 3 Baited remote video to investigate responses of free-living individual fish and crustaceans to impulsive and continuous sounds

Note

The work described in this chapter was undertaken as part of the SoundWaves consortium. The author was involved in all parts of the work, including experimental design, equipment development, software choice and training, data collection and analysis. The Plymouth footage was processed and analysed by IECS staff in-house, as part of DEFRA report ME5025 (SoundWaves, 2013).

Abstract

Free-ranging individual fish and crustaceans were observed using a baited remote underwater video system (BRUV) during sound playback experiments of synthetic short impulsive signatures and container ship noise. Data presented are limited due to logistical difficulties encountered, although the work has provided valuable information regarding the pros and cons of the techniques used. Pollack (*Pollachius pollachius*), and cuckoo wrasse (*Labrus mixtus*) were observed to respond to impulsive sound playbacks in the region of 163 and 167 – 171 dB re 1 µPa (peak-to-peak, conservative estimates). Thicklip grey mullet (*Chelon labrosus*) were observed repeatedly reacting to shipping noise of received SPL 142.7 dB re 1 µPa (RMS). Reactions exhibited were sudden directional changes and acceleration, and body spasms, and typically occurred at the maximum sound level produced. On one occasion European lobster (*Homarus gammarus*) was observed possibly reacting to playback, at an estimated level of 167 – 171 dB re 1 µPa (peak-to-peak). The methods described here were eventually successful and are fully reproducible, allowing future studies to examine the behaviour of free-ranging individual fish and invertebrates to playback of noise.

3.1 Introduction

The use of seabed camera systems, RUV or BRUV systems (remote underwater video or baited remote underwater video- either fixed station or diver-held systems) has increased notably within the last decade (for a comprehensive review see Mallet and Pelletier, 2014), due to the refinement of technology and the relatively inexpensive nature of cameras with technological advances. RUV and BRUV systems are non-destructive, can be used in a range of habitats and depths, provide permanent records, give potential for high replication and reduce the staff and boat time required for experiments (Ellis and DeMartini, 1995; Shortis *et al.*, 2007; Mallet and Pelletier, 2014). By using two cameras which have an overlapping field of view, a perception of depth can be obtained allowing the 3D co-ordinates of a subject to be calculated using stereophotogrammetry, making observations particularly useful for ocean work (first described by Harvey, 1995) .

Stereo RUV/BRUV systems have been implemented widely, for example, from estimating abundance, assemblage composition, richness and individual fish identification (Watson *et al.*, 2005; Langlois *et al.*, 2010; Wraith *et al.*, 2013; Unsworth *et al.*, 2014); and have been used in a range of depths from shallow water (Unsworth *et al.*, 2014) and natural-artificial reefs (Kemp *et al.*, 2008; White *et al.*, 2013; Wraith *et al.*, 2013) to the deep sea (Priede *et al.*, 2006; Cousins *et al.*, 2013). However despite the technique being widespread, Mallet and Pelletier (2014) found only six papers (at depths less than 100 m) that used these methods to investigate the effect of human disturbance, and of these only one was an acoustic study (Picciulin *et al.*, 2010). This is of relevance since, whilst the previous chapter emphasised the need to assess the impact of anthropogenic sounds on the behaviour of schooling fishes, there is also a need to describe the behavioural responses of individual fish, since many fishes are non-schooling or break away from schools at certain times of day (Hawkins *et al.*, 2012b). Furthermore, the responses of benthic invertebrates to noise exposure are not yet described under appropriate acoustic conditions.

There are very few data available regarding the effects of noise upon invertebrates such as decapod crustaceans, with comparatively few studies focussing solely upon them. Existing studies are hindered by a lack of understanding of the detection capabilities, although evidence supporting particle motion detection is increasing (Goodall, 1988; Goodall *et al.*, 1990; Popper *et al.*, 2001; Breithaupt, 2002; Hughes *et al.*, 2014) and there is some evidence of behavioural responses in conditions with high particle motion (Goodall, 1988; Christian *et al.*, 2003; Hughes *et al.*, 2014). In addition to this, sensitivity to substrate-bone vibration has been demonstrated (Roberts and Breithaupt, 2015), Section 4.5.2, Section 5.4.4. Whilst few data are available, changes in key behaviours have been demonstrated in response to noise, for example changes in acoustics, foraging and anti-predator activities (Chan *et al.*, 2010a; Chan *et al.*, 2010b; Staaterman *et al.*, 2011b; Wale *et al.*, 2013b;a). However such behaviours have not been observed in the field (Parry and Gason, 2006; Payne and Funds, 2007; Brack, 2010).

Typical immediate behavioural responses by fishes in tanks to underwater noise stimuli include swimming changes such as startle responses, increased speed and positional changes in the water column (Blaxter and Hoss, 1981; Engås *et al.*, 1995; Kastelein *et al.*, 2008). One type of behaviour described is the involuntary C-start response - this is a sudden C-shaped flexion of the body (Zottoli, 1977; Blaxter and Hoss, 1981). Another behaviour commonly exhibited is ‘milling’, an increased

swimming speed with random turns (Blaxter and Hoss, 1981). However it is widely accepted that behaviour within tanks is not an accurate representation of behaviour in the wild, e.g. Benhaïma *et al.* (2012). There are also difficulties of sound propagation within tanks, Section 1.11.2. There is therefore a need to describe behaviour of fishes and crustaceans in their natural environment during exposure to noise. The advantages of studying free-ranging animals rather than captive or penned animals will not be discussed further here. However use of cameras systems for this purpose may be problematic since the mobility of subjects makes monitoring a challenge for any given period of time. For this reason, many field-based studies have used captive animals in cages, nets or pens (Schwarz and Greer, 1984; Engås *et al.*, 1995; Engås *et al.*, 1998; Fernandes *et al.*, 2000; Sara *et al.*, 2007; Picciulin *et al.*, 2010; Fewtrell and McCauley, 2012a). For example during exposures to low frequency sonar (Popper *et al.*, 2007), vibro pile driving (Nedwell *et al.*, 2003a) and airgun arrays (Pearson *et al.*, 1992; Engås *et al.*, 1996; Hassel *et al.*, 2004). These studies are difficult to compare since they use dissimilar stimuli, sound levels and species (a common issue within the underwater noise literature, see Section 1.11.1). However, key responses exhibited in such playback conditions are directional avoidance, increased swimming speed, and variation of group density in response to airguns, boat engine and fishing trawler noise (Schwarz and Greer, 1984; Engås *et al.*, 1995; Sara *et al.*, 2007; Fewtrell and McCauley, 2012a).

Although captivity enables the subjects to be visible (and present) throughout the experiment and allows a detailed knowledge of the subjects, confinement may have a large influence on the response exhibited, and is likely to induce stress, damage and some behavioural changes (for example circling the tank, Kastelein *et al.* (2008). One solution is to film animals with distinct territories or nests, which would then naturally occupy the area for the duration of the experiment, eliminating the need for confinement. For example gobies and damsel fish (*Gobius cruentatus* and *Chromis chromis*), were filmed by divers on their natural reef when exposed to boat engine playbacks (Picciulin *et al.*, 2010). The fish were free-ranging and their behaviour was assessed using time budget analysis, which indicated reduced time caring for the nest. However the cameras were hand-held and may have influenced behaviour (Watson and Harvey, 2007). An alternative to diver-held cameras would be ROVs- although moving platforms such as these are also likely to attract attention and be intrusive. For example, it was found that the feeding rate of *Homarus americanus* decreased in the presence of a moving ROV with lights, compared to an unlit stationary camera (Spanier *et al.*, 1994). Hence the appropriate solution is to have an unattended RUV on the seabed. As such, areas of high fish aggregations must be targeted for deployments; for example by focussing on reef species (Wardle *et al.*, 2001; Picciulin *et al.*, 2010). The use of bait is another mechanism used to ensure high numbers of experimental animals (Løkkeborg and Bjordal, 1992; Watson *et al.*, 2005; Harvey *et al.*, 2007). Results from such BRUV systems (baited remote underwater video) are likely to provide constant observations of behaviour pre- and post-noise exposure, assuming the bait remains attractive throughout.

Processing camera data has previously been a highly labour intensive process. Where multiple individuals are on screen, the calculation of parameters such as directional changes, speed, and angular changes may be time consuming. In the past decade motion analysis software has been increasingly used for this purpose, for example for monitoring prey-predator interactions and schooling behaviour (Pohlmann *et al.*, 2001; Kawaguchi *et al.*, 2010) .

Depending on the experimental setup, video data available and the parameters to be calculated, there are a range of programmes that can track animal movement. These range from work frame-by-frame such as IMAGEJ (Abràmoff *et al.*, 2004) to more sophisticated packages able to track automatically in 3D (e.g. SIMI Reality Motion systems, GmbH, Unterschleissheim, Germany). Some of these rely on algorithms based upon contrast, that is, on dark objects on a light background or vice versa. These programmes are best suited for laboratory work such as tracking the movement of insects in a petri dish, for example IMAGEJ (Abràmoff *et al.*, 2004), LOLITRACK 1 (Loligo systems, Tjele, Denmark), MAXTRAQ (Innovision Systems, Columbiaville, MI) and ETHOVISION (Noldus Information Technology, Leesburg, VA). Other programmes require special markers and camera systems to function, such as the QUALISYS software (Qualisys, Gothenberg, Sweden) and VISUAL 3D (C-motion inc., Germantown, MD). These are costly and as a result some laboratories have developed their own programmes for such purposes (Kane *et al.*, 2004), which are not available for wider use.

Hence there are few programmes suitable for the purpose of fish studies in the wild: WINANALYSE (Mikromak, WinAnalyze 1.1, Weinberger, Karlsruhe, Germany), PROANALYST (Xcitex, Inc., Cambridge, MA, USA), VISUAL FUSION (Sanders-Reed, 1995) and SIMI MOTION 3D (SIMI Reality Motion systems, GmbH, Unterschleissheim, Germany). The more sophisticated programmes such as SIMI MOTION 3D were principally created for use in biomechanics, but have also been used to track the motion of organisms by looking for differences in contrast. Such motion analysis tools do not appear to have been used for the tracking of fishes and invertebrates in response to noise exposure- indeed a considerable amount of research was required here to obtain a programme able to fulfil this requirement.

Whilst the behaviours observed by a BRUV may be short lived, small changes may have knock-on implications for feeding, migration, reproduction and even interrupt predator-prey interactions (Chan *et al.*, 2010b; Simpson *et al.*, 2014). Indeed the extent to which noise affects migratory patterns, feeding, reproduction, communication, predator-prey interactions and navigation is relatively unknown (see Hawkins *et al.* 2014 for a review of information gaps), although a recent novel study has studied acoustics and predator-prey interactions (Hughes *et al.*, 2014). More direct observations of animal reactions in the wild are required to examine naïve fishes which have not been affected by the trauma of capture or handling. In addition to this as sound levels reduce with distance from the source, behavioural responses will vary magnitude- ranging from immediate c-start responses near the source and perhaps avoidance, to low level responses further away. It is therefore important to understand the threshold of such behavioural responses.

3.2 Aim, Objectives and null hypotheses

The aim of the current study was to investigate the behaviour of unrestrained individual fish and invertebrates in response to playback of noise signatures. Underwater cameras on a purpose-built fixed position frame (BRUV) were used to film behavioural responses of fishes and invertebrate species during control exposure experiments (CEE). The footage was analysed using motion analysis software, allowing the full quantification of swimming parameters such as speed, velocity, directional changes and distance to nearest neighbour pre- and post- stimulus. The species observed were wild, naïve and free to range throughout the experiment, allowing the observation of natural behaviours. A

fully calibrated purpose-built underwater sound projector array was used to accurately reproduce recorded shipping signatures and low frequency pulses similar to that produced by pile driving and airguns (as described in Section 2.3.3).

The combined field approach and other technical aspects such as the large-scale nature of the work, motion analysis tracking and the use of purpose-built projector array for example, made the current work both a challenge and innovative.

The species targeted included pollack (*Pollachius pollachius*), herring (*Clupea harengus*), bib (*Trisopterus luscus*), cod (*Gadus morhua*), wrasse (*Labridae* sp.), dab (*Limanda limanda*), dover sole (*Solea solea*), edible crab (*Cancer pagurus*), velvet crab (*Necora puber*) and European lobster (*Homarus gammarus*). These species have a wide range of hearing abilities, for example, lacking a swim bladder, flatfish are likely only to be sensitive to particle motion (Chapman and Sand, 1974; Karlsen, 1992; Berghahn *et al.*, 1995; Nedwell *et al.*, 2004). In contrast, pollack and cod, as Gadoids, are thought to be sensitive to pressure above 100 Hz (Chapman and Hawkins 1973, Mann and Song, 2009, Ladich and Fay, 2013).

As mentioned above the acoustic detection abilities of crustaceans are not well studied, but lacking air spaces, it is thought detection involves particle motion only (Goodall, 1988; Goodall *et al.*, 1990; Popper *et al.*, 2001). A recent AEP audiogram for the mud crab *Panopeus* sp., for example, has indicated sensitivity within the range of 75 – 1600 Hz (Hughes *et al.*, 2014), the sensitivity of crustaceans to particle motion is further explored in Chapter 4.

The null hypotheses addressed were:

1. The presence of sound exposure will have no relation to the occurrence of behavioural responses;
2. Response will not be variable with species, in terms of response exhibited and the sound level required to elicit such a response.

3.3 Methodology

Multiple fieldwork deployments were made in order to acquire and modify equipment and to develop the methodologies (Table A.3, appendix), (SoundWaves, 2013). The experimental procedure required a number of conditions to be successful. Weather and water visibility had to be sufficient with suitable fish aggregations present in the field of view pre-, during and post-exposures. The equipment required had to be purpose-built, robust, waterproof, and depth rated with low emission of sounds or lights (or anything that might influence behaviour), and had to perform reliably regardless of field conditions. The sound projector array was similarly purpose-built, had to perform in varied environmental conditions and be of a manageable weight for easy deployment. Furthermore, the deployment itself had to be an operation of precision, so that distances between the sound projector array and cameras were suitable, enabling ample received sound levels at the camera frame. In addition, the large-scale nature of the project meant that representatives from multiple organisations had to be present to undertake the fieldwork, which further increased the logistical difficulties.

It was a challenge to meet the above conditions, and, with this in mind, the focus of this chapter is the methodology rather than the results. The preliminary results of three field attempts in particular are described.

3.3.1 Experiment location

Experimental methodology development and equipment testing was undertaken at four locations (UK and Ireland) during a three year period (2011 - 2013). Initial playback experiments and equipment testing was undertaken at Lough Hyne, County Cork on the South West coast of Ireland (50° 30' N, 10° 18'W), a marine nature reserve well known for scientific study. Five trips to the Lough were undertaken that involved camera work (May 2011, August 2011, February 2012, May 2012, October 2012), with the aims principally being the testing of equipment and methods in calm conditions in preparation for full sea trials. Additional work was undertaken in the Holderness Coast area, Yorkshire, and in the Blyth harbour, Northumberland area (55° 07' N, 1° 14' W) during 2012 and 2013. The last experiment was undertaken in the Plymouth Sound (August 2014) (50° 21' N 4° 08' W).

3.3.2 Sound projector array and playback signatures

The sound projector array consisted initially of 2 – 4 purpose-built speakers (Subacoustech Ltd., 300^{+} kg) bolted together (Figure 3.1A). Later, six additional speakers, a third of the weight and an eighth of the occupied volume were built to aid deployment (Subacoustech Ltd., 15 kg, 2012, Figure 3.1B) which were suspended horizontally in the water at a depth of 3 – 5 m. The array produced source levels in the region of 186.0 dB re 1 µPa @ 1m. The same projectors were used in Section 2.3, with the use of a battery powered InPhase IPX2400 amplifier, Tascam DR05 recorder and an IBM laptop.

For initial shallow water work (< 30 m), a caged calibrated hydrophone (Reson model TC4014, sensitivity -186 dB re 1V/ µPa, 0.1 Hz – 400 kHz) was used to measure the received sound levels directly next to the BRUV. The signal from the hydrophone was amplified between 0 – 40 dB using a purpose-built battery-powered amplifier, digitised using a National Instruments type 6062E data acquisition device and stored on a laptop computer.

As deployment techniques improved, sound levels were measured precisely at the camera frame. For this purpose a purpose-built subsea recording pod (Subacoustech prototype 1/2) was used consisting of a steel pressure housing containing a purpose-built miniature battery amplifier and a digital recorder (Roland R-09HR or Tascam model DR05) connected to an external calibrated hydrophone (Brüel & Kjær 8105, -205 dB re 1V/µPa ± 2 dB, 0.1 Hz – 100 kHz). The data were amplified (20 dB) and digitised and stored at a rate of 96 kHz. A second purpose-built subsea recording pod was used as a backup consisting of an Aquarian Audio H2A hydrophone (uncalibrated, sensitivity -180 dB re 1V/ µPa, 10 Hz – 100 kHz) connected to a subsea housing, containing a Zoom H1 audio recorder. Both recording pods were synchronised with the video recorders and fixed to the camera frame, enabling the received sound levels to be recorded throughout the playback experiments. Both pods were able to record for 4 – 6 hours.

For synchronisation of playback noise with the video footage, an additional Aquarian Audio H2a hydrophone (uncalibrated, sensitivity -180 dB re 1V/ µPa, 10 Hz – 100 kHz) was connected to the video recorders on the camera frame, discussed later. It was not necessary for the hydrophone to be of a high specification since its primary purpose was to alert the viewer of playback occurrence, rather than for precise measurements.

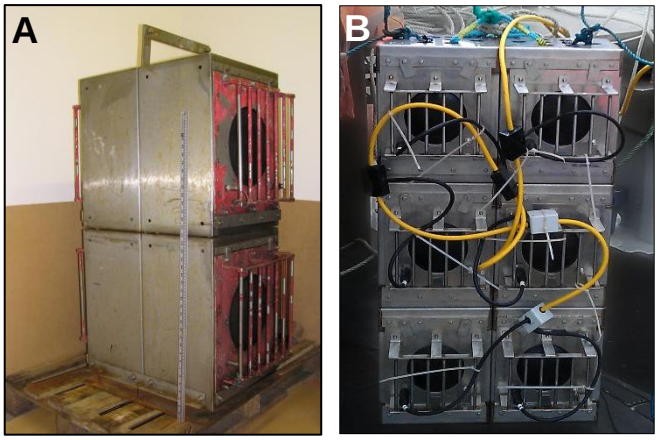

Figure 3.1 Initial prototype sound projector array, consisting of two sound projectors, 150 kg, metre rule shown for scale (A); Final transducer array consisting of six units bolted together, 15 kg each (B).

Playback signatures of shipping and a synthetic impulsive sound (twenty second duration) were used. The signals were presented at six amplitudes, varying incrementally 6 dB below the maximum level. The impulsive signature (as Section 2.3.3) was produced by filtering white noise to produce similar spectral characteristics to pile driving with a comparable exponential decay (decay constant 0.15 seconds). One playback consisted of ten sharp-onset, low frequency pulses, with a gap of two seconds in between each. The ship noise consisted of a twenty second recording of a large container ship, as recorded by Subacoustech Ltd. during routine noise monitoring. To avoid pseudoreplication (Hurlbert, 1984), six versions of each signature were produced by using a random seed in the production of the original white noise. Recordings of 'silence' were randomly interspersed to ensure that equipment alone did not influence subjects, referred to as control trials. All playback amplitudes were played in a fully randomised order.

For purposes of discussion, to allow comparison between published papers, it was necessary to convert between SPL peak, peak-to-peak and RMS values. For this purpose signals were assumed to have equal magnitude positive and negative peaks. This then allowed conversion from peak to peak-to-peak (subtraction of 6 dB) and from peak-to-peak to RMS (subtraction of 9 dB). Where this has been undertaken the new values are italicised in brackets, to denote the approximations.

3.3.3 BRUV system

A BRUV system, built to work in oceanic conditions, was purpose-built for the project, and evolved throughout the project in accordance with the conditions and locations of deployment.

During initial testing, the BRUV consisted of a slotted steel frame (approximately 1 m^3) equipped with two colour stereoscopic cameras and a hydrophone (Aquarian Audio H2A), connected to, and synchronized by a central subsea housing, (Figure 3.2A,B). Inside the subsea housing two video recorders (Mini DVR III HDVR720) and the necessary power supplies allowed the unit to record audio and video signals remotely for approximately 8 hours. The unit recorded continuously once deployed, with the audio of the cameras picking up playback noise sufficiently for synchronization purposes. A third colour bullet camera provided a live feed to the surface, to ascertain when fish were present in

the field of view. The system was adequate for the calm conditions of Lough Hyne where initial tests took place, however was deemed unsuitable for open sea conditions, prompting an enlargement. The principal issue with the initial prototype was the camera providing the live feed, which meant that the system, built to be remote, had to be within a short distance of the boat due to cable span.

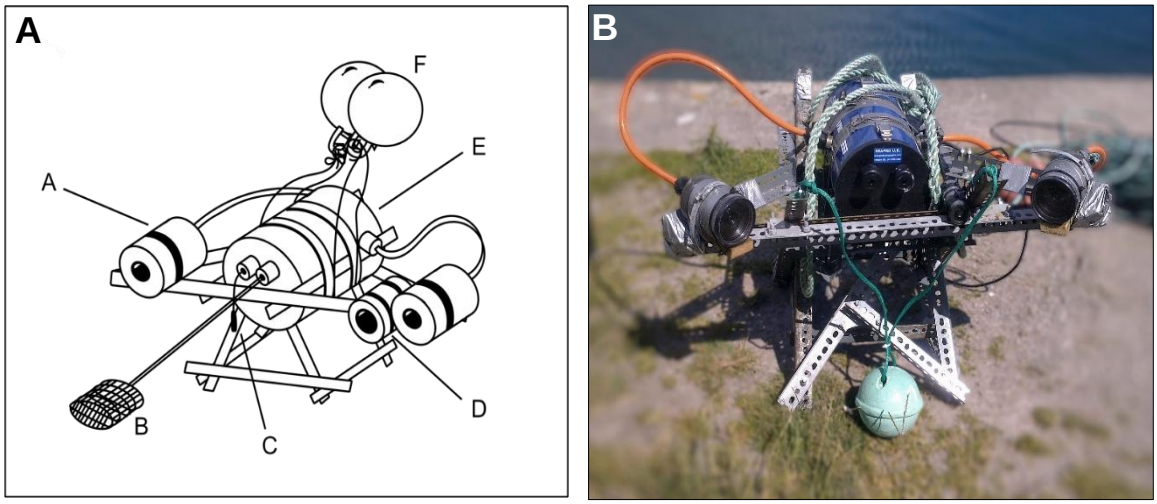

Figure 3.2 Initial prototype consisting of an iron frame equipped with two stereoscopic cameras and a hydrophone connected to a central subsea housing. A. stereoscopic cameras, B. Bait bag, C. Hydrophone connected to DVR recorders for synchronization of video and sound, D. Bullet camera for live video feed, E. Subsea housing containing mini DVR recorders and power supplies (A); Photo of the initial BRUV prototype at Lough Hyne during trials (B).

To resolve this, a buoy was purpose-built to transmit the signal from an IP camera, in a separate subsea housing, wirelessly to the vessel, (Figure 3.3A, B). The buoy supported a waterproof Pelicase, modified to contain the mini-recorders that had previously been housed in the subsea housing, and an aerial with a waterproofed wireless link. The transmission of the video feed was reliable up to 70 m, allowing the vessel to move clear of the experimental area. In this way the amount of noise in the experimental area was reduced, since the BRUV was entirely independent of the boat. The setup had the added advantage in allowing the distance between the projectors and the BRUV to be more carefully controlled without risk of cable entanglement. The surface buoy was a success when tested in the Blyth coastal area. The wireless network was detected by multiple computers on the boat and allowed the video to be viewed by multiple operators. The wireless buoy was used successfully for consecutive fieldwork attempts; however in some cases the signal incurred a 1 – 2 second delay, which proved disadvantageous for the experiment.

For the ocean trials the BRUV was considerably enlarged, consisting of a large steel frame (approximately 2 m x 1 m x 1 m) of a similar design as Langlois *et al.* (2010) and Cappo *et al.* (2007), (Figure 3.4A,B). The frame was equipped with the same stereoscopic camera heads and an Aquarian Audio hydrophone, but was connected to a Pelicase on deck containing the two video recorders (Mini DVR III HDVR720). Audio was synced to the video footage from an H2A Aquarian Audio hydrophone (uncalibrated). An observer on the vessel could therefore use the video recorders for live observations of the BRUV.

Due to logistical reasons the vessel was kept adjacent to the experimental area throughout the work. For this reason, the wireless IP camera link was exchanged for an armoured umbilical cable to the surface. In the meantime, attempts using the large vessel became increasingly more difficult due to the conditions required for work, and the experiment was subsequently downsized. To ease deployment of the BRUV from a smaller vessel, the central subsea housing was removed and all power was rewired to the surface (Figure 3.5A, B).

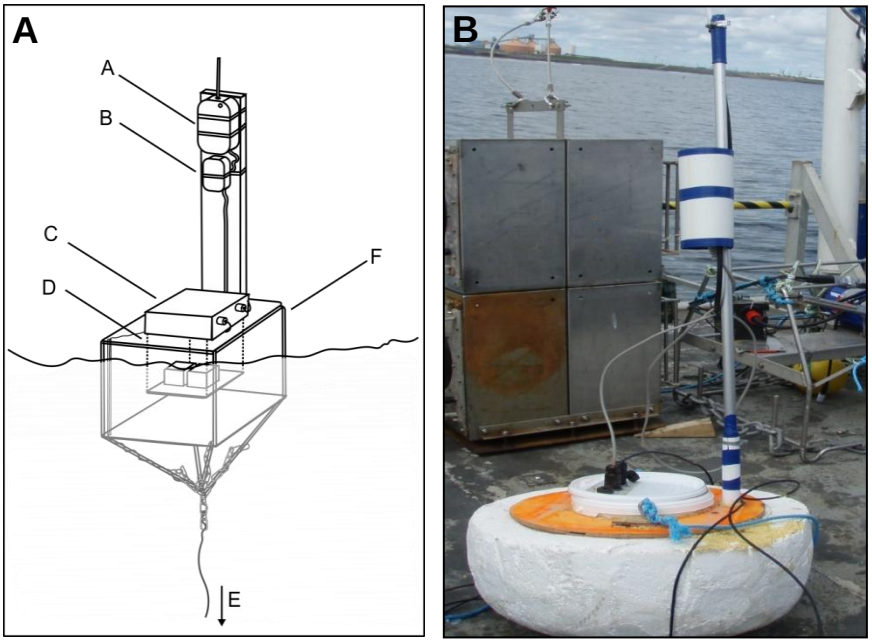

Figure 3.3 Buoy built to transmit a live IP camera signal remotely to the boat (A), consisting of power supply and waterproof wireless router. A. Wireless router, B. Power supply, C. Pelicase containing wiring, D. Battery supplies held inside the buoy, E. Umbilical cable attached to the BRUV on the seabed, F. Slotted steel frame containing polystyrene cube; Photo of the final prototype of the buoy, after rounding of corners (B).

For all fieldwork attempts the BRUV was baited using a combination of fresh herring, pilchards, mackerel, lugworm, and leftover fish scraps. In addition to this, to create a large plume in the water column, effervescent bait pellets were made, containing flour, chopped fish, sunflower oil, sodium bicarbonate, citric acid, fish oils and bloodworm, as in Stobart *et al.* (2007), (Figure 3.6). The effervescent plume lasted for approximately 1 hour. To attract fishes and invertebrates into the field of a view, a bait pole (approximately 1 m length) extended outwards from the camera frame. The bait was held either in a mesh bag or attached directly to the pole itself.

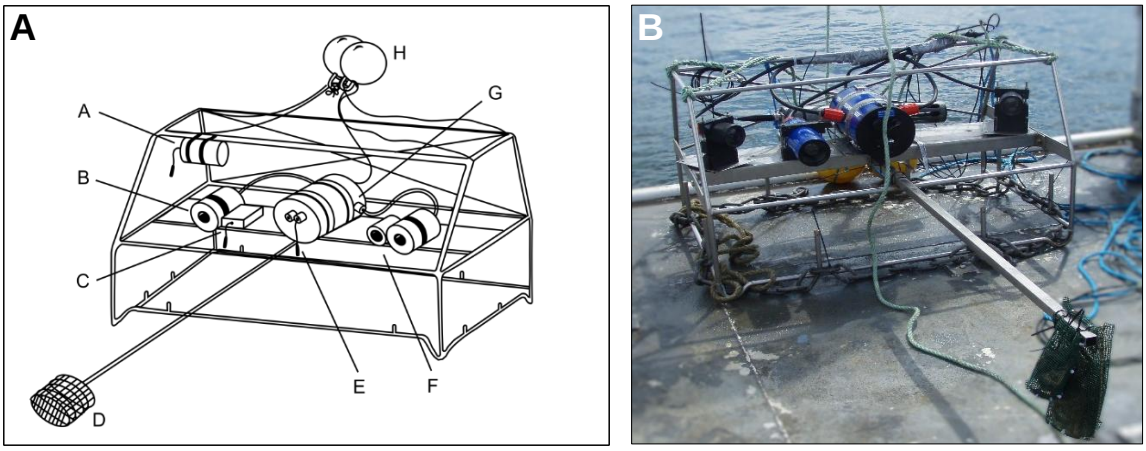

Figure 3.4 Second BRUV prototype (A), consisting of a larger steel frame equipped with two stereoscopic cameras and a hydrophone connected to a central subsea housing containing power and DVR recorders. Two remote recording pods with hydrophones recorded sound levels. A. Recording pod with Aquarian Audio hydrophone, B. stereoscopic camera (s), C. recording pod with Brüel & Kjær hydrophone, D. Bait bag, E. Aquarian audio hydrophone connected to DVR recorders for synchronization of video and sound., F. IP camera for live video link to surface. G. Subsea housing containing mini DVR recorders and power supplies, H. subsurface buoy. Photo of (A) on deck during the Blyth trials (B).

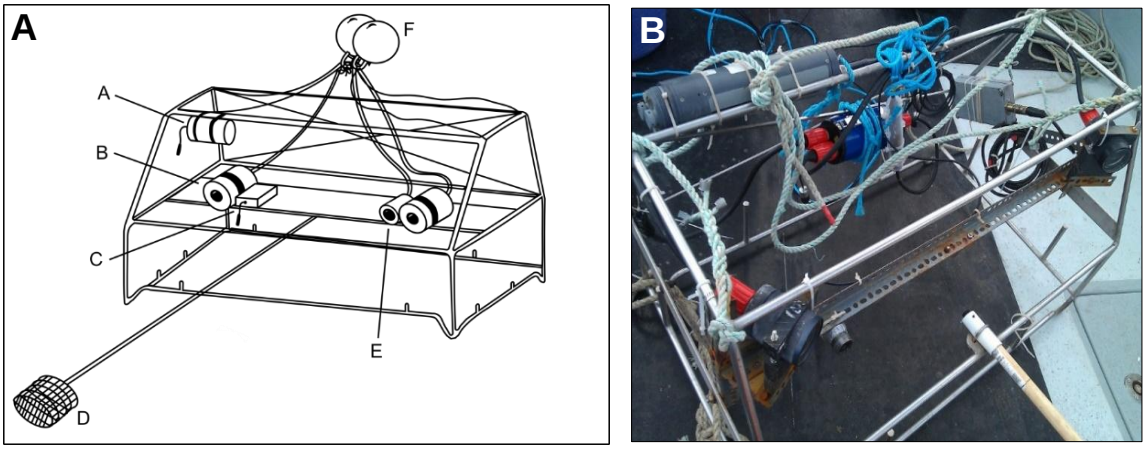

Figure 3.5 Third BRUV prototype (A), consisting of two stereoscopic cameras wired via an armoured ubilical cable to surface DVR recorders and power supplies. Two remote recording pods with hydrophones recorded sound levels. Letters as above except E. IP camera for live video link to surface, F. Subsurface buoy. Photo of Figure 3.5 on deck (B) during trials.

Figure 3.6 Digital still image taken during the Blyth trials, depth 10 – 5 m. An effervescent bait pellet, containing fish oils and bloodworm, was used to create a large fizzing plume.

3.3.4 Deployment method

The research vessel used for the sea trials was “The Princess Royal” (M.V.) a brand new 18.9 m catamaran of Newcastle University. For small-scale deployments along the Holderness coast, the Hull University RIB (approximately 8 – 10 m) was used, in addition to two local commercial boats (“Providence” Gemini catamaran 40’ x 17’ x 4’, “Kimberley” Cygnus Cyfish 40’ x 12’ x 3’6”). Work at Lough Hyne used the facilities of University College Cork, including the university RIB (approximately 6 m) but work was also undertaken from shore. The relocation of work from Lough Hyne to other areas was necessary to scale the experiment up to true oceanic conditions.

Prior to deployment drop-down cameras (Bullet camera, SeaViewer Drop-down camera and Drift HD action cameras) were used to ascertain whether visibility and bottom conditions were suitable. Additionally, during the Blyth work, two triangular camera frames, each equipped with colour camera in a subsea housing were deployed at various locations to assess abundance of species prior to the experiments. These recorded video unattended and provided information on the suitability of different locations for the experiment, aiding site selection.

Preferred locations for the work were those with a sandy, soft substratum, close to shore to minimise weather effects and a maximum of 15 – 20 m deep for suitable light levels and control of deployment. Flat substratum conditions were best due to the wide nature of the camera frame. Known areas of high fish aggregation, such as wrecks and reefs, were targeted when available.

The precise nature of deployment depended on the vessel size but typically consisted of:

- Anchor the vessel in the experimental area, wait until the vessel is stable with the currents.
- Link the BRUV to the A-frame of the vessel, lift over the port or starboard side (depending on currents). Lower the frame down to the seabed, deploy a recovery pellet and record position.
- Winch the anchor line in until the boat rests at 10 – 15 m from the BRUV.
- Using the A-frame, deploy the sound projector array from the bow, lower to half the depth or 10 m if total depth greater than 20 m.

- Begin acoustic testing.

This sequence was used to ensure that the vessel was moored 5 – 15 m away from the baited camera frame, allowing the sound levels to be sufficiently large in the experimental area, (Figure 3.7).

To allow the sediment around the camera frame to settle, experiments were initiated 10 – 15 minutes after deployment. The experimental vessel, when stationary with engine and generators running, was estimated to be at a consistent sound pressure level of 132 dB re 1 µPa RMS @ 1 m (data from Subacoustech Ltd., Blyth 2012). Where possible the vessel was therefore not powered throughout the experiments. It is of note that even with the engine off, wave slap against the vessel was audible- this is an issue with using large vessels for such experiments, and it may have had an influence on subjects.

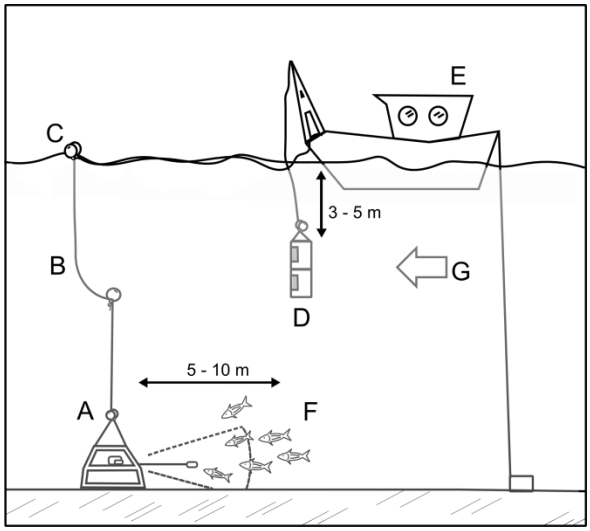

Figure 3.7 Deployment diagram of the BRUV and sound projector array from an anchored vessel. A. BRUV frame, B. subsurface buoy with umbilical cable to boat, C. Pellet for retrieval of frame, D. Transducer array (2 – 6 projectors), E. experimental vessel with A frame for deployment of equipment, F. Field of view, G. tidal direction.

Playbacks were undertaken when fishes and invertebrates were present in the field of view, although as discussed later this did not occur as often as expected. The experiment could be controlled by one operator, with consultation with the DVR footage and controlled selection of the playback signatures. Intervals between playbacks depended upon the availability of fishes and whether or not reactions occurred, but were typically 5 minutes. The precise time of playback (from the DVR), signature played, signature level and the behavioural response (if present) was recorded per playback.

3.3.5 Video analysis method

SIMI MOTION software was chosen for the current work since VISUAL FUSION, WINANALYSE and PROANALYST had fewer analysis functions and involved more manual input. This enabled calculation of locomotory changes such as acceleration, velocity, distance between neighbours and directional variation. The analysis required a system to calibrate both cameras in 3D. For this purpose, a PVC cube (55 cm^3) was used and the camera angles were fixed at 60 – 120°. A calibration sequence

consisted of simultaneous recording with the cube in the field of view of both cameras. Within the software, each corner of the cube was selected and labelled allowing the production of a 3D model within which 3D co-ordinates could be calculated accurately.

Due to time constraints the footage from the final experiment was not tracked, but behavioural responses were evident and quantification was not deemed necessary. Behavioural changes were recorded at playback occurrences, and were based on definitions from Slabbekoorn *et al.* (2010) and Van der Graaf *et al.* (2012). Behaviour was also monitored for 10 minutes post-playback. The behaviours noted were:

- Sudden movement (flinching/spasm) and duration of;
- Startle response - rapid movement/change in direction away from the bait/out of the frame;
- Slow movement of fishes away from the bait/out of the field of view;
- Time taken for fishes to return to the bait/into the field of view;
- Change in behaviour (e.g. fish ceases to feed, guard food, guard territory, display aggression towards other fishes);
- Time taken to resume behaviour.

3.4 Preliminary results

As mentioned earlier, numerous attempts were made to complete a successful playback experiment, the details of each individual attempt will not be described here. The results of three trials were notable, two attempts at Lough Hyne, where a small-scale playback experiment was successful, and the final trials at Plymouth Sound, where multiple playbacks were undertaken. There were imperfections within each trial, and therefore the results should be viewed with caution. In addition to the three notable trials, additional observations from other trials are also outlined (Section 3.4.4).

Unfortunately in most attempts, a combination of poor water visibility, lack of fish aggregations, equipment and bad weather prevented successful playback experiments. Indeed the requirements of the experiment were difficult to fulfil.

3.4.1 Sound Exposures

Overall, the synthetic impulsive sounds were an accurate replication of recorded piling strikes, with predominant energy within the 50 – 600 Hz band. The principal difference between the playback and an actual piling strike was an increased strike duration (0.16 s longer than an actual strike, 0.52 s longer than an airgun pulse), (Figure 3.8A). Similarly the playback of the large container ship sound was comparable to the original recording, with peak energy in the 100 – 500 Hz range, (Figure 3.8B).

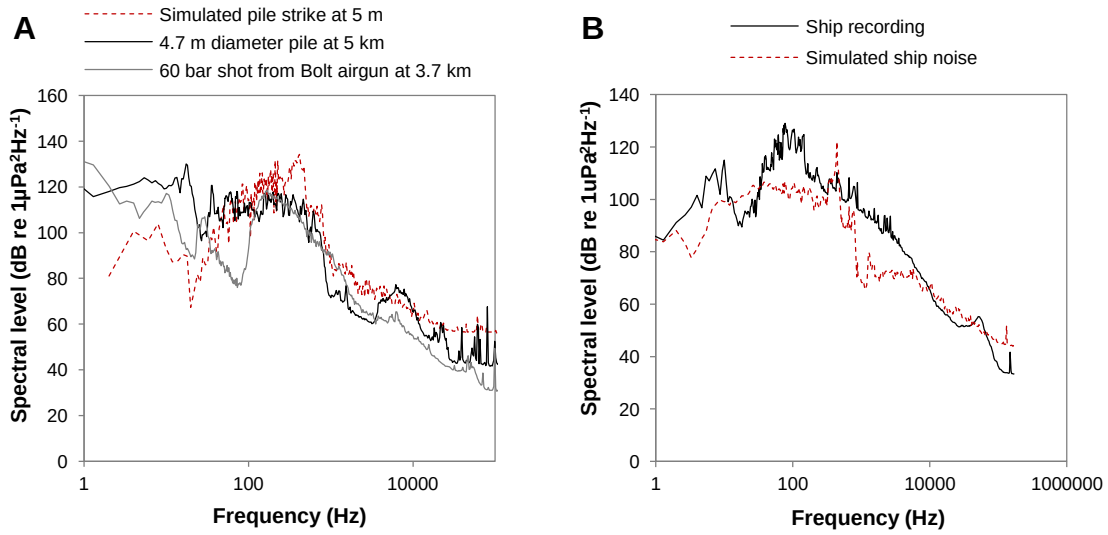

Figure 3.8 A comparison of playback of synthetic impulsive sound from the sound projector array to recordings of pile driving and airguns, data from Subacoustech Ltd. Piling data reanalysed from Nedwell *et al.* (2007) (A); A comparison of playback of boat noise to the original recording of a large container ship, data courtesy of Subacoustech Ltd (B).

3.4.2 Lough Hyne

Video observations were made during preliminary sound playback experiments at Lough Hyne (August 2011). Prior to deployment a survey of the Lough was undertaken to ascertain ideal locations for the BRUV system, which led to the deployment of the systems predominantly in the whirlpool area due to large aggregations of Pollack (*P. pollachius*), increased water visibility and easy access to the shore where equipment could be positioned, (Figure 3.9A). Position of the BRUV was adjusted until the field of view was clear of obstructions and fishes were present. The sound projector array was deployed at a distance of 5 – 10 m from the frame, in a depth of at least 10 m to enable sufficient sound propagation and sound level (judged by received sound levels), (Figure 3.9B). All playbacks were typically undertaken in less than 5 m of water to allow sufficient light levels for the cameras. Conditions of Beaufort sea state two and below were also necessary to ensure suitable water visibility. A number of playbacks were undertaken, of these only a small amount of footage was available when fishes were in the field of view and fewer of these were trackable.

A reaction of pollack (*P. pollachius*) to a stimulus was observed four times (Figure 3.10). The response was clear since swimming behaviour was observed at length prior to this, and was dissimilar, with no sudden sharp directional changes or accelerations. As a first indication, average swimming speed prior to the response was 0.02 m s^{-2}, immediately after the stimulus this increased to 7.21 m s^{-2} accompanied by a directional change.

The sound pressure level (received at the BRUV) produced on these occasions was 163.0 – 167.0 dB re 1 µPa (peak-to-peak) at its maximum, with a source level of 177.0 – 181.0 dB re 1 µPa, assuming spherical spreading. There was 1 – 2 dB variation between the amplitude levels (-6 dB from the maximum level). The variation of sound level was most likely due to the bathymetry of the lough and fractionally different deployment depths.

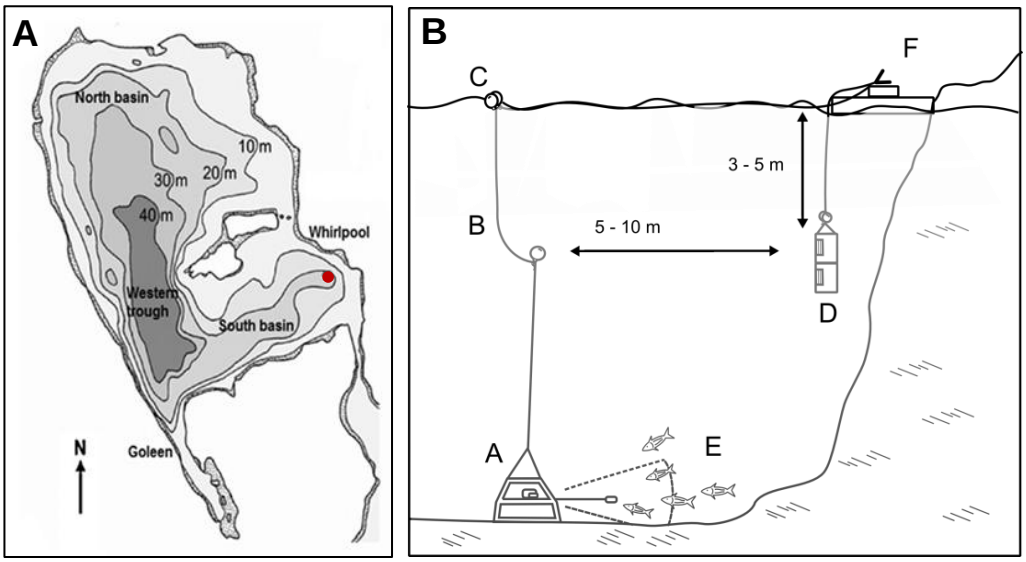

Figure 3.9 Sampling location for BRUV deployments in the Whirlpool area of Lough Hyne (A), circled in red, may 2012; Deployment diagram of the BRUV and sound projector array from the shore (B), A. BRUV frame, B. subsurface buoy, C. Pellet for retrieval of frame with umbilical cable to shore, D. Transducer array (2 – 6 projectors), E. Field of view, F. Observer station on shore with laptop and playback controls.

A number of other species were observed during the BRUV work, (Figure 3.11). Two-spotted gobies *Gobiusculus flavescens,* were very common in shallow areas (< 2 m) of the Lough. The prominent black spot on the caudal fin allowed the species to be identified easily in the footage obtained when testing the BRUV systems (proof of concept). SIMI was able to track the black spot almost entirely automatically, with some manual intervention (Figure 3.12A, B). Each goby was tracked individually, enabling parameters such as acceleration and velocity to be calculated and graphed. The two cameras were calibrated using the 'check calibration' function in SIMI Motion. The calibration cube proved sufficient to calibrate the cameras, since the values required (principal point, axes angle, captured points, as calculated within SIMI) were sufficient. The gobies did not respond to playback (L. Roberts *Pers. Obs*.) - possibly due to the shallow location of their habitat and being frequently disturbed by the laboratory RIB.

During the October 2012 Lough Hyne trip, playbacks were undertaken when thicklip grey mullet (*Chelon labrosus*) were in the field of view (19th – 26th October). In total 16.5 hours of useable footage from 19th, 22nd and 25th – 26th October was obtained, taken from the same location within the Lough (within the South Basin, near the shore, depth 2 – 3 m). Four hours of footage was discarded due to an unclear or obscured field of view. Six fish were observed circling the camera, revisiting the bait bag on each occasion. In this instance, a smaller tripod equipped with a colour bullet camera and a C55 hydrophone (Cetacean research technology) was used for the observations, which were undertaken in 2 – 3 m of water. The normal BRUV frame was used for the 25th – 26th October observations.

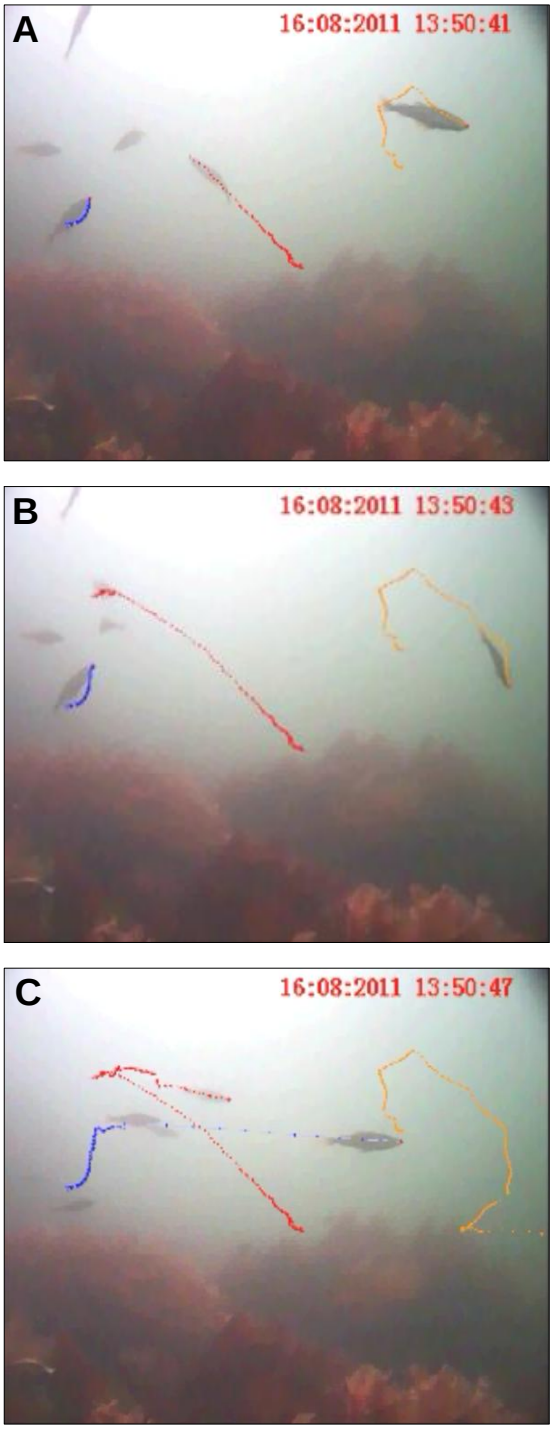

Figure 3.10 Digital stills taken from SIMI Motion Analysis software, tracking the movement of three pollack (*P. pollachius*) in response to playback noise (A – C). Each fish was shown to accelerate after exposure. For example in A., direction of a fish is shown by a red trace, after playback (B) the fish shows a sharp directional change (C). Similarly the fishes tracked in blue and yellow are shown to be heading in one direction (A - B) then sharply accelerates to the right.

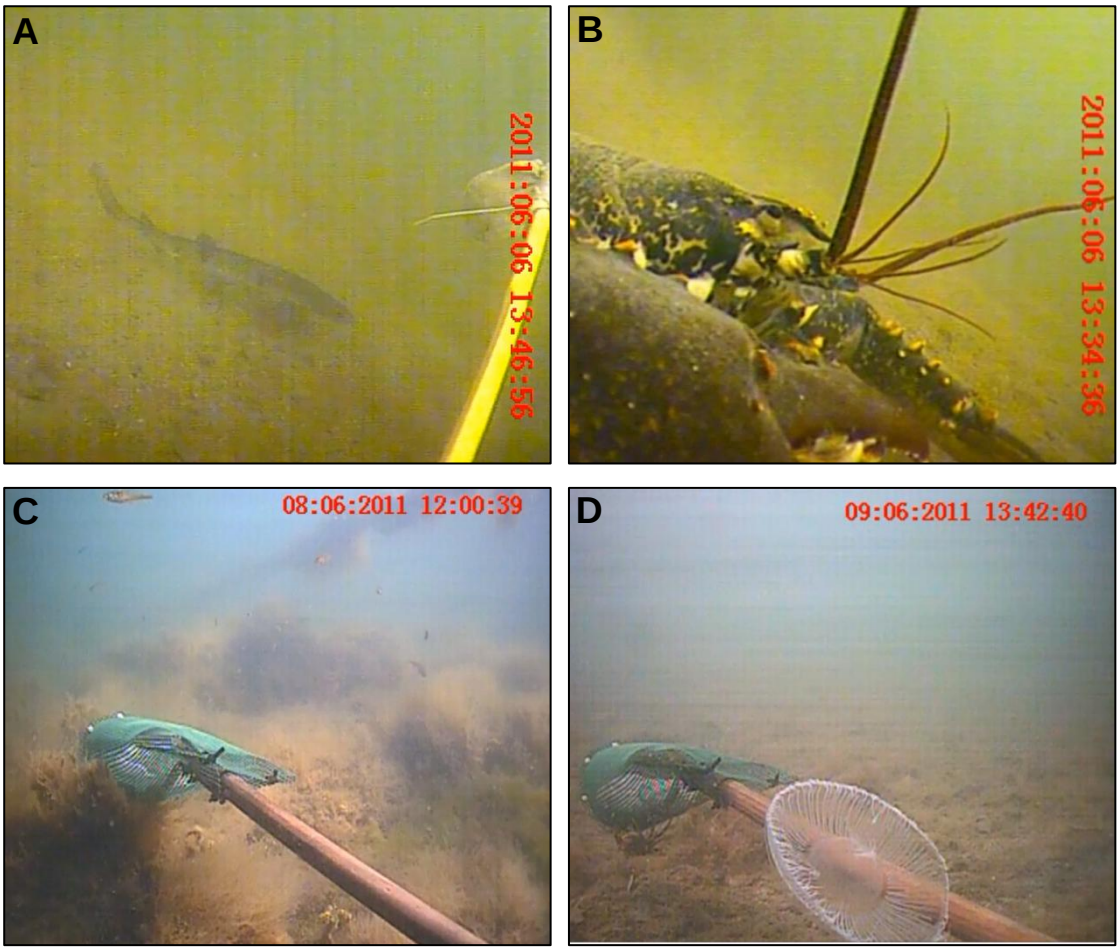

Figure 3.11 Digital still images indicating activity around the BRUV during the Lough Hyne trials (< 10 m) including small spotted catshark *S. canicula* (A), European lobster *H. gammarus* (B), unknown species of fishes, possibly *S. sprattus* (C) and moon jellyfish (*A. aurita*) (D).

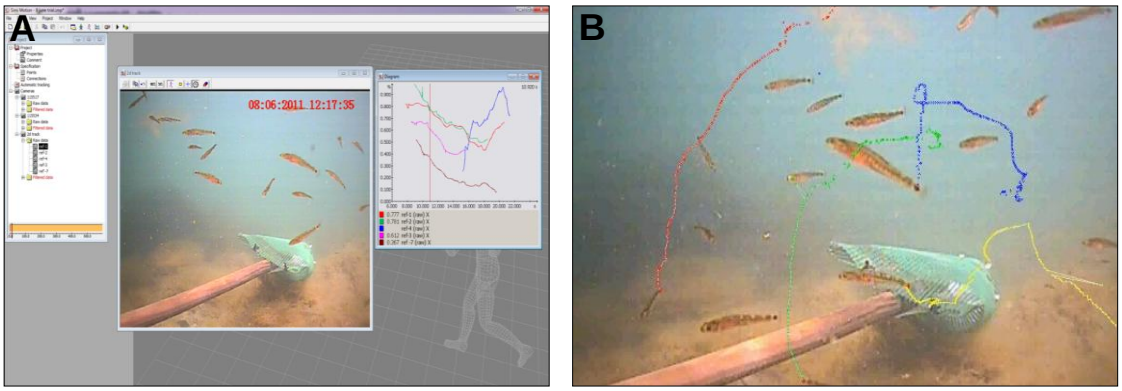

Figure 3.12 Digital stills taken from SIMI Motion Analysis software, tracking the movement of two-spotted goby (*G. flavescens*) around the BRUV bait bag during testing of the system. Digital still of motion analysis software showing the original video still and a graph of fish acceleration (A), with different lines representing the various gobies tracked in (B); Example tracks of four different gobies, shown in different colours.

Footage from 84 exposures of playback was obtained using the tripod frame, with 19 control exposures. Fish were exposed to a received SPL level of 128.1 – 141.9 dB re 1 µPa RMS (144.2 – 166.6 dB re 1 µPa peak-to-peak, 19th October) and 120.3 – 143.6 dB re 1 µPa RMS (144.2 – 166.6 peak-to-peak, 22nd October). Background levels were 105.7 and114.8 dB re 1 µPa RMS for 19th and 22nd October respectively (corresponding to 142.1 and 149.2 dB re 1 µPa peak-to-peak).

The footage from these exposures was comparatively poor quality in terms of sound and picture, but behavioural observations could be made on 15 occasions (for example, Figure 3.13). One reaction was observed at the maximum exposure level, shown as a sudden sharp directional change of two mullet, and a departure from the bait. No reactions were observed to control trials indicating that the equipment itself did not have an effect.

Additionally 101 exposures of grey mullet to shipping and impulsive sound were also undertaken using the BRUV frame, deployed from shore in water depth 2 – 3 m (Table 3.1). Shipping noise exposures ranged from received levels of 133.0 – 142.7 dB re 1 µPa RMS (162.1 – 167.1 re 1 µPa peak-to-peak) on 25th October and 115.6 – 135.7 dB re 1 µPa RMS (135.8 –152.6 dB re 1 µPa peak-to-peak) on 26th October. Impulsive sound exposures were a maximum received level of 163.4 dB re 1 µPa (peak-to-peak) with 1 – 2 dB variation with amplitude. Background levels were 114.0 dB re 1 µPa (RMS). Very few reactions were recorded, and of these only two were clear, shown as a sharp directional change at the onset of playback.

Table 3.1 Results from the October 2012 Lough Hyne playbacks of impulsive and shipping sound to grey mullet (*C. labrosus*) using the BRUV for observations.

Noise signature	Level (dB)	Occasions when fish observed	Responses[c]
Impulsive sound[a]	0 to -5	40	1 (4)
	-10 to -20	20	0
	-25 to -35	9	0
Shipping[b]	0 to -5	15	1 (3)
	-10 to -20	12	1
	-25 to -35	5	0

[a,b] Maximum sound level 163.4 (pk-pk) and 142.7 (RMS) re 1µPa respectively (0 dB)

[c] Total observations in brackets

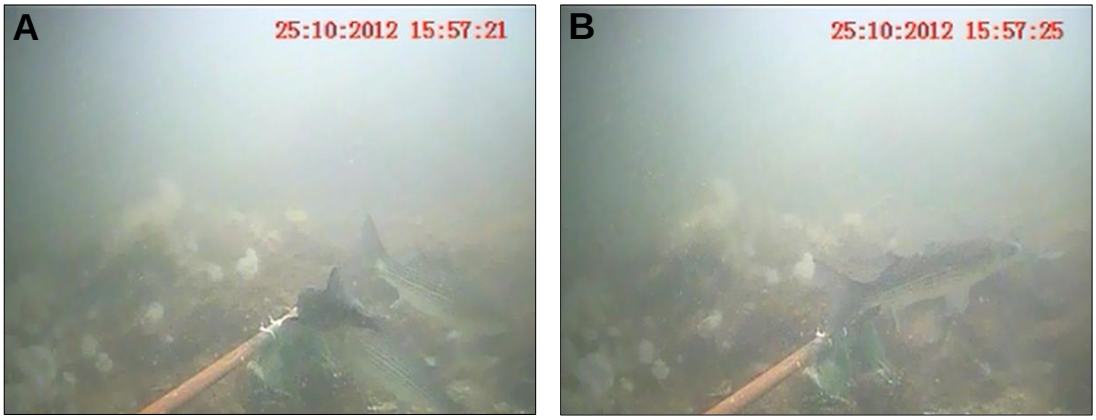

Figure 3.13 Digital still images of thicklip grey mullet, *C. labrosus*, reacting to playback of synthetic pile driving at Lough Hyne, October 2012. Multiple individuals were feeding at the bait at the onset of the noise see bottom right of (A), and startle after the first strike (B).

3.4.3 Plymouth

In the final stages of the project, work was undertaken in and around Plymouth sound (50° 19' 00. 7"N 4° 09 '20.1 "W). A total of 18 hours footage was obtained during the work, taken from 19 sites (depth < 24 m), (Figure 3.14), with 144 playbacks of the impulsive playback were undertaken, with 10 species recorded. Example digital still images of the species encountered are provided in Figure 3.15. The most abundant species were cuckoo wrasse (*Labrus mixtus,* Figure 3.15A) and pollack (*P. pollachius*, Figure 3.15C), with 213 and 106 fish observed respectively as part of groups or schools. Thirty-six of the 213 individual fish appeared to show a behavioural response related to the maximum level of noise, (Table 3.2). No responses were recorded for schooling species, although this was not the focus of the experiment.

The predominant reaction observed was a c-start most frequently by *L. mixtus*, but also exhibited by other species, (Table 3.2, appendix Table A.4, A.5).This was observed most often at the onset of exposure, however in some cases was exhibited halfway through, or even during periods of non-exposure. For example in some cases one fish responded whilst others continued feeding, and in other cases fishes responded to alternate pulses. Pollack were deterred by exposure on two occasions, and did not return to the bait- one individual continued to move towards the bait and swam past on the second strike, whilst the other swam away on the second pulse. It is of note that most responses were short term, with the fish returning to previous behaviour within a few minutes. For example a number of cuckoo wrasse were deterred from the bait upon exposure onset, but returned within 2 – 8 minutes.

Although the responses were observed at the top level of playback, the precise sound level was unable to be measured due to a malfunction in both recording pods on the camera frame. A speculative estimate of the sound levels at the frame would be a maximum received level of 167.0 dB re 1 µPa peak-to-peak, which was measured in previous trials when the cameras were approximately 10 m from the array (10 – 15 m depth). This estimate should be viewed with caution, since it is an estimate from previous trials in other locations (Blyth) where propagation conditions were different.

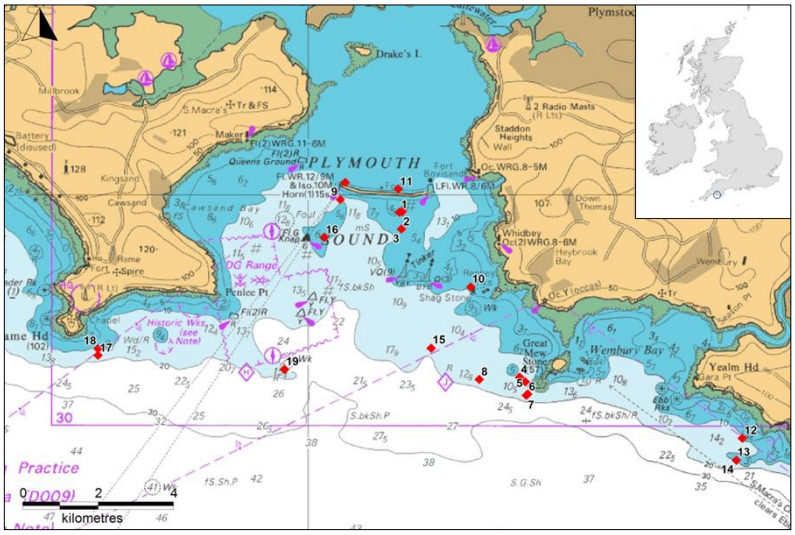

Figure 3.14 Sampling locations, represented by red markers, for BRUV deployments in Plymouth Sound, July 2013.

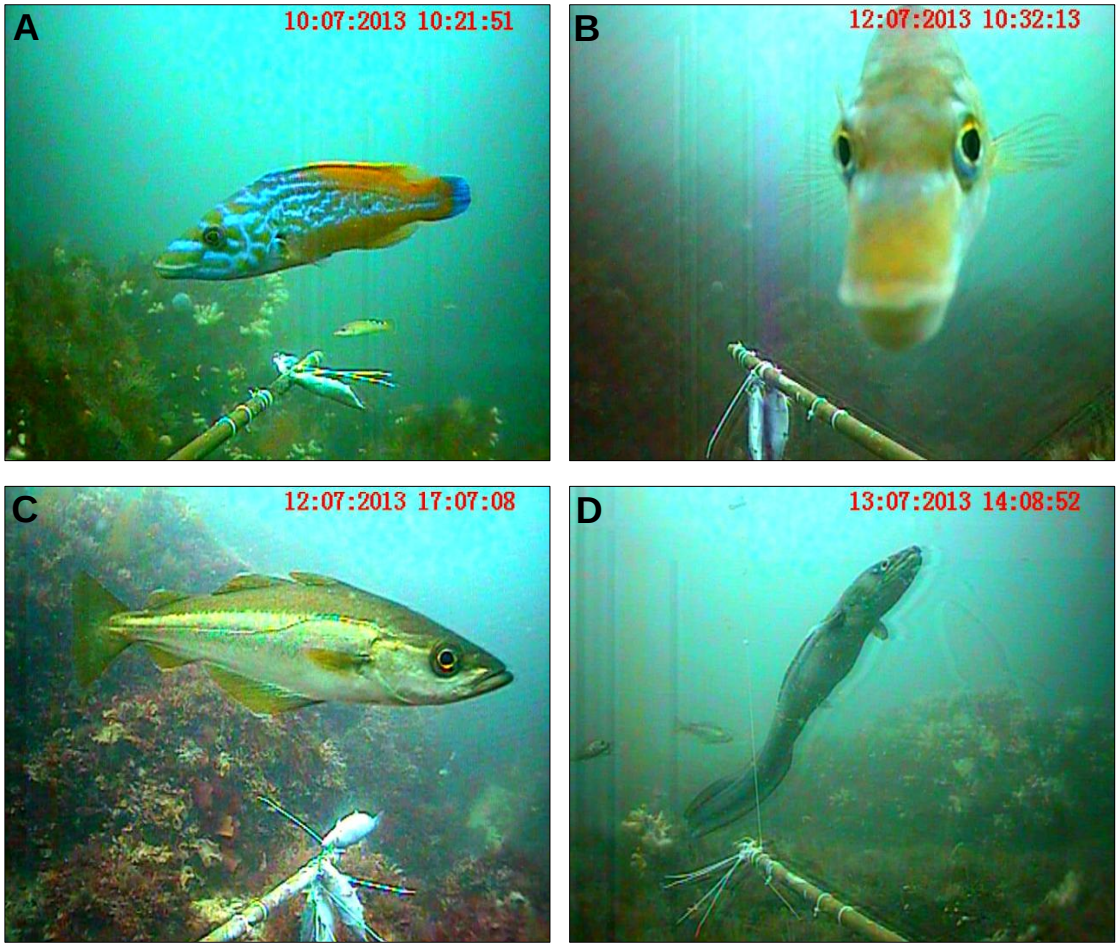

Figure 3.15 Digital still images indicating example activity around the BRUV during the Plymouth trials (< 24 m) including cuckoo wrasse *L. mixtus* (A), goldsinny wrasse *C. rupestris* (B), pollack (*P. pollachius*) (C), and European conger eel (*C. conger*) (D).

Table 3.2. Summary of behavioural responses to noise observed during the Plymouth 2013 trails. Behavioural responses are described as: moved out of frame, did not return (MON); moved out of frame with immediate return (MORI); Moved out of frame, returned within 2 – 8 minutes (MOR2); paused and immediately resumed behaviour (PI); no reaction (NR); continued normal behaviour/resumed behaviour (CN). All at the highest level, 0 dB estimated to be 167.0 dB re 1 µPa peak-to-peak, data from (SoundWaves, 2013).

Immediate Reaction	Occurrence frequency	Species exhibiting behaviour
Body spasm	3	cuckoo wrasse
		pollack, squid
MON	3	cuckoo wrasse
MOR2	20	cuckoo wrasse
PI	1	cuckoo wrasse
CN	0	n/a
NR	0	n/a
MORI	8	cuckoo /goldsinny wrasse

The estimated nominal value of the system at 10 m was 171.0 dB re 1 µPa peak-to-peak, but the conditions of deployment will affect this significantly. The lack of sound level measurement was detrimental to the work and is another reason (in addition to time constraints) why motion tracking of the Plymouth footage was not undertaken, since behavioural responses could not be linked to precise sound levels.

3.4.4 Additional observations

During the first four trips to Lough Hyne (May 2011, August 2011, Feb 2012, May 2012) trial playbacks were undertaken however most species were transient for playback experiments. Camera work was also undertaken in the Shetland islands in September 2012, using a small RUV. The purpose of the trip was to find areas where the BRUV could be deployed. Large numbers of cod, haddock and other species were found in the areas investigated, without the unit being baited; however the remote locations meant that the areas were deemed unsuitable for future experiments within the time frame of the current work.

During the Blyth sea trial (6th August 2012, appendix Table A.6), a solitary lobster (*H. gammarus*) was present at the bait bag during a playback of impulsive sound, at a received level of 167 dB re 1 µPa peak-to-peak (as estimated from the previous April trials). Immediately after the first impulsive sound strike the lobster appeared to back away from the bait bag, but returned and was in the same position by the third strike (Figure 3.16).

Figure 3.16 Digital still image of a lobster (*H. gammarus,* middle right) during playback of impulsive sound. This figure also illustrates the difficulty of seeing benthic organisms against the seabed.

3.4.5 Combined results from all trials

Table 3.3 summarises the successful playback experiment results from the above attempts, throughout the three year period, with the associated responses and exposure levels. Across all trials, key responses observed were directional changes, acceleration, c-start and avoidance to exposure levels of 142 – 167 dB re 1 µPa peak-to-peak. It is of note that the results here must be viewed with caution due to a lack of replicates and insufficient numbers of species. To extrapolate the results to different species, source types and exposure levels would be unwise.

3.5 Discussion

3.5.1 Reception abilities

The ability of organisms to perceive the sound affects behavioural responses. Pollack, in the Gadidae family, are likely to be able to detect both the particle motion and pressure component of a sound wave, due to the presence of a gas bladder. Without a physical connection between the gas bladder and the ear, the species hearing is more restricted in terms of frequency range compared to other, more sensitive species. Studies of other Gadidae species such as *Gadus morhua* (cod) have indicated sensitivity in the frequency range of 30 – 470 Hz (Chapman and Hawkins, 1973) with a sensitivity to infrasound (below 40 Hz) (Sand and Karlsen, 1986). The cuckoo wrasse (*L. mixtus*) family Labridae, another species with a gas bladder but without a connection to the inner ear, appears to be sensitive up to 1300 Hz (Tavolga and Wodinsky, 1963; Schuijf *et al.*, 1971). Finally the two spot goby (*G. flavescens*), family Gobidae, is thought to have sensitivity up to 400 Hz due to a gas bladder, but is less sensitive to sound than the aforementioned species (Lugli *et al.*, 2003). Therefore as indicated in the present work *G. flavescens* and *L. mixtus* were most likely to exhibit different responses to the playback sounds due to varied detection abilities. However it does not seem appropriate to deduce anything further due to few replicates.

It is of note that there is a lack of data regarding the hearing abilities of fish species (Section 1.4.2). Of the audiograms available, many have been created using AEP thresholds in standard laboratory

Table 3.3 Summary table for the results of all BRUV trials during the three year period. Behavioural responses are described as directional change (DC), acceleration (A), c-start (CS), moved out of frame and returned immediately (MORI), moved out of frame no return (MON).

Trial	Species	Frequency (total exposures)	Behaviour	Playback signature	Max. SPL dB re 1 µPa	Max. SL dB re 1 µPa	Background SPL dB re 1 µPa
Lough Hyne 2011	pollack	3 – 4	DC/A	Impulsive sound	167 (pk-pk)	181 @ 1m (pk-pk)	n/a
		1 (16)		Impulsive sound	Received 143.4 (RMS) 166.6 (pk-pk)	n/a	105.7 – 114.8 (RMS)
Lough Hyne 2012	thicklip grey mullet	2 (81)	DC/A/MORI	Shipping	Received 142.7 (RMS) 167.1 (pk-pk)	n/a	114.0 (RMS)
				Impulsive sound	Received 163.4 (pk-pk) 139.3 (RMS)	n/a	114.0 (RMS)
Blyth 2012	lobster	1 (5–10)	DC	Impulsive sound	Received 167 (pk-pk)	171(pk-pk) @ 10 m from speakers	Boat noise in the background 132 (RMS)
Plymouth 2013	cuckoo wrasse and pollack	36 (144)	MON/CS/DC/A	Impulsive sound			n/a

tanks with a large variation in methodology, and are not thought to be truly representative of hearing abilities (Ladich and Fay, 2013; Hawkins and Popper, 2014).

3.5.2 Responses of fishes

In the current work, pollack exhibited clear responses to impulsive sound at received sound levels of 163 – 167 dB re 1 µPa (peak-to-peak). Thicklip grey mullet were observed reacting to impulsive sound from received sound levels of 163.4 – 166.6 dB re 1 µPa (peak-to-peak). Cuckoo wrasse and pollack responded at received sound pressure levels estimated to be 167 dB re 1 µPa peak-to-peak. Therefore it seems that pile driving in the received SPL range of 163 – 167 dB re 1 µPa is sufficient to elicit behavioural responses from these species. However, these results are preliminary and should therefore be treated with caution. Furthermore, extrapolation beyond this work to other studies would be inappropriate since, as discussed in the previous section, hearing sensitivities of fishes vary considerably.

Indeed due to the low number of replicates here, even extrapolation to the same species could be misleading. In any case, experimental methods, conditions, noise sources, metrics reported, environmental parameters and histories of experimental subjects are also inconsistent between research groups. Low numbers of reactions were demonstrated in the current work. However the exposure levels were similar to the 50% response levels calculated in Section 2.4.5 (Chapter 2) for schools of sprat *Sprattus sprattus* and mackerel *Scomber scombrus*. Whilst the 50% level could not be calculated for the current work due to low numbers of replicates, it is likely that this would vary according to the species hearing ability and also with exposure context.

There are few studies that have observed free-living fishes during playbacks of sound. However there are similarities in other respects, for example in terms of exposure level. Thomsen *et al.* (2010) monitored captive fishes with cameras and acoustic tags during playback of piling signatures. Sole were observed to have significantly greater swimming speed and almost half of each species changed swimming direction during playback of the pile driving signature. Received peak levels of 140 – 160 dB re 1 µPa (146 – 166 dB re 1 µPa peak-to-peak) elicited increased swimming speed and directional changes in *G. morhua* and *S. solea.* These levels are similar to the levels in the current work. However the fish used were from fish farms, which may have influenced the behaviour patterns observed.

The replication of impulsive noise was accurate in the current work, therefore comparisons may be made between the current work and others using actual pile driving exposures, for example Nedwell *et al.* (2006) exposed caged brown trout (*Salmo trutta*) to pile driving. Received levels in the cages were not provided, but were calculated from the data later and estimated at 189 and 198 dB re 1 µPa (peak) (204 dB re 1 µPa peak-to-peak) for small and large diameter pile driving respectively (Hastings and Popper, 2009).

Other studies involving cages have similarly demonstrated unresponsive fishes, for example coho salmon (*Oncorhynchus kisutch*) exposed to piling, peak SPL 208 dB re 1 µPa (SEL 179 dB re 1 $\mu Pa^2 \cdot s$, SEL_{cum} 207 dB re 1 $\mu Pa^2 \cdot s$, peak-to-peak 214 dB re 1 µPa) (Ruggerone *et al.*, 2008); and brown trout (*S. trutta*) (Nedwell *et al.*, 2003a). However in the case of the latter, the sound levels at the fish cages were not measured, instead source levels at 400 m and 50 m from the piling were

provided, measured at 134 dB re 1 µPa and 180 – 190 re 1 µPa respectively. Popper and Hastings (2009) review four more studies which investigated the effects of piling (actual or projected) on the behaviour of fishes (Caltrans, 2001; Abbott and Bing-Sawyer, 2002; Abbott, 2004; Caltrans, 2004) but these either lack sound level data or have methodological problems. It is of note that all four studies involved the use of caged fishes as opposed to free-ranging fishes as in this work.

Since there are few similar studies to the current, an analysis of studies using other impulsive sounds (for example, airguns) may be valuable. For example, Wardle *et al.* (2001) found that individual tagged reef fishes did not react to airgun shots of received peak SPL 195 – 210 dB re 1 µPa (201 – 216 dB re 1 µPa peak-to-peak). Regular video recordings indicated that the noise did not disturb the daily patterns of the schooling and resident fishes, apart from the involuntary c-start response. The authors noted that the fish, as residents of the reef, may well have detected the sound but that it was not deemed a sufficient threat to leave the 'safety' of the home territory. This emphasises the importance of 'motivational state' of the animal upon the presence of reactions (Section 6.3, Chapter 6). Nomadic or migrating fishes would perhaps respond in a different way, perhaps as they did in the current work. It is of note that the camera system in Wardle *et al.* (2001) used a floodlight and this may have affected behaviour, although this may have been counteracted by the long duration of the system on the seabed allowing full acclimation of the fishes. At similar exposure levels (SPL 205 – 209 dB re 1 µPa, 211 – 215 dB re 1 µPa peak-to-peak), caged freshwater fishes did not react to airguns (Popper *et al.*, 2005). However at lower levels captive rockfish *Sebastes* sp. exhibited diving, startle and swimming changes (154 dB re 1µPa peak; 160 dB re 1µPa peak-to-peak) with the threshold of a startle response at 200 dB re 1µPa (peak) (Pearson *et al.*, 1992). The varied results from these studies at similar exposure levels are most likely emphasising the importance of experimental context for such experiments, but may also reflect the varied hearing abilities of the tested species.

Playback of shipping noise of received SPL 142.7 dB re 1 µPa (RMS) was sufficient to startle thicklip grey mullet repeatedly here. Similar levels of boat noise (142 – 162 dB re 1 µPa, RMS) have been demonstrated to alter the time budgets of reef fish such as damsel fish and red-mouthed gobies (*Gobius cruentatus* and *Chromis chromis*), which subsequently spent less time caring for nests (Picciulin *et al.*, 2010). However there is great variation in the frequency composition of boat engine noise, and therefore the exposures of the current work are unlikely to be similar. For example the signatures of Picciulin *et al.* (2010) had a main spectra energy content of below 1.5 kHz (peaks 1033 Hz and 602 Hz for a ferry and fiberglass boat respectively).

In the same paper, tourist ferry noise was described as producing 120 – 130 dB re 1 µPa (RMS) (at 82 m distance), and fiberglass boats as producing 140 – 150 dB re 1 µPa (RMS) (recorded at 1 m distance). Nedwell and Edwards (2004) described source levels of 170 –180 dB re 1 µPa @ 1m and 180 – 190 dB re 1 µPa @ 1m for small (< 50 m) and large (>100 m) boats respectively. The exposure levels of 142.7 dB re 1 µPa found in the current work to startle mullet would therefore be at an appropriate level encountered in the oceans.

In the current work, c-start responses were exhibited by cuckoo wrasse and pollack, in addition to directional changes and acceleration. The responses are in accordance with the previous studies (mentioned above) however since the sound levels in the current work are provisional it is difficult

to draw any further conclusions apart from that the exposures in this case was clearly sufficient to cause behavioural changes.

As discussed earlier, there are more playback studies involving captive fishes than free-living fishes. Although the results of these should be viewed with caution, they do indicate the types of responses exhibited. Captive studies show that that a wide range of reactions are likely, for example: 1) Increased acceleration and velocity, directional responses and changes in swimming depth (Blaxter and Hoss, 1981; Kastelein *et al.*, 2008; Fewtrell and McCauley), and 2) directional avoidance to noise, positional changes in the water column, increased swimming speed and startle responses (Schwarz and Greer, 1984; Engås *et al.*, 1995; Akamatsu *et al.*, 1997).

It is of note that the two spot gobies (*G. flavescens*) observed at Lough Hyne did not appear to respond to playback noise. This may be due to their location, underneath a floating jetty where the RIB was moored, or due to their reduced hearing sensitivity- further tests would be required to ascertain the reason. In this way they may have been habituated to noise disturbance (Chapman and Hawkins, 1969; Knudsen *et al.*, 1992; Peña *et al.*, 2013).

3.5.3 Responses of crustaceans

There was one notable playback occurrence when an invertebrate was present, *Homarus gammarus.* The lobster appeared to respond to the first strike of pile driving playback (estimated to be 167 dB re 1 µPa peak-to-peak) but did not respond to the remaining strikes. Without further replicates it is not possible to ascertain whether the response observed was related to the stimulus.

As discussed in Section 4.6 (Chapter 4) the detection abilities of crustaceans are still not fully understood, (Breithaupt, 2002). It has been conjectured that crustaceans respond to particle motion rather than sound pressure (Breithaupt and Tautz, 1990; Goodall *et al.*, 1990; Popper *et al.*, 2001), and thus are thought to be capable of vibration reception only (Tautz and Sandeman, 1980; Plummer *et al.*, 1986; Breithaupt and Tautz, 1988, 1990; Goodall *et al.*, 1990; Monteclaro *et al.*, 2010; Roberts and Breithaupt, 2015). As such the term ‘acoustic sense’ may be more appropriate when referring to crustaceans, rather than ‘hearing’ (Goodall, 1988).

It is likely that *H. gammarus* was sensitive to the particle motion produced by the playback array in the current work, however the levels of this were unquantifed and the exposure level is unknown. It is not known whether the projector produced particle motion levels in excess of the lobsters threshold of detection- it is of note that such a threshold is not documented, although may be estimated from behavioural observations of other crustaceans e.g. *N. norvegicus* (Goodall, 1988). Similarly, the pressure and particle motion produced from the source may also have propagated in the seabed, creating a substrate-borne component to the stimulus. Measurements of vibration on the seabed were not undertaken in this work, a deliberate omission due to logistical issues. It is highly likely that crustaceans such as *H. gammarus* are sensitive to such seismic waves. Most noise sources in the relevant literature are described in terms of pressure rather than particle motion- this issue is widespread and has led to a call for more descriptive sound level measurements so that impacts upon fishes and invertebrates can be understood (Hawkins and Popper, 2014; Popper *et al.*, 2014).

There is some evidence to suggest that crustaceans respond to noise stimuli, but there are few data to conclude anything for certain. Laboratory studies have indicated behavioural responses (leg withdrawal and movement) of snow crabs (*Chionoecetes opilio*) to sharp sounds (unquantified); however in the field, behaviour did not show such variation after exposure to airgun pulses (received sound level 201 dB re 1µPa, received energy level 150 dB re 1 µPa) (Christian *et al.*, 2003). The response in the laboratory compared to null response in the field supports the theory that crustaceans are sensitive to particle motion rather than pressure, since within the laboratory tank it is likely that particle motion would be greater. It is of note that acoustically tagged crabs moved away from shooting areas, although the tagging study had some logistical problems. Results from other species are similarly difficult to interpret- for example caged *Homarus americanus* exposed to airguns in the laboratory and field respectively (200 dB re 1 µPa and 230 dB re 1 µPa peak-to-peak with peak energy densities at 25 – 26 Hz) (Payne and Funds, 2007). There were no effects in terms of mortality or damage and no loss of limbs (indicative of stress) although laboratory animals indicated an increase in feeding and some serum biochemical changes. Changes in feeding behaviour have also been demonstrated in captive *Carcinus maenas* exposed to noise (Wale *et al.*, 2013a). Finally, Brack (2010) investigated the response of *H. gammarus* to airgun noise of 172.9 dB re 1 µPa by using a novel approach with cameras built inside lobster pots. There was no observable change in behaviour during or after exposure, and no significant impact upon survival rate. The authors of all the above studies emphasise the need for similar studies and further investigations.

Catch rates of crustaceans, measured pre- and post-exposure to noise have not demonstrated significant changes either (Christian *et al.*, 2003; Andriguetto-Filho *et al.*, 2005; Parry and Gason, 2006). However, as with catch rate studies with fishes (Skalski *et al.*, 1992; Engås *et al.*, 1996; Hassel *et al.*, 2003; Hassel *et al.*, 2004), there are difficulties of using such data unless the survey is specifically designed for purpose. For example, in the case of Parry and Gason (2006), seismic gun intensities varied greatly between the data sets which spanned 1978 – 2004, making comparisons of catch rates difficult.

Apart from Christian *et al.* (2003), there is little evidence to show a startle response in crustaceans (Payne *et al.*, 2008), although distinct behavioural changes in anti-predator behaviour have been shown in other species. For example after exposure to boat noise playback, *Coenobita clypeatus* (Caribbean land hermit crab) allowed a simulated predator to approach a greater distance before responding with withdrawal into the shell (Chan *et al.*, 2010b). Such a disruption of anti-predator behaviour may impede long-term survival of crabs, by increasing the likelihood of predation. Recent laboratory work with the marine crab *Carcinus maenas* has also indicated a slower retreat to shelter in response to a simulated predator after boat noise playback (Wale *et al.*, 2013a). It is likely, however, that the particle motion within such tanks was fairly high and erratic (Parvulescu, 1964b;a; Rogers, 2015), which may explain the reaction presence and it is unclear how representative the recordings were of the original recordings, or indeed to what aspect of the stimulus the crabs were responding. However even considering the work in terms of ‘noise’ and ‘no noise’, the results are indicative of change to anti-predator behaviour.

Changes in acoustic behaviour have been described in mantis shrimp (*Hemisquilla californiensis*), where 'rumble' dominant frequencies decreased in the presence of boat noise (Staaterman *et al.*, 2011a), although it was not clear if this was a result of masking. Furthermore, acoustic changes in frequency of 'snaps' thought to be produced by snapping shrimp (species unidentified) have been demonstrated at Lough Hyne during impulsive sound playbacks (Spiga, 2015). By disrupting acoustic communication and production, noise exposure may therefore have implications on a population level in addition to individual fitness consequences.

It is clear then, that data on the effects of anthropogenic sound sources upon crustaceans is limited, with only a small amount of extra information added from the current work. The available data are often in grey literature and a recurrent theme is that crustaceans are only mentioned briefly in addition to fishes (Wardle *et al.*, 2001; Moriyasu *et al.*, 2004). Overall there is not sufficient evidence to draw any firm conclusions. Of the existing studies, behavioural responses are difficult to compare since measurement units and methods are not well documented. Indeed in a review on the effects of explosives upon invertebrates, Moriyasu *et al.* (2004) found only thirty-five articles, more than half of these were incomplete or lacked sound measurements and others were anecdotal or mentioned unreferenced results. There is a clear need for studies which clearly state the sound levels and particle velocity used (frequency, duration, level, energy, distances from source) (Carr *et al.*, 2007) and for those investigating short and long-term behavioural changes in addition to physiological changes.

3.5.4 Replication of the stimulus

Replication of the impulsive sound is discussed more fully in Chapter 2, due to the identical sound transducer array used to produce the stimulus. To summarise, the sounds produced were an accurate representation of an actual pile driver and a large container ship in terms of energy peaks and spectra. In addition to this, the pulse like nature of the synthetic impulsive sound in this work is similar to that of airguns, providing a tentative indicator of responses to this source type. The water-borne SPL of piling may be in excess of 210 dB re 1 µPa peak-to-peak at 100 m from the source, and at 10 km may be over 140 dB re 1 µPa peak-to-peak (Nedwell *et al.*, 2003a), see Table 1.1, 1.2 (Chapter 1) for a summary key anthropogenic signatures source levels. Therefore responses observed in this study were elicited at levels that fall within the vicinity of a piling rig up to 10 km. The shipping noise in this study elicited responses at 142.7 dB re 1 µPa (RMS). Other continuous sources, such as drilling and wind turbines have been measured at 142 – 145 dB re 1 µPa (RMS) (Götz *et al.*, 2009), within a similar frequency range and therefore the species here may react in a comparable way to these sources.

However it is important to re-emphasise that whilst the water-borne component of the sound was accurately reproduced, many activities, such as piling, produce additional substrate-borne vibrations which the projector could not aim to mimic (Nedwell *et al.*, 2003). Longitudinal, shear and Rayleigh waves, as described by Hazelwood and Macey (2015), radiate outwards from piles driven into the seabed. This energy may travel along the seabed, and may even re-enter the water column away from the source (Nedwell *et al.*, 2003). Other sources may also indirectly induce motion in the substratum via the water column. 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, 2012; Hazelwood and Macey, 2015). This is important given that benthic and fishes and crustaceans were part of this work. These species are likely to be sensitive to such vibrations, being benthic or bentho-demersal. This may, for example, few reactions of benthic animals in the current work. It is also of note that operations vary between scenarios, for example in the case of pile driving varying with seabed composition, propagation conditions, pile diameter and duration of piling (Athanasopoulos and Pelekis, 2000; Harwood, 2002; Thandavamoorthy, 2004).

3.5.5 Critique of experimental setup

The experimental setup itself evolved rapidly throughout this work. Due to the purpose-built nature of the setup some of the problems encountered were a result of technical issues that were gradually resolved. The camera system was modified after each field attempt, depending on the conditions and subsequent deployment method. In terms of camera systems, there are commercially available units that could have been used for the project had cost been no option, which may have saved time. However the projector array had to be a purpose-built system since such a system did not previously exist, as it was intended to generate a plain progressive wave field and use inverse spectral processing to mimic the original signature as best as possible. Sound levels in excess of 210 dB 1 µPa were intended, and thus a custom build was necessary. The design evolution was thus an integral part of this project. For example the projector array was initially large and difficult to deploy, but by the end of the project the units were much lighter (15 kg versus 300 kg) and could be deployed without use of a winch. By the end of this study a set of small underwater sound projectors able to accurately reproduce anthropogenic signatures were available for future researchers.

One of the main issues encountered during this work was the difficulty of following each subject throughout the duration of a playback clip. Furthermore it was often unclear if that same individual was exposed multiple times to the same stimulus. This could be overcome by monitoring the organism movement in another way. For example on a smaller scale, acoustic tags can be used to study the behaviour of individual wild fish and indeed crustaceans, eliminating the need for confinement but maximising the traceability of the target (Engås *et al.*, 1998; Wardle *et al.*, 2001; Christian *et al.*, 2003). Unfortunately this technique is somewhat invasive and the tags and the hydrophone array would be costly. Nevertheless, once the animals have recovered, reactions to noise can be acquired with relatively little interference. For example, Engås *et al.* (1998) tagged cod *Gadus morhua* and monitored them pre-, during and post-trawler activity. Transmitters were hidden inside bait, which the cod voluntarily ingested, to minimise stress levels. However, since the fish were tracked using a stationary system, there were times when the trawling was occurring but the fish were not in range of the hydrophones. This problem was also described by Wardle *et al.* (2001) who used a similar approach. In this case the tagging was more invasive- pollack (*P. pollachius*) were caught by rod and line and then an acoustic ‘pinger’ was attached externally. A fixed array of seven hydrophones was used to track the fish movements. As in Engås *et al.* (1998) the tagged fish were not always in the ‘right’ area to be exposed to noise, but avoidance reactions were exhibited by two fish when they were within 10 m of the airguns. Use of tagging is valuable since the locomotory data can then be incorporated into individual-based models (IBM) of fish

behaviour (Willis, 2011; Willis and Teague, 2014), enabling the scaling of individual changes up the population level (Chapter 6, Section 6.5 for discussion).

A combinatorial approach may be best such as Thomsen *et al.* (2010) who used both tagging and RUV. However 'pings' between the acoustic transmitters were fairly widely spaced (every 22 seconds, with the position of each fish being every 90 seconds) meaning that movement tracking accuracy was poor. The use of tagging and net pens such as Thomsen *et al.* (2010) would have ensured the presence of animals throughout the current work. However the overarching aim here was the observation of free-ranging animals. Tagging is an invasive process and responses may not be fully representative of natural behaviour- additionally tagging poses limitations upon the species that can be used, and the numbers of animals that can be observed. Additionally the subjects are exposed to variable levels of sound as they move, which may affect the precision of calculated thresholds.

Whilst a range of bait types were used here, some deployments suffered from a lack of animals which was detrimental to the experiment. Bait has been used to attract organisms into the field of view in a number of video studies (King *et al.*, 2007; Stobart *et al.*, 2007) - a variety of bait is typically used, from mackerel (*Scomber scombrus*), to crushed sardines (Clupeidae) (Priede and Merrett, 1996; Watson *et al.*, 2005; Cappo *et al.*, 2007), and it is widely accepted that bait type has a significant effect on the fish assemblage attracted (Løkkeborg and Bjordal, 1992; Watson *et al.*, 2005; Harvey *et al.*, 2007; Wraith *et al.*, 2013). It is thought that sufficient care was taken to maximise the attractiveness of the BRUV to targeted species, but in some locations such as Lough Hyne, animals were transient only. The ideal scenario would not involve bait to attract animals, but it was clearly necessary to observe sufficient numbers of organisms.

Another method of increasing fishes in the experimental area may have been to use light as an attractant. However extra lights may attract zooplankton and affect behaviour (Juell and Fosseidengen, 2004; Raymond and Widder, 2007; Ryer *et al.*, 2009), and may also create a scattering effect in turbid water. As such this option was not explored in the current work.

3.5.6 Analysis

Motion analysis software was successfully used to track the movements of fishes in response to noise, when footage was available. The system was simple and was able to track movement automatically using pattern-matching with additional manual intervention. Digital stills of tracks and graphed parameters were straightforward to produce and cameras were accurately calibrated. Footage obtained during the field trials was trackable to varying degrees depending on the species observed, illustrating that for free-ranging experiments motion analysis software is an efficient tool. Indeed tracking software has been used to measure movement in a variety of different organisms ranging from spiders and crickets (Hall *et al.*, 2010; Sensenig *et al.*, 2010) to bats and chameleons (Schaub and Schnitzler, 2007; Fischer *et al.*, 2010).

Three-dimensional tracking programmes are not specifically designed for fishes, therefore the automatic algorithms used are frequently unable to pick up movement without the use of markers to target specific features. Many interpretations and standardisations have to be used for example choosing which part of the fish to track (eye, caudal or dorsal fin), the problem of fishes leaving the

field of view and more large-scale problems such as the influence of interactions between species within the footage. For example, in the case of the Lough Hyne footage, two-spotted gobies (*G. flavescens*) were automatically tracked due to the clear black spot on the caudal fin, however difficulties were encountered with more cryptic species. These issues with motion analysis software do not appear to be covered in published works.

Programmes are able to track fishes in 2D without markers providing there is suitable contrast in the footage (e.g. SIMI MOTION and ETHNOVISION). However the background of footage filmed in the field has additional moving features and highly variable light levels – the software is frequently unable to track a target automatically and manual intervention is required, for example in the case of pollack in the current work. Supervised tracking was also necessary to reduce software error. This issue was particularly prevalent when undertaking test tracks of invertebrates such as *Aurelia aurita* (moon jellyfish), *H. gammarus* (common lobster) *C. maenas* (shore crab), *Hyas sp*. (spider crab) and the common prawn (*Palaemon serratus*) observed at Lough Hyne- these species in particular, with pale or mottled colouration, were difficult to track against the seabed (Figure 3.16).

Although swimming changes such as the c-start response have been quantified using WINANALYSE for a few fish species (Domenici *et al.*, 2004; Weber, 2006; LeFrancois *et al.*, 2009; Fuiman *et al.*, 2010), such software does not appear to have been used for the analysis of responses to sound stimuli. This is of importance since the swimming parameters obtained from motion analysis could be translated into metrics (such as percent response, response latency), which could be compared across noise levels and signatures with parametric tests. Multimetric analysis, for example Principal Component Analysis (PCA) could be used to produce a 2D representation of the behaviour of the subjects. As with tagging data, swimming parameters could also be incorporated into IBM approaches (Willis, 2011; Willis and Teague, 2014) to link behavioural responses to population level.

3.5.7 Deployment

The playback of sound signatures is logistically difficult, indeed the setup of such a projector at the required depths and distances was a lengthy procedure initially, in addition to testing to ensure sound stimuli were of the required level. The deployment of the first sound projector array required use of a winch and two operators to unsure safe deployment without damage to the vessel.

The deployment of the BRUV had its own difficulties for example such landers are prone to instability on uneven substrata or when cables are taut. This was particularly relevant to the current BRUV, which was wide and heavy, with many cables and instruments upon it. If deployed upon uneven ground the lander was prone to fall forward, which risked damage. Although baited camera frames are used for a range of underwater environments from the deep sea (Priede *et al.*, 2006; Cousins *et al.*, 2013) to shallow water (White *et al.*, 2013; Unsworth *et al.*, 2014), the design of these is relatively standard. The principal difference in systems used in the literature is whether to have horizontally (e.g. Cappo *et al.*, 2007) or vertically mounted cameras (e.g. Cousins *et al.*, 2013, for a review see Mallet and Pelletier, 2014) . The design of horizontal camera landers, such as the current work, is common throughout the BRUV literature (Cappo *et al.*, 2007). A smaller footprint or a central anchor could have increased the stability of the current BRUV. Alternatively a pyramid-

shaped frame could have been used (Shortis *et al.*, 2007), but with outward facing cameras instead of downwardly facing ones. However since the BRUV in this study was successfully deployed it is not thought that the design was at fault.

Seabed rugosity posed a problem in terms of being a snag-risk for cables and the frame, which caused retrieval issues. Although drop-down cameras were used prior to deployment, these were at risk of entanglement with seabed debris and macroalgal fronds. It was advantageous to deploy near wrecks, as these are often areas of high fish aggregations. However, the balance between near a wreck and 'on' a wreck could be problematic, for example one small remote camera system was lost in the Blyth harbour area, thought to be tangled on debris. In situations like this the knowledge and expertise of the skipper was essential. Even when a suitable site was found, large macroalgal fronds could obscure the field of view. In this way the use of moveable camera heads, controlled from the surface, would have been valuable. In some cases the choice of site overruled other conditions required for the experiment, for example despite multiple attempts at the work away from boat traffic, the final experimental area in Plymouth was closer to the harbour than intended, simply due to higher fish aggregations in these areas. The success of a deployment was then, a trade-off between ideal bathymetry, camera picture, water visibility, ambient noise levels and numbers of species present. Time and budgetary constraints further restricted the numbers of deployments that could be attempted. It was not possible without long-term monitoring to know whether the particular area where the BRUV was of importance to the resident species- for example a key breeding or foraging area. In addition to this, as with all BRUV studies, the influence of species outside the field of view could not be ascertained or addressed.

Anchoring of the vessel near the BRUV required precise positioning, taking into account the currents and the distance from the camera frame. In the later trials, the umbilical cable from the camera frame also had to be suitably slack throughout the experiment to ensure stability of the BRUV. This meant, in some cases, continual adjustment of the equipment and the vessel. Modification of the buoyancy on the ropes was necessary to prevent the umbilical rope being lost. Such deployment concerns, whilst common to marine work, created difficulties in the field. There was concern that even with the engine off, the boat made noise (e.g. wave slap). For this reason it was suggested that a large buoy could be used to deploy the projector array remotely in a similar way to the camera but this was not attempted due to time constraints. Instead, for later attempts a smaller vessel was used, in fact multiple small vessels were used ranging from a RIB (Hull University) to a larger fishing vessel (Plymouth). This aided deployment, although meant heavy equipment had to be hand-deployed in some instances.

The greatest issue with deployment was that of water visibility which required constant monitoring of water conditions. By the end of the project, an 'on call' approach was used, with the intention that organisations could mobilise quickly, although the co-ordination of multiple organisations made this somewhat difficult. For this to be successful, Hull University personnel were therefore trained in the use of the sound projector array, enabling independence from Subacoustech Ltd. By the time of the Plymouth work, Hull University was able to mobilise independently from the SoundWaves consortium when conditions were suitable.

3.6 Conclusions and recommendations

The following recommendations can be made for future work using BRUV and sound projector arrays.

In order for this experiment to work the site choice is especially important. A reef or wreck is best since observations from this work indicate that nomadic species will not remain in the field of view. To maximise experimental time, the camera system should be deployed for a long period of time (e.g. weeks rather than each day), be monitored from the surface, perhaps from a floating platform or from shore. In order to do this, the power supplies of the cameras and hydrophones must be at the surface, such as the final set up in the current work. The best place may be in a sheltered area, or close to shore. A sea loch, such as Lough Hyne would be suitable for such shore work but multiple observations indicated that fish species in particular did not show sufficient interest in the bait for long enough that playbacks could occur. For invertebrates, however, this approach may work better. The difficulty of finding a suitable location and of all conditions being favourable should not be underestimated.

Timed bait release boxes could be used to refresh the bait supply as required, removing the need for the lander to be retrieved for bait refreshment, such as in many deep sea systems (Kemp *et al.*, 2008). However ideally fishes and crustaceans would be present without the need for bait, especially if the work was undertaken on a reef. In terms of the BRUV itself, this should have as small a footprint as practical (without compromising the stereo camera view) to reduce chances of entanglement and instability. Cameras pointing opposite ways would allow maximisation of the field of view (Gledhill *et al.*, 1996). Priority could be given to one side of the cameras only, with the other two providing the opportunity for fortuitous additional footage. A fifth camera could provide a live feed to the surface enabling the operator to observe the experiment. Motion analysis software such as SIMI is a logical and efficient way of analysing the large amounts of video produced. More long-term deployments would provide more flexibility regarding effective days and workload.

Finally, to overcome visibility issues sonar imaging may be used, such as in Chapter 2. Sound wavelengths are 2000 times longer than visible light in water, hence suspended particles are less likely to affect the sound waves than light waves. In low visibility conditions then, where cameras fail, sonar imaging can perform well. The DIDSON (Dual frequency identification sonar) system has been specifically designed for turbid water studies (Handegard and Williams, 2008), however it is of note that the narrow beam size of this system means it is most suited to monitoring narrow areas of water (such as the entrance to a river, for example).

The current work aimed to expose individual fish and crustaceans to a fully quantified sound source, monitored by a BRUV system. The limited results indicate avoidance responses of *P. pollachius* to impulsive sound signals of 163 and 167 dB re 1 µPa (peak-to-peak). Whereas *L. mixtus* exhibited avoidance and involuntary C-start responses to impulsive sound estimated (from other trials) to be at a level of 167 dB re 1 µPa peak-to-peak. *H. gammarus* appeared to respond to a single strike of impulsive sound at level 167 dB re 1 µPa peak-to-peak, although more tests would be required to ascertain this for certain.

These results are an initial step towards rejecting the first null hypotheses- there appears to be a relationship between behavioural changes and sound exposure. It is not possible to support or reject the second null hypothesis since the response of only two species was observed. Repeating the current work would allow sufficient replicates to further test the null hypotheses.

The wider implications of the behaviours observed in the current work are as yet unknown, especially the energetic cost of the involuntary c-start response or directional avoidance. These behaviours may divert time away from anti-predator behaviour, feeding, mating, reproduction and territorial behaviour, and may elicit physiological stress responses particularly if noise disturbance continues for long periods of time (Celi *et al.*, 2014; Simpson *et al.*, 2014). The population level consequences of such behaviour are relatively unknown.

Although the results here are preliminary, the current work was successful in designing the equipment and methodology required to undertake an experiment which has not, to this extent, been tried before. The use of a fully calibrated purpose-built projector array such as this, able to accurately reproduce sound playback at suitable sound levels is valuable to the field. Furthermore, this method shows that it is possible to observe reactions of individual fish to noise without the use of tags or captivity which may adversely affect behaviour. A further attempted experiment would have produced valuable data. Given the ambitious nature of the experiment it is not surprising that the outcome is future recommendations for other researchers, rather than firm results. Despite this, the results indicate that the 'ideal' playback experiment on wild fishes and crustaceans is feasible.

Chapter 4 Sensitivity and behavioural responses of the hermit crab *Pagurus bernhardus* to substrate-borne vibration

Abstract

Sensitivity of unconditioned *Pagurus bernhardus* to substrate-borne vibration was quantified by exposure to sinusoidal vibrations of 5 – 410 Hz of varied amplitudes using the staircase method of threshold determination, with two behavioural responses used as reception indicators. Impulsive vibration exposures were also undertaken, in addition to observations of startle response, used as a measure of 'personality' pre- and post-exposure. Behaviour varied according to the strength of the stimulus. There was a significant difference in average threshold values between the two indicators, with sensitivity ranging from 0.11 – 0.29 m s^{-2} (RMS, antennal change) and 0.09 – 0.44 m s^{-2} (RMS, movement). Time in the laboratory significantly affected average threshold values and startle response (s). Clear behavioural changes were exhibited after exposure to playback, with startle duration varying after exposure. There was no correlation between personality and average sensitivity threshold, however there was evidence of consistency of response within individuals in terms of both sensory threshold and startle responses. Sensitivity of *P. bernhardus* was shown to fall within the range of actual anthropogenic vibrations. The data are a first step towards understanding the effect of substrate-borne vibration on the behaviour of a common marine crustacean and indicate that the effects of substrate transmission should not be overlooked when investigating the effects of noise pollution on the marine environment.

4.1 Introduction

An underwater sound not only produces a pressure wave, but also a particle motion component; this can be propagated via the water and the seabed (Hazelwood, 2012; Miller, 2015). In solids, such as the seabed, these vibrations can travel as longitudinal (compressional 'P' waves), shear (transverse, 'S' waves), or surface (Rayleigh, 'ground roll') waves (Markl, 1983; Aicher and Tautz, 1990; Hazelwood and Macey, 2015). The speed of travel depends upon the substratum, frequency and the depth of the hard layers of the seabed. For Rayleigh waves the energy is confined to the surface without radiating energy up or downwards. This means that these surface waves are likely to propagate for large distances (over 1 km, possibly up to 2 km) from the source, especially at low frequencies (Hazelwood and Macey, 2015). This is of particular relevance when considering anthropogenic activities such as piling and drilling into the seabed. For example shell and auger piling, involves a metal 'shell' which is driven down into the ground in a similar way to pile driving, to line the borehole for construction use. Such activity is likely to produce large substrate-borne vibrations able to travel long distances. Measurements of particle motion in the seabed are not part of standard measurement procedures and hence there are few available data (Hazelwood and Macey, 2015; Miller, 2015), this makes the impacts of such vibrations on marine organisms difficult to ascertain.

The detection of seismic vibration has been described extensively in terrestrial organisms- for example in elephants, frogs, arachnids, scorpions, and lizards (Brownell, 1977; Brownell and Farley, 1979; Narins and Lewis, 1984; Lewis and Narins, 1985; Hebets *et al.*, 2008; Garstand, 2009), (for a comprehensive review see Hill 2001, Hill, 2009). Indeed, it is thought that 150,000 species of insects use vibration for the purposes of communication, for example in the fruit fly *Drosophila melanogaster* and arachnids such as the wolf spider *Schizocosa retrorsa* (Hill, 2009b;a; Fabre *et al.*, 2012). Substrate vibration may also play a role in the location and detection of prey in some species, for example in sand lizards and scorpions (Brownell and Farley, 1979; Hetherington, 1989).

There is little information on the ability of marine crustaceans to detect vibratory signals. Crustaceans appear to be responsive to particle motion rather than sound pressure (Breithaupt and Tautz, 1990; Goodall *et al.*, 1990; Popper *et al.*, 2001), and thus are capable of vibration reception (Tautz and Sandeman, 1980; Plummer *et al.*, 1986; Breithaupt and Tautz, 1988, 1990; Goodall *et al.*, 1990; Monteclaro *et al.*, 2010; Roberts and Breithaupt, 2015). A suite of cuticular mechanoreceptors have been described, for example sensory hairs on the carapace, telson, chelipeds, antennual flagellae, and second antenna (Wiese, 1976; Tautz and Sandeman, 1980; Derby and Atema, 1982; Sandeman and Wilkens, 1982; Breithaupt and Tautz, 1988; Goodall, 1988). In addition to this, Chordotonal organs in the joints of appendages, which signal joint position and stress, also appear to be sensitive to vibration, perhaps incidentally (Burke, 1954; Budelmann, 1992a). Furthermore the statocyst, a fluid-filled chamber with a mass loaded statolith inside (Cohen *et al.*, 1953; Cohen, 1955; Cohen and Dijkgraaf, 1961) may also be involved in the detection of particle motion in addition to gravity detection, as in the cephalopods (Mooney *et al.*, 2010). Although evidence for this is largely still lacking, there is evidence of such reception in crustaceans, for example Hughes *et al.* (2014) and Goodall (1988). Such detection is likely since

sound production is widespread in crustaceans, from snapping shrimp (Johnson *et al.*, 1947; Knowlton and Moulton, 1963; Schmitz and Herberholz, 1998; Versluis *et al.*, 2000) to lobster and crab stridulation (Moulton, 1957; Horch, 1971, 1975; Aicher *et al.*, 1983; Field *et al.*, 1987; Patek, 2001; Henninger and Watson, 2005; Patek *et al.*, 2009) and even rumbling of mantis shrimps (Order Stomatopoda) (Patek and Caldwell, 2006; Staaterman *et al.*, 2011b). Indeed water and substrate-borne motion reception, and perhaps communication, seems likely in marine invertebrates in general since vibrations can propagate long distances through solids, making the seabed an ideal medium for animals to use (Taylor and Patek, 2009).

Focus upon such reception abilities have been predominantly directed towards semi-terrestrial fiddler crabs, which use vibration for communication and mating rituals (Aicher and Tautz, 1990). Thresholds of sensitivity have been determined using electrophysiological techniques (Salmon and Horch, 1973; Salmon *et al.*, 1977; Aicher and Tautz, 1984) and behavioural observations (Salmon and Atsaides, 1969) or a combination of both (Salmon, 1971; Salmon *et al.*, 1977). In marine crustaceans such techniques have demonstrated sensitivities to particle motion within the range of 0.01 – 0.81 m s^{-2} (20 – 200 Hz) for *Crangon crangon* and *Nephrops norvegicus* (Heinisch and Wiese, 1987; Goodall, 1988; Goodall *et al.*, 1990; Berghahn *et al.*, 1995). Most recently, Hughes *et al.* (2014) demonstrated sensitivity of the mud crab *Panopeus spp.* in the range of 0.01 – 0.2 m s^{-2} (75 – 1600 Hz).

Establishing the sensitivity of an organism to an acoustic or vibratory stimulus typically involves the production of a threshold curve spanning a range of frequencies, e.g. Fay and Popper (1974). On an electrophysiological level, methods involve isolation of particular sensory detectors, for example the statocysts, thorax hairs, antennules or chelae mechanoreceptive hairs (Mellon, 1963; Tautz and Sandeman, 1980; Breithaupt and Tautz, 1988; Monteclaro *et al.*, 2010). For whole animals, sensitivity curves may be produced from conditioning responses or by the auditory brainstem response (ABR) technique, often using a staircase method of presentation to determine the threshold level (Cornsweet, 1962). The technique in invertebrates does not typically involve the brainstem and thus can be called AEP (auditory evoked potential). For cephalopods, and indeed some crustaceans, the AEP technique has been successful applied (Lovell *et al.*, 2005; Mooney *et al.*, 2010), but it is now accepted that thresholds determined in this manner are likely to differ from behavioural thresholds (Ladich and Fay, 2013).

Conditioning of crustaceans has been undertaken using operant and classical conditioning (Offutt, 1970; Abramson and Fieinman, 1990; Feinman *et al.*, 1990; Burnovicz, 2010). However whilst a successful attempt of crustacean conditioning to sound has been published (Offutt, 1970), the heart rate is naturally erratic and cessation may be elicited by shadows, footsteps and light level changes within the laboratory (Florey and Kriebelm, 1974) making such attempts difficult. As an alternative to the conditioning approach, small behavioural changes may be indicative of stimulus reception; and this approach has been used for examining exposure to unconditioned animals vibrations. For example Heinisch and Wiese (1987) and Berghahn *et al.* (1995) observed a reliable flicking of the second antenna in *C. crangon* exposed to vibration, and Tautz (1987) described antennual movements in the crayfish *Orconectes limosus* (Spiny cheek crayfish). Other postural changes

such as abdominal extension, clawing and leg movement have also been used as markers for stimulus reception (Goodall *et al.*, 1990; Breithaupt, 2002).

It is of note that previous investigations of sensory thresholds of animals have not considered individual differences in response between individuals, which may be termed personality of the animal (Dingemanse and Réale, 2005; Briffa *et al.*, 2008; Dingemanse and Wolf, 2010; Briffa *et al.*, 2013). This may be of importance since individuals may differ from each other in terms of their behavioural responses, with individuals displaying the same behavioural tendency in different contexts, for example in hermit crabs (Briffa *et al.*, 2008) and in spiders (Wright *et al.*, 2014). Further variation in response may be explained by duration in captivity prior to tests, since time in the laboratory may increase the possibility of acclimation to background noise levels and disturbance stimuli. Such habituation has been demonstrated in fishes (Chapman and Hawkins, 1969; Knudsen *et al.*, 1992; Peña *et al.*, 2013). It is therefore important to consider these factors when investigating behavioural sensory thresholds.

There are few studies exposing crustaceans to noise, and yet such energy is likely to have strong particle motion components and therefore to be within the detection capabilities of marine crustaceans (Popper *et al.*, 2001; Hazelwood and Macey, 2015). Behavioural work has focussed upon fisheries catch rate changes (Andriguetto-Filho *et al.*, 2005; Parry and Gason, 2006) or effects upon early developmental stages (Radford *et al.*, 2007; Radford *et al.*, 2008; Stanley *et al.*, 2010; Pine *et al.*, 2012), with only Stanley *et al.* (2010) detecting behavioural changes such as a delay in the metamorphosis of postlarvae. Recent work undertaken in the laboratory has indicated increased oxygen consumption, decreased feeding and changes in anti-predator behaviour of *Carcinus* maenas (Wale *et al.*, 2013b;a) in response to ship noise. However such small tank studies should be viewed with caution (Section 1.11.2). Another recent laboratory study has also indicated reduced foraging of *Panopeus sp.* (mud crab) in response to predatory fish sound (Hughes *et al.*, 2014). Changes in acoustic behaviour have been reported in the mantis and snapping shrimp when exposed to boat playback (Staaterman *et al.*, 2011a; Spiga, 2015). There also is some evidence to suggest that anti-predator responses of crustaceans may vary after exposure to noise sources (Chan *et al.*, 2010a; Chan *et al.*, 2010b; Stahlman *et al.*, 2011; Wale *et al.*, 2013a) and may also vary with individuals and context (Briffa *et al.*, 2008). In general, existing data are often in the grey literature and a recurrent theme is that crustaceans are mentioned briefly as a side-line to fishes (Wardle *et al.*, 2001; Moriyasu *et al.*, 2004). This has led to a call for data to fill the deficit in the invertebrate literature (Hawkins *et al.*, 2014a). Underlying this requirement is a need for a greater understanding of reception capabilities of crustaceans.

Whilst particle motion detection by crustaceans is indeed possible in both water and solids, it has been suggested that water-borne detection of natural cues may only be possible close to source (Goodall, 1988; Popper *et al.*, 2001). However the extent to which motion is detected and used through the substrate is relatively unknown and can only currently be estimated from semi-terrestrial species. It is therefore clear that there is a need for studies investigating this reception, using behavioural techniques in relatively unconfined animals. Furthermore, there is a need to relate such data to specific seabed vibrations produced by anthropogenic activities.

4.2 Aim, objectives and null hypotheses

The aim of the current work was to begin to understand the effects of the vibration component of noise upon marine crustaceans. To do this, the precise sensitivity and response of a common decapod species to a sinusoidal signal was undertaken in controlled laboratory conditions, followed by exposures to recordings of piling (an impulsive vibration). Postural changes and antenna movement were used as indicators of reception, since preliminary tests indicated that occurrence of each varied with amplitude of exposure. Startle responses were also measured to investigate flexibility in response between stimulus, situation and individual.

The threshold experiments used a fully calibrated vibration source, a purpose-built tank to minimise external vibration, and two sensors recording the exposure levels and ambient levels in all three axes of motion within the experimental tank. Subjects were unconditioned since simple behavioural indicators could be used to determine sensitivity, with the animals free to move within the tank, enabling a more representative threshold than other restrictive methods. Threshold values were related to duration in the laboratory prior to tests, morphological parameters, startle response behaviours (personality, as discussed later), threshold values from the literature and measurements of anthropogenic vibrations in the field.

The species investigated was *Pagurus bernhardus* (*L.,* family Paguridae) a marine intertidal hermit crab that shows a clear anti-predator mechanism (withdrawal into the shell) in stressful conditions (Elwood and Briffa, 2001; Chan *et al.*, 2010a; Chan *et al.*, 2010b). The sensitivity of *P. bernhardus* to vibrations (natural or anthropogenic) is unknown, although the sensitivity may be similar to the semi-terrestrial hermit crabs such as *Uca sp.* (Salmon and Atsaides, 1969; Aicher and Tautz, 1990), or marine crustaceans such as *N. norvegicus* and *C. crangon* (Heinisch and Wiese, 1987; Goodall *et al.*, 1990). It is assumed that, like many Decapoda, the presence of a statocyst, and a suite of mechanoreceptors may facilitate possible reception capabilities although the structures within *P. bernhardus* specifically for such detection have not been described.

The null hypotheses that were tested in this work were:

1. Characteristics of the vibration stimulus (frequency and amplitude) will not affect response.

2. The threshold sensitivity will be the same regardless of the behavioural indicator used to calculate it.

3. Time in the laboratory prior to tests will have no effect upon sensitivity thresholds nor upon startle response behaviour.

4. There will be no consistency in thresholds per individuals.

5. Morphology variation between individuals will have no relation to the sensitivity nor to the duration of the startle response.

6. The threshold of response will not be correlated with startle response (s) (personality). There will be no consistency in startle response between different individuals and scenarios.

7. Playback of an anthropogenic vibration will not affect the behaviour of tested individuals, in terms of key behavioural changes and more specific changes such as startle response duration.

4.3 Materials and Methodology

Hermit crabs, *P. bernhardus* occupying mixed *Littorina sp.* shells (shell height 15.9 – 23.3 mm), were collected by hand from the intertidal area of Scarborough bay (54° 16' 15.3"N 0° 23' 17.1"W) and transported in seawater to holding tanks within a room with minimal disturbance. The holding tanks were in a temperature controlled room under a 12 hour light 12 hour darkness regime, with a water temperature range of 11 – 12°C. All animals were starved for 24 – 48 hours before tests.

The crabs were subsequently fed every 48 hrs on a diet of mixed shellfish. Food was placed in two areas in each tank, and excess cleared within 4 – 8 hrs. Partial water changes (25%) were undertaken every 2 – 3 days to ensure that water conditions were suitable, and levels of salinity, nitrates, nitrites and ammonia were monitored throughout.

Within the holding tanks crabs were free to move and interact, hence shell swapping may have occurred during this period. To reduce conflicts, the tanks were furnished with shelters, rocks and a small number of spare shells. Individuals with missing appendages were withheld from tests, as were those that moulted. A minimum period of 24 – 48 hours was allowed between collection from the shore and experimental testing to allow for sufficient acclimation.

4.3.1 Experimental setup

The experimental tank (500 x 400 mm initially, changed to 400 x 600 mm after the first experimental run for logistical reasons) had a water depth of 150 mm and a substratum of depth 30 mm, consisting of fine white aquarium sand. This was chosen to increase visibility of movements. A circular plastic arena (100 x 100 x 50 mm), assumed to be acoustically transparent, was at one end of the tank. Within this arena, the subject moved freely throughout the experiments. An acclimation period of 12 – 14 hours inside the tank was used prior to threshold determination.

A purpose-built base supported the experimental tank, consisting of a layered structure built to minimise external ground vibrations entering the experimental tank (Figure 4.1). A steel frame held an electromagnetic shaker above the experimental tank, with a purpose-built carbon fibre rod (with associated spigot) descending vertically to the substratum. A plastic cap, fixed with epoxy onto the end of the rod, was buried in the substrate. This increased the vibration propagation. The steel frame was weighted for stability, and was separate from the base of the tank. A small black plastic screen shielded the arena from any visual disturbance created by the stinger rod movement.

An underwater camera (initially a SeaViewer SeaDrop camera, later changed to a Microsoft Lifecam web-camera in a subsea housing) was situated above the arena allowing the behaviour of the subject to be monitored, and the presentation signal to be modified accordingly. The camera was connected to a monitor which was situated away from the experimental tank. The behaviour of the subject could then be observed without disturbance by the experimenter. This was important since the staircase method required adjustment of the signal according to prior responses.

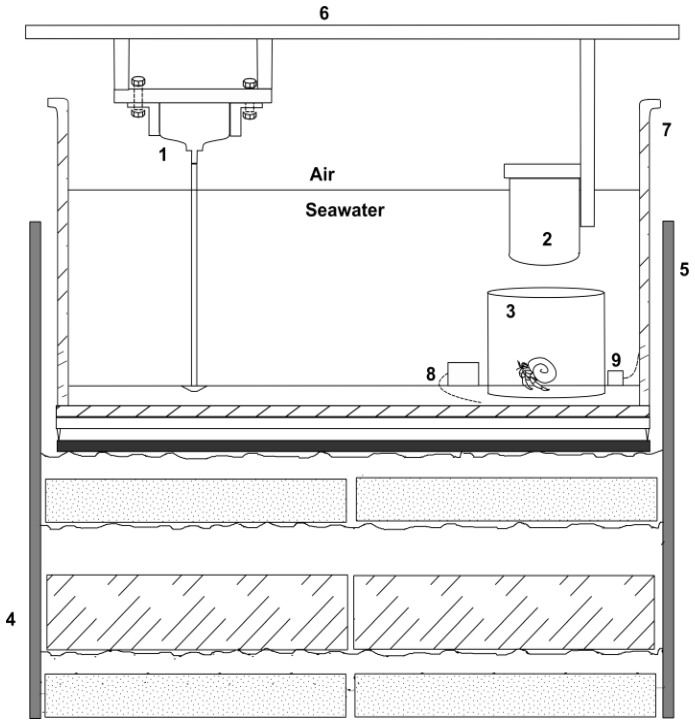

Figure 4.1 Schematic of experimental setup, consisting of electromagnetic shaker and stinger rod (1), underwater camera (2), experimental arena (3), layered base made up of mixed hard and soft insulaton and concrete (4), wooden support structure (5), steel frame separate from the base (6), experimental tank with needlepoint legs (7), position of geophone system (8), position of accelerometer (9).

4.3.2 Vibration stimuli

The experimental setup aimed to test the effects of primarily substrate-borne particle motion, using a shaker system to provide sinusoidal stimuli, with a limited pressure gradient and waterborne particle motion elsewhere in the tank. Sine waves of 8 second duration (with a 1 s rise and decay time to prevent distortion of the signal) were presented in the range of 5 – 410 Hz, at 11 different amplitudes (in increments of 6 dB below the maximum level) and seven frequencies. Signals were generated in AUDACITY (version 2.0.5), exported on an SD card and played back through a Roland R-09HR MP3 recorder. The recorder was connected to a car amplifier (JL Audio XD 200/2 200 W 2 channel, full range 12 – 22 kHz) and an LDS v101 electromagnetic shaker (sine force 8.9 N, frequency range 5 – 12,000 Hz, calibration date 31st May 2012).

4.3.3 Experimental sessions

Several experimental sessions were undertaken over a period of 12 months to determine threshold values with a total of 45 crabs tested throughout the study. An additional 30 crabs were exposed to playback of impulsive vibration. There was a delay of 8 months between the first threshold tests and the other sets. This was due to unforeseen external construction work which negated the quiet conditions required for testing.

To test hypotheses 1 – 6 from Section 4.2, the following experimental sessions were undertaken (Figure 4.2).

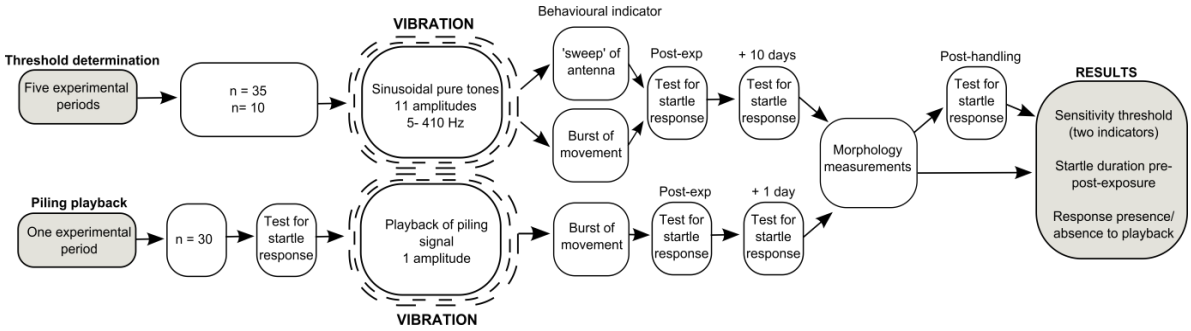

Figure 4.2. Flow diagram of the experiments undertaken exposing *P. bernhardus* to vibration.

4.3.4 Hypothesis 1 & 2

Extensive preliminary tests indicated that individuals exhibited a suite of responses after exposure to vibration stimuli, ranging from full retraction into the shell to smaller 'flinch' type responses. As such, two different behavioural indicators were used to calculate threshold values (hypothesis 2). The first indicator used was a 'sweep' of the first antenna, which was clearly visible at onset of the stimulus, often with additional movement of the antennules (experimental sessions 8th April – 5th May 2013, 8th – 18th December 2013; 11th – 20th February; 21st February – 6th March 2014). The second indicator used was a burst of movement which occurred at the onset of the stimulus (experimental sessions 18th March – 11th April; 2nd – 11th April). Only one indicator was used per set of crabs- i.e. several groups were tested for the threshold either by using the first or the second indicator as a response.

The staircase method of threshold determination was used to determine the threshold (Cornsweet, 1962). The experimental procedure consisted of exposing the subject to the signal, observing the response and then choosing the next signal accordingly. A positive response to the signal initiated a reduction of the signal amplitude, and vice versa. This procedure continued until two amplitudes were repeatedly presented, with positive and negative responses consistently i.e. that the staircase reached a plateau (Figure 4.3). Presentation of these two amplitudes was undertaken until ten repetitions had been tested. The threshold value was then calculated as the average of these ten iterations (Cornsweet, 1962).

One crab was tested per day with the presentation of frequencies fully randomised. Each crab was tested only once, apart from in the re-test experiments. Amplitudes were presented two minutes apart since preliminary testing indicated that responses lasted up to 1 – 2 seconds after each stimuli ended. A period of 10 – 20 minutes was given between frequencies to allow recovery of the subject. At the end of each experiment, the water was partially changed in the experimental tank (10 L), with organic debris removed.

Control observations were made during each day of experiments, at a random time throughout the day. Behaviour was observed when exposed to five 'blank' signatures.

In addition to the determined threshold values, the literature was searched for known threshold values of crustaceans to particle motion (water and substrate).

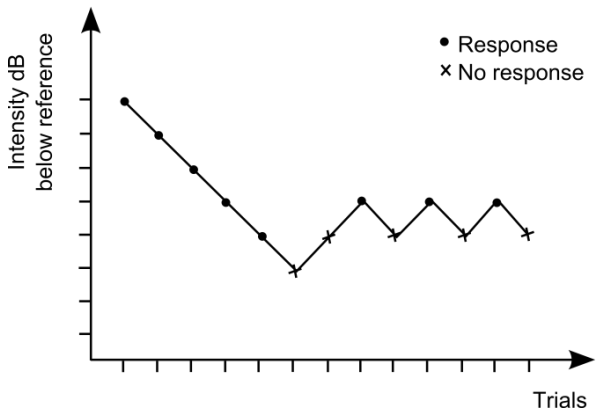

Figure 4.3. Example data for a typical sensitivity threshold by the staircase-method. Amplitude of the signal is reduced with every positive response (circle), and increased when a negative response is observed (cross), this continues until there are consecutive iterations of positive-negative (shown by the last six points). An average of ten iterations is used to calculate the threshold of response.

4.3.5 Hypothesis 3

Hypothesis 3 (time in the laboratory) was tested by using all threshold data sets which used the antennae movement as a response (8th April – 5th March 2013, 8 – 18th December 2013, 11th February – 6th March 2014) but subdivided into two groups according to duration in the laboratory. Group 1 (Spring 2013, Spring 2014 crabs 1 – 10) and group two (December 2013 and February 2014 crabs 11 – 20) were then compared, being 66 – 74 days and 0 – 9 days in the laboratory respectively. The thresholds using movement as the response indicator were not included since all crabs had approximately the same duration in the laboratory prior to tests.

In addition to this, the February data (11th February – 6th March 2014) was analysed separately since the data consisted of 20 crabs, 10 having been in the laboratory for 74 days prior to tests, and 10 having been in the laboratory for one day only prior to tests.

4.3.6 Hypothesis 4

Hypothesis 4 was tested by re-testing a set of crabs for the threshold, to investigate whether the threshold would vary each time. Crabs were tested for the threshold using movement as the response indicator on 18th March – 11th April 2014, and then re-tested on 2nd – 11th April 2014. In this way the consistency in response of each individual was tested i.e. to investigate whether the average threshold per individual was consistent or variable.

4.3.7 Hypothesis 5

To test hypothesis 5 (morphology variation and threshold values), shell and claw morphology data were related to threshold values for all crabs (n = 25). Morphological measures were taken of each individual after testing for the threshold, using callipers to measure to the nearest millimetre. Cheliped length (maximum anterior-posterior axis of chela) and width (maximum lateral axis, mm) were measured, in addition to shell height, width and aperture height of the occupied *Littorina sp.* shell (Figure 4.4A, B). The animals were then placed in a separate holding tank (200 x 200 x 150 mm) for their remaining time in the experimental room.

4.3.8 Hypothesis 6

Observations from the threshold experiments indicated that behaviour appeared to vary significantly between individuals when first placed in the arena- some crabs explored the area thoroughly and throughout the experiments, whilst others remained stationary throughout. To quantify such individual variation, crabs were tested for 'personality' as defined in Briffa *et al.* (2008). The test consisted of lifting the crab from the substrate by hand, inverting for ten seconds then replacing the crab upside back on the substrate with the shell aperture upwards. This caused the crab to withdraw into the shell fully. The time taken for the crab to replace all appendages back onto the substrate was timed using a stopwatch. The startle response can be used as a measure of 'boldness' (associated with bolder behaviour in general such as exploring new environments and objects more readily), where 'bold' is defined as a short startle response after being upturned, and 'shyness' as a longer recovery time (Briffa *et al.* 2008). Crabs used in the threshold experiments (December, February and March, indicators 1 and 2) were tested for startle response after the threshold tests in different situations.

The startle response test was undertaken straight after exposure to vibration (e.g. at the end of the days threshold testing) for all three groups. Additionally for the February and March groups crabs were also tested for the startle response after 10 – 17 undisturbed days, and then immediately after being handled for measurement purposes (Figure 4.4). The data from the February and March experiments was not pooled, since crabs differed in terms of duration in the laboratory prior to tests (being 74 days and 26 days respectively).

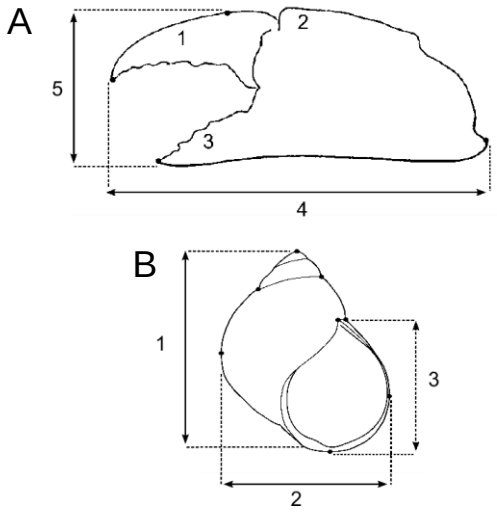

Figure 4.4. Morphology of *P. bernhardus* cheliped (A) Dactyl (1), Manus (2), Propodus (3), cheliped length (mm, 4), cheliped width (5); Morphology of *Littorina sp.* (B), shell height (1), shell width (2), aperture height (3).

The startle durations were used as a measure of plasticity of personality between situations where 'situation' in this case was defined as the occasion the startle test was undertaken (e.g. post exposure, post handling). Startle durations were also compared to average thresholds per crab to investigate whether sensitivity could be linked to personality.

The startle response times were then converted into a ranking of 'boldness', where a ranking of '1' denoted 'boldness' (short startle duration) and the lowest ranking of 'shyness' (long startle duration). The rankings within each group were compared to investigate consistency of rankings between tests. For example, in the March group the crabs were tested three times for startle response, these three rankings were then compared. Additionally average threshold, as defined in Section 4.3.4 was compared to personality rankings for all three test groups (December, February, March).

Table 4.1 Groups of crabs tested for personality (Briffa *et al.*, 2008; Briffa *et al.*, 2013), numbers of test given below.

Exposure type	Test group	Test	Total tests
Sinusoidal (5 – 410 Hz)	December n = 10	Post-exposure	1
	February	Post- exposure	
	n = 15	Pre- handling[a]	3
		Post- handling	
	March	Post-exposure	
	n = 10	Pre- handling[b]	3
		Post-handling	

[a] 17 days post-exposure

[b] 11 days post-exposure

4.3.9 Hypothesis 7

To test hypothesis 7 (playback of an anthropogenic source) a recording of shell auger piling (from now on termed impulsive vibration) was played through the electromagnetic shaker. The signal (15 seconds) had been recorded by Subacoustech Ltd. using a Vibrock geophone (sensitivity 0.023 V/mm s^{-1}, 10 kHz) at 23 m from a piling operation. The amplitude of the playback was adjusted accordingly so that the level within the tank approximated the actual recorded level (0.0005 m s^{-1}). The amplitude of the signal was then kept constant throughout the experiments.

Thirty crabs (fresh for experiments) were used in the tests, with ten crabs tested per day. The day before testing, each crab was tested for startle response/personality as previously outlined, after 1 hour of acclimation. After 4 minutes, crabs were exposed first to a 15 second recording of 'silence' and then, one minute later, the impulsive playback signal (15 s). Behaviour was monitored throughout and for 5 minutes after the exposure. A startle response test was undertaken straight after the test, and the next day prior to morphology measurements and weighing (AND EK-300 I, 0.01g balance). There were therefore three personality/startle response tests applied - denoted from now on as pre-exposure, post-exposure, and post-exposure + 1 day. The video was scored for response (presence or absence, binary) live by the experimenter, and also by an independent

observer, who recorded reactions and described behavioural responses at a later date. The two sets of scores were then reviewed, with conflicting scores discussed and resolved.

4.3.10 Statistical analysis: all hypotheses

Simple descriptive statistics were calculated in EXCEL software (version 2007), further analysis was undertaken with SPSS (version 19). 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. A Levene's test for homogeneity of variance was used where appropriate.

Hypotheses 1 and 2

Threshold values (RMS), as defined earlier, were calculated and plotted against frequency and average background levels. Comparisons between the data were undertaken using a Mann Whitney U-test. Data were compared as a whole and also subdivided by frequency. A Kruskal-Wallis test was used to compare background vibration levels between experimental periods.

In order to compare sensitivities to vibration from actual anthropogenic sources, the literature was searched for publicly available vibration data from sources such as drilling and pile driving. The values in the literature are typically provided as velocity (m s^{-1}). It is of note that the vibration levels summarised in the current work were given in terms of maximum peak amplitude across all axes- the axis of the maximum was not provided and therefore it is not known which axis is predominant in the given signals. Since anthropogenic signals cannot be considered sinusoidal, the measurements were not converted to acceleration, as ideally they would be differentiated with respect to time. Even an approximate conversion, using the sinusoidal equation was not undertaken since most of the data did not include peak frequency data to allow accurate conversion.

Instead, 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:

$$A = 2\pi fV \qquad [10]$$

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

Hypothesis 3

To test whether time in the laboratory prior to experiments affected results, an independent t-test was used to compare average threshold values between the two groups (i.e.- short and long duration in the laboratory prior to tests), both with the data grouped altogether and subdivided by frequency.

Hypothesis 4 & 5

A paired t-test was used to compare the mean threshold between the first and the second test per crab. Data were analysed as a whole, and subdivided by frequency. Pearsons correlation was used to investigate the relationship between shell height (mm), aperture (mm), cheliped width (mm).To

compare morphology data with average thresholds, Pearsons R correlation with morphology measures and the average threshold per crab (for both threshold indicators, m s^{-2}) was calculated. Data were tested as a whole and subdivided by frequency.

Hypothesis 6

Startle response (s) measured immediately after threshold tests was averaged throughout the three groups (December, February and March, n = 33) and compared to threshold sensitivities (m $s^{-2,}$ averaged per crab across all frequencies) by means of pearsons correlation. The data were further subdivided by group and the analysis repeated. Kendall's coefficient of concordance was calculated to test the degree of consistency in response between rankings of individuals in each personality test. Startle times across each testing situation were then compared within the February and March groups, used as an indicator of plasticity of behaviour across situations. A repeated measures ANOVA with sphericity assumed was used to test for differences in startle times between tests. Posthoc testing, pairwise comparisons (supported by pairwise t-tests) were undertaken where appropriate. Correlation analysis was undertaken to compare duration in the laboratory prior to threshold tests (in days) and startle response for the three groups, using non-parametric correlation (Spearmans).

Hypothesis 7

In order to test the effect of impulsive vibration upon responsiveness, number of responses (binary data) between control and exposure periods were compared using a chi-square test. Startle times (pre-, post- and post- exposure + 1 day) to playback were compared using a repeated measures ANOVA. Kendall's coefficient of concordance was calculated to test the degree of consistency in response between rankings of individuals in each personality test. Pairwise comparisons were undertaken where appropriate, with paired t-tests to corroborate the findings.

4.3.11 Signal analysis all hypotheses

Vibrations within the tank substrate (in the vertical axis, sampling rate 1 K/s) were measured continuously using a Brüel & Kjær piezo-electric accelerometer (Type 4333, sensitivity 20.6 mV/g) waterproofed with Milliput epoxy putty. The signal was fed through a battery powered calibrated Brüel & Kjær charge amplifier type 2635 (calibration date 5th July 2013), an ADInstrument Powerlab data acquisition system and a laptop computer with CHART 5 software (version 5.5.6) installed. 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. At the end of each set of experiments, measurements were taken inside the arena whilst sweeping the shaker through the range of test signals. This enabled the calculation of a correction factor for received vibrations inside the arena from the measurements taken next to the arena.

Since particle motion is a vector quantity, a waterproof geophone system was also used (Sensor Nederland, SM-7 375 ohm, IO, sensitivity 28.8 V/m/s), consisting of three orthogonally mounted geophones embedded inside epoxy resin. This allowed the vibration in all three axes of motion to be measured, using the same ADInstrument Powerlab module for data acquisition. The geophone

was adjacent to the arena throughout the experiments. 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.

Data from the accelerometer and the geophone system was recorded simultaneously (and continuously) using CHART 5.5 software. The exact amplitude of each shaker signal for each threshold 'step' was measured using the hand tool and the Datapad calculation function. The root mean square of the signal (RMS), as defined by CHART 5.5 was calculated as 'the square root of the sum of the squared amplitude values of the points'. All four sensor channels were selected at once, allowing RMS calculations for the accelerometer and the three 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 received vibration the subject would receive inside the arena), and then were averaged to calculate the threshold value for each frequency.

It is of note that the accelerometer malfunctioned half way through the threshold experiments (indicator 2) and therefore the thresholds for that period (18th March – 11th April 2014) were calculated using accelerations for each shaker amplitude, calculated from other threshold exposures. The average acceleration was calculated from each respective shaker amplitude, by using data most recently obtained from the tank. The geophone was not used for this purpose, since calculations of the thresholds using this sensor revealed an underestimation of the thresholds compared to the accelerometer outputs (i.e. animals appeared to be more sensitive to stimuli when thresholds taken from the vertical axis of both sensors were calculated). This was thought to be due to calibration inaccuracies, rather than sensor malfunctions- therefore data from one sensor only was used in the threshold calculations with the geophone being used to demonstrate the relative proportions of the stimulus in each axis.

For the impulsive vibration playback experiments, the geophone system was used to measure vibration levels within the tank. Peak amplitude (taken from the maximum pulse per exposure) and background level (RMS, 1 minute) were exported for each playback test (n = 30). Background levels and peak amplitudes were then averaged across all thirty days. Conversion to acceleration (and therefore calibration) of the velocities was not undertaken due to the frequency dependent nature of the sine wave equations, and the non-sinusoidal nature of the playback signal.

4.4 Calibration of sensors

4.4.1 Accelerometer calibration

The 4333 accelerometer and the tri-axial geophone were calibrated using a type 4370 accelerometer (Brüel & Kjær, sensitivity 80 mV/g, 24th February 2014) which was used for the sole purpose of calibration. Two calibrations were thus undertaken, of the 4333 accelerometer, and of the geophone system. The methods for these differed slightly, as outlined below.

The calibration of the type 4333 accelerometer used a charge amplifier (type 2635, calibration date 5th July 2013) and an electromagnetic shaker (LDS v101, used also for the threshold experiments,

calibration date 31st May 2012). The type 4370 accelerometer was not a waterproof sensor, therefore the calibration was undertaken outside of the experimental tank.

The shaker was secured to a metal frame, with the excitation spigot (length 18 mm) pointing upwards (Figure 4.5). Bolted onto the spigot was a purpose-built, suitably rigid, acrylic platform (65 x 31 mm x 5 mm). The 4370 was attached to one end of the platform, in an upright position, using a Brüel & Kjær 4 mm attachment screw and nut, hand tightened. A mounting disc was placed between the two mating surfaces, maximising the transmission of high frequencies to the accelerometer. The 4333 was attached at the other end of the platform using epoxy putty. The cables from both sensors were attached to the adjacent table, allowing for slight movement when the platform vibrated, ensuring that there was no significant cable interference. As with the threshold experiments, the sensors were attached to a charge amplifier (Brüel & Kjær type 2635), a data acquisition module and a laptop computer. The exciter was the LDS v101 electromagnetic shaker as used in the previous section, and the signals were identical to the threshold experiments, although the amplifier gain was reduced due to the close nature of the accelerometers to the shaker. Due to the lightweight nature of both accelerometers (54.7 g and 12.7 g for the 4370 and 4333 respectively) the side to side instability of the platform was considered to be negligible especially due to the size of the platform itself.

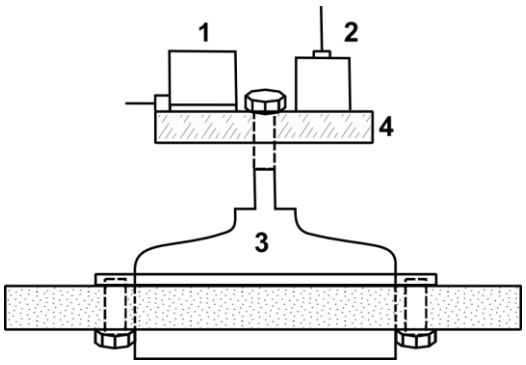

Figure 4.5 Schematic of accelerometer calibration setup, consisting of Type 4333 accelerometer (uncalibrated, 1), 4370 accelerometer (2), electromagnetic shaker (3), acrylic platform (4).

Once the sensors were attached to the platform, the shaker was swept through a test range of signals (all seven frequencies 5 - 410 Hz, eleven amplitudes) with sufficient time in between the signal for the platform to settle to the 'at rest' position. Since only one calibrated charge amplifier was available, signals were recorded consecutively for each sensor. In between the two sets, all fastenings were checked for continuity. The whole process (recording from each sensor) was repeated five times, to check the repeatability of the calibration, with the average taken as the final value after continuity had been assessed.

Sensor outputs were recorded in CHART software (sampling rate 1 k/s), as for the thresholds, and were exported in a similar way using the selection tool and the data pad calculation window. Six seconds of each signal was selected for each amplitude and frequency. The RMS values from each sensor were then exported to EXCEL, and the two sensor outputs were plotted, with the ensuing line graph then used as a 'calibration line', (Figure 4.6). The line equations from (Table

4.2) were then used to adjust any future values measured by the 4333 accelerometer. The intercept of 40 and 90 Hz were negative and therefore were adjusted to zero to prevent the calculation of incorrect negative accelerations.

A frequency response curve was produced from the calibration data, using three acceleration constants (0.1, 0.5 and 5 m s^{-2}) as measured by the 4370 accelerometer, and calculating the predicted 4333 response at these values (Figure 4.7,Table 4.2). The values at 90 Hz were higher, it was thought this was due to an error, or internal resonance/issue of the shaker at this frequency which could not be removed. The equation of the 90 Hz calibration line was adjusted to allow values to fall within the expected range. For this reason, 90 Hz threshold values were carefully assessed in the final results.

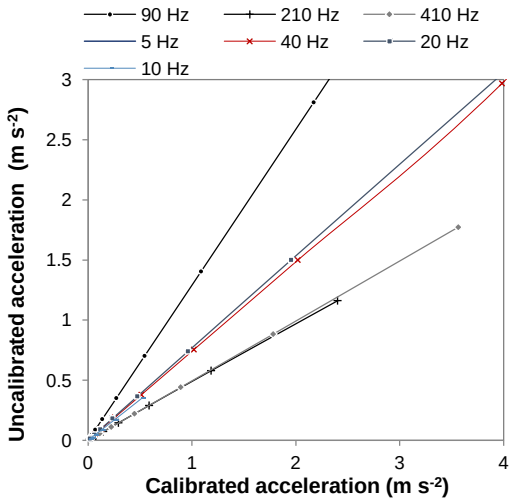

Figure 4.6. Calibration chart, as derived from calibration measurements, for the type 4333 Brüel & Kjær accelerometer used in the current work.

4.4.2 Geophone calibration

Calibration of the geophone system (sensitivity 28.8 V/m/s) was initially undertaken in a similar setup as Section 4.4.1, however two different methods were used- one in dry conditions and one within the experimental tank to allow comparisons. The calibration was based upon the assumption that each geophone within the system (i.e. each axis) would need to be calibrated to the same degree.

The method for the dry calibration was similar to that of the accelerometer, however due to the increased weight (74 g) of the geophone a slightly larger platform was purpose-built (acrylic, 100 mm x 90 mm, 10 mm thickness) (Figure 4.8). The geophone system was fastened securely to the centre of the platform with the 4370 attached using temporary epoxy putty. The associated cables were again fixed loosely to the adjacent table. Both sensors connected to the Powerlab data acquisition module, with the signals recorded in CHART (sampling rate 1 K/s), and the signals from both recorded simultaneously. As above, the shaker was swept through the test sequence (all frequencies and amplitudes). The procedure was repeated in triplicate. RMS velocities were exported into EXCEL and converted to acceleration using the sine wave equations.

Table 4.2. Slope and intercepts for the calibration lines, as shown in Figure 4.6.

Frequency (Hz)	Slope	Intercept
5	0.62	0.007
10	0.67	0.0003
20	0.76	0.002
40	0.80	0.00[a]
90	0.68	0.00[a]
210	0.48	0.0028
410	0.50	0.0003

[a] Adjusted to pass through the origin

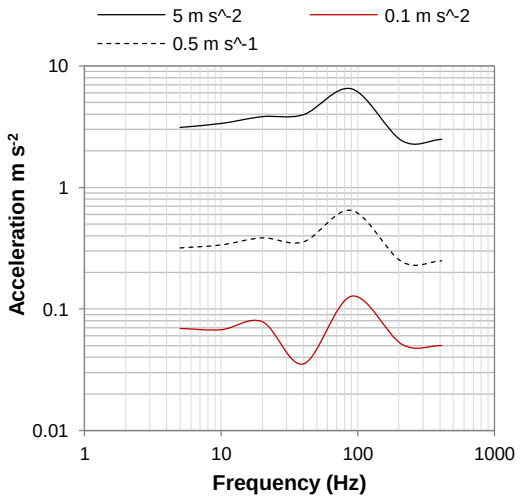

Figure 4.7 Frequency response chart for type 4333 Brüel & Kjær accelerometer (5 – 410 Hz), calibrated using a type 4370 Brüel & Kjær accelerometer. Three reference accelerations are provided.

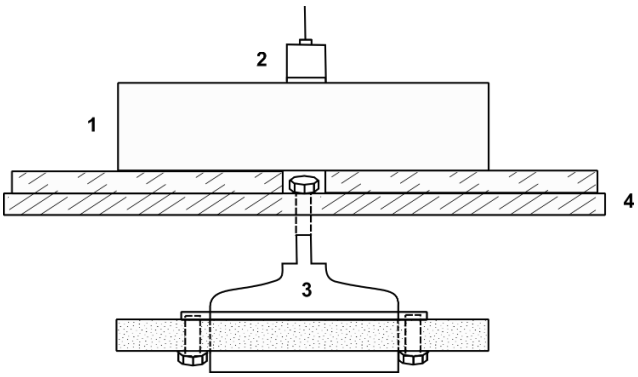

Figure 4.8 Schematic of the geophone calibration setup, consisting of geophone system (1, uncalibrated), 4370 accelerometer (2), electromagnetic shaker (3), acrylic platform (4).

The accelerations, derived originally from the geophone, were then plotted against the calibrated acceleration values (Figure 4.9), for the vertical axis.

The consequential calibration curves for the geophone system were highly variable, thought to be due to flexibility in the stinger rod of the shaker producing resonance with the greater weight of the geophone on the platform. Due to this, data were used from when the geophone and 4333 accelerometer were recording simultaneously inside the water filled tank. For these measurements the accelerometer was inside the arena and the geophone was outside (calibration date 7th March 2014). By replacement of the geophone values with the associated acceleration values for each value, the final calibrated values could be derived using a plot of geophone data (derived from velocities), against the 4333 data (after adjusted for calibration) (Figure 4.9, Table 4.3). It is of note that this calibration method was undertaken for the vertical axis of the geophone only.

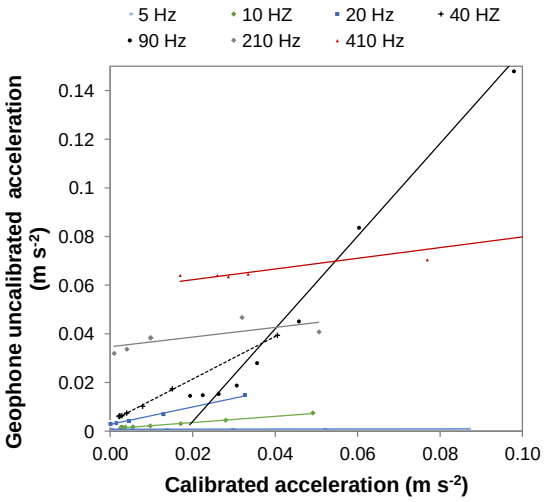

Figure 4.9 Calibration chart for the vertical axis of the geophone system (SM-7 375 ohm, IO, sensitivity 28.8 V m s^{-1}).

Table 4.3. Slope and intercepts for the geophone calibration lines, as shown in Figure 4.9.

Frequency (Hz)	Slope	Intercept
5	0.001	0.008
10	0.126	0.001
20	0.359	0.003
40	0.818	0.004
90	1.905	-0.034
210	0.211	0.035
410	0.219	0.0579

4.4.3 Experimental signal & background levels

Spectra of the excitation signals were calculated from the time courses using a 1024 FFTs, Blackman window (1 k/s).

Average background vibration levels were calculated for each day of experimentation. A six second portion of background levels (taken from between exposures) was randomly selected for each frequency and day. This was then averaged to calculate background levels (RMS) across each day and each experimental period. Background levels were calibrated approximately using the equation of a generalised calibration curve (an amalgamation of all calibration curves for each frequency, for the accelerometer data):

$$y = 0.8338x - 0.026 \qquad [11]$$

With y being the uncalibrated and x being the calibrated accelerometer, allowing calibration of the uncalibrated sensor.

4.5 Results

4.5.1 Stimulus and background levels

The output signal showed an approximate halving of amplitude (6 dB) per input step (Figure 4.10), as intended. The excitation signals inside the tank were sinusoidal, with a prominent peak at the desired frequency with slight variation of signal with frequency per day (Figure 4.11). It is of note that in some cases at 40 Hz there were harmonic peaks due to resonance or interference. At the maximum these peaks were 10 – 30% of the maximum peak amplitude. The 40 Hz results were viewed with some caution.

The signal was greatest in the vertical axis and increased with input amplitude (Figure 4.12). However at the highest amplitude of 5 Hz the z axis was marginally greater, this is thought to be due to interference. Threshold sensitivities were below this value hence this was deemed insignificant.

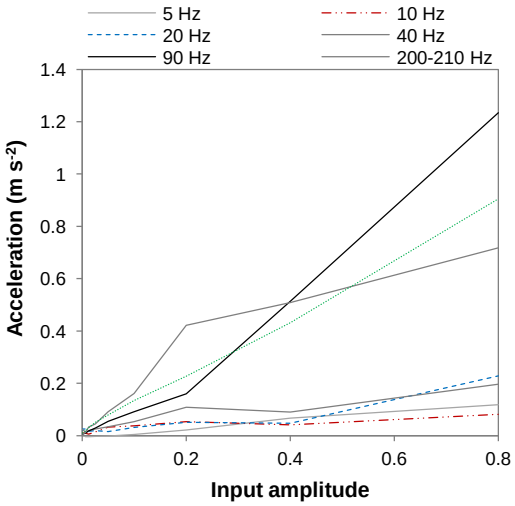

Figure 4.10 Dynamic range of the shaker excitation system (m s^{-2}).

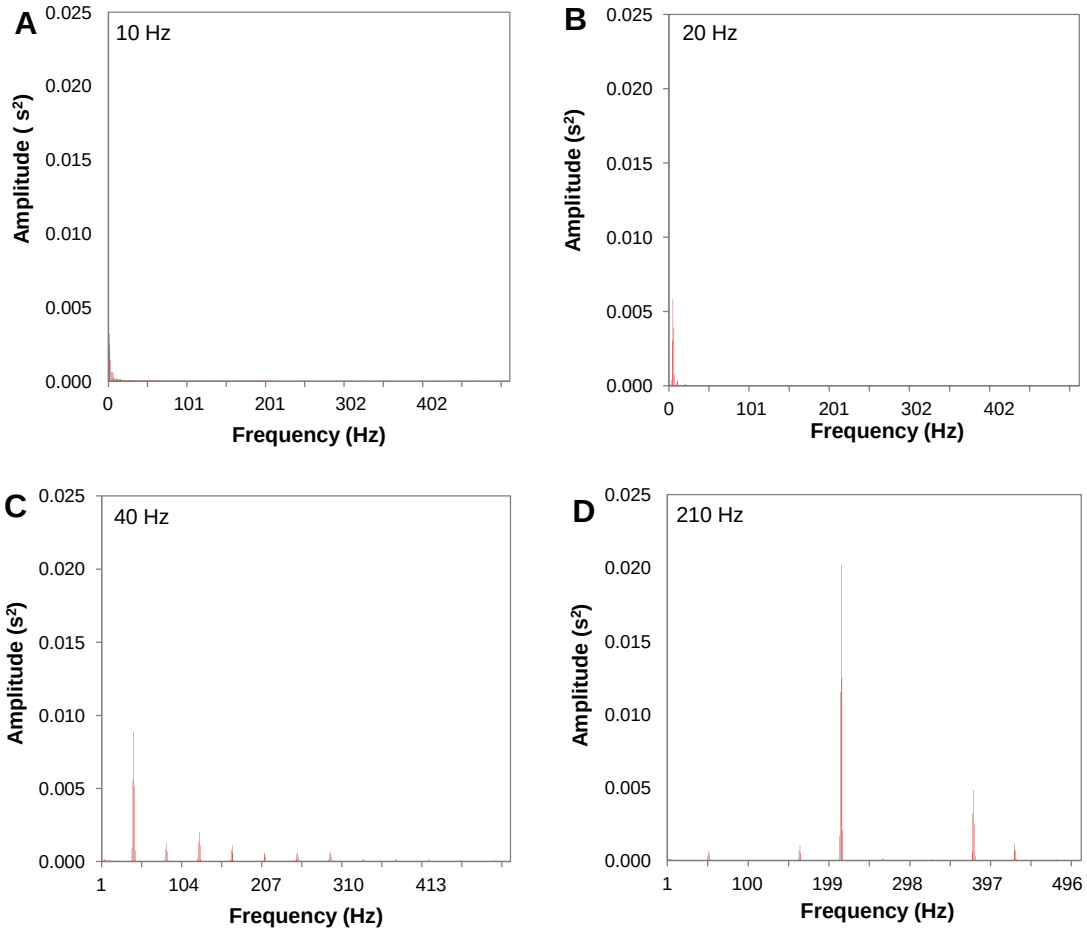

Figure 4.11 Example spectra of measured shaker signals from 8th and 9th December 2013, 10 Hz (A), 20 Hz (B), 40 Hz (C) with small resonant peaks, 210 Hz (D).

Average background vibration levels within the tank were calculated for the three main experimental periods, denoted as spring 2013, winter 2013 and spring 2014 (RMS, Table 4.4). Most measurements were within the 0.0005 – 0.01 m s^{-2} region. There was no significant difference between background levels for the three periods of experimentation (H = 0.68, df = 2, p = 0.71). For this reason the average background level across all periods (0.0074 m s^{-2}, RMS) was compared to threshold values.

Table 4.4. Average background vibration levels (RMS, m s^{-2}) between the three main experimental periods.

	Spring 2013	Winter 2013	Spring 2014
Average	0.0068	0.0079	0.0075
SD	0.0008	0.0032	0.0029
Total samples	63	66	151
S.E.	0.0001	0.0004	0.0002

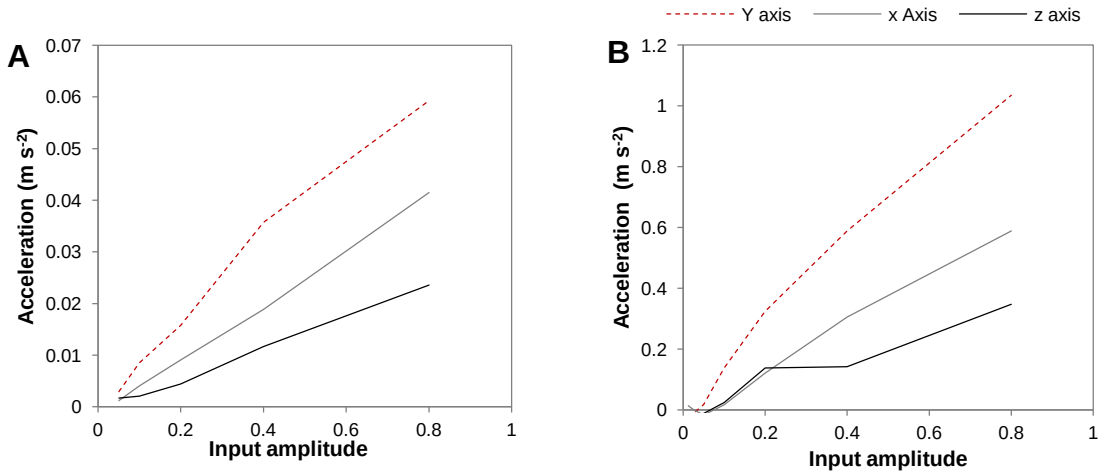

Figure 4.12 The relative proportion in each axis (x, y, z) of the sinusoidal signal, for example at 40 Hz (A) and at 210 Hz (B). Maximum input amplitude (x axis) is 0 dB, denoted as 1, reducing in -6 dB steps.

4.5.2 Behavioural responses to vibration

At onset of the stimulus, or within a second of onset, clear behavioural changes were observed; with the type of response varying according to the amplitude of the stimulus. At the lowest levels of exposure, a clear movement of the second antenna occurred at the onset of the signal (indicator 1). The movement consisted of a 'sweeping' backwards of both antennae towards the shell, accompanied by 'flicks' of the antennules and rapid movement of the maxillipeds. The movement of the second antenna typically occurred once or twice at the onset of the vibration, but the movement of the antennules and maxillipeds lasted for the duration of the exposure. The movement of these body parts was not accompanied by any other sort of movement.

At the highest amplitudes a burst of movement was seen (indicator 2). This behaviour occurred at the onset of the vibration (or within 1 – 2 seconds), and consisted of forward locomotory movement

until the end of the exposure. In animals already moving at the onset of the signal, the vibration typically induced a cessation of movement for the duration of the signal. Therefore regardless of the activity level of the individual, this behavioural indicator was clearly defined. It is of note that indicator 2 was often accompanied by antenna and antennule movements as of indicator 1, however indicator 1 often occurred without indicator 2. Since the responses were clear, it was therefore possible to find the threshold of sensitivity using the two respective indicators (antennae movement and burst of movement) of behavioural change. Example digital stills are provided in Figure 4.13.

Between the two indicators there was a suite of other postures which clearly began at the onset of the stimulus; these included a clear 'flinch' of all legs, and a sudden burst of digging in the sand. All these changes appeared to be indicative of a response, since non-exposed crabs did not exhibit such clear 'startle' type behaviour. In preliminary tests a semi- or full retraction into the shell was elicited a number of times but was not common during the experiments.

Figure 4.13 Digital stills taken from threshold determination experiments. Crabs were unrestrained inside an arena.

On a number of occasions crabs appeared to lift the shell from the substrate during the stimulus, and in other cases to exit the shell, examine it thoroughly and return. On a couple of occasions the most extreme response was seen, where the crab fell onto its back at the onset of the stimulus. It is of note that no crab permanently left the shell, although in preliminary tests involving the vibration box of Section 5.3.3 (Chapter 5) this response was observed multiple times due to a greater vibration amplitude.

Control observations indicated that the onset and offset of the shaker signal (a 'silent' audio file) did not elicit a response, hence the experimental setup did not appear to affect the animals.

4.5.3 Threshold determination

A total of forty five hermit crabs were tested for sensitivity (5 – 410 Hz). Thirty five of those (cheliped width 2.13 - 6.00 mm) were tested using antennae movement as response (indicator 1). Ten crabs (cheliped width 2.13 – 5.9 mm) were tested using a burst of movement as the indicator (indicator 2), with only five frequencies tested (20 – 410 Hz) since movement was not elicited at the two lowest frequencies. No mortality was observed during the experiments, crabs were active throughout and fed normally afterwards.

When indicator 1 was used to calculate the threshold, the greatest sensitivity (i.e. - the lowest average threshold of response) was measured at 90 Hz, with an average threshold value of 0.11 m s^2 (n = 35, RMS) in the vertical plane. An approximately flat response was obtained overall throughout the frequency range, with a range of 0.11 – 0.29 m s^{-2}, and a reduced sensitivity at 210 Hz (0.29 m s^{-2}, RMS). Across all frequencies, the average sensitivity value was 0.20 m s^{-2} (SD = 0.44, n = 35, RMS) (Figure 4.14).

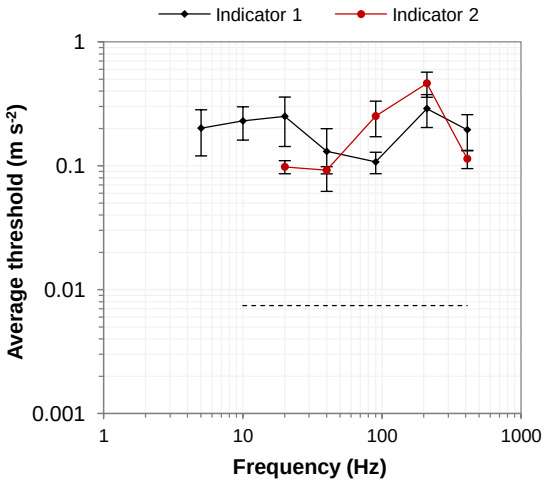

Figure 4.14 Average behavioural thresholds for *P. bernhardus* (n = 45, +/- SE, RMS) to substrate vibration in terms of vertical acceleration (m s^{-2}). Average background levels are denoted by a dashed line. Indicator 1 was defined as a 'flick' of the antenna, and indicator 2 as a burst of movement.

When indicator 2 was used to calculate the threshold the greatest sensitivity (i.e. - the lowest average threshold of response) was at 40 Hz (0.09 m s^{-2}), with an overall threshold range of 0.09 – 0.44 m s^{-2} (RMS). The response curve was more irregular, with a similar, but larger peak at 210 Hz. Across all frequencies the average threshold was 0.21 m s^{-2} (SD = 0.23, n = 10) (Figure 4.14).

It is of note that threshold values were higher for indicator 2 (movement) than indicator 1 (antennae movement) at 90 and 210 Hz, whereas the opposite pattern was observed at the other frequencies (Figure 4.14). Threshold values varied significantly between the two indicators when all data were grouped ($U = 3634$, $p < 0.001$) and when subdivided by frequency ($U = 66, 102, 129, 142$; $p < 0.05$ for 40, 90, 210, 410 Hz respectively). However the difference at 20 Hz was non-significant ($U = 216$, $p = 0.11$).

4.5.4 Comparison to anthropogenic values

After conversion to velocity, the lowest threshold of sensitivity ranged from 0.00004 – 0.0064 m s^{-1} (Table 4.5). These values are well within the exposure levels that anthropogenic activities may produce at large distances from source (Section 1.9, Table 4.6). One publicly available source was found with the rest of the data being obtained from personal communication, grey literature or conference proceedings. For example at 400 m from a pile driving rig the vibration levels are estimated to be 0.001 m s^{-1}, a lot higher than those we would expect to see a behavioural reaction such as in the current work. Similarly the levels of vibration at 220 m from dredging have been

measured at 0.00015 m s^{-1}, (Table 4.6). Furthermore, even at 410 m from a shell auger operation the levels are estimated to be 0.00011 m s^{-1}, again well within the detectable range of the animals in the present study. This is of particular relevance as, with an intertidal distribution, *P. bernhardus* is likely to be in areas in the vicinity of many anthropogenic activities. Furthermore, it is likely that the vibrations summarised in. Table 4.6 would also be detectable by other crustaceans (discussed in Section 4.6.5, Table 4.13).

Table 4.5. Threshold sensitivities (RMS) of *P. bernhardus* converted to velocity for purposes of comparison to anthropogenic vibration sources. The conversion between acceleration and velocity was undertaken using equation [2].

Indicator type	Frequency (Hz)	Threshold (m s^{-2})	Threshold (m s^{-1})
Antennae change	5	0.201	0.0064
	10	0.230	0.0037
	20	0.251	0.0020
	40	0.130	0.0005
	90	0.107	0.0002
	210	0.289	0.0002
	410	0.195	0.00001
Movement	20	0.098	0.0008
	40	0.092	0.0004
	90	0.251	0.0004
	210	0.462	0.0004
	410	0.114	0.00004

4.5.5 Time in the laboratory prior to tests & threshold

Mean threshold varied significantly according to duration in the laboratory prior to tests ($t = 6.73$, $df = 270$, $p < 0.05$, indicator 1, log transformed, RMS), with crabs of short duration in the laboratory being most sensitive to vibration (Figure 4.15).

When subdivided by frequency, all frequencies indicated a significant difference between means with a reduced sensitivity in crabs which had spent longer in the laboratory prior to experiments (10 Hz $t = 3.84$, $p < 0.05$; 20 Hz $t = 2.13$, $p < 0.05$; 40 Hz $t = 2.13$, $p < 0.05$; 90 Hz $t = 4.75$, $p < 0.01$; 210 $t = 2.79$, $p < 0.05$; 410 Hz $t = 3.04$, $p < 0.05$; all $df = 38$, apart from at 5 Hz $t = 1.33$, $df = 31$, $p < 0.05$) (Figure 4.15). Background levels between experimental runs did not vary significantly between experiments.

However for the February data alone, the mean threshold did not vary significantly according to duration in the laboratory prior to tests ($t = 1.78$, $p = 0.08$, $n = 135$, all frequencies grouped together). Hence, although the larger data set should be considered more reliable due to a higher number of replicates, more data would be required to fully explore the hypothesis.

Table 4.6. Summary of the available vibration levels (measured or modelled) from the literature, provided in terms of the maximum amplitude across all three axis.

Activity	Distance (m)	Vibration levels (m s^{-1})	Measurement type	Frequency range (Hz)	Method	Source
Drilling	23	1E-02 – 7.0E-04		< 100		
Shell auger	70	3.66 – 9.42E-05		Unspecified		
	109	1.18E-05	RMS			
	198	5.5E-04 – 1E-03				
Shell auger piling and drilling	23	2.7E-3 – 6.0E-3		Unspecified	Measured	
	64	7.74 – 6.74E-05			Vibrock geophone	Subacoustech Ltd.-S.Cheesman *Pers. Comm.* (2014)[3]
	410	8.78E-06 – 1.1E-04			Sample rate - 10 kHz	
Pile driving	17	4.1E-03	Peak	5 – 50	Sensitivity of 0.023 V mm s^{-1}	
	34	1.7E-03	Peak		Amplifier gain – 40 dB	
Dredging	5	3.8E-04 (8.0 E-05)		1 – 30		
	50	2.6E -04 (2.3E-05)	Peak (RMS)			
	175	2.9E -04 (1.3E-05)				
	220	1.5E-04 (3E-06)				
Tunnel boring machine	Above TBM	6.8E-05	RMS	Unspecified		East and Collett (2014)
Blasting	24.25	6.0E-02	Peak	Unspecified	Measured Instantel Minimate Plus and DS-077 Seismographs	Edwards and Kynoch (2008)
	296.75	< 1E-03	Peak		Seismographs on land	
Pile driving	< 5	5.0E-03 – 0.1	Unspecified	Unspecified	Modelling	Miller (2015)
	150	0.01				
	400	0.001				

[3] Mr S.Cheesman, Acoustic consultant, Subacoustech Ltd., Southampton, UK.

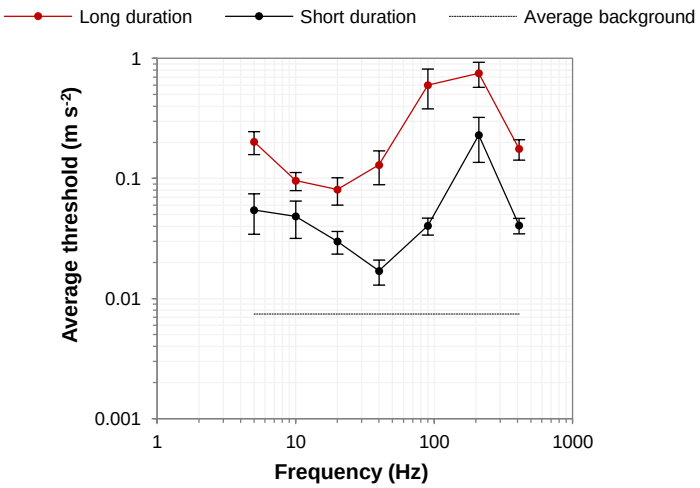

Figure 4.15 Average behavioural thresholds for *P. bernhardus* (n = 10 per group, +/- SE, RMS, indicator 1) to substrate vibration given in terms of vertical acceleration (m s^{-2}), for two groups with different amounts of time in the laboratory prior to tests. Average background levels are denoted by a dashed line.

4.5.6 Consistency of response

There was significant consistency in response between re-tested crabs on the whole (t = -0.34, df = 28, p = 0.73, indicator 2, log transformed). When subdivided by frequency there was also no significant difference (20 Hz t = 0.70, df = 6, p = 0.51; 40 Hz t = -0.42, df = 4, p = 0.70; 90 Hz t = -0.87, df = 4, p = 0.43; 210 Hz t = -0.36, df = 7, p = 0.73; 410 Hz t = 0.39, df = 3, p = 0.72). Overall this indicated that the threshold did not appear to change significantly upon re-testing and indicates a consistency in sensitivity and response per individual. However, there were fewer responses on the re-test in general (Table 4.7).

Table 4.7. Total number of responses between *P. bernhardus* (n = 10) tested for the threshold (using a burst of movement as the response) with a ten day gap between re-tests (1 and 2).

Frequency (Hz)	Test 1	Test 2
20	8	9
40	10	3
90	9	6
210	10	10
410	7	6
Overall	44	34

4.5.7 Morphology

Tested crabs ranged in cheliped width from 2.13 – 6.00 mm ($\bar{x}$ = 4.34, SD = 0.93, n = 30), and cheliped length 3.72 – 12.6 mm ($\bar{x}$ = 7.79, SD = 2.08, n = 30). Shells occupied were typically

Littorina sp. (*L. obtusata, L. littorea, L. saxatilis*), varying in width from 10.12 –18.8 mm ($\bar{x}$ = 13.75, SD = 2.36, n = 30), and height from 15.9 – 23.3 mm ($\bar{x}$ = 20.34, SD = 2.02, n = 30). All shell morphology measurements were correlated with each other and were also correlated with the size of crab (cheliped width) occupying the shell (Table 4.8).

Thresholds using indicator 1 (all frequencies combined) significantly increased with cheliped width whereas no significant correlation was observed with other morphological parameters (Table 4.9, log transformed). When subdivided by frequency, cheliped length was significantly correlated with 90 Hz threshold (Table 4.10).

Table 4.8. Correlation coefficients between *P. bernhardus* morphology parameters (occupied shell and cheliped, mm). Shell height (SH), shell aperture (SA), cheliped length (CL), cheliped width (CW). Statistical significance is denoted by asterisks (* p < 0.05, ** p < 0.01).

	Correlation coefficients (n = 30)			
	SH	SA	CL	CW
SH	-			
SA	0.40*	-		
CL	0.2	0.45*	-	
CW	0.45*	0.50**	0.92**	-

Table 4.9. Correlation coefficients between claw morphology of *P. bernhardus* and shell morphology of occupied *Littorina* sp. shells related to average threshold values (across all frequencies, n = 10, thresholds calculated using two behavioural indicators). Statistical significance is represented by asterisks (* p < 0.05, ** p < 0.01). Shell height (SH), aperture (SA), cheliped length (CL), cheliped width (CW). Full p values provided in appendix Table A.7.

Variable	**Indicator 1**	**Indicator 2**
SH	0.33	0.03
SW	-0.04	-0.27
SA	-0.01	0.06
CW	0.53*	0.34
CL	0.34	0.36

Table 4.10. Correlation coefficients of claw morphology of *P. bernhardus* and shell morphology of occupied *Littorina* sp. shells related to average threshold values (separated by frequency, thresholds calculated from two behavioural indicators). Statistical significance is represented by asterisks (* p < 0.05, ** p < 0.01). Shell height (SH), aperture (SA), cheliped length (CL), cheliped width (CW). Full p values provided in appendix Table A.8.

	Frequency (Hz)											
	Indicator 1							Indicator 2				
Variable	5	10	20	40	90	210	410	20	40	90	210	410
SH	0.22	-0.24	0.32	0.27	0.32	0.16	0.07	-0.04	0.38	0.62	-0.12	0.16
SW	-0.78	0.10	-0.55	0.06	0.32	-0.30	0.34	-0.40	-0.12	0.11	-0.46	-0.59
SA	0.20	-0.47	0.32	0.29	0.12	-0.11	-0.22	0.10	0.23	0.42	-0.40	-0.46
CW	0.68**	-0.04	0.34	-0.31	0.01	0.42	-0.31	-0.17	0.45	0.64	-0.12	0.04
CL	0.63*	-0.03	0.23	-0.37	-0.18	0.26	-0.38	-0.16	0.55	0.70*	-0.13	-0.04

4.5.8 Startle response and personality

Upon first introduction to the arena, some subjects were more 'curious' than others, exploring for most of the experiment, with multiple attempts to climb the arena walls. In contrast, other subjects did not explore and stayed within the same position inside the arena for the duration of the exposures. More active crabs were more likely to stop moving in response to the stimuli, rather than increase movement. Similarly, recovery time after stimulus cessation also varied between individuals. This is consistent with the idea of variation between individual responses, and personality (Briffa *et al.*, 2008; Briffa *et al.*, 2013).

There was no significant correlation between startle response and average sensitivity threshold ($m\ s^{-2}$), ($r = 0.02$, $n = 33$, $p = 0.90$) when data from all three groups was combined. As such there was no significant correlation between personality and average sensitivity threshold ($m\ s^{-2}$), (rho = 0.01, $p = 0.96$, $n = 33$) when data from all three groups was combined. That is, the most 'bold' were not most sensitive to vibration or vice versa. The same trend was present when the data were separated by group (February, March, and December).

For the March group the average startle response decreased with time after vibration exposure, but this difference was non-significant ($F = 0.45$, $df = 3$, $p = 0.72$, log transformed), (Figure 4.16A). However for the February group average startle response significantly increased across the three tests (being post exposure, 1 day post-exposure and post-handling), ($F = 10.73$, $df = 2$, $p < 0.001$, log transformed), (Figure 4.16B). Pairwise comparisons indicated significant differences between post-exposure and pre-handling, and post-exposure and post-handling ($t = -2.91$, $df = 13$, $p < 0.01$; and $t = -4.40$, $df = 13$, $p < 0.001$ respectively).

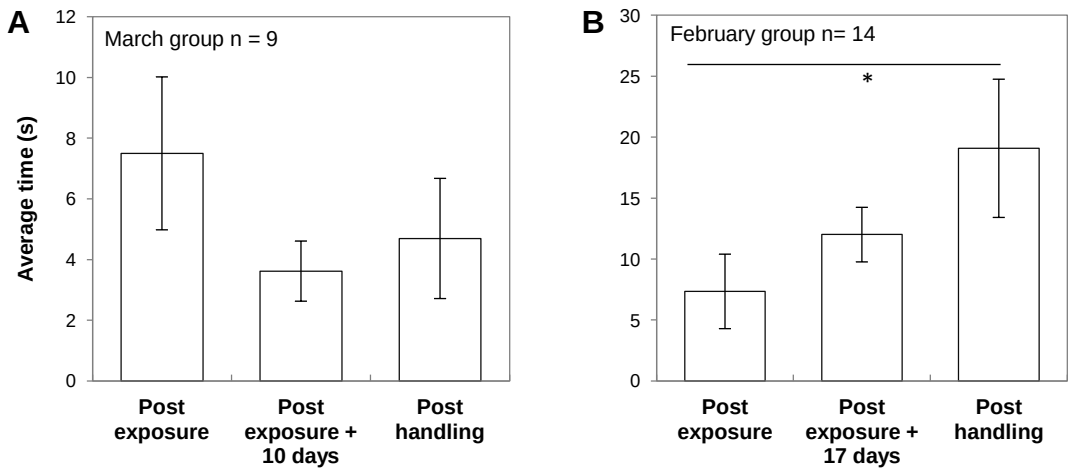

Figure 4.16 Average startle response time (s, +/- SE) of *P. bernhardus* after vibration exposure, and 10 - 17 days after exposure to impulsive vibration playback (substrate-borne), March (A), February group (B), asterisks denote significance, * p < 0.05.

However when rankings of personality were compared within each test group (February, March), there was no significant correlation between each startle test, (Table 4.11). This indicates a lack of consistency in response (personality) per individual, that is, the 'boldest' individual was not the 'boldest' in each scenario. December data were not included in analysis due to only one personality test being undertaken.

If the middle test value (after 10 – 17 undisturbed days, Figure 4.16A, B) is interpreted to be the 'normal' startle response, it appeared to be influenced both by exposure to vibration, and by handling, with a reduction and increase in duration respectively. This may indicate plasticity in behaviour according to the situational context, confirmation would require repetition of the work.

Table 4.11. Kendalls tau results for *P. bernhardus* tested for personality. Tests were undertaken immediately after vibration exposure, after a period of non-exposure and after handling during morphology measurements.

Group	Variable	Variable	τ	p	n
February	Post- exposure	Pre- handling	-0.28	0.15	15
		Post- handling	0.16	0.40	
	Pre- handling	Post- handling	0.11	0.59	
March	Post- exposure	Pre- handling	0.24	0.33	10
		Post- handling	-0.16	0.53	
	Pre- handling	Post- handling	0.24	0.33	

There was a significant correlation between time spent by crabs in the laboratory prior to tests and startle response (rho = 0.47, df = 31, $p < 0.01$), with the startle response increasing with duration in the laboratory, i.e.- crabs taking longer to re-emerge after being upturned. In terms of personality this could be described as becoming more 'shy' according to time spent in the laboratory. This may also be an indicator of adaptation to experimental conditions.

4.5.9 Impulsive vibration playback

Thirty hermit crabs (cheliped width 2.36 – 6.60 mm, weight 1.20 – 6.71 g) were exposed to impulsive vibration (piling) playback over the course of three days (May 20 – 22nd 2014). No mortality was observed during the experiments and crabs fed normally after tests.

The playbacks of the vibration recorded were similar to the signal from the recording piling exposure, having a characteristic sharp onset and decay, with predominant energy in the 50 – 200 Hz band (Figure 4.17). The maximum peak amplitude averaged across all presentations was 0.0005 m s^{-1} in the vertical plane (SD = 0.0001, n = 30), 0.0003 m s^{-1} (x, SD= 0.000095, n = 30) and 0.0005 m s^{-1} (z, SD = 0.001, n = 30. The original recorded level was 0.0005 m s^{-1} at 23 m from the operation (data courtesy of Subacoustech Ltd.). Average background level across all presentations was 0.00007 m s^{-1} (SD = 0.00018, n = 30).

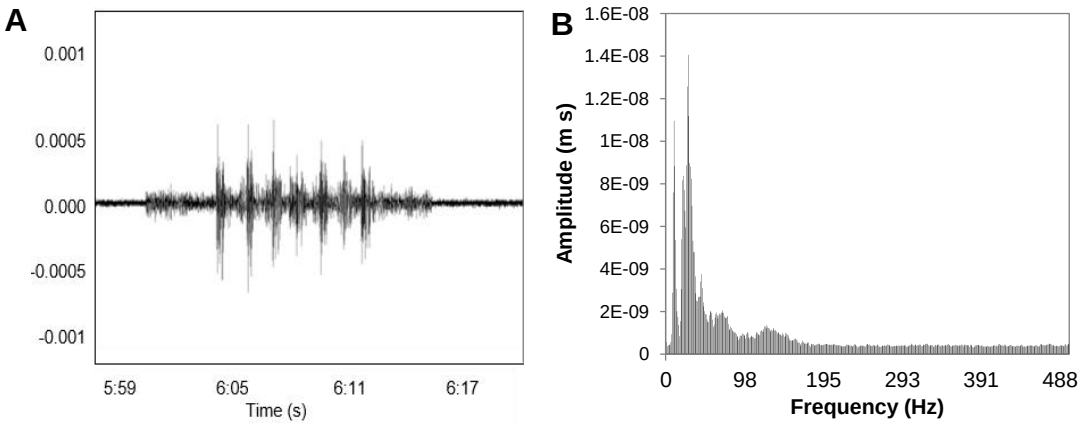

Figure 4.17 Time history of impulsive vibration exposures (shell auger piling) within the experimental tank (A); Amplitude spectrum of the playback signature (B).

Clear behavioural changes were observed at the onset of playback, these manifested as short bursts of movement, lasting typically 10 seconds up to 1 minute after the beginning of the exposure. This was often accompanied by a sweep of the large antennae, and vigorous sampling with the maxillipeds. In less active crabs, a small 'flinch' (partial retraction of legs into the shell) and vigorous movement of the maxillipeds was exhibited at onset, rather than movement changes. Again, individuals responded differently upon introduction to the arena, with some being active and others remaining stationary throughout. Responses were clear, demonstrating that in laboratory conditions, amplitudes similar to actual shell auger piling are likely to elicit responses.

There was a significant difference between response rate in control (without vibration) and exposure trials (χ^2= 34.74, df = 1, p < 0.001), (Table 4.12). There was no correlation between morphology parameters and likelihood of response (χ^2 = 2.45, df = 4, p = 0.65).

There was a reduction in startle duration between the three startle response tests, with the startle immediately after exposure being shorter than pre-exposure (average 3.45 and 2.08 s pre- and post-exposure respectively). There was a return to pre-exposure levels a day after exposure (average 3.46 s), (Figure 4.18). The trend was strong but proved non-significant (F = 2.88, df = 2, p = 0.07 and F = 0.95, df = 2, p = 0.49, sphericity assumed), although pairwise comparisons indicated a significant decrease after exposure (t = 2.05, df = 28, p < 0.05) and a significant increase one day later (t = -2.28 df = 28, p < 0.05). This indicated that exposure to vibration may reduce the startle response.

Table 4.12. Number of responses of *P. bernhardus* after exposure to substrate-borne impulsive vibration, total number of exposures provided in brackets.

Trial	Response
Control	0 (30)
Exposure	22 (30)

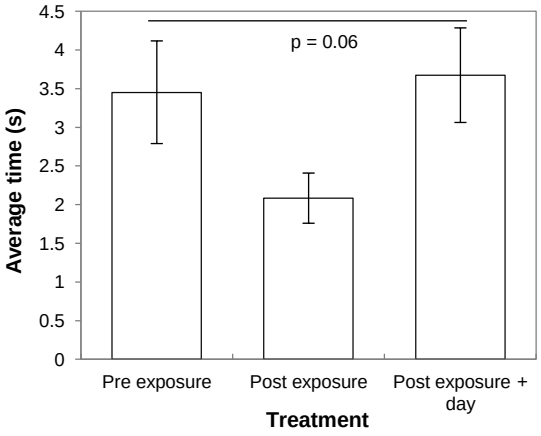

Figure 4.18 Average startle response time (+/- SE) between *P. bernhardus*, pre-, post and post + 1 day after exposure to substrate-borne playback of impulsive vibration.

When the startle response data were ranked, there was significant consistency between two of the personality rankings, but not of the other (τ = -0.07, 0.19, 0.35; df = 29; p = 0.60, 0.15, 0.01). The two consistent rankings were those after the stimulus, suggesting that the vibration exposure may have affected the rankings (i.e. all crabs displayed unusual behaviour in relation to their normal behaviour/personality). In terms of personality, the results here could be interpreted as plasticity between situations, that is, crabs became temporarily more 'bold' as a result of exposure.

4.6 Discussion

4.6.1 Sensitivity of P. bernhardus *to vibrations*

The ability of *P. bernhardus* to detect the signals in the present work depends upon the capabilities of the sensory system involved. An acoustic stimulus consists of a water-borne compressional pressure wave; it also produces a back and forth particle motion which propagates both through the water column and the seabed. In crustacea it is widely agreed that a mechanism to convert pressure into mechanical displacement is not present since there is a lack of air filled cavities. Therefore when discussing acoustic or vibratory reception (see Chapter 1 for a definition of these terms), particle motion is thought to be the main stimulator. This has been demonstrated in complementary field and laboratory studies (Tautz and Sandeman, 1980; Breithaupt and Tautz, 1990; Hughes *et al.*, 2014), for example Goodall (1988) demonstrated in *N. norvegicus* reception of an acoustic stimulus at 0.9 m but not at 1 m indicating predominantly near field particle motion reception. Detection of such motion is thought to involve a suite of mechanoreceptors consisting of surface receptors, internal statocyst receptors and the chordotonal organs (see Section 1.6.3, Budelmann (1992a) for a comprehensive review, Wiese (1976); Goodall (1988); Breithaupt and Tautz (1990), although the role of each type within detection abilities of vibration is relatively unknown. For example the chordotonal organs, located within the joints of appendages may detect vibration in addition to joint extension (Burke, 1954; Horch, 1971; Salmon *et al.*, 1977; Barth, 1980; Aicher and Tautz, 1984; Budelmann, 1992a). Similarly it has been suggested that the statocyst, in addition to its role as an equilibrium receptor (Budelmann, 1988; Fraser, 1990; Budelmann, 1992a) may also be sensitive to particle motion, and may be involved in acoustic detection (Cohen, 1955; Breithaupt and Tautz, 1988; Nakagawa and Hisada, 1990).

When comparing the results here to the literature it is important to note that it is widely accepted that electrophysiological thresholds provide lower threshold values than behaviourally determined values, and that the two measures are not equivalent descriptions of auditory/receptive response (Ladich and Fay, 2013).

P. bernhardus in the current work were shown to be sensitive to sinusoidal vibrations in the region of 0.11 – 0.29 m s^{-2} at the lowest sensitivity (using antennae movement as the indicator) with greatest sensitivity (0.11 m s^{-2}) at 90 Hz. These values are within the region of previously reported sensitivities to vibration. For example Salmon (1971) reported a threshold of 0.06 m s^{-2} for *U. pugilator. Goodall (1988)* demonstrated a sensitivity of 0.01 m s^{-2} (20 Hz) and 1.4 m s^{-2} (200 Hz) for *N. norvegicus* using behavioural methods. Another marine species, *H. americanus*, was demonstrated to be of much greater sensitivity that the current species (0.0002 m s^{-2} at 75 Hz) although the study used conditioned animals rather than the unconditioned here. Of most importance are studies involving vibration stimuli and marine species such as Berghahn *et al.* (1995) and Heinisch and Wiese (1987), which both demonstrated marginally reduced sensitivities than the current work. This may be attributed to the application of the vibration stimulus, and the different species tested. Other threshold values from the literature are shown in Table 4.13.

A comparison of particle motion sensitivity curves (RMS only, Figure 4.19) indicates that as expected, electrophysiology methods yield greater sensitivities- for example comparing the curves

Table 4.13 Thresholds of greatest sensitivity to vibration for a variety of crustacean species. Units of measurement are given as originally stated (acceleration or displacement), those marked (asterisk) have been converted. For comparative purposes the results of the current work are shaded in grey.

Reference	Vibration presentation	Threshold (m s^{-2})	Threshold (μm)	Frequency (Hz)	Species	Method of determination
Salmon and Atsaides (1969)		0.07*	0.03	400	*Uca pugilator*	Behavioural
Salmon (1971)		0.04 0.06		30 60	*Uca pugilator* *Uca rapax*	Behavioural
Salmon and Horch (1973)	Substrate-borne	0.01		90		Electrophysiology
		0.02*		50	*Uca minax*	Behavioural
Barth (1980)		0.0002	0.4	20 – 200	*Carcinus maenas*	Electrophysiology
Aicher and Tautz (1984)		0.005		20	*Uca pugilator*	
Heinisch and Wiese (1987)		0.81	0.7	170	*Crangon crangon*	Behavioural
Berghahn *et al.* (1995)		0.4*		20 – 200	*Crangon crangon*	
The current work (2014)		0.11		90	*Pagurus bernhardus*	Behavioural
Offutt (1970)	Water-borne	0.0002		75	*Homarus americanus*	Behavioural
Horch (1971)		0.12		400	*Ocypodde ceratophthalmus*	
Wiese (1976)			0.1	5 – 40	*Procambarus clarkia*	Electrophysiology
Tautz and Sandeman (1980)			0.2	150 – 300	*Cherax destructor*	
Breithaupt and Tautz (1988)		0.14*		20	*Orconectes limosus*	
Goodall *et al.* (1990)		0.01		20	*Nephrops norvegicus*	Behavioural
		1.4		200		
Breithaupt (2002)		0.01		3	*Orconectes limosus*	Behavioural
Hughes *et al.* (2014)		0.001		75	*Panopeus sp.*	Electrophysiology

of two *Uca* species (Salmon and Atsaides, 1969; Aicher and Tautz, 1984). Yet it should be noted that the collated literature here measures a combination of water and substrate-borne particle motion, and is focussed mostly upon semi-terrestrial species, making further comparisons difficult. For example whilst threshold values obtained from the semi-terrestrial *Uca sp.* are similar to the present work in the 100 Hz region, behavioural tests have indicated sensitivities up to an order of magnitude lower for example 0.0175 m s^{-2} at 50 Hz (Salmon, 1973). However it is likely that *Uca sp.* would have a greater sensitivity than *P. bernhardus* since this species communicates by 'drumming' the substratum. Such activity has not been observed in hermit crabs, although stridulation has been described (Field *et al.*, 1987).

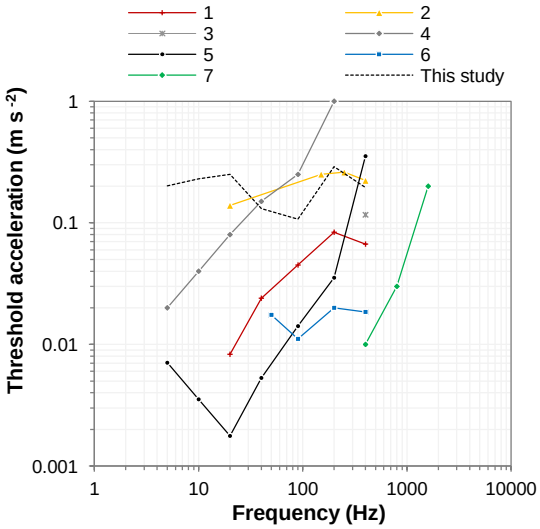

Figure 4.19 Behavioural thresholds to vibration (water and substrate-borne) for crustaceans (mixed species), values taken from the literature and compared to those of the present work (RMS only), Salmon and Atsaides (1969) (1), Breithaupt and Tautz (1988) (2), Horch (1971) (3), Breithaupt (2002), (4), Aicher and Tautz (1984) (5), Salmon and Horch (1973) (6), Hughes *et al.* (2014) (7).

The current results show that sensitivity of *P. bernhardus* appears to be independent of frequency up to the 410 Hz tested, with an approximately flat response overall (Figure 4.19). Similar results have also been shown for *Orconectes limosus* (Breithaupt and Tautz, 1988) and *Uca sp. (Salmon and Horch, 1973; Salmon et al., 1977)*, (Figure 4.19). However it was expected that sensitivity would reduce with increasing frequency, especially above 100 Hz (Salmon and Atsaides, 1969; Aicher and Tautz, 1984; Breithaupt, 2002). This trend is clear in Figure 4.19, where a steep increase is slope may be observed in four of the eight trend lines (Salmon and Atsaides, 1969; Aicher and Tautz, 1984; Breithaupt, 2002; Hughes *et al.*, 2014), in some case this increase begins as low as 5 Hz. A doubling effect such as this has been demonstrated in water-borne particle motion thresholds of cephalopods and fish (Packard *et al.*, 1990; Hughes *et al.*, 2014), and is thought to be indicative of directionally sensitive cells within a receptor system involving the statocyst (Budelmann, 1979; Hughes *et al.*, 2014). However the results of the current work do not support this reduction in sensitivity with increasing frequency. Indeed the threshold values of 5 and 410 Hz are similar. The possible discrepancy between the literature and the current work could be explained if there were an artefact or additional resonances within the exposure stimuli here;

However contrary to this, signals at 210 and 410 Hz were comparatively more 'pure' in terms of the frequency composition compared to the other frequencies (that is, had fewer resonant peaks than at other frequencies). Furthermore behavioural responses were the most pronounced within the upper frequency range. It is possible then, that differences may be attributed to the species tested, and the methods used in obtaining the threshold. In the current work the crabs were unrestrained. This was more natural than in electrophysiology studies and in behavioural studies which restrict movement. In the case of the *P. bernhardus* the resonance of the shell itself may also have implications to the frequencies received. It is also possible that the sensitivity of *P. bernhardus* may reduce above the frequency range tested here (> 410 Hz), or that in fact marine species such as this do not have increased sensitivity in the lower frequency range compared to the upper.

Behavioural thresholds of other marine species are similar to those observed in the current work. For example sensitivity of *N. norvegicus* was demonstrated in the region of 0.01 – 1.40 m s^{-2} (20 – 200 Hz, water-borne) (Goodall, 1988). Berghahn *et al.* (1995) found a behavioural threshold of 0.4 m s^{-2} in the 20 – 200 Hz (substrate-borne) range for *C. crangon,* whilst Heinisch and Wiese (1987) demonstrated a higher threshold of 0.81 m s^{-2} at 170 Hz. Other marine organisms thought to detect motion, the cephalopods, have been shown to range in sensitivity from 0.0003 – 0.043 m s^{-2} (50 – 280 Hz, *Octopuso cellatus*), 0.002 – 1 m s^{-2} (1 Hz – 100 Hz, *S. officinalis*) to 0.8 – 1.1 m s^{-2} (at 100 Hz, *Loligo vulgaris*) (Packard *et al.*, 1990; Kaifu *et al.*, 2008; Mooney *et al.*, 2010).

The sensitivity of fishes to particle motion is greater than shown in the current work (i.e.- they are more sensitive) (Chapman and Hawkins, 1973; Chapman and Sand, 1974; Sand and Karlsen, 1986; Karlsen, 1992; Fay and Simmons, 1998; Sigray and Andersson, 2011); for an audiogram comparison of fishes without a swim bladder see Popper and Fay (2011). Of higher relevance would be benthic fishes, such as flatfish, which appear to detect motion within the region of 0.001 m s^{-2} up to 100 Hz (Chapman and Sand, 1974; Sigray and Andersson, 2011). A threshold of 0.1 m s^{-2} at 170 – 200 Hz has been demonstrated for plaice and sole (Berghahn *et al.*, 1995). For crayfish, it has been suggested that detection sensitivities to water-borne motion are similar to the fish lateral line (Wiese, 1976; Goodall, 1988; Monteclaro *et al.*, 2010), with capabilities to sense close range stimuli only. For example *N. norvegicus* sensitivities are thought not to be sufficient to detect predatory fish sounds (Goodall, 1988). However it may be that sensitivity to waves within the substratum may be different, for example *Uca sp.* have been shown to be sensitive to vibrations up to 75 cm away from source (Horch and Salmon, 1972).

In the current work, threshold values (indicator 1) were correlated with cheliped width; this may indicate that sensory hairs on the chelae were involved within the reception in addition to the statocyst, although it is of note that no such correlation was detected when considering the thresholds of indicator 2.

4.6.2 Behavioural responses

Responses here were clear and occurred at onset of the stimulus (within 1 – 2 seconds), appearing to take a somewhat hierarchical form varying with the amplitude of the stimulus; the two different behaviours could therefore be used as indicators. Threshold values were consistent when individuals were re-tested. At the highest amplitudes, vibration elicited a burst of movement for the

duration of the vibration. At the lowest levels a clear 'sweep' of the second antenna directly at onset appeared to be indicative of reception. This was most often accompanied by movement of the antennules and the mouth parts. In crayfish, sweeping movement of the second antennae is common during exploration behaviour (Krång and Rosenqvist, 2006), since there are sensory hairs located there to detect tactile and chemo-mechanical cues. Antennae movement in response to vibration has been demonstrated in a range of other crustaceans (Meyer-Rochow, 1982; Heinisch and Wiese, 1987; Tautz, 1987; Berghahn *et al.*, 1995). Postural changes and movement of appendages have also been documented (Goodall, 1988; Goodall *et al.*, 1990; Breithaupt, 2002). In the current work, a similar range of startle-type responses were seen as in *Uca sp.* (Salmon and Atsaides, 1969). Crabs were unresponsive during control trials indicating that the experimental setup itself did not have an effect.

The current work used unconditioned animals rather than conditioned to determine thresholds. There is only one record of conditioning a crustacean to an acoustic stimulus (Offutt, 1970), and such training has not been successful since (T. Breithaupt, *Pers. Comm.*[4]). The use of conditioned animals has an advantage in that it reduces the chances of habituation, which has been demonstrated in fishes (Schwarz and Greer, 1984; Knudsen *et al.*, 1992). There are few data available on habituation in crustaceans with one recent work being inconclusive about the presence of habituation (Wale *et al.*, 2013b). To minimise the chance of habituation in the current work, stimuli were widely spaced and there were large gaps between frequencies (20 minutes); this method was successful since crabs stayed responsive throughout experiments enabling the staircase method of determination to be used (Cornsweet, 1962). Although habituation within trials was not demonstrated, the data from the current work are indicative of habituation across a longer time period- that is, crabs exhibited reduced sensitivity to vibration after a long duration (weeks) in the laboratory prior to tests. This is of significance to future researchers repeating the current work.

It is of note that the average threshold was higher (i.e. – reduced sensitivity) for indicator 2 than for indicator 1 at 90 and 210 Hz only, otherwise the curves were similar. A difference between the two indicators was expected, since indicator 2 may be described as a more 'energetic' response and may require a stronger vibration to be triggered. The use of the two indicators in this way demonstrates how this method could be applied to provide threshold values for a suite of behavioural responses. These thresholds could then be integrated with actual exposure levels, and vibration propagation information, to produce models such as the zone of influence model (Richardson *et al.*, 1995).

The energetic consequences of the behavioural responses in the present work are unknown. Frequent bursts of movement are likely to interrupt natural behaviour and could dramatically change the time energy budget of *P. bernhardus.* Similar time budget disruptions have been seen in reef fishes (Picciulin *et al.*, 2010) in response to playback sounds. The clear 'sweep' of the second antennae seen in the current work may also be accompanied by internal changes- for example heart beat, production of stress proteins and oxygen consumption changes (Florey and Kriebelm, 1974; Wale *et al.*, 2013b; Celi *et al.*, 2014). However such changes are difficult to

[4] Dr. T. Breithaupt, Lecturer, School of Biological, Biomedical and Environmental Science, Hull University.

measure, since heart beat in particular is often erratic in crustaceans and cessation can be elicited even by passing shadows, footsteps and light level changes in the laboratory (Florey and Kriebelm, 1974).

Previous investigations of sensory thresholds have not taken into account individual differences in personality. Since personality has been described previously for *P. bernhardus* (Briffa *et al.*, 2008; Briffa *et al.*, 2013), it was particularly valuable to investigate this in the current work within the context of vibration exposure. The results from the current work did not demonstrate a correlation between personality and average sensory thresholds, however personality was demonstrated in the animals tested. For example, 'bolder' crabs explored the arena thoroughly and stayed active during the experiments, whereas 'shy' crabs did not explore and stayed centralised within the arena with minimal movements. This difference was also reflected in the variation of startle response within the tested crabs.

In the crabs exposed to impulsive vibration playback there was a significant consistency between responses of individuals in the tests undertaken in periods of minimal disturbance. That is, when the crabs were ranked, the same 'bold' crabs were always quicker to respond per scenario than the 'shy' crabs. Regardless of ranking, the duration of response varied according to the situation (pre- or post-exposure), 'bold' crabs showing a reduced response time after exposure, and 'shy' crabs an even greater reduction. Similar evidence of personality in crustaceans has been demonstrated after exposure to visual predators (Vainikka *et al.*, 2011; Watanabe *et al.*, 2012), and in field and laboratory conditions (Briffa *et al.*, 2008). The threshold data here also indicated a consistency in response with crabs demonstrating a significantly consistent threshold when tested twice. This is in accordance with the idea of consistency of response within individuals, i.e. - personality. However no such correlation was found in rankings of crabs in the February and March data. It seemed that the response of the individual was variable according to the situation, although the influence of the stimuli within the tests may have had an effect. It is of note however, that the crabs in February and March had a different duration in the laboratory prior to tests and therefore acclimation may have affected individual responses, masking the effect of personality differences. Alternatively, it may be that the exposure to vibration changed 'normal' behaviour of the crabs, and therefore disrupted the startle response data. For example all crabs were seen to modify their behaviour after exposure, being quicker to respond after being turned over in the impulsive vibration experiments. The startle response time then returned to 'normal' after a period of minimal disturbance. Data from the February group also suggested the same trend. This is of importance, since when upturned, the crab is more vulnerable to predation, hence the longer it remains in that state the increased risk of mortality. In terms of personality, in some cases vibration therefore appeared to make crabs 'bolder', and the data may indicate a plasticity in behaviour according to the situation.

It is of note that the relation of personality and anti-predator responses to vibration and sensory thresholds, as in the previous section and here, is a novel approach, although recently the effect of other physical stimuli, such as temperature upon hermit crab personality has been investigated (Briffa *et al.*, 2013).

Exposure to acoustic stimuli has been shown to affect the anti-predator behaviour of other crustaceans. For example in Caribbean land crabs (*Coenobita clypeatus*) exposed to boat sound

playbacks. It was found that a simulated predator was able to get much closer to the crabs during playbacks (Chan *et al.*, 2010a; Chan *et al.*, 2010b; Stahlman *et al.*, 2011), it was also found that the distance between the visual stimulus and the auditory distractor was important (Ryan *et al.*, 2012). A 'distracted prey' hypothesis was suggested, that a stimulus is capable of distracting attention and therefore reducing its ability to respond to a predatory stimulus. Furthermore, anti-predator behaviour of *Carcinus maenas* was also found to vary according to the presence or absence of noise (Wale *et al.*, 2013a).

In several cases crabs were seen lifting their shell from the substrate during vibration exposures. This behaviour may have been a way of reducing exposure levels. In stridulating terrestrial hermit crabs, lifting of the shell from the substrate has been shown to reduce vibrations reaching the sand (Field *et al.* 1989). In the current work, crabs may have lifted the shell to reduce vibrations reaching the body. It was also found that the *Trizopagurus* sp. shell amplified specific frequencies (Field *et al.*, 1987), e.g. 100 – 300 Hz being of most relevance. Shell resonance was not investigated here, but broken shells were discounted in tests to eliminate inconsistencies. On a number of occasions individuals were seen exiting the shell, examining it thoroughly before returning. It is possible that these individuals supposed that another crab was 'tapping' the shell to initiate agonistic behaviour (Briffa *et al.*, 2008; Briffa *et al.*, 2013). The two previous behaviours illustrate the importance of examining threshold behaviours in conditions were the animal is unconstrained. The observation of such behaviours would not have been observed had the crabs been fixed to a point or held in a sling such as Horch and Salmon (1972).

The responses of crustaceans to noise are discussed in Section 3.5.3 (Chapter 3) and will not be discussed again here, since there are few that consider the effects of vibration within the noise signal.

4.6.3 Critique

In the current work, the animals were free to move within the arena and free to make postural and movement changes. As such, the received vibration may have varied between individuals and presentations of the stimulus. Although beyond the scope of the current work, laser Doppler vibrometry can be used to measure the received stimulus on test animals, for example in Breithaupt (2002) and Aicher *et al.* (1983). This would be beneficial to understand the precise value of the stimulus that elicits response. Furthermore, the current work used unconditioned animals, however conditioned animals may have shown greater sensitivity such as in Offutt (1970). Such experiments are likely to yield more natural, greater sensitivity curves than AEP and unconditioned work due to the conditioning of the animal. Although the current experiment did not aim to interfere with natural behaviour of *P. bernhardus,* it would be valuable to attempt conditioning of *P. bernhardus* and re-test the sensitivity here to allow comparison between conditioned and unconditioned animals.

It is of note that whilst the stimulus was predominantly exciting the substrate, it is possible that the signal also elicited water-borne particle motion, and perhaps even pressure within the tank due to the stinger rod used. There is no evidence yet to suggest crustaceans can detect pressure (Goodall, 1988), therefore the latter may be of little consequence. However the former may have

had an effect. Nevertheless, the boundaries of water-borne and substrate-borne particle motion in water are somewhat unclear, as this depends upon the liquidity of the substrate. A specially designed tank could be used to control both particle motion and pressure further, to allow the acoustic field to be more precisely measured. Future plans for such a tank were made within this project, but were not pursued due to time constraints (A. Hawkins, *Pers. Comm.*[5], SoundWaves consortium). Other works have indicated the need for such a tank (Hazelwood and Macey, 2015), and similar standing wave tanks have been built in the past with varied levels of success (Tautz and Sandeman, 1980; Plummer *et al.*, 1986; Breithaupt, 2002); for a comprehensive discussion see Hawkins and MacLennan (1975). Most recently, Bolle *et al.* (2011) built the 'larvaebrator', a similar tank on a smaller scale designed to investigate the effect of vibration upon fish larvae.

The proposed tank would enable the magnitude, direction and characteristics of the particle motion and pressure components of a sound to be manipulated and accurately measured. The tank would consist of a thick-walled (> 20 mm) open-ended steel tube which can be sealed to create a 'pressure' mode (minimal particle motion), or opened to create a 'particle motion' mode, up to 1 kHz (Figure 4.20). An electromagnetic shaker (of greater sine force than the current work) would drive a diaphragm at the bottom of the tank in a vertical direction towards the water surface. Accelerometers, or a laser Doppler vibrometer, would be used to measure exact vibration in all three directions to ensure that the signal was fully described. Such a tank would allow investigation of responses within a fully quantified acoustic field.

4.6.4 Stimulus presentation

The sinusoidal waves used here had predominant peaks in the region of the intended frequency. The coupling between the stinger rod and the substrate was adjusted during preliminary tests to minimise distortion of the waves. There were however some harmonic peaks at 40 Hz thought to be due to resonance within the tank. In audiometry studies of fishes, waveforms must be as pure as possible for example as in Chapman and Hawkins (1973). The current setup was a trade-off between purity of signal and a tank setup that would allow animals to display natural behaviours.

The stimuli were greatest in the vertical axis for most of the exposures, but the extent of this varied marginally with frequency. At 5 Hz the z axis (horizontally across the tank) was marginally greater. The positioning of the geophone was such that the x axis was between the electromagnetic shaker and the arena, and the z axis perpendicular to this. It should be noted however that whilst the vertical axis was predominant, the other two axis were also of notable strength, that is, the stimulus was not constrained to one axis. It is not possible then to determine resolutely to which of the three planes the crabs were sensitive to, however the signal could be described as predominantly vertical. The variation of the signal in the three planes with frequency highlights the need to measure all three axes in studies such as this, given that particle motion is a vector quantity, and it is important to understand the entirety of the signal received. It is of note that the vector amplitude of the three axis was not calculated, since the geophone measurements were being compared to the accelerometer measures in the vertical axis only.

[5] Prof. A. Hawkins, Loughine Ltd, Director of Loughine Ltd., Aberdeen.

In a solid, particle motion can travel as longitudinal (compressional), shear or surface (Rayleigh) waves (Section 1.3.1) (Markl, 1983; Hill, 2009b) with energy being transmitted in one or multiple waveforms depending on the substrate type, boundary layers, and connection to the substrate to name but a few (for a useful review see Aicher and Tautz (1990). In dry conditions, Brownell (1977) measured two types of waves in a sandy substrate; Rayleigh (surface) and compressional, with the Rayleigh wave being likely the only detectable wave at distance due to greater attenuation of the compressional waves.

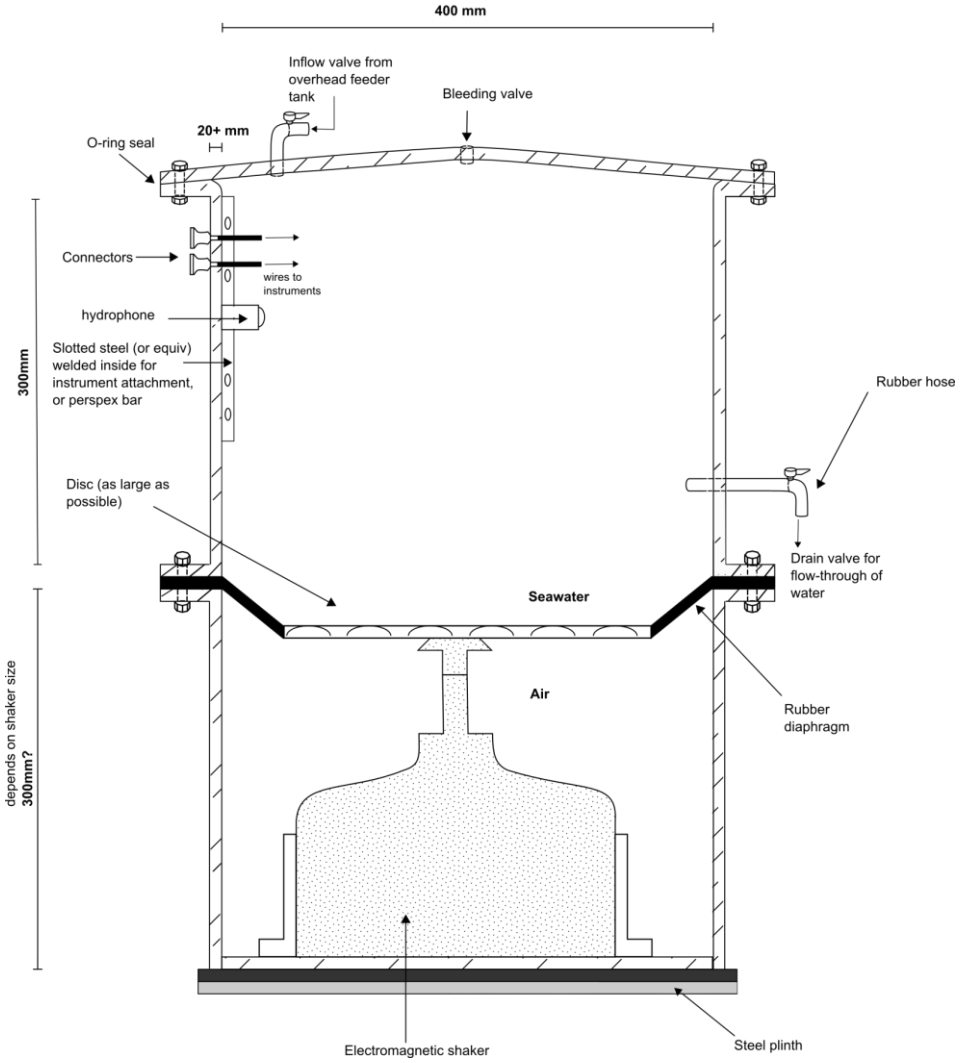

Figure 4.20 Schematic of a tank specifically designed to investigate the response of crustaceans to the particle motion component of sound (based on discussion with A. Hawkins *Pers. Comm.*[6], as part of the SoundWaves consortium). The tank would be constructed from a thick walled steel tube, with a diaphragm at the base connected to an electromagnetic shaker.

These waves also decay the slowest of all the wave types; it is likely then that these will be most important in affecting vibration sense. Recent work by Hazelwood and Macey (2015) has supported this, indicating that Rayleigh waves are most likely the waves involved in long distance vibration

[6] Prof. A. Hawkins, Loughine Ltd, Director of Loughine Ltd., Aberdeen.

propagation from anthropogenic sources, being low in frequency and speed, and therefore being trapped in the surface layer of the sediment. It is not clear whether the waves here were Rayleigh (surface waves), however they comprised predominantly of vertical motion. These types of waves have been shown to be detectable by crustaceans such as the fiddler crab *Uca pugilator* (Aicher *et al.*, 1983; Aicher and Tautz, 1984, 1990) and are thought to be a predominant wave type produced by anthropogenic signals (Hazelwood and Macey, 2015). The current work has indicated reception abilities of crustaceans to such low frequencies (< 100 Hz) which are particularly accentuated in the propagation of surface waves, and hence the effect of seismic waves cannot be underestimated (Hazelwood, 2012; Hazelwood and Macey, 2015).

An adjusted experimental setup could be used to constrain the motion to strictly one axis, such as the work of Heinisch and Wiese (1987), or with the use of a shaker table (Mooney *et al.*, 2010). In the current work the vibratory stimulus was presented using a stinger rod. This is not representative of a typical anthropogenic source of vibration, such as an impact pile driver, since the vibration may propagate through the substrate in a number of complex ways. However, for the purposes of threshold determination this was deemed sufficient. Measurement of the vibration in three planes ensured that the vibration field was understood within the tank, and it could be argued that the more technical the setup the more unnatural the behavioural responses would be. For example, the only way to ensure that the hermit crabs received the desired signal consistently between individuals would have been to constrain the animal (Salmon and Atsaides, 1969; Salmon, 1971) and, in the case of the hermit crab, take into account the different resonant properties of occupied shells. Similarly, a hermit crab may be in or outside the shell and this too could be standardised-which would require fixing of the crab posture each experiment. Therefore the 'perfect' experiment in terms of standardising response may not be representative of natural behaviour.

The precise stimulus strength and frequency composition received may have been affected by, for example, the type of shell occupied, the size, volume, and shell wall thickness. For this reason, crabs occupying damaged shells were not used in the experiments. Similarly crabs that moulted within the holding conditions, or that had missing appendages were discounted from tests-particularly since Offutt (1970) noted that the response threshold was affected after moulting. Furthermore the 'fit' of the shell may have had an effect on the resonance of the shells (i.e. whether the crab was in a shell suited approximately for its size). In the present work a significant positive correlation was found between chela size and shell size, which indicated that crabs were in fact occupying shells appropriate to size. The properties of a shell may affect resonance, in addition to its contact area with the substrate (Field *et al.*, 1987). Similarly it is possible that the vibration field varied slightly throughout experimental arena, and crabs were free to move throughout tests. However there was no indication of a preference inside the arena, and the spatial area encompassed by the arena was only 100 x 100 x 50 mm.

In the present work external vibrations were dampened by using a purpose-built base to the tank consisting of foam, wood and concrete layers. Similar structures have been used in other studies for example using dampeners and rubber gaskets (Mosher, 1972; Mooney *et al.*, 2010). Such structures are important since it has been shown that thresholds may be affected by background

levels, for example in fishes (Hawkins and Chapman, 1975). It is therefore important to measure background levels throughout vibration exposures, as in this work.

4.6.5 Relation to anthropogenic vibration levels

The current work suggests that the sensitivity of crustaceans to substrate vibrations is sufficient to enable detection of anthropogenic disturbances propagated through the seabed (Table 4.6). This is of great relevance since the core energy of many sources is low frequency (Nedwell *et al.*, 2003a; Nedwell *et al.*, 2003b) and would be detectable. For example, measurements taken with a geophone at 220 m from a dredging operation (core energy < 100 Hz) were 0.00015 m s^{-1} (presumed to be in the vertical plane), which, within the context of the present work, would be detectable by hermit crabs. Furthermore, levels at 410 m from a shell auger and piling operation were calculated as 0.00011 m s^{-1} (S. Cheesman, *Pers. comm*[7]). Sensitivities can also be related to water-borne measurements of particle motion, for example at full power a wind turbine was found to produce dominant tones at 29, 36 and 178 Hz in the region of 0.0001 m s^{-1} (Sigray and Andersson, 2011) with this energy possibly translating to motion in the seabed. Although it has been shown that detection of water-borne particle motion may only be at close range (Goodall, 1988), it is possible that the detection of substrate vibrations may be at a greater range, the current study supporting this hypothesis. It is clear then, that besides determining whether detection is possible, the bigger question is the extent of behavioural disruption these sources would cause. Crabs in this work responded to playback of a key anthropogenic signal by a burst of movement- a response such as this would have energetic consequences if regularly repeated.

It is of note that in general there is a shortage of publicly available underwater vibration measurements (Hazelwood, 2012; Hazelwood and Macey, 2015; Miller, 2015). Indeed, due to the complexities of underwater sound measurement, standard protocol involves predominantly pressure data rather than water-borne particle motion, let alone substrate-borne. Additionally there are not yet international standards for measuring particle motion, although the ISO () has recently proposed 1 p m, 1 nm s and 1 µ m s^{-2}. The measurement of vibration is, at least, easier to measure with three dimensional seismic sensors and directional accelerometers, whereas measurement of water-borne vibration is more complex, with sensors not yet commercially available. Two solutions to the problem exist, i.e. to measure motion with the dual hydrophone method e.g. Popper *et al.* (2005); (Zeddies *et al.*, 2010) or to use purpose-built sensors consisting of a motion sensor inside a neutrally buoyant casing, e.g. Kaifu *et al.* (2008); Zeddies *et al.* (2012). The implications of such measurement uncertainties are that data, where available, are stated in dissimilar units, making comparisons challenging. For example, in the case of seabed vibration the measurements of anthropogenic signals obtained from the literature were not fully described in terms of frequency composition- this made conversion to acceleration impossible. Many activities specifically contact the seabed, such as drilling and piling, but without measurements the consequences of these to marine organisms cannot be fully understood. To address this issue, a modelling approach may be used to estimate seabed vibrations such as from piling (Hazelwood and Macey, 2015; Miller, 2015). For example at 5 m from a pile driving operation levels are estimated to be 0.05 – 0.1 m s^{-1},

[7] Mr S. Cheesman, Acoustic consultant, Subacoustech Ltd., Southampton, UK.

dropping to 0.001 at 400 m from the pile (Miller, 2015) . In the current work, *P. berhardus* was sensitive to levels as 7.5 x 10^{-5} m s^{-1} at 410 Hz, and even if a higher level of behaviour is taken as reception (indicator 2) a sensitivity of 9.9 x 10^{-5} m s^{-1} was recorded at 40 Hz.

In addition to this, within the measurements of anthropogenic sources available, levels of vibration will fluctuate according to a number of factors, for example, type of source, parameters of the source (for example diameter and shape of pile), depth, propagation conditions, speed of, duration of operation (Section 1.9) (Athanasopoulos and Pelekis, 2000; Kim and Lee, 2000; Thandavamoorthy, 2004). As such, measurements are scenario specific and it would be unwise to generalise between sources and conditions. The speed of Rayleigh waves in particular vary with properties of the solid, frequency, the depth of the sediment hard layer and the poisson ratio (Hazelwood and Macey, 2015). These factors all affect the level of the sound produced, and the frequency spectrum of the signal. Indeed at low frequencies (< 100 Hz) and low speeds the energy of such waves is confined within the seabed-water boundary and is therefore able to propagate long distances for example up to 2 km (Hazelwood and Macey, 2015). Furthermore it is not sufficient to focus solely upon substrate vibration since such disturbance, for example pile driving, also causes a pressure component and a water-borne particle motion, both of which would reach the seabed indirectly. Additionally, activities that are not directly in contact with the seabed (for example shipping) are also likely to induce motion in the seabed (Hazelwood, 2012; Hazelwood and Macey, 2015; Miller, 2015). In order to fully investigate the response to such sources then, exposures must be undertaken in the field with actual sources, since even sophisticated playback systems such as those used in Chapters 2 and 3 cannot replicate the strong ground borne component produced by many activities.

In the current work, *P. bernhardus* was seen to respond to impulsive vibration playback. Whilst it may be argued that such playback, in a small tank, is not a truly accurate representation of the original source (Section 1.11.2), the signal was approximately representative in terms of spectrum and had a similar rise and decay time. Most importantly, responses were seen at levels of playback corresponding to levels at 23 m from the piling operation.

Of even more relevance is the sensitivity of *P. bernhardus* to natural vibrations, which may be useful for prey location, predator detection, reproductive display, communication and advertisement as seen in terrestrial organisms such as insects and scorpions (Brownell, 1977; Lewis and Narins, 1985; Hetherington, 1989; Hebets *et al.*, 2008; Hill, 2009b;a; Fabre *et al.*, 2012). However evidence for communication using the substrate has only been demonstrated in the semi-terrestrial Uca and Ocypode sp. (Salmon and Atsaides, 1969; Salmon, 1971; Horch and Salmon, 1972; Salmon and Horch, 1973; Aicher and Tautz, 1990). Indeed seismic reception (and communication) in crustaceans is not often discussed (Taylor and Patek, 2009), although there have been suggestions that sounds produced by crustaceans are likely to travel predominantly through the substrate (Sandeman and Wilkens, 1982; Field *et al.*, 1987). High levels of background sound in the marine environment may mean that detection and use of vibration signals would also be advantageous to marine invertebrates, given the improved propagation conditions of solids compared to water.

The frequency composition and strength of naturally produced substratum vibrations is not widely documented, although it is known that drumming in fiddler crabs produces energy in the region of 340 - 370 Hz (Aicher and Tautz, 1990). In the current work, the walking of *P. bernhardus* around the arena, at approximately 100 mm from the sensor was measured at a level of 0.003 m s^{-2} (RMS) which is well within the detectable range of *P. bernhardus* (< 500 Hz). Breithaupt (2002) compared the carapace vibrations of lobster to sensitivities of crayfish to particle motion and found that the vibration was perceivable at a distance of under 300 mm only (assuming the lobster was acting as a dipole source), or less depending on modelling conditions. Similarly Goodall (1988) proposed near field detection of stimuli only. Results here indicate that in fact *P. bernhardus* may be able to detect vibrations from greater distances, for example that of anthropogenic sources. Results here indicate sensitivity to substrate-borne vibration, and this may in fact open up the possibility of detection of incidental conspecific signals.

It is likely that *P. bernhardus* would be sensitive to a wide range of vibrations, occupying the rocky shore in areas of high wave action, for example. However there are few measurements of shore vibrations, although acoustic recordings upon the shore have indicated energy predominantly at 60 – 100 Hz (Ellers, 1995).

4.7 Conclusions and recommendations

The present work has indicated that *P. bernhardus* are sensitive to substrate-borne vibration within the range of 5 – 410 Hz. More behavioural tests would be required to ascertain the effect of the stimulus on both short and long-term behaviour, and to extend the frequency range. In terms of postural changes it would be valuable to determine the threshold required for the animals to abandon the shell, since such behaviour is likely to induce a physiological stress response and increase the susceptibility to predation. Furthermore, vibration may also affect movement, feeding, avoidance, habituation, agonistic behaviour and shell choice; in a similar way as acoustic studies with fishes have demonstrated (Hawkins *et al.*, 2014b; Simpson *et al.*, 2014; Voellmy *et al.*, 2014a; Voellmy *et al.*, 2014b). Anti-predator response, defined as time taken to withdraw into the shell, could be measured as in land hermit crabs (Stahlman *et al.* (2011). Additionally physiological measures of stress could be incorporated, for example, urea/ammonia excreted into the water, glucose or blood parameters, and oxygen consumption rates (Celi, 2013; Celi *et al.*, 2014). Such measures would help link behavioural changes to fitness implications, and therefore to the population level.

The responses observed may vary according to the characteristics of the exposure, for example, duration, substrate type, rise time duration and waveform. A repetition of the current work could therefore include a suite of different stimuli, and also could extend the frequency range upwards to investigate whether sensitivity reduces. Additionally, the directionality of response could be measured since it is likely that benthic organisms may be able to use surface waves for directional orientation (Hazelwood and Macey, 2015).

Background vibration levels were below average threshold values, however a valuable next step would be to vary background levels using white noise and study the variation in threshold (e.g. Wysocki and Ladich (2005). In the current work, time in the laboratory was shown to significantly

raise the threshold (i.e. reduce sensitivity to vibration) when all data were analysed together. Future work could repeat the test to increase replicates, however it is likely that the former trend is representative, particularly as background levels have been shown to affect thresholds of fishes, for example (Hawkins and Chapman, 1975).

It is of note that laboratory conditions cannot replicate the vibroacoustic conditions of the sea shore or the ocean. Indeed even in terms of behavioural experiments there is a need to observe behaviours in the natural environment if results are to have any meaning (Huber, 1988). The current work should be replicated in the field, in terms of reproducing a sensitivity curve and studying the flexibility of behaviour. In the current work, preliminary tests were undertaken on the seashore, using the electromagnetic shaker mounted inside a rigid frame to expose hermit crabs to sinusoidal signals. Preliminary work was undertaken to this effect- but it was found that the logistics of creating a vibration in a free vibratory field required more powerful equipment than was available (L. Roberts *Pers. Obs.*). One alternative to the shaker would be to mimic drilling and piling on a small-scale. Additionally a playback system (Chapter 2, 3) could be used to investigate the effect of water-borne signals which may also elicit vibration in the seabed at close range. For example lobster pots mounted with cameras could be used, an approach trialled by Brack (2010). For such experiments precise measurements upon the seabed would need to be undertaken in all three planes of motion. Small studies such as this would enable the behavioural responses of crustaceans to be more precisely described and understood.

There are few data investigating the response of invertebrates to vibration and acoustic sources, and fewer focussing upon anthropogenic signatures. Future studies must focus upon a range of other species, for example the common mussel *Mytilus edulis* (Chapter 5) and other molluscs, sea urchins and crustaceans, in addition to pelagic invertebrates, such as crab and bivalve larvae.

The experimental method undertaken in the current work was successful in establishing behavioural thresholds for the hermit crab *P. berhardus* to vibration. The thresholds obtained begin to provide an understanding of the levels of vibration that could potentially cause behavioural changes in the natural environment, an area of research that has been neglected in recent years. Behavioural responses were clear and allowed a sensitivity curve to be obtained. Sensitivity thresholds indicated an approximately flat threshold of response, suggesting that response was independent of frequency, however the amplitude of the stimulus did affect responses observed. This leads to partial acceptance of the first null hypothesis (i.e. frequency did not affect sensitivity). The threshold values were approximately lower when calculated using the first behavioural indicator (antennae movement) rather than the second leading to rejection of the second null hypothesis. That is, the crabs were, at the lowest amplitudes of vibration, more likely to respond with small antennae changes rather than movement. It is likely that at the higher amplitudes the vibration was interpreted as a threat (perhaps as an approaching predator), whereas at the lower amplitudes as a change of environment. This may explain the antennae movement, which may be the crab testing the surrounding water for chemical changes. Thresholds appeared to be correlated with time in the laboratory, with crabs being less sensitive to vibration after a longer duration in the laboratory prior to tests, although more data would be required to further understand the

hypothesis. There appeared to be a correlation between morphology measurements and sensitivity. This lead to rejection of the third and fifth null hypothesis.

In periods of minimal disturbance there appeared to be a consistency in behaviour within individuals, consistent with the idea of personality. This was supported by a consistency in threshold in re-tested crabs, and by observations of greatly differing behaviour between individuals upon introduction to the experimental arena. However, after exposure to vibration, personality rankings in two of the data sets appeared to be different indicating that vibration may have been modified 'normal' behaviour. Indeed crabs were quicker to recover from being upturned after exposure to vibration. Therefore the fourth null hypothesis cannot be rejected or accepted in full. Despite evidence for personality in the tested crabs, there was no link between sensitivity threshold and 'boldness', leading to acceptance of the sixth null hypothesis. Finally, playback of an impulsive vibration signal was shown to significantly affect hermit crab behaviour in terms of behaviour exhibited immediately at the onset of playback, and also in terms of startle response tested later. This allowed rejection of the seventh hypothesis.

Popper *et al.* (2001) concluded that the sensitivities of many semi-terrestrial decapods to vibrations were sufficient to detect predators, mates and conspecifics at close range. The present results have shown that a marine crustacean is sensitive to vibration under 500 Hz and may use vibratory cues in a similar way- this is likely since sound production is widespread in the marine environment. The upper or lower limit of the frequency response is not yet known.

The results of the current work must be viewed within the context of the experimental conditions- involving a particular stimulus, species, frequency range, substrate type and amplitude range. Similarly, behavioural responses of an individual may vary according to environmental parameters, energy availability, and perhaps even personality (as demonstrated in the current work). This must be taken into consideration when extrapolating the results into a wider context. There has been insufficient work, especially recently, on the detection capabilities of crustaceans to conclude anything for certain without more research, the current work provides reliable data within this context. Threshold values, responses to impulsive piling playback, and collated measurements of actual anthropogenic vibrations indicate that *P. bernhardus* may be sensitive to anthropogenic vibrations. Indeed may be sensitive at a great distance from source (e.g. up to 400 m from drilling). These results must now be translated into actual conditions, in the vibroacoustic free field, to fully understand the effect of such activities. This is of importance since many anthropogenic activities involve direct contact with the substrate and other activities may also induce particle motion indirectly. The effects of substrate transmission should not be overlooked when investigating the effects of noise pollution on the marine environment.

Chapter 5 Sensitivity and behavioural responses of the mussel *Mytilus edulis* to substrate-borne vibration

Note

The work described in this chapter was undertaken by the author together with two students. The threshold experiments were solely undertaken by the author. The response determination experiments were devised, designed and overseen by the author, but undertaken by two undergraduate students. Data were re-analysed by the author.

Abstract

Sensitivity of *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, with valve closure used as the reception indicator. The variation of behavioural response (valve closure duration) was further investigated using a mixed frequency vibration source (5 – 450 Hz), with duration of response compared between solitary and grouped mussels. Sensitivity ranged from 0.06 – 0.55 m s^{-2} (RMS) with an approximately flat sensitivity curve. As exposure level increased the incidence of responses increased. The 50% incidence rate of response was 0.53 m s^{-2} (RMS) for solitary mussels, and response likelihood varied according to the size of mussel. The duration of response increased with stimulus strength, and did not vary according to the whether the mussels were grouped or solitary. Preliminary data also indicated sensitivity of the barnacle *Balanus crenatus*. The thresholds of *M. edulis* were shown to be within the levels measured in the vicinity of actual anthropogenic vibrations. The data provided are a strong indication of the sensitivity and response of *M. edulis* to vibration, and should allow further expansion of a novel research area.

5.1 Introduction

Whilst an underwater sound source creates a water-borne pressure wave, it also produces a back and forth particle motion which propagates both through the water column and the seabed (Hazelwood, 2012; Miller, 2015). Once in the solid seabed, the energy may be propagated as longitudinal (compressional 'P' waves), shear (transverse, 'S' waves), or surface (Rayleigh, 'ground roll') waves (Markl, 1983; Aicher and Tautz, 1990; Hazelwood and Macey, 2015), with the signal changing in terms of amplitude with attenuation, depending upon frequency. For Rayleigh waves, the energy is confined within the surface of the seabed and the waves are likely to propagate for large distances from source (Hazelwood and Macey, 2015). Anthropogenic activities, especially those directly in contact with the seabed, are likely to produce such substrate-borne vibrations, but 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.

In terrestrial organisms, detection of substrate-borne vibration (from now on referred to as vibration) has been described in various organisms such as spiders, snakes, lizards, scorpions and insects (Hetherington, 1989; Cocroft *et al.*, 2000; Hill, 2001; Hebets *et al.*, 2008; Fabre *et al.*, 2012). Uca sp. (semi-terrestrial fiddler crabs) have also been shown to be receptive to, and indeed to communicate by using such vibrations (Salmon and Atsaides, 1969; Salmon, 1971; Salmon and Horch, 1973; Aicher and Tautz, 1984). In the marine environment there is evidence that other crustaceans have detection systems for particle motion, which may also be used for substrate-borne vibrations (Tautz and Sandeman, 1980; Plummer *et al.*, 1986; Breithaupt and Tautz, 1988, 1990; Goodall *et al.*, 1990; Monteclaro *et al.*, 2010; Roberts and Breithaupt, 2015). Indeed vibration reception, and perhaps communication, seems likely in marine invertebrates since vibrations can propagate large distances through solids, making the seabed an ideal medium for animals to use (Taylor and Patek, 2009).

The ability of an organism to respond to particle motion (be it solid or water-borne) depends upon the detection abilities of the sensory system. The presence of mechanoreceptors which may be stimulated by the movement of dense masses such as the statocyst organs (Williamson and Budelmann, 1985; Neumeister and Budelmann, 1997; Kaifu *et al.*, 2008; Mooney *et al.*, 2010), or by cilia cells on body parts and other receptors on the body and appendages (Cragg and Nott, 1977; Charles, 1980; Zhadan, 2005) and in some cases specialised abdominal sense organs (Zhadan, 2005) in molluscs indicates that reception is likely in bivalves and gastropods. The specific receptors, if present, to detect acoustic and vibrational stimuli are relatively unstudied although there is some evidence of detection to unspecified levels of vibration (Mosher, 1972; Kastelein, 2008), and of detecting particle motion rather than pressure (Ellers, 1995).

In molluscs, work has largely focussed upon cephalopods, where sound-induced trauma has been linked with exposure to low frequency and impulsive sounds (André *et al.*, 2011), and behavioural responses such as ink jetting (McCauley and Fewtrell, 2008; Fewtrell and McCauley, 2012a). Studies indicate reception abilities in the frequency range of 1 – 300 Hz, with threshold amplitudes ranging from 0.0003 – 1.1 m s^{-2} (Kaifu *et al.*, 2008; Mooney *et al.*, 2010), which may be indicative of the capabilities also of other molluscs. There has also been investigations focussed upon the statocyst and lateral line systems of cephalopoda, for a review see Budelmann (1992b). Threshold

sensitivities of other invertebrates such as crustaceans are reported to be in the range of 0.005 – 0.81 m s^{-2} (20 – 400 Hz) (Salmon and Atsaides, 1969; Salmon, 1971; Salmon and Horch, 1973; Barth, 1980; Aicher and Tautz, 1984; Heinisch and Wiese, 1987; Berghahn *et al.*, 1995). These values may provide an insight into the detection capabilities of other invertebrates.

There are few studies exposing bivalves to vibration stimuli, or indeed underwater sound stimuli (Mosher, 1972; Kowalewski *et al.*, 1992; Ellers, 1995; Zhadan, 2005; Kastelein, 2008). Responses described include siphonal retraction, closure of the valves and, in the more active Pectinids, jumping from the substratum (Mosher, 1972; Ellers, 1995; Kastelein, 2008). Typically work has focussed on larval mortality (Kowalewski *et al.*, 1992), behaviour (Mosher, 1972) and directional sensitivity (Zhadan, 2005) of bivalves, rather than a precise understanding of the sensitivity range or key behavioural responses. Incomplete or undisclosed descriptions of the exposures make it difficult to fully interpret the results, for example when comments about sensitivity to vibration are provided without data to confirm these observations (Hughes, 1970; Kádár *et al.*, 2005). Kosheleva (1992) described the exposure of caged bivalves and gastropods (*M. edulis* and *Littorina obtusata, L. littorea*) to airguns (source levels 220 – 240 dB re 1 µPa), and reported a lack of response however the original full document is untraceable. Regardless of the data being incomplete, the reference is widely cited as an example of invertebrate exposure to noise (Moriyasu *et al.*, 2004).

Despite the lack of information, the behavioural responses of bivalves to vibration may be compared with exposure to other stressors, for example chemical pollutants (Manley and Davenport, 1979; Akberali and Black, 1980; Kramer *et al.*, 1989; Salanki and Vbalogh, 1989; Curtis *et al.*, 2000; Kádár *et al.*, 2005), predators (Toomey *et al.*, 2002; Robson *et al.*, 2010) or varying environmental parameters (Englund and Heino, 1996; Newell *et al.*, 2001; Gnyubkin, 2010). The monitoring of heart rate, oxygen consumption, carbon dioxide production, movement and siphonal changes may indicate the effects of such stressors. Rather than being open or closed, the valve gape angle of bivalves may be a graded response according to the perceived risk or benefit of a stimulus (Dolmer, 2000; Newell *et al.*, 2001; Toomey *et al.*, 2002; Robson *et al.*, 2007; Robson *et al.*, 2010). Behavioural responses may also vary according to group size (Wilson *et al.*, 2012).

The standard method for determining the sensitivity of an organism to a sound stimulus is to produce a curve of sensitivity across all frequencies. For acoustics, the threshold (the lowest level of sound for each frequency measured) is presented as an audiogram, using the staircase method of presentation to determine the threshold (Cornsweet, 1962). The threshold is measured either by behavioural conditioning (Chapman and Hawkins, 1973) or by auditory brainstem response (ABR or AEP) technique (Nedwell *et al.*, 2007) when the acoustic receptors are stimulated by sounds (Smith *et al.*, 2004). For cephalopods, and indeed some crustaceans, the AEP technique has been successful (Lovell *et al.*, 2005; Mooney *et al.*, 2010). The behavioural conditioning technique trains the animal to respond to a stimulus, using a mild electric shock. Conditioned subjects associate the sound stimulus with the shock, and the sound elicits a cardiac change. However for invertebrates such conditioning is difficult, although attempts in crustaceans have been successful (Offutt, 1970). An alternative to this approach is to use small behavioural changes as markers for reception. For example postural changes, antenna movement and displacement of the walking legs are commonly used as indicative of response in crustaceans (Heinisch and Wiese, 1987; Goodall,

1988; Breithaupt, 2002) (Section 4.5, Chapter 4), and monitoring of respiratory action has also been used in cephalopods (Kaifu *et al.*, 2008). Work such as this must be undertaken in controlled conditions, with close monitoring of behaviour, an adjustable stimulus and measurements of accuracy. Once baseline sensitivities levels are known, responses to vibrations can be fully described. In order to understand the reaction of bivalves to vibrations, threshold experiments such as these are required using behavioural changes as indicators of reception.

5.2 Aim, objectives and null hypotheses

The aim of the current work was to examine the effects of the substrate-borne component of anthropogenic sound upon the behaviour of a common intertidal bivalve, with particular focus on the precise sensitivity and response to a repeatable, quantifiable source. Once the sensitivity threshold was obtained, behavioural tests were undertaken (using levels within the reception range) to investigate the variation of responsiveness in relation to stimulus changes, at individual and group level.

Specifically, the threshold experiments used the widely accepted staircase method of threshold determination (Cornsweet, 1962). A purpose-built tank was constructed with external vibration dampening. A fully calibrated source was used to generate the sinusoidal stimulus, and particle motion sensors were deployed to measure the received vibration in all three planes of motion. Behavioural changes were recorded using live observations and video techniques. The follow-on response determination work used a quantifiable vibration source and simple behavioural measures to investigate variations in response at variable stimulus levels.

The species investigated in this study was *Mytilus edulis* (L., family Mytilidae), a marine intertidal bivalve which dominates both exposed and wave exposed shores (Seed and Suchanek, 1992), creating a stable habitat for many other organisms (Lintas and Seed, 1994; Borthagaray and Carranza, 2007). *M. edulis* is also a common biofouling species and of great commercial importance. *Balanus crenatus* (L., family Balanidae) encrusting on many of the test subjects, was also investigated as a side study. *B. crenatus* is a common sublittoral barnacle species, typically found on hard substrata including shells, cobbles, molluscs and rocks (Newman and Abbott, 1980) with a widespread distribution around UK shores. The sensitivity of these two species to substrate-borne vibration has not been documented in detail in the literature, but mechanoreceptors appear to be involved in reception capabilities (Cragg and Nott, 1977; Zhadan, 2005). It is likely that *M. edulis*, being an organism adapted to the high energy shore line, would be sensitive to vibrational changes.

The null hypotheses tested in this study were:

1. Species response will not be related to the characteristics of the vibration stimulus (frequency and amplitude).

2. The sensitivity of the tested species, in terms of threshold responses and presence of response will not be related to morphological parameters.

3. The occurrence and duration of responses will not be related to the presence and amplitude of vibration.

4. Response presence or level will not vary between solitary and grouped mussels.

5.3 Materials and Methods

Two sets of experiments were undertaken to test the proposed hypotheses: these were 'threshold determination' experiments and 'response determination' experiments (Figure 5.1). The aims of these were to quantify the sensitivity of *M. edulis* to vibration, and to explore the variation of behavioural responses to vibration. The threshold experiments were undertaken in one session (May 1st – 10th 2014), whilst the response experiments were undertaken in two sessions (February – March 2013, 2014).

Mussels for each experimental session (February 2013, February 2014, May 2014) were collected from the intertidal area of Filey Brigg shore, Filey (54° 13 ' 02.5"N 0° 16' 28.3"W), ranging in shell length from 31.3 – 69.5 mm. 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 algae were present in the water. 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.

The two experiments partially share methodologies as described in the sections below.

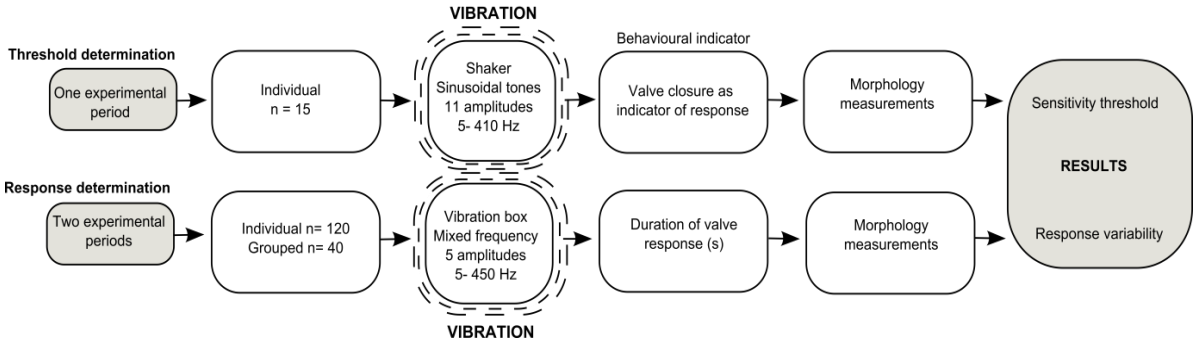

Figure 5.1 Flow diagram of the two sets of experiments undertaken with *M. edulis* in the current work: threshold and response determination.

5.3.1 Experimental setup and experimental procedure

(i) Threshold determination

The tank setup and method for threshold determination are identical to Section 4.3 (Chapter 4) and will be summarised here. Each mussel was acclimated in the experimental tank for 1 hour prior to threshold determination. Inside the arena each mussel was placed with the umbo into the substratum and the exhalant siphon pointing upwards, and was not restrained in any way. As described in Section 4.3, subjects were exposed to sinusoidal signals (rise and fall time 1 s) of

varied amplitude and frequency (5 – 410 Hz) using the staircase method of presentation (Cornsweet, 1962). This method involves the presentation of stimuli of decreasing amplitude until response ceases, the signal is then increased until a response is exhibited. The experimental procedure consisted of exposing the subject to the signal, observing the response and then choosing the next signal accordingly. A positive response to the signal initiated a reduction of the signal amplitude, and vice versa. This procedure continued until two amplitudes were repeatedly presented, with positive and negative responses consistently i.e. that the staircase reached a plateau. Presentation of these two amplitudes was undertaken until ten repetitions had been tested. The threshold value was then calculated as the average of these ten iterations.

A threshold value was calculated at each frequency. At a random point across each test session animals were exposed to a 'blank' sound clip to investigate the effect of the equipment itself (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 (1^{st} – 10^{th} May 2014), one per session (morning and afternoon) respectively.

In order to compare sensitivities to vibration from actual anthropogenic sources, the literature was searched for publicly available vibration data from sources such as drilling and pile driving.

Barnacles, identified as *B.crenatus,* were encrusting on the mussel valves. It was noted that these opened and closed in response to vibrations, both independently and in association with the reaction of the mussel. As such, responses could be used to calculate the threshold of response. To increase visibility of the cirri against the background, a ring of black plastic was placed around the base of the mussel. For the purposes of analysis one barnacle per group was observed throughout exposures to ease live observations. Video recordings were also stored for future analysis of group responses but were not analysed due to time constraints. A response was defined as a cessation of activity or an increased beating of the cirri.

Response data were recorded and an approximate threshold was calculated from six days of observations. It is of note that presentations of the stimulus and the corresponding amplitude adjustments (using the staircase method of presentation) were made in response to the mussel behaviour rather than that of the barnacles. The resulting barnacle threshold, made up of these *ad hoc* observations, was therefore not calculated with the same accuracy as the mussel threshold. The threshold values for barnacles should therefore be considered approximate. The threshold values per frequency were calculated as the average acceleration value (m s^{-2}) that a response was observed, rather than the 50% response level. It is of note that the vibration the barnacle received may have been distorted due to propagation across the valves and the precise value of signal, at the barnacle, was not measured.

(ii) Response determination

A different setup was used in the response determination experiments (Figure 5.2). Three plastic vessels (100 mm height) (five in the first study, February 2013) filled with seawater and a thin layer of sand were placed in an arc around the vibration source, with a distance of 220 mm from the

centre of each cup to the centre of the box. Each vessel or 'arena' was secured to minimise side-to-side vibration and the sides were screened to eliminate visual stimuli. One mussel was placed in each arena, without touching the sides, and allowed 5 - 10 minutes acclimation time prior to the start of each experiment. Inside the arena each mussel was placed with the umbo buried in the substratum and the exhalant siphon pointing upwards. The seawater within each arena was refreshed between subjects, and was taken from the holding tanks to ensure environmental continuity. Each arena was not specifically acoustically isolated from the surroundings, although the work was undertaken in an area of minimal disturbance.

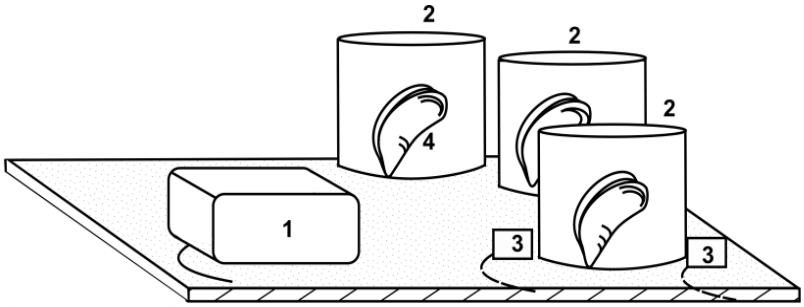

Figure 5.2 Schematic of experimental setup used to expose *M. edulis* to vibration, consisting of three arenas in an arc around a vibration source, vibration source (1), experimental arena (2), position of acceleration and velocity sensors (3), *M. edulis*, one in each arena for solitary tests four per arena for grouped tests (4).

To test the responses of groups, clumps of four mussels were tested in a larger arena. Each group was tested individually, at a distance of 100 mm from the vibration source. This distance was reduced from 220 mm as preliminary tests indicated lower numbers of responses at the original distance. The groups were given 5 – 10 minutes acclimation time. Grouped experiments were undertaken in both experimental sessions (February 2013, 2014). However the data from February 2013 could not be validated therefore these results must be viewed with caution.

A purpose-built vibration box (200 x 150 x 100 mm) was used as a vibration source in the response determination experiment, as inspired by a preliminary work with cockles, *Cerastoderma edule* (Kastelein 2008) and from discussion with R. Kastelein (*Pers. Comm.*[8]). The vibration source was a miniature drill motor (MFA/Como drills, 6 V DC) with a purpose-built eccentric fly-wheel (a 'K'nex' toy wheel, weighted) attached to the spindle. This produced an irregular spin of the wheel and therefore a strong vibration. The motor was encased in an IP67 weatherproof box (100 mm^3), inside a weighted plastic container with foam surround. The vibrating box had been used for preliminary work of Section 4.3 (Chapter 4) hence the redundant waterproofing. The motor was attached to a desktop power supply (Maplin 36W DC Variable Voltage bench power supply) via a miniature panel-mounted variable speed voltage regulator (6 – 15 V, MFA/Como drills) allowing the rotations per minute (R.P.M) of the motor to be adjusted. Five settings of R.P.M. were marked on the voltage regulator dial, denoted as vibration levels 1 – 5 in the current work (five being the maximum).The output vibration was an uncalibrated mixed frequency signal, omnidirectional, with

[8] Dr R. Kastelein, SEAMARCO (Sea Mammal Research Company), Director of SEAMARCO, Netherlands.

an irregular waveform. This was sufficient for the purposes of the experiment, which did not seek to replicate anthropogenic vibrations, nor to estimate precise sensitivity, but merely to elicit a behavioural change in response to a specific amplitude.

It is of note that when the term 'vibration level' is used from now onwards, it is described as either being in terms of the vibration box (levels 1 – 5), or being in terms of acceleration (m s^{-2}) or velocity (m s^{-1}), as appropriate to the calculation method.

Mussels in the arenas were each simultaneously exposed to varied levels of vibration (level 1– 5) for 10 seconds, in a random order. A control trial, when the power was disconnected from the vibrating box, was also undertaken to ensure the equipment had no effect upon behaviour. The interval between stimuli presentations ranged in duration, being at a maximum five minutes after the last mussel reopened. Within each arena mussels were unrestrained.

Since the mussels were separately enclosed and were not able to influence each other, the tests were run simultaneously (e.g. 3 – 5 mussels at a time in the case of solitary tests). Group tests were undertaken with one group at a time to allow full observation of all individuals.

5.3.2 Recording responses

Preliminary tests prior to both experiments indicated that a response of an individual to vibration could be classed as full closure or partial closure of the valves, termed 'startle response' for the remainder of this work. Additionally, if the foot were extended during the exposure this could be retracted, partially retracted or remain active outside the valves, and a 'twitch' of the valves was observed in some cases. Although in some cases digging of the mussel into the sandy substratum occurred, this was not monitored since it was a gradual process.

For the purposes of threshold determination, closure or clear partial closure of the valves was used as a reception indicator. For the response experiments the time taken to reopen (duration of the startle response, to the nearest five seconds was recorded), where 'open' was defined as visible lightness (i.e. the light coloured respiratory siphons being visible). The number of null responses was also recorded. A response of a group was classed as the mean value time taken for the first two of the mussels to reopen.

All experiments were filmed throughout, allowing any uncertainties to be revisited if necessary. Morphological measures were taken after testing, using callipers to measure to the nearest millimetre. Shell length (defined as the maximum anterior-posterior axis) and shell width (maximum lateral axis) were measured, and length-width ratio was derived (Figure 5.3). After measurements mussels were placed in a smaller aerated holding tank (200 x 200 x 150 mm) for the remaining time in the experimental room.

5.3.3 Statistical analysis

Simple descriptive statistics were calculated in EXCEL software (version 2007), further analysis was undertaken with SPSS (version 19). 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. A Levene's test for homogeneity of variance was used where appropriate.

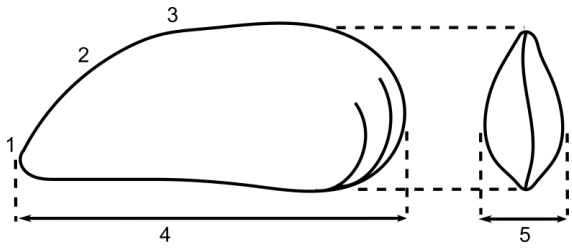

Figure 5.3 Anatomy and measured parameters of *M. edulis*, umbo (1), anterior side (2), dorsal side (3), shell length (4), shell width (5).

(i) Threshold determination

Mussel thresholds were averaged across individuals at each frequency. A Mann-Whitney U test was used to compare average threshold values between mussels and hermit crabs (data from threshold of indicator 1 Section 4.5.3) both with data grouped and subdivided by frequency. The threshold values per frequency were compared between barnacle and mussels. Statistical comparisons were not undertaken with the barnacle data due to the differing methodologies and the approximate nature of the barnacle sensitivities.

For comparison with the threshold data, morphology data consisting of width (mm), length (mm) and shell length/width ratio were correlated with average threshold values (m s^{-2}) by using Pearsons correlation. Width (mm), length (mm) and shell length/width ratio were correlated with threshold values (m s^{-2}) (split by frequency) using Spearmans Rho correlation.

The values of anthropogenic vibration in the literature were typically provided as velocity (m s^{-1}). Since anthropogenic signals cannot be considered sinusoidal, the measurements were not converted to acceleration, as ideally they would be differentiated with respect to time. Even an approximate conversion, using the sinusoidal equation was not undertaken since most of the data did not include peak frequency data to allow accurate conversion. Instead, 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:

$$A = 2\pi fV \qquad [12]$$

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

(ii) Response determination

Vibration (m s^{-2}) either side each arena were compared using a paired t-test. Paired t-tests were also undertaken using the vibration levels (m s^{-2}) next to each arena, to investigate whether levels varied between the arenas.

For the purposes of analysis the two sets of data (2013, 2014) were grouped together due to similar experimental conditions. Frequency response was defined as the number of responses divided by the total number of exposures. Pearsons correlation was used to relate the number of responses with exposure level. A linear regression was undertaken with response rate, calculated as the number of responses out of total presentations, and acceleration (m s^{-2}). The 50% response level was calculated from the equation of the regression line.

Binary logistic regression was performed on response (presence or absence) data with length of mussel (mm) and vibration level (1 – 5, categorical) as predictors, with vibration level 5 as the reference category. Chi-squared was used to assess the difference in frequency of response per size category of mussel (mm). The data was grouped by vibration level (1 – 5 combined) and subdivided. Post-hoc tests (standardised residuals) were used to investigate differences further.

A factorial ANOVA (two-way) was then performed with response data only, to compare startle duration (s) with the size of mussel (categorical, seven categories of 5 mm span, 30 – 70 mm) and vibration level (1 – 5, categorical).

An independent t-test was undertaken to compare the mean duration of response between solitary and grouped mussels. Since during the grouped experiments the vibration levels were higher than in the solitary experiments, data from comparative velocities was used rather than input vibration box levels. In terms of velocity, this meant a comparison in mean response duration at 0.0002 m s^{-1} and 0.0003 m s^{-1} respectively. A repeated measures one-way ANOVA with a Greenhouse-Geisser correction was performed on grouped mussel data to compare startle duration (s) among vibration level (1 – 5, categorical) as the independent variable. The data violated Mauchleys test for Sphericity hence Greenhouse-Geisser corrections were used. Bonferroni pairwise comparisons were used to compare further between the vibration levels (1 – 5).

5.3.4 Stimulus measurements and signal analysis

Measurements of the vibration emitted during the threshold experiments and the response experiments were measured in the vertical axis (m s^{-2},1 k/s sampling rate) using a Brüel & Kjær type 4333 piezo-electric accelerometer (sensitivity 20.60 mV/g) connected to a battery powered Brüel & Kjær Charge Amplifier type 2635. A three-dimensional geophone system (SM-7 370 ohm, IO, 28.8 V/m/s) was also used simultaneously to measure velocity in all three planes (m s^{-1}). Sensors were connected to an ADInstrument Powerlab module and to an IBM Laptop with CHART software (v 5.5.6) installed.

In the case of the threshold experiments, all vibration levels (m s^{-2} and m s^{-1}) were measured inside the tank using waterproofed sensors. In the case of the response experiments measurements were taken next to the experimental arena (in dry conditions).

Signal analysis was undertaken as described in Section 4.3.11. In CHART 5 software, twenty seconds of each signal was selected for each of the sensors, with the RMS calculated for that period. Spectra were calculated using a Blackman window, 1024 FFTs, sampling rate 1k/s.

Geophone measurements were exported from CHART 5 into Excel. Conversion to acceleration (and therefore precise calibration) of the velocities was not undertaken due to the frequency dependent

nature of the sine wave equations and the mixed frequency nature of the vibration source. However whilst inconvenient, the calibration of the geophone system was not strictly necessary, since the data were used to demonstrate the respective influence of each plane of motion in comparison with each other, rather than being using for calculation of the threshold (i.e. to determine that energy was predominantly in the vertical axis compared to the other two axis).

(i) Threshold determination

The precise measurement of signals, calibration of sensors and calculation of background levels for the threshold experiments are fully described in Section 4.3 and Section 4.4 (Chapter 4).

Signal analysis was undertaken as described in Section 4.3.11 although here the accelerometer malfunctioned part-way through the experiments, and therefore sensitivities were calculated from the input shaker values rather than being calculated from the precise exposure level measured next to the arena. To do this, the average acceleration was calculated from each respective shaker amplitude, by using data most recently obtained from the tank. The measured amplitude of the shaker varied marginally across the test days, and therefore the implication of this method of calculation may have been a minor loss of accuracy in the threshold.

(ii) Response determination

The overall vibration levels of the vibration source were measured at the end of the experimental period, under the assumption that the drill motor produced consistent acceleration levels. A generalised calibration equation was used to calibrate the accelerometer, rather than the method of Section 4.4.1, to account for the mixed frequency nature of the signal, this was calculated by averaging the slope and intercept values from the calibration equations of Section 4.4.1, resulting in the following calibration equation:

$$y = 0.8338x - 0.026 \qquad [13]$$

With y being the uncalibrated and x being the calibrated acceleration, allowing calibration of the uncalibrated sensor. During the calibration measurements the acceleration either side of each arena was measured, due to space constraints within each arena. There were therefore six vibration measurements (two per arena) taken for each of the five vibration levels.

Ambient vibration level measurements were undertaken simultaneously to the vibration box measurements using the geophone system. Ten 8 – 10 second samples of the background levels were recorded, with the RMS values calculated in CHART 5, and averaged to calculate the overall level. It is of note that background measurements were not taken continuously throughout the experiment, for logistical reasons, but any obvious external stimuli were documented. The positioning of the geophone was such that the x axis ran between the arena and the vibration box, the y axis was vertical, and the z axis perpendicular to this.

5.4 Results

5.4.1 Stimulus and background levels

(i) Threshold determination

The stimulus and background levels are outlined fully in Section 4.5.1. Average background levels were not significantly different between three core experimental periods tested during the experiments of Chapter 4 and hence were assumed to be similar for the current work, being on average 0.0074 m s^{-2}.

The input signals were sinusoidal, with predominant energy in the desired frequency and in the vertical plane of motion.

(ii) Response determination

The stimulus was approximately sinusoidal, with a frequency range of 5 – 450 Hz, and predominant energy produced at 5 – 200 Hz. Peak frequencies varied with vibration level, for example levels 1 – 3 had predominant energy in the range of 5 – 80 Hz, whereas levels 4 and 5 had peak frequencies of 120 – 240 Hz. The output accelerations of each vibration level increased with a positive linear trend (Figure 5.4).

Vibration levels (m s^{-2}) did not vary significantly either side of each arena ($t = -2.18$, $df = 4$, $p = 0.1$). For this reason the data between front and back of each arena were averaged (Figure 5.4). There was no significant difference of acceleration between arena 1 and 2 ($t = 0.96$, $df = 2$, $p = 0.44$) and arena 2 and 3 ($t = 2.92$, $df = 2$, $p = 0.1$); however there was a significant difference between arena 1 and 3 ($t = 8.71$, $df = 2$, $p < 0.01$) with the mean acceleration in arena 1 being 0.34 m s^{-2} versus 0.42 m s^{-2}. Although this difference was significant, the responses of mussels from the third arena did not appear to differ markedly from any other arena, so all data were grouped. For all the above, it was assumed that similar relationships would be seen when there had been five arena in the first experimental session.

The stimulus was greatest in the vertical (y) axis, ranging linearly from 0.0003 – 0.0002 m s^{-1} (RMS, levels 1 – 5), with the vibration in the x and z axes remaining approximately constant (in the region of 0.00005 m s^{-1}, RMS) (Figure 5.5). The trend was most clear at 220 mm from the vibration box. For the grouped experiments the distance between the vibration box and the area was reduced to 100 mm, and the velocity levels were higher (Figure 5.5A).

Average background noise measurements were 0.00003 m s^{-1} in the x, y and z planes (SD = 8.9 x 10^{-6}, 6.3 x 10^{-6} and 8.4 x 10^{-6}, n = 10) (Figure 5.5). This may explain why there were fewer responses at level 1 of the vibration box, since the signal was close to background range (3.51 x 10^{-5} m s^{-1}).

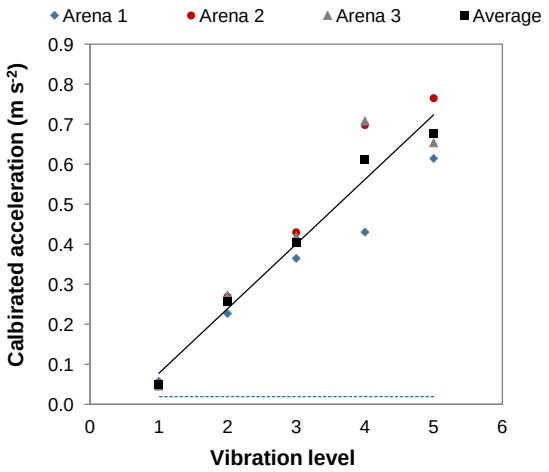

Figure 5.4 Average acceleration (m s^{-2}) corresponding to levels of vibration (1 – 5), for each of the three arena (1 – 3), and average (m s^{-2}, RMS), data from February 2013. The lowest threshold of *M. edulis*, as found in Figure 5.7 is represented by a dotted line.

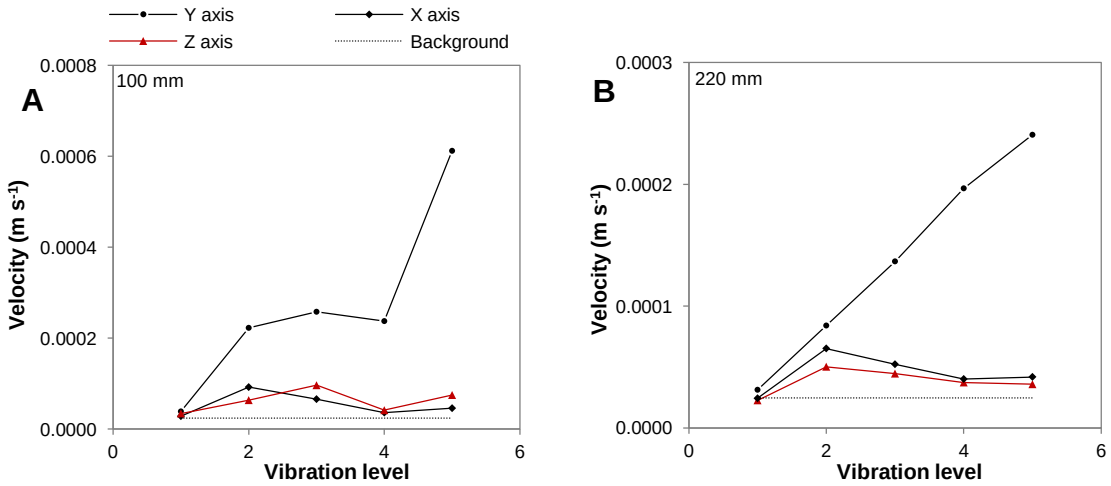

Figure 5.5 Velocity measurements of the five vibration levels (1 – 5), taken at either 220 mm (A) or 100 mm (B) distance from the vibration box. A dashed line indicates average background levels (m s^{-1}).

5.4.2 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. Details of the stimuli levels and background levels are provided in Chapter 4 Section 4.5.1. Fitness of the mussels was deemed satisfactory since valve gape was frequent, gills and siphons were visible (Figure 5.6B), and exploration of the area was undertaken with the foot, sometimes leading to partial digging behaviour (Figure 5.6A, C). No mortality was observed. Clear behavioural changes were observed in all mussels in response to the vibration stimulus. No reactions were observed during control trials. Full and partial closure of the valves was frequent and clearly visible throughout the experiment.

On average each mussel reacted to five out of the seven frequencies tested (n = 15, SD = 1.24), 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), and was approximately constant throughout the duration of each experiment being on average 12 individuals reacting out of 15 for every presentation across each experimental session ($\bar{x}$ = 11.57, SD = 1.13), (Table 5.1).

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 5.7A).

Figure 5.6 Digital stills taken during threshold determination experiments (May 2014). *M. edulis* with a wide valve gape, and inhalant and exhalant siphons clearly visible. *B. crenatus*, encrusting upon the valves were also visible against black plastic surround (cirri visible lower), (A) *M. edulis* with exhalant and inhalant siphons visible (B) Siphons visible and the foot extended (bottom right) (C).

Table 5.1 Descriptive statistics for the mussel *M. edulis* threshold experiments, with closure and partial closure used as the indicator of response n = 15.

Frequency (Hz)	$\bar{x}$ threshold (m s^{-2})	SD	Response frequency
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	0.56	0.092	12
410	0.12	0.014	9

Table 5.2 Threshold comparisons between hermit crabs, *P. bernhardus* and mussels, *M. edulis*. Statistical significance is denoted by asterisks (* p < 0.05, ** p < 0.01).

Frequency (Hz)	t	df
5	0.03**	42
10	0.31**	48
20	-2.82**	48
40	-3.33**	48
90	-0.81	48
210	-2.81	46
410	-1.08*	49

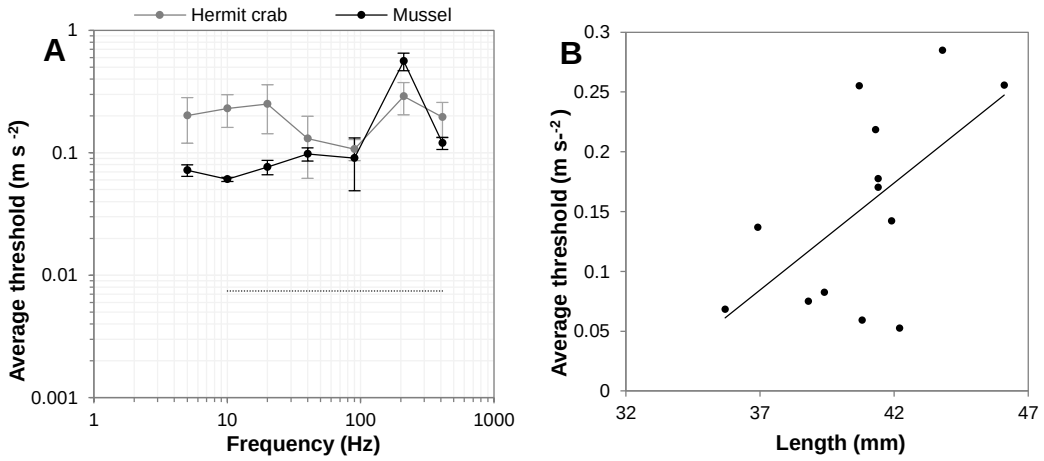

Figure 5.7 Sensitivity threshold (m s^{-2}, RMS) of *M. edulis* (n = 15 +/- SE) compared to *P. bernhardus*, (n = 10, +/- SE) to sinusoidal vibration. Average background levels are denoted by the dotted line (A). Correlation of shell length (mm) and average threshold (m s^{-2}), (B).

Average threshold (across all frequencies) was significantly different between *M. edulis* and *P. bernhardus* thresholds (U = 9881, p < 0.001; *P. bernhardus* values from Chapter 4 Section 4.5.3), with mussels being more sensitive to vibration below 90 Hz. When subdivided by frequency there was a significant difference at all frequencies apart from 90 Hz and 210 Hz (Table 5.2, Figure 5.7A). There were insufficient data available in the literature to compare the current results to known threshold values of bivalves.

There was a significant correlation between length of mussel (mm) and average threshold value (m s^{-2}) (r = 0.59, n = 13, p < 0.05, log transformed), (Figure 5.7B). No correlation was found between width (mm), length/width ratio and average threshold values (all frequencies together, 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 frequency (Hz) category, there were no significant correlations between the threshold and the morphological variables, (Table 5.3).

Table 5.3. Correlation coefficients (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). Full p values are provided in appendix Table A.9.

Frequency (Hz)	Length (mm)	Width (mm)	Length*width ratio
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

5.4.3 Comparison to anthropogenic values

The threshold of sensitivity ranged from 0.002 – 0.00005 m s^{-1}, after conversion to velocity, (Table 5.4). This is within the levels produced by typical anthropogenic sources, (Table 4.8, Chapter 4). For example, behavioural changes of mussels could be elicited at 23 m from drilling, dredging up to 185 m, blasting at 296 m and shell-auger drilling up to 64 m.

Table 5.4. Average acceleration thresholds for *M. edulis* provided in terms of velocity in the vertical axis (m s^{-1}, RMS) for comparison with anthropogenic signals, typically measured in velocity.

Frequency (Hz)	Threshold (m s^{-2})	Threshold (m s^{-1})
5	0.072	0.0022
10	0.061	0.0009
20	0.077	0.0006
40	0.098	0.0004
90	0.090	0.0002
210	0.559	0.0004
410	0.120	0.00005

5.4.4 Threshold determination in B.crenatus

Eleven groups of *B. crenatus* were observed encrusting on tested *M. edulis* (5^{th} – 10^{th} May 2014). At the three highest stimulus levels, barnacle cirri were observed retracting simultaneously with closure of the associated mussel valve, although the barnacles usually re-emerged more quickly after retraction. In this case it was difficult to tell whether the animal was reacting to the movement of the valve itself. However in other cases the barnacles responded (to the highest exposure levels) when the mussel did not react, in particular appearing to beat the cirri faster during stimuli, although more data would be required to support this observation. It is of note that even when the

barnacle appeared to be reacting independently of the mussel valve movement, the received vibration (in terms of amplitude and waveform) may have been distorted with propagation across the valve.

Numbers of reactions were low per individual, with typically between 1 – 6 responses throughout the whole session per barnacle. Individuals typically responded to 2 – 3 of the 7 frequencies presented to them ($\bar{x}$ response = 2.55, SD = 1.67, n = 11). Despite the low response rate, the responses observed were clear and were sufficient to calculate an approximate threshold. Not all individuals responded to exposures, hence threshold values were calculated from 2 – 6 responding individuals (different individuals per frequency) only (Figure 5.8). A similar threshold level was shown at 5, 20, 40 Hz and 410 Hz with threshold values of around 0.1 m s^{-2}, whereas there was a large peak of 0.90 m s^{-2} at 210 Hz (n = 2), and an increase to 0.38 m s^{-2} at 90 Hz (n = 13). It is possible that the vibration at these frequencies was attenuated or distorted more as it travelled across and through the mussel shell. *B. crenatus* showed generally reduced sensitivity compared to *M. edulis* at all frequencies apart from at 410 Hz and 40 Hz (Figure 5.8), but the curves were approximately similar on the whole.

Comparisons of *B. crenatus* thresholds with anthropogenic data were not undertaken due to the preliminary nature of the data.

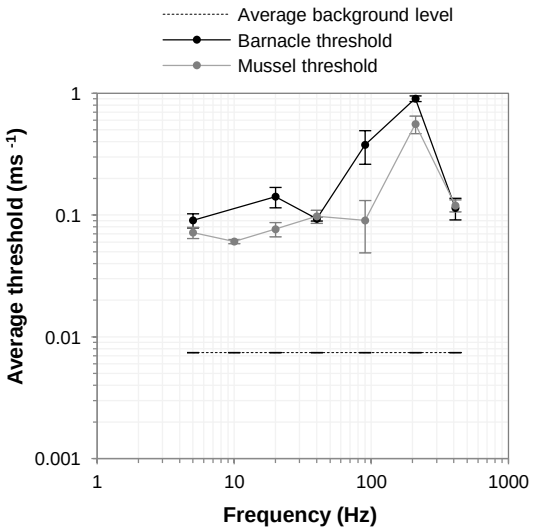

Figure 5.8 Average sensitivity threshold (m s^{-2}, RMS) of *B. crenatus* (n = 11, +/- SE) (encrusting on *M. edulis* valves) to sinusoidal vibration in the vertical axis (5 – 410 Hz). Average sensitivity threshold for *M. edulis* is also provided (n = 15, +/- SE). Average background levels are denoted by a dotted line.

5.4.5 Response determination

The exposure levels were well within the sensitivity range of *M. edulis,* as found in Section 5.4.2.

(i) Solitary mussels

A total of 120 solitary mussels, in the range of 31.3 – 69.5 mm (average shell length 49.8 mm, SD = 8.60, n = 120) were tested in two experimental sessions (60 in each, February 2013 and 2014). Closure of the valves was clear and quantifiable, apart from 19 occasions when a partial closure was observed (Table 5.5). Ten of the partial closures were elicited at the highest vibration level.

Mussels were deemed in suitable condition for the tests since valves opened and closed, siphons and gills were often visible and no mortality was observed. Any other type of behaviour was classed in the 'no response' category, and for the purposes of statistical analysis 'partial closure' was classed as 'no response' since the timing of the behaviour was difficult to measure. No responses were observed during control trials, indicating that the equipment had not had an effect on the results.

The numbers of responses significantly increased with vibration level in acceleration ($r = 0.81$, $n = 250$, $p < 0.001$). The regression line between response rate and acceleration was $y = 0.66 x + 0.1532$, $r^2 = 0.79$, $n = 5$, $p < 0.05$), allowing a 50% response rate to be calculated as 0.53 m s^{-2} (Figure 5.9). Overall, there was a significant increased likelihood of response with increased shell length (mm), (Table 5.6). When separated by vibration category (1 – 5) the likelihood of response at vibration levels 1 and 3 was reduced compared to levels 2, 4 and 5. Few responses were observed at the lowest vibration level, with a 10.0% response compared to a 54.2% response at the top level of vibration.

Table 5.5. Summary values from the exposure of *M. edulis* to a vibration source (levels 1 – 5, m s^{-2} RMS, vertical axis). Time to reopen (s) was measured after response (valve closure).

Vibration level	Calibrated vertical vibration (m s^{-2})	Number of responses	No response	Average time to reopen (s)
1	0.05	12	107	62.25
2	0.26	55	63	59.05
3	0.41	48	71	104.69
4	0.61	70	45	71.84
5	0.68	65	45	113.26

Table 5.6. Binary logistic regression of response data (presence or absence) with *M. edulis* shell length (mm) and vibration level (1 - 5, reference category 5) as predictors. Statistical significance is denoted by asterisks (* p < 0.05, ** p < 0.01), B is the regression coefficient.

Predictor	B (SE)	95 % CI for odds ratio		
		Lower	Odds ratio	Upper
Length (mm)	0.03 (0.01)**	1.01	1.03	1.06
Vib 1	-2.31 (0.35)**	0.05	0.10	0.20
Vib 2	-0.34 (0.26)	0.43	0.71	1.19
Vib 3	-0.55 (0.26)*	0.35	0.58	0.97
Vib 4	0.17 (0.26)	0.71	1.19	1.99
Constant	-1.47 (0.55)**		0.23	

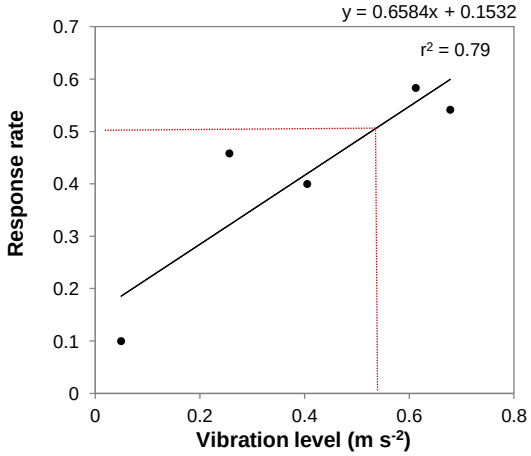

Figure 5.9 Regression line, equation and r^2 ($p < 0.05$) for calculation of the 50% response rate of *M. edulis* to vibration (m s^{-2}).

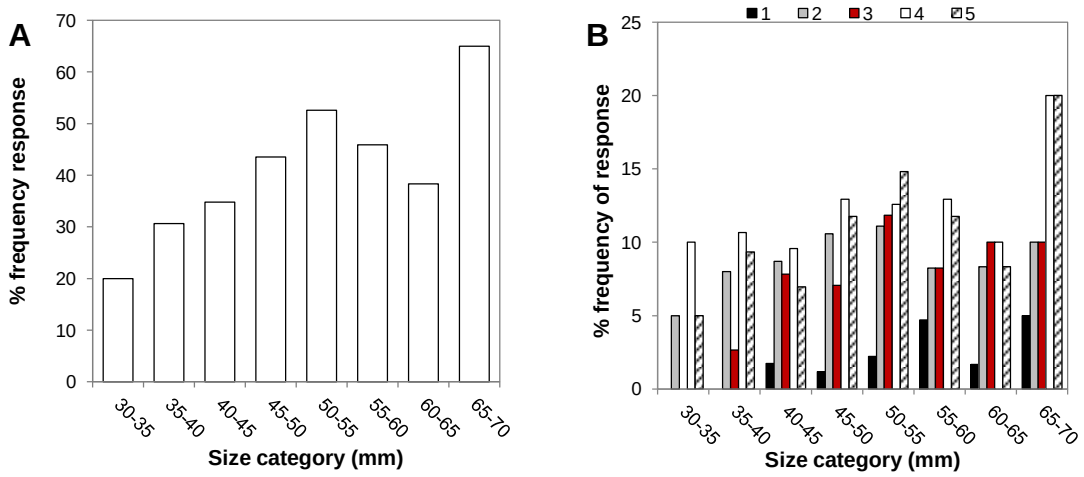

Figure 5.10 Variation in response frequency for *M. edulis* with size category (mm) for vibration levels combined (A) and vibration level 1 – 5 (B).

When frequency of response (decimal) was analysed with size category (shell length) there was a significant positive association between size of mussel and response ($\chi^2 = 21.89$, df =7, $p < 0.05$; all vibration levels combined), with size classes 50 – 55 mm and 65 – 70 mm accounting for the association (standardised residuals 1.9 and 1.6 for the two categories respectively; combined vibration levels) (Figure 5.10A). Similar results were obtained when analysing vibration levels (1 – 5) separately, although the pattern was unclear at vibration level 1 (Figure 5.10B).

There was a significant effect of size on the duration of startle response when the data were separated by size categories ($F = 2.45$, df = 7, $p < 0.01$), but a non-significant main effect of vibration level and the interaction of vibration level and size category ($F = 0.79$, df = 4, $p = 0.54$ and $F = 0.47$, df = 23, $p = 0.98$, log transformed) (Figure 5.11, Table 5.7, 5.8).

Table 5.7. Results from pairwise comparisons after a factorial (two-way) ANOVA (p values) for *M. edulis* of eight size categories exposed to vibration levels (1 – 5), statistical significance is denoted by asterisks (* p < 0.05, ** p < 0.01).

Size category	1	2	3	4	5	6	7
1	-						
2	1.0	-					
3	1.0	0.95	-				
4	1.0	0.9	1.0	-			
5	0.93	0.02**	0.07	0.12	-		
6	0.98	0.17	0.61	0.70	0.99	-	
7	0.96	0.11	0.42	0.50	1.0	1.0	-
8	0.79	0.07	0.25	0.29	0.99	0.92	0.99

Table 5.8. Pairwise comparisons after a factorial (two-way) ANOVA (p values) for *M. edulis* of eight size categories exposed to vibration levels 1 – 5. Statistical significance is denoted by asterisks (* p < 0.05, ** p < 0.01).

Vibration level	1	2	3	4
1	-			
2	0.99	-		
3	0.77	0.81	-	
4	0.95	0.10	0.93	-
5	0.63	0.568	1.0	0.73

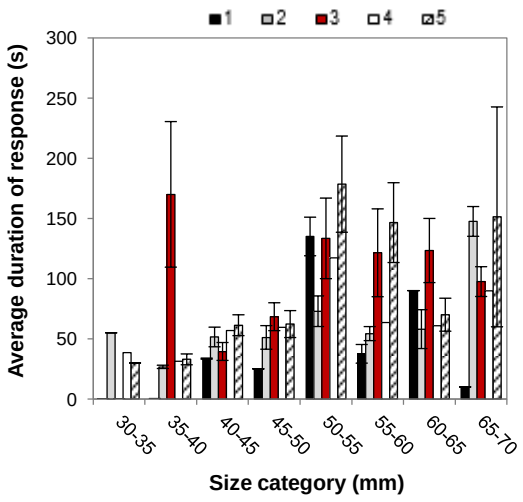

Figure 5.11 Duration of startle response (s) of *M. edulis* size category (mm) in relation to vibration level (1 – 5), +/- SE.

(ii) Grouped mussels

A total of forty groups of mussels (n = 160) were exposed to all vibration levels. Note that the vibration levels for the grouped tests were higher than for the solitary tests since the groups were at half the distance from the vibration box. Responses of every group were recorded at all levels apart from the lowest level when no responses were observed, hence four vibration categories were used in the analysis.

Duration of response ranged from 50.5 – 63.4 s, and increased with increasing vibration level ($F = 5.82$, $df = 3$, $p < 0.05$, log transformed), with pairwise comparisons indicating significant differences between most vibration levels, (Table 5.9, Figure 5.12).

There was no significant difference in response duration between grouped and solitary mussels ($t = 0.627$, $df = 69$, $p = 0.53$ and $t = 0.90$, $df = 62$, $p = 0.37$ for grouped and solitary respectively, log transformed), although mussels in groups appeared to be marginally quicker to recover after exposure than solitary. Additional summary data from February 2013 grouped trials (not included in the above analysis) did not show the same trend- with mean response times of 8.76 s and 54.95 for grouped and solitary mussels respectively. However these data could not be validated therefore these results must be viewed with caution.

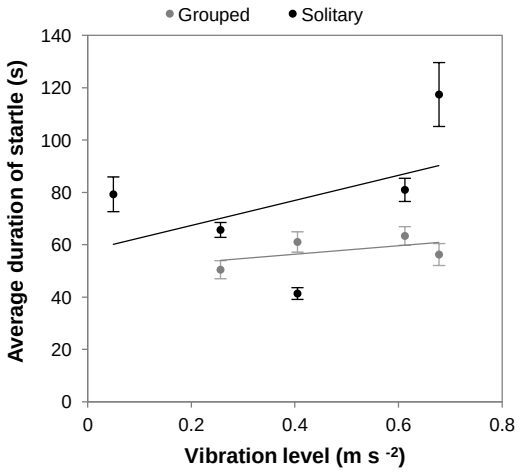

Figure 5.12 Changes in startle duration (s) with vibration level (m s^{-2}), data for solitary *M. edulis* and for groups (+/- SE).

Table 5.9. Pairwise comparisons (with Bonferroni correction) after a repeated measured ANOVA for *M. edulis* exposed to vibration (levels 2 – 5), statistical significance is denoted by asterisks (* p < 0.05, ** p <0.01).

Vibration level	2	3	4
2	-		
3	0.01	-	
4	0.00**	0.28	-
5	0.11	0.29	0.02*

5.5 Discussion

5.5.1 Sensitivity of solitary mussels to vibrations

It is largely understood that a range of sensory systems exists in bivalves, consisting of chemoreceptors and mechanoreceptors (Olivo, 1970; Lacourse and Northrop, 1977). A statocyst is present, functioning principally as an equilibrium receptor (analogous to the otolith of fish). It is likely that vibration may stimulate movement of the statolith, as it travels through the body, there is evidence that water-borne particle motion may be detected in this way in bivalves (Ellers, 1995).

M. edulis in the current work were shown to respond to sinusoidal signals in the vertical plane of frequency range of 5 – 410 Hz. Response was approximately constant across all frequencies, with a prominent decrease in sensitivity at 210 Hz (0.55 m s^{-2}). An explanation for the 210 Hz peak has previously been described in Section 4.6 and will not be discussed again. There have been few studies investigating sensitivity of bivalves to vibration (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. Therefore there are insufficient data available to compare the current sensitivity results to other works, indeed the exposure levels of the summarised literature are incomplete (Table 5.10).

The remaining works do not provide details of the vibration stimulus in terms of amplitude, but are indicative of the frequency range of reception to which bivalves may be sensitive. Of most relevance to the current work is Kastelein *et al.* (2008). *C. edule* (Cardiidae) were exposed to vibrations from a vibration box (as in the current work) which was placed on the substratum, of mixed frequencies and amplitudes (uncalibrated, ranging from 100 – 64 kHz). Siphons, visible on the top of the substrate, were monitored as the frequency and amplitude of the signal was varied, until a threshold value was obtained for response. Unfortunately the behavioural threshold of response was not published (R. Kastelein *Pers. Comm.*[9]), but at a certain frequency and amplitude the cockles were seen to retract the siphons and close the valves. Similarly, Mosher (1972) exposed a small aquarium containing *Macoma balthica* (Tellinidae) to vibration from a solenoid unit, and studied the digging behaviour using a myograph. The amplitude of the signal was at a constant (unspecified) amplitude but the frequency of the signal could be varied. It was found that *M. balthica* responded equally to signals between 2 – 50 Hz (duration above 5 seconds), but not to impulsive vibrations. An additional unpublished source indicates similar digging in *M. balthica* after exposure to 50 – 200 Hz, in addition to strong individual differences between responses (Jumers, 1998). Zhadan (2005) observed a reaction of *Mizuhopecten yessoensis* and swift scallop *Chlamys swifti* at a frequency range of 30 – 1000 Hz and 20 – 50 kHz when presented with water-borne sinusoidal vibrations, although the focus of the study was directional sensitivity (using 140 Hz as a threshold value) rather than threshold across the frequency range. Finally, Ellers (1995) presented

[9] Dr R. Kastelein, SEAMARCO (Sea Mammal Research Company), Director of SEAMARCO, Netherlands.

Table 5.10 Thresholds of greatest sensitivity to vibration for a variety of bivalve species. These studies use various vibroacoustic stimuli, a wide range of frequencies and species. For comparative purposes the results of the current work are shaded in grey.

Reference	Vibration Presentation	Threshold of sensitivity ($m\ s^{-2}$)	Frequency (Hz)	Species	Behaviour	Source type	Notes
Zhadan (2005)	Water	n/a	140	*Mizuhopecten yessoensis*	Tentacle contraction	Oscillator-sinusoidal generators 140 – 40000 Hz	Focus was directional sensitivity
				Chlamys swifti	Valve closure		
Ellers (1995)	Substrate-Water	n/a	n/a	*Donax variabilis*	'Jumped'	Underwater speaker 40 –830 Hz	Most responsive to wave playback
					Siphon elongation		
					Knocking on tank		
Mosher (1972)	Substrate- Water	n/a	n/a	*Macoma balthica*	Digging with delay same across all frequencies	Solenoid unit-kymograph	No habituation
						2 – 50 Hz	
Kastelein (2008)	Acoustic (water)	n/a	n/a	*Cerastoderma edule*	No reaction	Speakers	Pure tones-
						100 – 64 000 Hz	broadband
	Substrate				Retracted siphons and closure at specific threshold	Vibration box	Results undisclosed
	Solid	58.86	8000–14000	*Dreissena polymorpha*	Could be detached from surface	Piezoelectric shaker 5000N	Rod (< 20 cm) to apply vibration to pipe
Kowalewski *et al.* (1992)	Air/Water	3433.5		(Juvenile)	Mortality 100 % at 11 kHz Closure	40 – 45000 Hz	
The current work	Substrate 0 – 1.2 $m\ s^{-2}$	0.06	10	*Mytilus edulis*	Closure	Piezoelectric shaker 8.9 N 5 – 450 Hz	

Donax variabilis (the 'swash rider' clam, which rides incoming waves on the shore) with a frequency range of 40 – 300 Hz from an underwater speaker buried in the sand. Responses were observed in response to wave sounds and to knocking on the tank, although these results should be viewed with caution; due to the complexities of acoustics in small tanks (Section 1.11.2) the received signal may not have been representative of the intended waveform.

Studies of bivalve larvae sensitivity may also indicate reception abilities of adult bivalves. For example settlement of larvae has been induced by playback of vessel and reef noise (100 – 1000 Hz, 126 dB re 1 µPa and 118 – 124 dB re 1 µPa respectively) (Wilkens *et al.*, 2012; Lillis *et al.*, 2014). Indeed Lillis *et al.* (2014) claim this is the first work to describe such auditory stimulation in bivalves. It was suggested that the vessel playback was of a similar frequency range to ambient reef sounds, which may explain the attractant properties of the stimulus. Other studies with crustacean, bivalve and fish larvae have indicated similar results (Radford *et al.*, 2008; Simpson *et al.*, 2008; Vermeij *et al.*, 2010). Inhibition of juvenile zebra mussel *Dreissena polymorpha* settlement has been demonstrated with the use of sinusoidal vibrations (5000N force shaker), however, whilst the vibration levels were fully quantified, the high exposure levels (1471.5 m s^{-2}, 8 – 10 kHz) were causing 75 – 95% mortality rate rather than inducing behavioural changes (Kowalewski *et al.*, 1992)

In the current work lowest and highest sensitivity were exhibited by mussels at 210 Hz (0.55 m s^{-2}) and 10 Hz (0.06 m s^{-2}) respectively. As expected, exposures at higher levels (as in the response experiments) also elicited responses, often of longer duration than during the threshold determinations. The 50% response level of mussels to the stimulus was estimated to be at 0.53 m s^{-2} (n = 5). As previously discussed, specific thresholds of bivalves to particle motion (water or substrate-borne) are not well documented. However threshold values are available for other molluscs, for example cephalopods. Sensitivity of conditioned *Octopuso cellatus* exposed to water-borne particle motion (50 – 283 Hz) ranged from 0.00034 m s^{-2} (50 Hz) to 0.043 m s^{-2} (283 Hz) (Kaifu *et al.*, 2008). Similarly, sensitivity of *Sepia officinalis* was demonstrated in the range of 0.002 – 1 m s^{-2} (1 Hz – 100 Hz respectively), and for *Octopus vulgaris* and *Loligo vulgaris* water-borne sensitivity ranged from 0.004 – 0.006 m s^{-2} (3 Hz) to 0.8 – 1.1 m s^{-2} at 100 Hz (Packard *et al.*, 1990; Kaifu *et al.*, 2008). Data from Mooney *et al.* (2010) later corroborated these findings, with thresholds ranging from 0.05 – 0.17 m s^{-2} in a slightly higher frequency range of 10 – 10 000 Hz. Although the authors noted differences between the two papers near 100 Hz and under 30 Hz, this was thought to be due to the dissimilar techniques used. Indeed it is widely accepted that electrophysiological thresholds provide different threshold values than behaviourally determined values, and that they are not equivalent descriptions of auditory response (Ladich and Fay, 2013). These studies indicate that cephalopods have greater sensitivity to particle motion than *M. edulis* in the current work, but the values here are within the range that other molluscs have been shown to detect. It is of note that the cephalopod studies use water-borne stimuli rather than the vibration stimuli of the current work, but that many cephalopods are benthic and are therefore likely to be sensitive also to substratum stimuli.

Mussel and hermit thresholds were significantly different, although the departure between the two was at the lower frequencies only (5 – 20 Hz). Occupying a similar environment of varying wave

action, for example, it was expected these animals would demonstrate similar sensitivities to vibration. The difference may be attributed to the varied life styles of the two, or perhaps due to different methods of threshold calculation in the mussel experiments.

5.5.2 Behavioural responses

In the current work, responses were clear and occurred at onset of the stimulus (within 1 – 2 seconds). Valve responses included full closure and retraction of the valves, partial closure (with inhalant and exhalant siphons visible) and also a 'twitch' of the valves at the onset of vibration. A partial opening and closing of *M. edulis* has been described in response to pollutants, and is proposed to be the mussel 'testing' levels in the water (Manley and Davenport, 1979). In the current work this behaviour was described as a 'twitch' and it is possible that *M. edulis* was 'testing' the water for chemical cues of predators or damaged conspecifics in response to the stimulus.

The response of *M. edulis* to fully calibrated vibration sources has not been recorded previously in the literature, although responses observed may be similar across bivalves. Full valve closure with siphon retraction has been described in *C. edule* in response to vibration stimuli (Kastelein, 2008), and similarly full shell closure of *Bathymodiolus azoricus* has been described in response to shipment and laboratory manipulations (Kádár *et al.*, 2005). Burrowing bivalves such as *Macoma balthica* have responded by digging further into the substratum, and not only closing the valves (Mosher, 1972).

In the current work, the foot was observed partially retracting back into the valves at the onset of the stimulus, however at other times it remained actively exploring the substratum. This may have been in preparation for digging behaviour, and in some cases by the end of the session mussels were slightly deeper in the substratum, although this was not quantified.

Whilst the effects of man-made vibration upon bivalves is little studied, the effects of other man-made stressors, such as chemical pollutants are well understood and have indicated that gape width varies with stressor (Manley and Davenport, 1979; Akberali and Black, 1980; Kramer *et al.*, 1989; Salanki and Vbalogh, 1989; Curtis *et al.*, 2000; Kádár *et al.*, 2005). For example valve gape and heart rate of *M. edulis* has been demonstrated to decrease with increasing copper concentration (Curtis *et al.*, 2000). Similar responses to copper have been described for zebra and blue mussel (Kramer *et al.*, 1989), *Scrobicularia plana* (Akberali and Black, 1980) and *Anodonta cygnea (Salanki and Vbalogh, 1989),* in addition to the bivalves *Modiolus modilous, Chlamys ioercularis, Crassostrea gigas, Anadara senilis,* and *M. demissus (Manley and Davenport, 1979)*.

In the present work, valve gape was classified as either 'open' or 'closed'. Partial gapes were also recorded, but due to difficulties quantifying these, were not included in the final results as positive responses. Borcherding (2006) and Englund and Heino (1996) have used a similarly simple approach to recording valve gape; for example Borcherding (2006) described the *Dreissena* monitoring system, which monitors valve response (open or closed) in zebra mussels, enabling the mussels to be used as a biological warning system. A similar digital gape system has been used to record valve movement of *Anodonta anatine* (Englund and Heino, 1996). Indeed valve gape of bivalves has been studied using a variety of other methods (Ameyaw-akumfi and Naylor, 1987; Kramer *et al.*, 1989; Newell *et al.*, 2001; Riisgard *et al.*, 2003; Gnyubkin, 2010; Robson *et al.*,

2010). Initial attempts of quantifying gape involved video cameras (Newell *et al.*, 2001; Riisgard *et al.*, 2003) and strain gauges (Ameyaw-akumfi and Naylor, 1987), but more recent methods have involved the use of magnetic fields (Kramer *et al.*, 1989; Borcherding, 2006; Robson *et al.*, 2010) and digital systems (Englund and Heino, 1996) to measure gape angle more accurately. Indeed the use of valve gape in addition to measures of energy loss via waste and excretion for example, may be incorporated into a calculation of 'scope for growth', which is defined as the energy status of the animal which can then be related to levels of stressors such as chemical pollutants (Widdows *et al.*, 1984; Widdows *et al.*, 1997; Widdows *et al.*, 2002).

More detailed quantification of valve response in this way has shown valve gaping to exhibit considerable periodicity (Kádár *et al.*, 2005) with active and inactive periods that can be disrupted by stressors. Indeed gaping, rather than being a simple 'open' or 'close' behaviour appears to be a complex response, with angles of valve gape being linked to different levels of biotic and abiotic cues (Englund and Heino, 1996; Dolmer, 2000; Newell *et al.*, 2001; Gnyubkin, 2010; Robson *et al.*, 2010). For example gape has been found to vary according to food presence with a larger gape angle and longer gape duration occurring in increased food and seston concentrations (Dolmer, 2000; Newell *et al.*, 2001; Robson *et al.*, 2010). Gape has also shown to vary according to environmental change (Englund and Heino, 1996) and light levels (Gnyubkin, 2010; Robson *et al.*, 2010). A decrease in the area of the exhalant siphon has also been exhibited in response to current velocity variation (Newell *et al.*, 2001).

Valve gape of *M. edulis* may also vary according to perceived risk of threat , with mean gape angle decreasing as a simulated predatory risk increased (Robson *et al.*, 2007), which may be a way of reducing predation success. In the current work numbers of responses (and response rate) increased significantly with increasing vibration level. This may be because increased vibration was perceived as a greater threat to the mussels. On the shore, substratum vibration may indicate wave action or predator approach, with strength of the stimulus representing distance of the threat or stimulus from the individual. For example, the oystercatcher *Haematopus ostralegus* probes the sand with its bill, thrusting into the mud to extract *Scrobicularia plana* and hence *S. plana* is highly sensitive to the vibrations associated with the bird walking (Hughes, 1970). If *M. edulis* perceives a strong vibration as a greater predation risk, then perhaps more of them would respond- as observed in the current work.

Furthermore, the duration of startle response significantly increased with increasing vibration strength here. This is in agreement with other studies, for example startle response duration of freshwater pearl mussels *(Margaritifera margaritifera*) and zebra mussels (*Dreissena polymorpha)* appears to vary according to the perceived risk of predation (Toomey *et al.*, 2002; Wilson *et al.*, 2012). Shorter responses were observed in response to a simulated distant predator (a shadow) compared to a direct tapping on the shell. Toomey *et al.* (2002) also found that movement of *D. polymorpha* was adjusted according to the perceived risk of predation (in this case exposure to chemical cues of injured conspecifics), with those exposed to chemical cues moving shorter distances than those in control tanks. In the current work, *M. edulis* may have perceived lower vibration levels as a predator at a greater distance from them, and recovered more quickly from the stimulus.

Reacting to a stimulus is a trade-off between energy-expensive predator avoidance and the requirement of food (Wilson *et al.*, 2012). But, as the predatory threat increases, it is advantageous for the mussel to suspend feeding for a longer duration until the threat has passed. The above studies, in addition to the current results, indicate that anti-predator responses of bivalves appear to be flexible according to the stimulus characteristics and therefore perceived risk of predation (Wilson *et al.*, 2011; Wilson *et al.*, 2012). The closure response thus seems to be a trade-off between predatory risks and respiration/excretory needs. To support this hypothesis further, it is of note that the duration of response was shorter during the current threshold experiments than the response experiments, a result that was possibly due to the signal being of lower amplitude, and therefore perceived as less of a threat, in the former experiments.

Wilson *et al.* (2012) also found that solitary behaviour of bivalves was consistent between scenarios (light, touch and vibration stimuli), this idea of consistency of response with individual, or 'personality' has also been documented for other invertebrates for example hermit crabs (Briffa *et al.*, 2008; Briffa *et al.*, 2013) (Chapter 4). Preliminary data has indicated strong variation among *M. balthica* in response to vibration, although variation within each individual was also reported (Jumers, 1998). The current work on *M. edulis* is unable to support or reject the proposition of personality in bivalves. However, if such consistency within individuals is present, the thresholds of each solitary mussel may be expected to be consistent with repetition of the experiment. Similarly, if individuals exposed to the vibration box had been ranked, one might expect that ranking to be consistent upon re-testing. It would be valuable to explore this further.

In the current work *M. edulis* closed the valves in response to the vibration. The closure of the valves in response to a stressor is a costly behaviour in terms of energy, respiration rate reduction and an impaired ability to remove waste products (Wilson *et al.*, 2011). Although bivalves are able to respire anaerobically when closed, eventually faeces, pseudofaeces and waste gases must be evacuated (Di Iorio *et al.*, 2012). This is illustrated best during exposure to increasing levels of pollutants when a 'testing' behaviour is exhibited more often at high concentrations (Manley and Davenport, 1979), since the need to remove these waste products becomes more urgent. In high pollutant concentrations death occurs due to the build-up of waste products (Akberali and Trueman, 1979). Indeed valve closure of three hour duration has been demonstrated to halve oxygen within the shell and increase carbon dioxide levels by two hundred percent (Akberali and Trueman, 1979). Since energy is gained via feeding and lost via 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.*, 1997; Widdows *et al.*, 2002; Mazik *et al.*, 2013). Furthermore, long-term closure may disrupt heart rates, indeed in some cases can involve heart cessation (Curtis *et al.*, 2000). It is therefore possible that the valve closures exhibited here were having an effect on the overall fitness of the individuals involved, with long-term implications to the animal and the population (Widdows *et al.*, 1984).

Response of mussels to a stimulus (simulated predator) may be correlated with mussel size, with larger, older mussels re-emerging quicker after disturbance than smaller (Wilson *et al.*, 2012). In terms of energy costs, larger mussels require more food and therefore may 'risk' feeding longer

when a threat is perceived than smaller. In the current work larger solitary mussels were in fact slower to recover after vibration, with a significantly longer startle duration. Overall large mussels (60 – 70 mm) were more responsive than the smallest mussels- although in actual fact medium-sized mussels (50 – 55 mm) also contributed to the trend. In the threshold experiments, larger mussels ceased responding at a greater amplitude than smaller, with a higher average threshold overall. These data could be interpreted as a reduced sensitivity of larger mussels, or an artefact of the experiment- for example the larger valve size may affect the propagation of the vibration across the shell, increasing the strength.

5.5.3 Sensitivity of M. edulis *groups to vibration*

In the current work there was no significant difference in duration of response between solitary and grouped mussels, although data indicated that grouped mussels were marginally quicker to recover after exposure. It was expected that mussels in groups would be quicker to reopen: they may exhibit this behaviour because sensing their conspecifics, they may perceive the overall predation threat as lower, and hence be willing to open more quickly (Wilson *et al.*, 2012). Furthermore, food availability in groups is lower so those in groups will need to feed more often, which in turn means more valve gaping. The trade-off between predation risk and the need to feed in sessile organisms, where one activity inhibits the other, has been discussed at length within the context of barnacle behaviour (Mauck and Harkless, 2001).

The results here are in disagreement with Wilson *et al.* (2012) who showed that solitary freshwater pearl mussels, *Margaritifera margaritifera* took longer to recover after predator cues than when grouped. Similar responses have been observed in barnacles, for example barnacles exhibit a shorter startle response when in a group (Mauck and Harkless, 2001). It is of note that summary data (unconfirmed results) from the current work supported the literature, although the raw data were unavailable to allow further confirmation.

Living in a group is advantageous since the overall risk of predation is spread across an increased number of individuals (Lima and Dill, 1990). However group living comes at a cost, being a reduction in food availability and space, an increased risk of parasite dispersal and increased competition growth, as shown, for example in fish schools, (Hamner and Hamner, 2000; Hawkins *et al.*, 2012b). It appears to be a cost benefit trade-off; for example it has been shown that *M. edulis* on the edge of groups have an increased risk of predation but overall are likely to have greater reproductive success and more space to grow (Okamura, 1986). Cote and Jelnikar (1999) found that formation of *M. edulis* groups was greatest with increased predatory chemical cues in the water. If vibration presence is perceived as a predatory threat by solitary *M. edulis,* then continuous vibration may perhaps induce grouping behaviour. In addition to this, the closure response may also be quicker in response to increasing vibration, as Robson *et al.* (2007) found when exposing mussels to chemical cues of conspecifics.

5.5.4 Sensitivity of B. crenatus *to vibrations*

Encrusting barnacles, *Balanus crenatus* were observed fortuitously during the threshold experiments and responded to vibrations, both independently of, and in agreement with, the

mussel host. The response level was low, deemed to be 1%, but responses were observed at all frequencies (5 – 410 Hz). The threshold curve was more irregular than that of *M. edulis,* with predominant peaks at 90 and 210 Hz.

The larger values may be accounted for by the irregular nature of the threshold presentations (not tailored to the barnacles specifically) which may have affected the results. However sensitivity at 20, 40 and 410 Hz was more stable, in the region of 0.1 m s^{-2}. The data indicate a wide threshold range of 0.1 – 1 m s^{-2} across all frequencies. Given the continuity in the threshold curves between the mussel and the barnacle thresholds at 20 – 410 Hz, it may be that the barnacle sensitivity here is in fact representative of true values. Living in a coastal environment, and being exposed to similar environmental conditions on the shore may mean that barnacle and mussel would have similar sensitivity to substrate-borne vibrations. However it cannot be excluded that, by encrusting on the mussel valve, the barnacles were responding to the valve movement rather than the original stimulus, although during the experiments barnacles were observed to react also independently.

As crustacea, barnacles are likely to detect vibrations in a similar way to the postlarvae of decapod crabs despite the lack of a specific statocyst-receptor. Much of the literature on the sensitivity of barnacles to vibration is focussed solely upon the larval stages (Branscomb and Rittschof, 1984; Guo *et al.*, 2011a; Guo *et al.*, 2012; Choi *et al.*, 2013). Sound may be advantageous to barnacle cyprids, acting as an attractant to habitats in a similar way as for bivalve and crab larvae (Simpson *et al.*, 2004; Montgomery *et al.*, 2006; Lillis *et al.*, 2014). Work has previously focussed upon the inhibition of settlement upon man-made marine structures, due to the widespread biofouling threat barnacles pose to man-made marine structures. Focus has been upon the cyprid stage, which is known to explore the substratum before metamorphosing into the sessile adult. For example settlement of *Amphibalanus variegatus* and *Elminus sp.* has been reduced by using low frequency vibrations (Choi *et al.*, 2013), with the higher frequencies being more successful, indicating a sensitivity of the cyprids to such frequencies. Similarly attachment of *Balanus amphritrite* may be prevented using a biofouling-prevention oscillator, with 30 Hz being better than other frequencies (Branscomb and Rittschof, 1984). At the opposite end of the spectrum, ultrasound has been equally effective at preventing settlement (Guo *et al.*, 2011a), for example sinusoidal energy at 23, 63 and 102 kHz (Guo *et al.*, 2011a). At low amplitudes ultrasound significantly affected exploratory behaviour and reduced basal areas in those that metamorphosed upon the substratum, although Guo *et al.* (2012) were unable to repeat such results. The above studies indicate that the larval stage of barnacles may detect and be affected by sound; this has been attributed to sensitive sensory organs used for exploration (Rittschof *et al.*, 1998; Maruzzo *et al.*, 2011). However, in the case of ultrasound it may in fact be the side effects of the waves (the creation of cavitation bubbles at the water-solid boundary), causing damage rather than the characteristics of the wave itself.

Vibration might also be useful to adult barnacles, which have been observed to retract in response to tactile and vibrational stimuli (Crisp and Southward, 1961). Since intertidal predators such as dog whelk (*Nucella lapillus*) and sea stars (e.g. *Asterias rubens*) would be moving across barnacle colonies when feeding at high tide, by detecting their approach early, and retracting into the shell, *B. crenatus* could avoid mortality. By responding to vibrations the barnacles may also be in tune with the tidal cycles upon the sea shore which would increase feeding success. In response to

vibrations in the current work, most barnacles exhibited full retraction of the cirri, briefly (< 2 s) at the onset of the cue, before resuming beating behaviour. In some cases, the cirri were observed to beat faster for a short period before returning to 'normal' rhythm. Cirral activity can be divided into four or five classes, depending on the dominant function (respiratory or feeding) as described in detail by Crisp and Southward (1961). These behaviours range from 'testing' behaviour, where the valves are open, the operculum moving but cirri are withdrawn; to full extension of the cirri without beating, known as 'extension'. 'Normal' beating behaviour, without pauses, typically settles into a regular rhythm consisting of activity with a short rest period in between (Southward and Crisp, 1965). The rhythm appears to vary according to the natural environment of the barnacle, with the Balanidae for example, exhibiting faster beating (perhaps according to water current variation) (Southward and Crisp, 1965). It is likely that two types of cirral activity observed in the current study were the 'normal' beating activity, consisting of a strong movement of the operculum and cirri beating, and the 'fast beating', when the valves stay open, and cirri sweep rapidly without withdrawal into the shell. With the current experimental setup it was difficult to see the movement of the operculum, although cirral movement was clear. In some cases, onset of vibration initiated a period of faster beating for a short time. This may be because the vibration affected water currents within the arena, or it may have been incidental to the stimulus, and be part of normal removal of internal acid build-up which occurs sporadically (Southward and Crisp, 1965).

A 'shock' reaction is described as full operculum closure with full retraction of the cirri (Crisp and Southward, 1961) which was often observed in this work. This response has been observed in response to vibrational cues. Whilst *B. crenatus* was clearly sensitive to the stimulus, the precise sensitivity of adult barnacles to sound and vibration does not appear to have been reported in the literature, although observations of retraction at vibration onset have been described (Crisp and Southward, 1961; Southward and Crisp, 1965). It is of note that only one barnacle was observed per group in the current study, but that actually neighbourly responses may also have influenced the observed responses.

It is of note that the implications of vibration exposures on adult barnacles could be extrapolated both within the context of anthropogenic pollution, and also to the fouling of vessels where, for example, vibration could be used to disrupt adult barnacle feeding and cause mortality. As such it would be valuable to extend the work of this study further.

5.5.5 Critique

Mussels are relatively straightforward to maintain and test in laboratory conditions, however laboratory conditions may affect behaviour, for example light levels have been shown to affect movement, gape angle and circadian rhythms (Toomey *et al.*, 2002; Gnyubkin, 2010; Robson *et al.*, 2010). *M. edulis* may also be more active during the night time when exposed to natural light regimes (Robson *et al.*, 2010) whereas many studies, the current included, run experiments during the day. It is of note that in some studies bivalves are exposed to constant light during tests, e.g. Ameyaw-akumfi and Naylor (1987), which may affect natural valve movements. In the current work, *M. edulis* were in the laboratory for a period of at least 3 days prior to experiments, allowing time for acclimatisation, and had a natural lighting regime (12 hours darkness, 12 hours light).

Feeding regimes in the laboratory may also affect the periodicity of bivalve feeding, and therefore periods of acclimatisation prior to experiments should be undertaken (as here), (Robson *et al.*, 2010). In the current work the mussels were not actively fed, but were kept in unfiltered seawater which had natural levels of algal growth. There was no mortality and individuals appeared to be filter feeding regularly, with gills and siphons visible. Within the experimental tank, a partial water change was undertaken between each experiment, this ensured that parameters in the water were not depleted. All mussels therefore appeared accustomed to their environment and were assumed to be fit for tests. For the vibration box experiments, mussels were kept in the arena for a short amount of time and since valve gape was varying according to the stimulus, were assumed to be deemed fit for testing.

With all behavioural experiments involving presentation of stimuli there is a risk of habituation (becoming unresponsive to repetitive sound exposure after a period of time), as observed in fishes (Chapman and Hawkins, 1969; Knudsen *et al.*, 1992). The present work is no exception however in order to minimise this effect 10 – 15 minutes were left between frequency presentations during the experiments and there was no evidence of habituation (for example in the sensitivity experiments, response did not differ throughout the day). This is consistent with observations of *Macoma balthica* which did not appear to habituate after exposure to vibration stimuli up to 30 times in one week (Mosher, 1972). A lack of habituation is likely to be advantageous in such bivalves to avoid predation.

Finally, there may be concerns that the valve closures exhibited in the experiments undertaken in this study were natural rhythms of feeding rather than true responses. However the large number of replicates in the experiments, and the inclusion of control trials significantly reduced the likelihood of this occuring. Valve closure was clearly pronounced at the onset of the stimulus and therefore it seemed unlikely to be random.

5.5.6 Stimulus presentation

In a substrate, particle motion can travel as longitudinal, shear or surface waves (Markl, 1983; Hill, 2009b) with energy being transmitted in one or multiple waveforms depending on the substratum boundary layers, and connection to the substratum (for a review see Aicher and Tautz (1990). 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 know the wave type present without further investigation. It has been shown that scorpions use Rayleigh waves to locate prey and for mating (Brownell, 1977; Brownell and Farley, 1979). These types of waves have also been shown to be detectable by crustaceans such as the fiddler crab *Uca pugilator* (Aicher *et al.*, 1983; Aicher and Tautz, 1984, 1990), by using receptors in the walking appendages. It is plausible that these waves could be detected by, and are of relevance to bivalve molluscs.

Sinusoidal stimuli were used in the current tests. Since the ability of mussels to detect vibrations is relatively unstudied, it seemed logical to present pure tones when investigating their sensitivity. This is common practise in threshold investigations of other marine organisms for example, Chapman and Hawkins (1973). For both experiments in this work the signal was sinusoidal (more

so in the threshold experiments), greatest in the vertical axis and was in the intended range of 5 – 410 Hz. However for the more precise threshold determination work, the frequency of the signal was adjustable and therefore the experiment was more controlled than with the vibration box. This is appropriate since the two experiments had different aims- with the response determination work to study specific responses, and the sensitivity experiments to quantify precise sensitivities. The mixed frequency signal of the vibration box was therefore sufficient, for the purposes of investigating response rates.

It is of relevance that whilst the vibratory signal here was predominantly substrate-borne, there is a possibility that this signal also had a water-borne particle motion and perhaps even pressure component within the experimental tanks. By using a shaker directly contacting the substratum, the pressure and interference phenomena found in small tanks are likely to be minimal. However this issue is particularly relevant in the response experiments, where the substrate vibration propagated through the base of the arena prior to being received, so that the sides of the arena may also have been excited. Furthermore the experimental arena were small which may have changed the frequency composition of the signal. In the threshold experiments the stinger rod of the shaker may also have caused compressional waves on the sand-water boundary (Brownell and Farley, 1979) and perhaps also changes within the water column. Although water-borne particle motion was not measured in the current work, a tri-dimensional geophone system enabled the measurement of the stimulus in all three axes, and indicated that the energy in the vertical plane was predominant. Rayleigh waves, whilst involving circular motion of particles, excite the substratum in the vertical plane in addition to the horizontal hence it may be that these waves are most relevant to the current work (Hazelwood, 2012; Hazelwood and Macey, 2015).

Due to the small nature of the arena in the response determination work, measurements were taken adjacently- therefore the stimulus characteristics must be viewed with caution in comparison to the more accurate threshold measurements. However the accelerations next to the arenas give an approximation of sufficient detail for the experiment, which did not aim to quantify precise sensitivity but rather to explore response variation.

For the threshold setup, a purpose-built base dampened external vibrations entering the tank. The setup was not available for the response experiments, since both experiments ran simultaneously. As such, the bench which the arena were placed on was not specifically dampened from external vibrations, although the strong nature of the stimuli may have reduced the influence of this. Nevertheless it is of note that behavioural responses were clearly at the onset the stimulus and did not occur spontaneously.

5.5.7 Relation to anthropogenic vibration levels

The frequency range tested in the current work (5 – 500 Hz) was chosen since energy of key anthropogenic signatures is low frequency (Nedwell *et al.*, 2003a; Nedwell *et al.*, 2003b) as are natural sounds. In terms of vibrations, the longer wavelengths of low frequencies propagate further and therefore are perhaps more likely to be present in the vicinity of, and at greater distances from anthropogenic operations.

It is difficult to relate thresholds to actual values of anthropogenic signals since there is a shortage of seabed measurements, with many of these not being publicly available (Hazelwood and Macey, 2015; Miller, 2015). Due to the complexities of underwater sound measurement, many studies only measure pressure, without considering water-borne particle motion, or the energy in the seabed. Of man-made activities, those that specifically contact the seabed are of most relevance to the current work- for example pile driving and airguns, which produce vibrations as compressional, Rayleigh and shear waves (Athanasopoulos and Pelekis, 2000; Thandavamoorthy, 2004; Hazelwood, 2012; Hazelwood and Macey, 2015).

Despite the lack of vibration data available, the results of the current work clearly indicate that *M. edulis* is likely to be able to detect vibrations produced by anthropogenic sources at short and long range. For example vibrations at 17 m and 34 m distance from a piling operation were 0.0001 m s^{-1} and 0.0006 m s^{-1} respectively (S. Cheesman, *Pers. Comm.*[10]) and these levels may be sufficient to induce closure of the valves in *M. edulis* as demonstrated here (Table 4.8, Chapter 4). Since there are few data available of seabed vibration, modelling may be able to provide an estimation of the levels that marine animals may encounter. For example vibration levels at 5 m from pile driving have been estimated to be 0.05 – 0.1 m s^{-1}, decreasing to 0.001 at 400 m from the pile (Miller, 2015). These levels are much higher than those applied in this study and it is therefore likely that behavioural reactions in mussels may be more pronounced (with the possibility of damage to the animal). Disruption of mussel behaviour by vibration may have other implications- for example *M. edulis* is frequently used as a biomonitor of pollutant levels (e.g. Mazik *et al.* 2013), however if areas of high vibration are those being monitored for chemical pollutants, measures of the energy of the animal are likely to be affected by the vibration alone, in addition to the chemical pollutants. Multiple animal indicators should be used for this reason.

It is of note however that the 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 (Nedwell and Howell, 2004; Thandavamoorthy, 2004). Therefore whilst the data here indicates reception 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 pressure component which were deliberately not replicated in the current work. Furthermore, the noise from activities which do not have specific contact with the seabed (such as shipping) are also likely to produce seismic waves in the seabed after propagation through the water (Hazelwood, 2012; Miller, 2015) and therefore are also of relevance, although levels of these are relatively unknown. Notably, the current work used a relatively short stimulus, whereas actual vibrations may be of much longer duration (for example piling), and this may affect the responses.

Of even more relevance to *M. edulis* is their sensitivity in relation to natural vibrations, which are most frequently encountered by the species. Common underwater acoustic sources include crustaceans, predatory fish, the scraping of mollusc feeding apparatus and valve movement of bivalves (Vermeij, 2010; Di Iorio *et al.*, 2012) in addition to abiotic sources such as wave action,

[10] Mr S.Cheesman, Acoustic consultant, Subacoustech Ltd., Southampton, UK.

rain, turbulence and flow motion. It is likely that organisms will be adapted to detect such vibrations, for example reception has been described for the anemone *Anthopleura elegantissima* and the sea urchin *Strongylocentrotus purpuratus*, within the frequency range of waves on the shore (Ellers, 1995). However levels of these, in terms of seabed motion, are not well documented. There are also few data about the frequency range and amplitude of shore vibrations, or within rock pools for example. Acoustic recordings of waves upon the shore have indicated energy in the 40 – 300 Hz range, predominantly at 60 – 100 Hz (Ellers, 1995),and are hence fully within the sensitivity range determined for mussels in the present study.

The method of threshold determination and the caveats of other determination methods will not be discussed again here (Section 4.6). However *M. edulis* in this work responded clearly to the stimulus and did not habituate, enabling an accurate threshold to be calculated. Mooney *et al.* (2010) tested cephalopod sensitivity to particle motion using both a shaker table and a standing wave acoustic tank, and they found comparable thresholds with both methods. It may be that the thresholds in the current work could therefore be replicated using other methods.

5.6 Further research development and conclusions

Although the methodology here was successful in demonstrating sensitivity and behavioural responses to quantified stimuli, the ideal experiment would be undertaken in the natural environment, in the acoustic free field. This would allow the vibration (and the water-borne component) to propagate freely without boundaries. However, it is of note that *M. edulis* is found predominantly on rocky shores, residing often in rock pools, and the tanks used in this work may have similar characteristics to small rock pools to a crude extent, for example having boundaries and a sandy substratum. Nevertheless future work monitoring mussel beds using cameras and valve gape monitors in response to small-scale vibration events (for example a stake driven into the ground) could be undertaken. This type of approach was trialled in Lough Hyne, Ireland during the present work, and could be pursued further. Moreover, the use of a laser Doppler vibrometer would be valuable to measure the precise stimulus upon the valves, and on solitary barnacles, which was beyond the budget of the current work.

Furthermore, since many anthropogenic signals are continuous rather than impulsive, it would be valuable to investigate the vibrations produced by these sources and the behavioural changes they elicit. Indeed the current methodology could be adjusted to incorporate any number of vibration stimuli, including different anthropogenic recordings, and variation in pure tones such as the duration of the signal. The responses to longer, more continuous stimuli could be investigated in terms of inducing clumping behaviour. Further work could use a higher level of vibration to test whether habituation occurs in bivalves. This would be step towards understanding the implications of these behavioural changes.

Valve closure, as observed here, may be extrapolated to a direct energetic consequence to the animal, since it is linked to a reduction in filter feeding. Another way to investigate fitness consequences of the exposure would be to monitor physiological parameters such as heart rate, oxygen consumption and byssal thread production in relation to vibration exposures, which was beyond the scope of the current work. A number of indices may also be used to monitor animals

such as scope for growth, mantle condition and body condition index, which reflect energy status, nutritional state, stress and reproductive condition (Widdows *et al.*, 1984; Widdows *et al.*, 1997; Widdows *et al.*, 2002; Mazik *et al.*, 2013). These could then be linked to long-term changes, for example changes in energy consumption. In turn these could have consequences to mussel beds and to the population level, for example. The difficulties of extrapolating behavioural changes to population level are discussed in Section 6.5, (Chapter 6).

The use of the staircase method (Cornsweet, 1962) to determine the precise threshold of *B. crenatus*, as for *M. edulis* in this chapter, would also provide a more accurate estimation of sensitivity levels of these. In order to do this, a high resolution camera would be useful, in addition to a non-biological substratum choice to prevent bias. Motion of the cirri could be tracked using Motion Analysis software (Chapter 3) which could provide an accurate record of beating frequency. Barnacles were not the focus of the current study, but work such as this would be valuable due to the biofouling nature of these organisms having commercial implications. The same applies to *M. edulis* which is also a key biofouling species.

The current work was successful in determining the sensitivity and behavioural responses of a common marine bivalve, *M. edulis*, to vibration. Responses were measured as full or partial closure of the valves, with an approximately flat sensitivity curve being obtained in the frequency range of 5 – 410 Hz, aside from a prominent peak at 210 Hz. This finding led to rejection of the first null hypothesis, that response would not be dependent upon frequency or amplitude. Preliminary data also supported a sensitivity of adult *B. crenatus* to vibrations. The second null hypothesis was also rejected since responses were associated with changes in vibration level, with the duration of response also increasing with increasing stimuli. In turn the third null hypothesis could not be accepted or rejected, due to conflicting evidence as although there was a significant correlation between the size of mussel and response, there was no relationship between the size and the average threshold value itself. This may be attributed to a small test group (n = 15) for the threshold experiments, therefore further investigations are needed to test this hypothesis. The final null hypothesis was accepted here since the startle duration between solitary and grouped mussels was demonstrated to be similar.

As with all vibrational and acoustical studies, the results here should be taken within the context of the experimental setting, involving a particular exposure duration, frequency range, substratum, vibration stimulus, and species. To extrapolate the results further would be unwise since propagation of vibration energy varies according to, for example, substrate, environment, and propagation conditions (Kim and Lee, 2000; Hill, 2009b). Furthermore, behavioural responses of an individual may be the results of other individual-specific cues such as energy availability, size, respiratory requirements, interactions with conspecifics and perhaps even consistent individual behavioural tendencies (Jumers, 1998; Briffa *et al.*, 2008; Briffa *et al.*, 2013). 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.

Despite this, the present work has provided a valuable first estimation 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 fully quantified in three axes; this

should allow comparisons with future studies. Vibration sensitivity may be important since many anthropogenic activities in the oceans involve direct contact with the seabed (for example pile driving, drilling), creating radiating particle motion waves. By comparing sensitivities to actual measurements, this chapter has shown that *M. edulis* is likely to detect such vibrations at large distances from anthropogenic sources, and is likely to exhibit behavioural changes at these levels. Furthermore, other sources of noise in the ocean, such as shipping, which do not contact the seabed directly, may propagate energy into the seabed indirectly via the water-borne component of the energy. If noise such as this translates into seabed vibrations, the productivity of mussel beds may be affected which could have both ecosystem and commercial implications. Additionally, as a common biomonitoring species, disruption of *M. edulis* behaviour by ambient vibration levels may also have implications for the monitoring of other pollutant levels if baseline data is disrupted. Therefore the effects of vibration may be far-reaching, and the work here has made an important initial step towards understanding the effects of such stimuli upon a common bivalve species.

Chapter 6 General discussion and conclusions

6.1 Outcomes related to objectives

Chapter 1 highlighted large 'information gaps' in the underwater bioacoustics research field (Hawkins *et al.*, 2014a) for example a lack of reliable audiograms for fishes and invertebrates, of standards for the measurement of underwater particle motion and pressure, and data regarding sensitivity to seabed vibration. The overarching theme across these factors is that there is a need for behavioural studies undertaken on fishes and invertebrates during and after exposure to noise and vibration stimuli. Indeed in terms of behavioural reactions, the extent to which noise affects migratory patterns, feeding, reproduction, communication, predator-prey interactions and navigation is relatively unknown compared to other stressors- with many of the available data produced by small-scale laboratory studies or field studies with captive animals. As such, the consequences on a population level of acoustically exposed organisms have not been investigated in detail. More direct observations of animal reactions in the wild are required to examine naïve fishes and invertebrates which have not been affected by the trauma of capture or handling.

The current work sought to inform the research field using four objectives:

- The behavioural responses of free-ranging fish schools to acoustic playback will be measured and linked with specific exposure levels to predict the exposure levels that will elicit responses.
- The responses of individual crustaceans and fishes to acoustic playback will be measured and linked with exposure levels to predict the exposure levels that will elicit behavioural responses.
- The behavioural responses of benthic marine invertebrates to substrate-borne vibration will be measured and interpreted with vibration level data to predict the levels of response, and to calculate threshold sensitivity.
- The data from all objectives will be synthesised to discuss the overall impact of noise upon the behaviour of marine species, from individual to population level.

Using a combination of laboratory and field work the current work has demonstrated the sensitivity of various species of marine fishes and invertebrates to noise, both in terms of acoustics and vibration (Objectives 1 – 3). Acoustic imaging observations within controlled exposure experiments have demonstrated specific exposure levels of synthetic impulsive sound that will elicit behavioural responses in pelagic fish schools (Objective 1). BRUV (Baited Remote Underwater Video) work allowed observations of individual fish during playbacks of the same noise signature which indicated a number of key responses at specific exposure levels (Objective 2). The sensitivity of three key coastal invertebrates to substrate-borne vibration was demonstrated (Objective 3), and the provided sensitivity curves may be used to achieve Objective 4. The current chapter aims to combine and produce a synthesis of the previous results and discusses the difficulty of Objective 4, although data from the current work, such as the dose response data and threshold curves may be used for impact assessments, for example.

6.2 Results summary

Free-ranging, wild schools of Atlantic mackerel *Scomber scombrus* and sprat *Sprattus sprattus* were observed responding to playbacks of impulsive sounds (Chapter 2). The synthetic noise signature was representative of a piling operation at distance, in terms of the water-borne component. As sound level increased there was an increased likelihood of response for both species. Dose response curves indicated 50% response levels of 163.2 dB and 163.3 re 1 µPa peak-to-peak for sprat and mackerel respectively. The particle velocity that 50% of the mackerel responded to was 80.4 dB re 1 m s^{-1}, it is likely that this stimulus is more relevant since they lack a swim bladder (Iversen, 1969; Hawkins *et al.*, 2014b). The response to the stimulus itself varied with species, being predominantly school density changes and dispersal for sprat, and depth change for mackerel. This is most likely due to the different lifestyles of the two species, with mackerel being more mobile predatory fish able to change depth rapidly, compared to sprat which need to expel air to change depth making it a more energetically costly procedure (Knudsen *et al.*, 2009). Similar response levels have been demonstrated for captive fish (e.g. Blaxter and Hoss, 1981, Engas *et al.* 1995), and horizontal and vertical avoidance has been observed using sonar systems (Engås *et al.*, 1996; Slotte *et al.*, 2004). Changes in density and vertical displacement in response to noise has been described in captive studies of fish schools, e.g. Jorgenson and Gyselman (2009), Rosen *et al.* (2012), Jorgenson and Gyselman (2009); Doksaeter *et al.* (2012). The decision of a schooling fish to respond is likely to vary according with hearing ability, environment, physiological state, parasite load, and motivational state in addition to the characteristics of the stimulus (Lima and Dill, 1990). For example in this study individual fish were unresponsive at night time when feeding (Knudsen *et al.*, 2009; Hawkins *et al.*, 2012b). This is important since schooling is thought to reduce the success of predatory attacks and aid foraging (Grunbaum, 1998).

Preliminary results from BRUV studies of individual, free-ranging fish such as pollack *Pollachius pollachius,* thicklip grey mullet *Chelon labrosus* and Cuckoo wrasse *Labrus mixtus* re-emphasised that behaviours exhibited in response to playback depended upon species, context and sound level (Chapter 3). Individual fishes and invertebrates were exposed to playback of impulsive sound, with responses seen from 163.4 – 167.0 re 1 µPa peak-to-peak. A range of behavioural changes were demonstrated, including directional changes, accelerations, involuntary body spasms and avoidance. It is of note that many fishes continued feeding which reiterates the importance of motivational state and context as also demonstrated in Chapter 2. Analogous responses by individual fishes have been exhibited in captive studies at levels of 146 – 166 re 1 µPa peak-to-peak upwards (Thomsen *et al.*, 2010). Similar camera work based upon reefs has indicated changes in time budgets and involuntary c-start responses in resident fishes (Wardle *et al.*, 2001; Picciulin *et al.*, 2010). Here, a response of European lobster *Homarus gammarus* was seen at an estimated level of 167 dB re1 µPa peak-to-peak, but the particle motion level for the particular playback was unrecorded. There are few studies exposing crustaceans to noise, and of those available there are few measuring the particle motion of the stimuli which is most likely the appropriate (Goodall, 1988; Breithaupt and Tautz, 1990; Popper *et al.*, 2001). Behavioural reactions have not been exhibited in acoustic studies, possibly due to this reason (Christian *et al.*, 2003; Andriguetto-Filho *et al.*, 2005; Parry and Gason, 2006; Brack, 2010). The BRUV experiments

are an example of how a logistically difficult playback experiment upon free-ranging fishes and invertebrates can be undertaken, and the discussion will inform future researchers of methodologies.

In the laboratory, the hermit crab *Pagurus bernhardus* was sensitive to sinusoidal vibration within the frequency range 5 – 410 Hz (Chapter 4). A suite of postural changes were exhibited by unconditioned animals in response to vibration, which varied with amplitude, enabling threshold curves of sensitivity to be produced using two behavioural indicators. Thresholds appeared to be independent of frequency, with sensitivity in the range of 0.11 – 0.29 m s^{-2} and 0.09 – 0.44 m s^{-2} (RMS) for the two indicators respectively. These sensitivities fall within the range of those previously described for semi-terrestrial crabs exposed to vibrations (Salmon and Atsaides, 1969; Horch, 1971; Salmon and Horch, 1973; Aicher and Tautz, 1984), and for water-borne particle motion sensitivity of other crustacean species (Breithaupt and Tautz, 1988; Breithaupt, 2002). Sensitivities were also comparable to other marine species for example *Nephrops norvegicus* and *Crangon crangon* (Goodall, 1988; Berghahn *et al.*, 1995), although there are few studies directly comparable to the current work since sensitivity of marine species to substrate-borne vibration is relatively unstudied. Here, demonstrated sensitivities were also shown to fall within the vibration levels produced in the vicinity of anthropogenic activities indeed clear behavioural changes occurred after playback of a piling signature at an equivalent level within the laboratory. Sensitivity was demonstrated below 500 Hz, and so it is therefore likely that this species is able to detect signals produced both directly and incidentally by other marine invertebrates, enabling the detection of conspecifics, prey and predators as seen in terrestrial organisms (Brownell, 1977; Lewis and Narins, 1985; Hetherington, 1989; Hebets *et al.*, 2008; Hill, 2009a; Fabre *et al.*, 2012). There was a consistency in response to vibration within each individual, and observations indicated that there were clear differences between individuals in terms of exploration of the experimental arena, indicative of the concept of individual 'personality' (Briffa *et al.*, 2008; Briffa *et al.*, 2013). There was no correlation between average threshold and startle duration, but there was a significant correlation with time in the laboratory prior to tests. Crabs exposed to playback of impulsive vibration had a reduced startle duration post exposure. Similar changes in anti-predator response after vibroacoustic stimuli have been shown in other crustaceans such as semi-terrestrial hermit crabs (Chan *et al.*, 2010a; Chan *et al.*, 2010b; Stahlman *et al.*, 2011; Ryan *et al.*, 2012) and marine species (Wale *et al.*, 2013a). However the sensitivity of marine species and the concept of behavioural and personality changes after exposure to substratum vibration has not been investigated before to this extent.

Further work in the laboratory demonstrated sensitivity of another coastal invertebrate, the mussel *Mytilus edulis,* to sinusoidal vibration in the region of 0.06 – 0.55 m s^{-2} (RMS) with greatest sensitivity at 10 Hz (Chapter 5). There are few similar studies (Mosher, 1972; Kowalewski *et al.*, 1992; Ellers, 1995; Zhadan, 2005; Kastelein, 2008), and only one fully describes the exposure stimulus making comparisons difficult. This study supports the literature which indicate a similar frequency range of reception (Mosher, 1972; Zhadan, 2005; Kastelein, 2008). The sensitivities of other molluscs to water-borne particle motion, for example the cephalopods, have also been demonstrated within a similar range (Packard *et al.*, 1990; Mooney *et al.*, 2010), with a greater

sensitivity for some species (Kaifu *et al.*, 2008).The reception range was shown to fall within the range produced within the vicinity of anthropogenic vibrations (e.g. Edwards, 2008; East, 2014). Additional behavioural tests indicated that number of responses increased with vibration level, and that the likelihood of response increased with size of mussel. Furthermore, the duration of the valve closure ('startle' response) increased with vibration amplitude, but did not vary between grouped and solitary mussels. This may be due to an association of greater vibration and predation risk, indeed such variation in startle response with predatory risk has been demonstrated in other bivalves (Toomey *et al.*, 2002; Robson *et al.*, 2010; Wilson *et al.*, 2012). Similar variation in gape has been recorded in bivalves exposed to other anthropogenic stressors such as chemical pollutants (Manley and Davenport, 1979; Akberali and Black, 1980; Kramer *et al.*, 1989; Salanki and Vbalogh, 1989; Curtis *et al.*, 2000; Kádár *et al.*, 2005). Indeed gape itself may be a complex response varying in angle and duration of closure, with natural stimuli such as food concentrations and environmental fluctuations (Englund and Heino, 1996; Dolmer, 2000; Newell *et al.*, 2001; Robson *et al.*, 2010). For sessile invertebrates in particular, the response to a stimulus is a trade-off between perceived risk and respiratory and excretory needs. Overall, disruption to natural valve movements may be of importance to the fitness of individuals, for example by disrupting feeding or disrupting the energy status of the animal, and may translate into population implications- for commercially farmed species such as *M. edulis* this is especially of importance (Widdows *et al.*, 1984; Widdows *et al.*, 1997; Widdows *et al.*, 2002; Mazik *et al.*, 2013).

Additional preliminary data from *Balanus crenatus* encrusting on the valves of *M. edulis* indicated a reduced sensitivity of 0.1 – 1 m s^{-2} with a more irregular threshold curve (Chapter 5). Barnacles appeared to beat the cirri faster during exposures although more data would be required to confirm this. The sensitivity of larval barnacles to vibration is well studied due to interest in biofouling prevention (Branscomb and Rittschof, 1984; Guo *et al.*, 2011a; Guo *et al.*, 2011b; Guo *et al.*, 2012; Choi *et al.*, 2013), however there are few data on the abilities of adult barnacles to detect vibration. Retraction in response to unquantified vibrational stimuli has been described (Crisp and Southward, 1961), and it is likely that the detection of this would be highly advantageous on the seashore, an area of high vibration, for example caused by wave action. The vibration source appeared to affect feeding behaviour, and therefore the use of such stimuli to disrupt adult barnacle behaviour could have commercial importance for example by reducing fouling of boat hulls.

6.3 Synthesis of results

There are three key themes running through these results: the first is that exposure to vibroacoustic stimuli appears to elicit and affect anti-predator responses in all the species investigated. For example, impulsive sound caused disruption of schools (Chapter 2), vibration affected startle responses in crabs (Chapter 4) and caused closure of the valves in mussels (Chapter 5). Such changes in response to noise have been demonstrated for other species, for example in eels, three-spined sticklebacks and semi-terrestrial hermit crabs (Chan *et al.*, 2010a; Purser and Radford, 2011; Simpson *et al.*, 2014). In the case of Chapter 2, the types of responses seen were similar as described in response to a predator (Pitcher *et al.*, 1996; Misund *et al.*, 1998; Wilson and Dill, 2002; Southall *et al.*, 2009). By responding to the stimuli, these species were also 'distracted' from normal behaviour such as feeding- for example crabs exhibited a sudden burst of movement

which stopped them from previous activity (Chapter 4), mussels closed (Chapter 5), and fishes were deterred from a source of food (Chapter 3). This is consistent with Chan *et al.* (2010a) who suggested the 'distracted prey' hypothesis to describe how hermit crabs were distracted after noise exposure, allowing, in that context, a predator to approach.

In all cases (Chapters 2 – 5) the responses exhibited varied according to the strength of the stimulus. Whilst this is a simple concept, this too may be linked to anti-predator behaviour, since if the stimulus is perceived as a predator, then responses will increase as perceived threat increases (Toomey *et al.*, 2002; Robson *et al.*, 2010; Wilson *et al.*, 2012). For example hermit crabs were shown to exhibit a suite of postures which depending largely upon the amplitude of the exposure (Chapter 4) and duration of mussel valve closure increased with greater vibration amplitude (Chapter 5). Fish schools exhibited different responses according to sound level, from increasing packing density of the school to full dispersal (Chapter 2).

Secondly, response to a stimulus also depends upon the context of the noise exposure and the motivational state of the organism (Lima and Dill, 1990). For example, fishes that were responsive in schools during the daytime were unresponsive when feeding individually at night time (Chapter 2), and many individual fishes did not respond to playback whilst feeding in front of the BRUV whereas conspecifics did (Chapter 3). In the case of vibration, there was some preliminary data to suggest that mussels in groups varied in response to individuals (Chapter 5), and hermit crabs varied the duration of startle response depending upon the preceding situation (for example exposure to vibration, or handling) (Chapter 4). Furthermore reactions of mackerel and sprat varied according to whether they were part of a group, as did that of mussels (e.g. Mauck and Harkless, 2001; Wilson and Arnott, 2012). This highlights the difficulty of behavioural studies, since responses are contextual, indeed the importance of context for management purposes has recently been recognised for marine mammals (Ellison *et al.*, 2011).

Thirdly, throughout the current work, reaction levels have been shown to be within levels produced by anthropogenic activities. For examples the sensitivities of key invertebrates species were demonstrated within the levels experienced in the vicinity of anthropogenic operations such as pile driving and drilling (Chapters 4 and 5), and by providing specific exposure levels, the results of Chapter 2 can be directly related to actual anthropogenic exposure levels. Furthermore, using playback of actual anthropogenic stimuli, with a fully quantified playback source, the results of this study may be considered reliable.

6.4 Conceptual modelling of anthropogenic stressors using individual changes

The results from the current work have demonstrated behavioural responses to anthropogenic acoustic and vibratory stimuli (Chapters 2 – 5). To indicate the relevance of these, and other behavioural, physiological and physical factors at population and ecosystem level, a conceptual model may be used (Olla *et al.*, 1980; Hawkins, 2011a; Tuomainen and Candolin, 2011). Behaviour is the first factor to change in relation to an environmental variation and acts as an early-warning response to a stressor (Olla *et al.*, 1980; Tuomainen and Candolin, 2011) and the response depends upon the ability to detect the stimulus or not. After behavioural changes, physiological changes may occur, leading to an adjustment of sensory behaviour depending upon the recipient's

reaction abilities, or an overall adjustment of fitness. Individuals that are capable of adjusting to the presence of the stimulus are likely to prevail over other species (Tuomainen and Candolin, 2011). Changes in behaviour of one species may also have implications for other species, causing community level changes. For example, Frid and Dill (2002) discuss that disturbance stimuli are a form of predation risk (discussed previously) and propose a conceptual model that incorporates the effects of such risk (fleeing, habitat selection changes, mating displays, parental changes).

A conceptual model is proposed here (Figure 6.1), which attempts to link the processes involved after exposure to noise. At each stage of the model the data requirements are listed to understand the exposure fully and to scale effects up to the population level. The areas which the current work contributes to are denoted with asterisks, although it is of note that the data from the current work are only applicable to the specific species, context and source type.

In many cases the long-term consequences of behavioural changes are lacking, which makes the application of such models difficult. There is a need for data linking individual short term behavioural variation to long-term impacts. For example, Picciulin *et al.* (2010) observed changes in time budgets of gobies and damsel fish, with less time spent attending to the nest and shelter areas. Most recently European eels exposed to noise have been shown to be 50% less likely to show a startle response to a predator in laboratory studies (Simpson *et al.*, 2014). These types of behaviours can be linked to overall fitness. In bivalves, implications for overall fitness are somewhat simpler to calculate, for example by calculating energetic status of individuals by monitoring in and outputs of the valves (e.g. Widdows *et al.,* 2002).

There are indications that individual anti-predator behaviour in crustaceans is modified after exposure to noise. For example Chan *et al.* (2010a) demonstrated that semi-terrestrial hermit crabs allowed a simulated predator to approach closer after noise exposure before withdrawing into the shell. Wale *et al.* (2013a) studied the anti-predator behaviour of *Carcinus maenas* after exposure to boat noise. Whilst the ability to detect a simulated predator was the same in quiet and noisy conditions, crabs exposed to noise were significantly slower to relocate to safety. In the current work, disruption of anti-predator behaviour was demonstrated for pelagic fish (Chapter 2), for crustaceans (Chapter 4) and for bivalves (Chapter 5). The implications of such disruption are an increased risk of predation, leading to an increase in mortality risk, which would have population level consequences. It is clear then, that the links between individual behavioural changes and fitness implications must be further explored for fishes and invertebrates (discussed in Hawkins *et al.,* 2014a; Morley *et al.,* 2014) ideally within the natural environment (Olla *et al.*, 1980).

6.5 Population level and ecological effects

The effects of noise and vibration upon fishes and invertebrates may range from death, injury and damage to hearing, loss of communication to distributional changes, predator-prey modifications, reduced feeding or problems with orientation (Engås *et al.*, 1996; McCauley *et al.*, 2003; Smith *et al.*, 2004; Popper *et al.*, 2007; Wale *et al.*, 2013a; Hawkins *et al.*, 2014b; Simpson *et al.*, 2014).

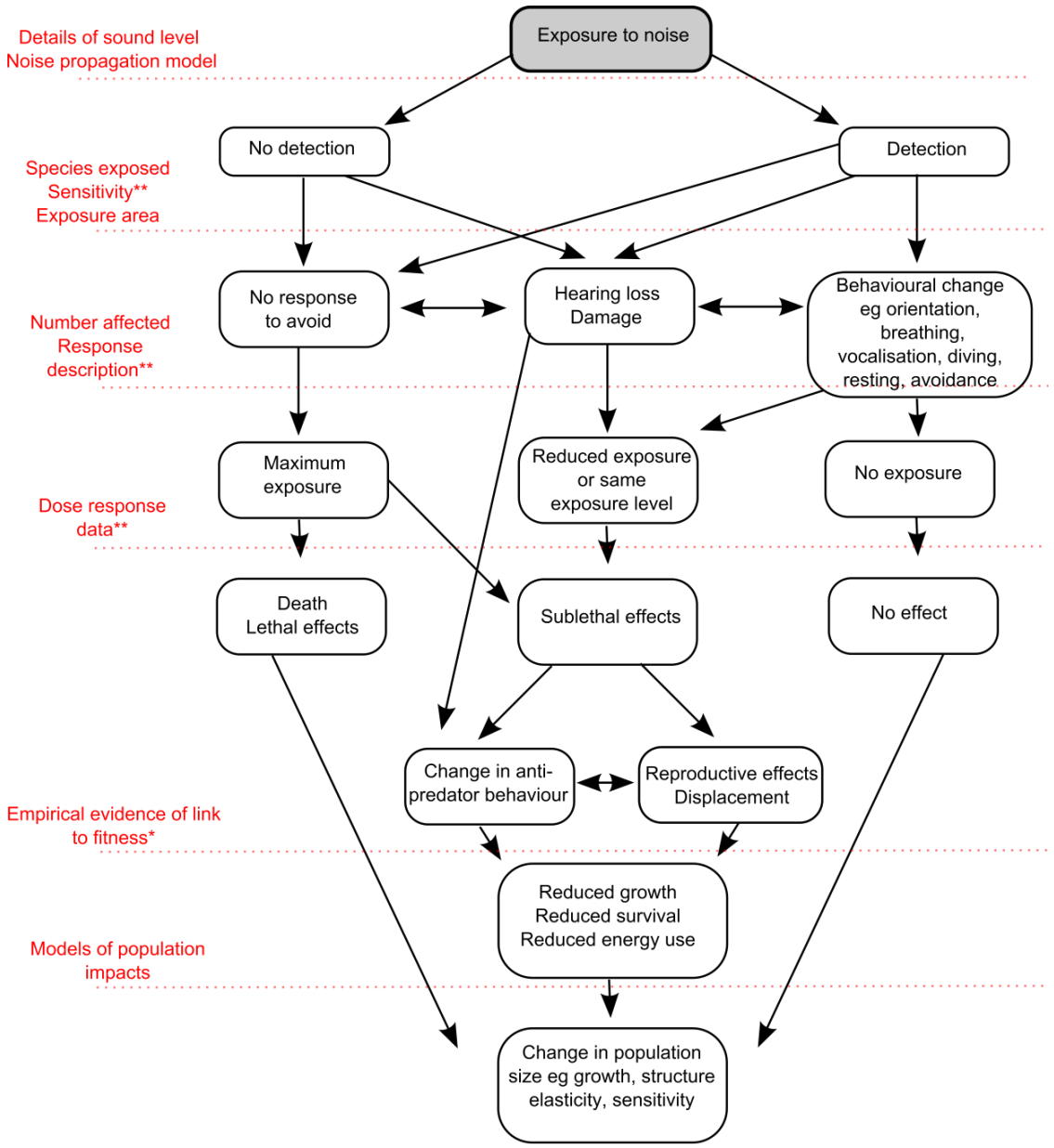

Figure 6.1 A conceptual model outlining the possible mechanisms by which animals may be affected by exposure to noise pollution, from an individual to a population level. Dotted lines subdivide the model into sections, with the associated labels listing the required data for that section. Double asterisks (**) denote areas which the current work could begin to inform for the tested species and source types, single asterisk (*) denote areas in which the current work provides some preliminary data. Figure adapted and expanded from Olla *et al.* (1980), NRC (2005) and Hawkins (2011b).

At some point the fitness of an individual may be affected by exposure noise, and thus it is important to understand the threshold at which it becomes a problem to the individual, since there are likely to be population level effects such as declines, abandonment of key habitats, or even regional extinctions (Blickley and Patricelli, 2010; SoundWaves, 2012). It is difficult to understand the significance of individual responses on a population level, due to the lack of data regarding the effects of underwater noise, and the variation in response that will be seen between and within species (discussed in Blickley, 2010) . For example, bursts of sudden movement such as those exhibited by hermit crabs in response to vibration (Chapter 4) are likely to have energetic

consequences and may also elicit a stress response, these factors may affect overall fitness of the animal if such processes are triggered more often than usual. In bivalves, such energetic costs are simple to measure using valve movements, faecal intake and gas exchanges (Widdows *et al.*, 1984; Widdows *et al.*, 1997; Widdows *et al.*, 2002), however for more complex, more mobile animals such measurements are more difficult.

Initial attempts to assess impacts of noise exposure involved the use of the source-path-receiver model (Richardson *et al.*, 1995; Tasker *et al.*, 2010), with the source being the noise source, the path being the propagation pathway and the receiver being the affected organism. However as discussed previously, each of those steps have complexities hence a more detailed model is required (Tasker *et al.*, 2010). To estimate the impact zone around an anthropogenic source, Richardson *et al.* (1995) proposed the zones of noise influence model. This consists of concentric circles with the effect upon the animal ranging from injury at the centre to lower responses such as detection and masking in the outer regions (the largest zone of influence). However whilst the model is still used in impact assessments, it does not take into account the propagation of sound which varies with environmental conditions in a 3D manner (Tasker *et al.*, 2010).

In a more detailed effort to link individual changes to population consequences, the Population Consequence of Acoustic Disturbance (PCAD) was devised for marine mammals (NRC, 2005). The model aims to address the complexities of linking individual responses to fitness implications by the use of four transfer functions. For example step one involves a description of the sound (e.g. frequency, duration, level, source), step two lists the possible behavioural changes, and the next stage describes life functions which influence the vital rates, which in turn will have a population effect. However whilst it is a more detailed model, in many cases there are insufficient data to support each transfer function and it was noted when proposed that it was conceptual only (NRC, 2005; Tasker *et al.*, 2010). Most recently the PCOD (Population Consequences Of Disturbance) model has been released to transfer the PCAD into a workable mathematical version and to extend to consider other disturbances (Harwood and King, 2014). In order to apply the model to a situation involving two hypothetical wind farms and five marine mammal species, expert opinion was required to fill in data gaps, indeed the model was further simplified to incorporate the lack of empirical data. A list of nine requirements was given for implementation of the approach - this includes sound field measurements, threshold sound levels (dose response) causing permanent threshold shift and behavioural changes, estimations of numbers exposed to cause PTS and behavioural changes, potential effects upon vital rates, and key population parameters.

Harwood and King (2014) found it difficult to apply the model to marine mammals, therefore to apply this approach to fishes and invertebrates, where fewer data exist, would be difficult. Indeed for example, dose response data for fish species are largely lacking due to the logistical difficulties of such experiments (Chapter 3). In the current work, dose response curves for two pelagic fish species exposed to impulsive sound are provided (Chapter 2). In addition to this the methodology used was reliable and will therefore allow repetition with other fish species. It is clear then that data such as this is key to informing models such as the PCAD, which require detailed information about responses. Additionally Chapter 3 outlines a method that, with repetition in a suitable location, would also obtain thresholds of response. It is of note that whilst such models may be simplified by

subdividing fishes into functional groups relating to hearing (Popper *et al.*, 2014), there are few reliable audiograms available (Ladich and Fay, 2013) and this research area must be explored further before models may be applied with any meaning.

Costa (2012) suggests a method of developing the PCAD model by using bioenergetics. It is possible that this method of supporting the transfer functions may be more applicable to fish, by assessing activities such as growth, reproduction and swimming behaviour; similarly bioenergetics of bivalves could easily be translated to the PCAD in this way by the monitoring of energetic status in response to noise. Such an approach has already been used for other pollutants (Widdows *et al.*, 1997; Widdows *et al.*, 2002; Mazik *et al.*, 2013).

For invertebrates there is relatively little known about the reception abilities, physical and behavioural responses to noise (reviewed in Popper *et al.,* 2001, Chapter 4). For these species, there are many more steps to overcome before models such as PCAD could be applied. The first stage must be investigating sensitivity to stimuli to which they are likely to be susceptible. Chapters 4 and 5 of the current work are a step towards understanding this sensitivity. Another set of experiments, investigating behavioural responses in the field, with incorporation of other species would be required before data would be ready for predictive models such as PCAD. It is of note that the current work tested vibration only, and more research would be required to study water-borne stimuli.

Another approach to measuring the impacts of noise upon populations is to predict population changes using individual-based modelling (IBM) (NRC, 2005; Willis, 2011). These models incorporate physiological and behavioural traits of individuals with environmental parameters enabling the prediction of responses to stressors (Willis, 2011; Rossington *et al.*, 2013; Willis and Teague, 2014). The advantage of such models is that they are based upon 'rules' defining movement and physiological requirements rather than past data trends (Willis, 2011). If such models are able to be validated with field data they would prove useful for monitoring population level effects of a stressor. Such approaches have been used for ecotoxicological studies, for example, with larval fishes (Alvarez *et al.*, 2006). Willis and Teague (2014) used an IBM to predict the response of a fish when approaching a barrier to migration; it is clear that such an approach could be applied to noise. Indeed Rossington *et al.* (2013) used IBM to predict the response of cod to noise from an offshore wind farm. Two models, one for sound propagation (HAMMER, HR Wallingford, Hydro-Acoustic Model for Mitigation and Ecological Response, 2014) and one for ecological response were combined to predict movement responses. However whilst the approach is clearly useful, the 'rules' required a value of exposure level to which 'fish' in the model responded to, which re-emphasises the requirement of empirical data.

To overcome a lack of dose response data, an interim method using hearing weighting criteria has been applied to predict the thresholds of response of harbour seals ($dBht_{species}$ and M-weighting) using Generalised Additive Modelling (GAM) and noise propagation models (INSPIRE, Subacoustech Ltd.). However application of the model depended solely upon expert opinion. The limitations of such weighting criteria have been discussed previously (Chapter 1), however there has been a recent call for reappraisal of such weightings due to inaccuracies with methodologies, and differences between research groups (Hawkins and Popper, 2014).

Dose response and behavioural response data are required for models such as PCAD, IBM and GAM. Data from Chapter 2 of this work provides data that could be incorporated into such models. Furthermore, had the methods of Chapter 3 been more successful, the motion analysis software would have calculated detailed locomotory parameters of the fish such as swimming speeds, directional and angular changes which could inform IBM approaches. There is no reason why IBM approaches could not be applied to benthic invertebrates such as crustaceans; for example the data from Chapter 4 provides sensitivity curves of response and details of behavioural responses. An extension of the work to produce a dose response curve to substratum vibration in the field would provide data for modelling, and behavioural observations from the current work are useful to predict responses. For example, hermit crabs exhibited a clear burst of movement in response to vibratory stimuli. In the case of bivalves such as mussels, where sensitivity to vibration has now been demonstrated (Chapter 5), a 50% response level was estimated in the current work which would be informative to IBM approaches. Over longer ranges, 2-D or 3-D hydroacoustic tracking would also be another way of obtaining reliable movement data to inform such models (Bolland *et al.*, 2008; Noble *et al.*, 2014), although it is more invasive than the techniques used in the current work.

It is of note that in all cases of modelling the impacts of underwater noise, suitable noise propagation models are also required. There is some uncertainty within these types of models, which must be appropriate to the system applied to, indeed Harwood (2002) list five key influencing factors relevant to propagation which include bathymetry, sound speed, and properties of the medium. The relationship between particle motion and pressure also varies with distance from source and with the number of and proximity to reflective boundaries such as the seabed and sea surface for example (Harwood, 2002). Additional models are required for the propagation of vibration through the seabed as there are few publicly available (Hazelwood, 2012; Hazelwood and Macey, 2015; Miller, 2015). There are some existing models, such as the INSPIRE model, which may be used to understand the impact ranges of noise sources, which then may be validated against field data (Subacoustech Ltd.) (Hawkins *et al.*, 2012a). In combination with the $dBht_{species}$ (Nedwell and Turnpenny, 1998; Nedwell *et al.*, 2007; Hawkins *et al.*, 2012a) these have been used to predict the impact range for dab, cod and herring of a specific source. For example, an impact range of 850 m from an airgun was predicted for dab, compared to 2400 m for cod and 4300 for herring, attributed to the varied hearing abilities of these species. Data from Chapters 2 and 3 in the current work provide measurements from two playback signatures, which provide an idea of levels produced by underwater sound transducer arrays. Furthermore, Chapters 4 and 5 assimilate vibration measurements from the literature and commercial reports, allowing levels of vibration from various sources to be understood. These data will be informative to propagation models.

A different approach to predicting impacts of noise may be to use a risk assessment framework (Tasker *et al.*, 2010; Hawkins and Popper, 2014). This may consist of hazard identification, exposure assessment, exposure response assessment, risk characterisation and risk management (Tasker *et al.*, 2010). The applicability of this approach to marine species is discussed by Hawkins and Popper (2014) by using a specific experimental case study involving fish schools exposed to impulsive sound (data from Chapter 2, Hawkins *et al.* (2014b). The results from the current work,

as with any empirical study undertaken in appropriate experimental conditions, could inform such approaches. Alternatively, Ellison *et al.* (2011) propose a deviation away from dose-response based predictions, towards a contextual approach involving many factors rather than amplitude of exposure alone.

6.6 Mitigation of underwater noise

Mitigation is the minimisation, elimination or control of the impacts of an anthropogenic stressor (Harwood, 2002), in this case underwater noise. Whilst the aim of such processes are clearly to reduce the impact as much as possible, mitigation measures must also be economically viable and be accompanied by evidence that they will be successful (Ducrotoy and Elliott, 2008; Normandeau Associates, 2012), since measures are likely to have a cost to the noise-producer. Indeed 10-tenets have been proposed to manage one or multiple stressors on the marine environment (Elliott, 2013) which suggest that management measures should also be, for example, 'ecologically sustainable, legally permissible, administratively achievable and politically expedient'. There must also be incentive for such measures or legislation to support their effective use. For any mitigation measure the process is the same, being the setting of specific standards or criteria ('rules') and the enforcement of such standards.

There are many difficulties to consider when considering the mitigation of underwater noise, most of these are largely underpinned by a lack of information about specific sound levels required to elicit different responses in fish and invertebrates. For the purposes of this discussion behavioural responses of fishes and invertebrates will be considered rather that physical damage since this is more appropriate to the current work. It is of note that whilst many of the considerations below apply to physical damage and physiological responses, for fishes at least there are some known thresholds for damage and mortality caused by noise (Popper *et al.*, 2014). Mitigation measures may involve control of the noise source itself, engineering changes to reduce noise production, and monitoring of noise levels. In terms of the 10-tenets, for example, management of noise must be economically viable, i.e. realistic in terms of the impact upon the industries making noise in the ocean; technologically feasible i.e.- using technology to the advantage of reducing noise; and culturally inclusive i.e – noise reducing measures must be accepted by society and communities if they are to be successful (Elliott, 2013).

6.6.1 Difficulties of mitigation

The first issue with setting mitigation measures for noise relates to the lack of biological and experimental information available about the effects of underwater noise. Behaviour is difficult to study, it varies for example, with size, physiology, individual, age, species, context, environmental parameters and motivation (Ellison *et al.*, 2011; Normandeau Associates, 2012; Hawkins *et al.*, 2014a). Furthermore to observe the behaviour of marine organisms in the wild is costly and logistically difficult, and requires carefully controlled experiments in suitable conditions (Hawkins and Popper (2014); Chapter 3), and behaviour of captive organisms is not always consistent with animals in the wild (Benhaïma *et al.*, 2012). The range of behavioural responses described in response to noise is also highly variable, for example, ranging from startle responses (Wardle *et*

al., 2001; Hassel *et al.*, 2004), increased speed and positional changes (Blaxter and Hoss, 1981; Engås *et al.*, 1995; Kastelein *et al.*, 2007; Kastelein *et al.*, 2008), changes in schooling position and swimming parameters (Misund *et al.*, 1996; Pitcher *et al.*, 1996; Vabø *et al.*, 2002; Gerlotto *et al.*, 2004), and changes in foraging and anti-predator responses (e.g. Wale *et al.,* 2013; Simpson *et al.,* 2014). The difficulty then, is understanding which are important to the animal in terms of overall fitness and long-term implications, but data and information for this are lacking.

Most specifically to underwater noise is the hearing capability of the receiving organism; for the majority of the 32 000 fish species there are few data available (see Chapter 1 for review). Indeed of the data available, methods between laboratories vary and many audiograms have been produced in inappropriate acoustic conditions making comparisons and use of the data more difficult (discussed in Chapter 1, Ladich and Fay, 2013; Hawkins, 2014). In the case of invertebrates, little is known about the vibroacoustic detection capabilities (discussed in Chapter 4). Of those fish species studied, it has become apparent that there is a wide range of hearing abilities ranging from those with specialised connections to the inner ear enabling pressure detection (Enger, 1967; Blaxter and Hoss, 1981; Nedwell *et al.*, 2004), to those which only detect particle motion (Chapman and Sand, 1974; Hawkins and MacLennan, 1975; Berghahn *et al.*, 1995; Nedwell *et al.*, 2004; Sigray and Andersson, 2011); to species that are able to detect ultrasound (Mann et al 2001) and those only detecting a restricted frequency range. This makes extrapolation between species inappropriate when considering the effects of noise, unless the hearing mechanisms are similar and understood (Hastings and Popper, 2009). Due to the small amount of data compared to the number of taxa species, when considering exposure thresholds it is therefore necessary to group fish approximately according to hearing ability (Popper *et al.*, 2014).

The second consideration is that impacts of a noise vary with, for example, background levels, propagation conditions, and the noise properties (source type, source level, duration, repetition) (Kastelein *et al.*, 2008; Götz *et al.*, 2009). It is difficult to measure the effects since every sound has sound has distinct characteristics, varying in source level, frequency content, pattern of occurrence and movement (stationary or mobile) (Tasker *et al.*, 2010). For example short sounds may lengthen during transmission over distance due to refraction and absorption (Nieukirk *et al.*, 2012). Additional energy may be propagated through the seabed as well as the water column, which may affect a wider range of organisms (Hazelwood and Macey, 2015). There is a need to fully characterise the acoustic fields of a variety of sources, and further, to fully investigate the types within each source. In the case of piling, for example, measurements of the sound produced by a range of hammer types and pile diameters are required in addition to measurements of the efficacy of mitigation measures such as ramp-up. In addition to this acoustic sources must be described using appropriate metrics (e.g. Chapter 2, Hawkins and Hughes, 2012) and in terms of water-borne and substrate-borne energy.

Further exacerbating the issue is that effects of noise, as with other anthropogenic stressors, may be cumulative or in combination with other influences (Crain *et al.*, 2008; Halpern *et al.*, 2008; Normandeau Associates, 2012; SoundWaves *et al.*, 2012). For example pile driving not only creates noise, but the end-product may be a new physical structure in the ocean, which may induce local environmental changes such as artificial light and chemical variations. Other sounds

are intentionally produced, such as seismic surveys, or are incidental to human processes such as the transport of goods (shipping) or the construction of a wind farm (piling) (SoundWaves *et al.*, 2012). Indeed, a comprehensive management of multiple environmental stressors is necessary to evaluate impact upon the marine environment (Halpern *et al.*, 2008; Elliott, 2014).

Taken in combination, the result is a lack of information of the effects of fully quantified noise sources upon the behaviour of fishes and invertebrates, this has led to a call for studies undertaken by experts with representative hearing abilities, reliable methods with control observations, appropriate statistical methods, peer reviewed published results, fully quantified acoustic fields in laboratory or field studies methods (Rogers, 2015). This will allow the link between specific behaviours and sound level to be formed, enabling the development of mitigation measures. In the meantime, precautionary mitigation measures must be used (COM, 2000; SoundWaves *et al.*, 2012), where the developer has to demonstrate no effect of the stimulus, whereas the regulator has to demonstrate the opposite.

6.6.2 Developing sound exposure criteria

In order to inform mitigation strategies the sound exposure criteria that elicit behavioural responses are required for fishes and invertebrates. In the case of marine mammals, there is sufficient data to propose sound exposure guidelines (Southall *et al.*, 2007). This is made easier by much fewer marine mammal species compared to fishes (130 versus 32 000), a lack of particle motion detection in such species and arguably due to increased research interests in larger more charismatic species. Southall *et al.* (2007) proposed two criteria using peak sound pressure and energy, with whichever exceeded first used as the criteria. For behavioural results, the difficulties of assigning criteria were discussed and only given in terms of temporary hearing shift.

Due to the lack of data, standards have previously been set in an arbitrary way, for example for pile driving, a value of below 180 dB re 1 µPa SPL has been used in the commercial industry however the origin of such a number is largely unknown (discussed in Hawkins, 2011b) . Rather than setting specific levels, other researchers have proposed the weighting of sound levels using the $dBht_{(species)}$ (Chapter 1, Nedwell and Turnpenny, 1998; Nedwell *et al.,* 2007). It has been suggested that 90 dB above the hearing threshold of a species is likely to cause strong behavioural avoidance, 110 dB above is thought to be the upper limit of tolerance and above 130 dB causing damage (Nedwell and Turnpenny, 1998; Nedwell *et al.*, 2007). The principal of frequency weighting is similar to that of M-weighting for marine mammals (Southall *et al.*, 2007), but these weightings rely on audiograms which are not always a reliable indicator of hearing (Ladich and Fay, 2013).

Most recently, sound exposure guidelines for fishes and turtles have been proposed (Popper *et al.*, 2014). For the purposes of defining criteria, fishes and turtles were classified into three groups according to approximate hearing ability, with sea turtles and fish eggs/larvae being the other two groups. Four specific types of sound were incorporated (explosions, piling, seismic airguns, sonar) in addition to continuous sound as a whole. Effects of exposure were divided into mortality, recoverable injury, changes in hearing sensitivity, masking and behavioural effects. To overcome the deficit of data, where data were lacking the effect was rated as 'high, moderate or low' at three distances from the source. It is of note that in the case of behavioural changes the effects are

always rated, apart from for mid and low frequency sonar where the exposure level causing behavioural changes in one of the fish categories has been defined (Popper *et al.*, 2014). Overall, the criteria are suggested to be guidelines in the interim period before further research is able to inform exposure criteria (Popper *et al.*, 2014). There are currently no guidelines for the exposure of invertebrates to noise although the need for such data has been highlighted (Popper *et al.*, 2001; Morley *et al.*, 2014).

6.6.3 Types of mitigation

A number of the proposed, and practised mitigation methods are outlined below, with associated caveats (Table 6.1). There are principally two types of mitigation method, changing the source itself or using biology to minimise the effects (Normandeau Associates, 2012). For example, louder sources could be replaced with quieter technologies, and passive listening can be applied to detect marine mammals before they are disturbed. The efficacies of such measures are not well understood, however an extension of the current work, for example the methods of Chapter 2, could investigate the effect of mitigation measures such as ramp-up with adjustment of the noise stimuli. There is also a need to determine the effects of other measures that are used on marine mammals, such as acoustic deterrents, on slower moving fish and invertebrates; such devices would need to be specifically designed to fish for example fish guidance systems (Maes *et al.*, 2004; Taylor *et al.*, 2005).

In 2010 a EU regulatory framework, the Marine Strategy Framework Directive (MSFD), was put in place with the aim to achieve Good Environmental Status (GES) in European seas by 2020 (Tasker *et al.*, 2010; Van der Graaf *et al.*, 2012). The framework comprises of eleven qualitative descriptors, of these the eleventh refers to underwater noise, defined as “the introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment” (Tasker *et al.*, 2010; Van der Graaf *et al.*, 2012). There are two indicators relating to noise, relating to low and mid frequency impulsive sounds (indicator 11.1.1) and low frequency continuous sound (indicator 11.2.1), (Table 6.2). These must be defined and monitored with time. It is recognised that the proposed indicators do not, and do not seek to, cover all anthropogenic sources, for example an indicator to cover acoustic deterrent devices has been suggested for the future (Tasker *et al.*, 2010).

However implementation of such a strategy is difficult, as discussed in the current work and by Van der Graaf *et al.* (2012). International standards are lacking for the terminology describing underwater sound, there is little baseline data and the effects of noise upon marine organisms is relatively unknown compared to other pollutants. For this reason the working group (Van der Graaf *et al.*, 2012) did not attempt to identify all the gaps in knowledge, but focussed on the knowledge required to implement the MSFD. As discussed in Borja *et al.* (2010), the difficulty of implementing the MSFD is being able to define GES. The same issue applies to the European water strategy framework (WFD), which overlaps spatially in UK coastal regions, and aims to achieve ‘good ecological status’ (GEcS) (WFD, 2000). The two frameworks suggest different mechanisms of assessing GES or GEcS, and the WFD itself defines GEcS by using five Biological

Table 6.1 Examples of mitigation methods and measures applicable to anthropogenic sources developed from Nedwell *et al.* (2003a); Nedwell *et al.* (2003b); Normandeau Associates (2012); SoundWaves *et al.* (2012).

Method	Mitigation Example	Relevant Activity	Problems
Stop sound emission.	Cease activity. Use alternative technology.	All	Not always possible. Alternative technology not always economically feasible.
	Use alternative foundation types e.g. gravity foundation. Use alternative piling types e.g. vibroesis.	Pile driving	Dependent upon the seabed conditions.
Minimise sound output.	Bubble curtains, soft pads, foam.	Pile driving	Difficult to install pile sleeves at sea. Producing sufficient bubbles to achieve success at all frequencies.
	Reduce vessel speed, quieter ships.	Shipping	No evidence that quieter vessels are quieter, may be more noisy in other ways.
Trade intensity for duration or size for duration.	Increase number of strikes but reduce driving force. Use many smaller piles instead of one large.	Pile driving	Relationship between diameter of pile and sound level not well defined.
Safety exclusion zones.	Exclude activity from sensitive areas or at specific times.	All	Depends upon sufficient biological knowledge of relevant area.
Exclude or drive animals away.	Soft-start, ramp-up, acoustic deterrents.	Pile driving Seismic shooting Sonar	No evidence of the efficacy of such measures. No evidence that effective with slowly moving fishes and invertebrates.
Activity restricted to sighting-free periods.	Passive acoustic monitoring. Active acoustic monitoring.	Pile driving Seismic shooting	Depends upon successful detection.

Quality Elements which has been suggested to provide an incomplete picture of marine system complexity (Borja *et al.*, 2010).

The key step for each indicator of the MSFD is to define 'GES' in terms of exposure level (Borja *et al.*, 2010; Tasker *et al.*, 2010). Van der Graaf *et al.* (2012) discuss three options for setting the exposure criteria of impulsive sound (indicators 1 and 2,Table 6.2) within the paucity of data - the first is to use two exposures defined by Tasker *et al.* (2010) (183 dB re 1µPa^{2}m^2 or zero to peak source level 224 dB re 1µPa^{2}m^2), the second is to use a risk assessment approach to estimate threshold values, and the third is to estimate threshold levels for each source individually. It is proposed that option 2 is best since it would incorporate a more solid scientific foundation. Once a threshold is decided for a source, a register of impulsive sounds would be created to enable enforcement. For continuous low frequency sound (indicator 3, Table 6.2), it is proposed that, due to the costly nature of ambient noise monitoring, areas of high shipping traffic are to be monitored and modelled (Van der Graaf *et al.*, 2012). This approach has been questioned within the underwater acoustics research community, for example Merchant *et al.* (2014) argue that by representing high traffic areas only, changes in areas of lower pollution will be overlooked.

Table 6.2. The three underwater noise indicators proposed within the MSFD, (Tasker *et al.*, 2010).

Indicator	Source type	Definition
Indicator 1	Low and mid impulsive sounds	'The proportion of days within a calendar year, over areas of 15'N x 15'E/W in which anthropogenic sound sources exceed either of two levels, 183 dB re 1µPa^2•s (i.e. measured as Sound Exposure Level, SEL) or 224 dB re 1µPa peak (i.e. measured as peak sound pressure level) when extrapolated to one metre, measured over the frequency band 10 Hz to 10 kHz'.
Indicator 2	High frequency impulsive sounds	'The total number of vessels that are equipped with sonar systems generating sonar pulses below 200 kHz should decrease by at least x% per year starting in [2012].'
Indicator 3	Low frequency continuous noise	'The ambient noise level measured by a statistical representative sets of observation stations in Regional Seas where noise within the 1/3 octave bands 63 and 125 Hz (centre frequency) should not exceed the baseline values of year [2012] or 100 dB (re 1µPa RMS; average noise level in these octave bands over a year).'

It is of note that the current work was part of a larger project funded by DEFRA and was intended to be informative to the achievement of GES by providing data of behavioural changes at specific behaviour levels. Most recently, a summary of the intended monitoring programmes was released (DEFRA, 2014). For underwater noise, the proposed monitoring includes a noise register of noise-producing activities and ambient noise monitoring in the field. The noise criteria for impulsive sounds are those proposed by Tasker *et al.* (2010), there are no targets for ambient noise until sufficient baseline data has been collected. Other approaches to achieve GES include suggestions

of ecosystem-based management (EBM) using existing local information on ecosystems to allow a national a regional assessment of GES (Breen *et al.*, 2012).

6.7 Critique

Individual limitations of the current work were discussed in each chapter. As with all behavioural studies there is a possibility that the behaviours exhibited were unrelated to the stimulus however repetition of experiments minimised this risk, for example by observing hundreds of fish schools in the field (Chapter 2) and hundreds of mussels in the laboratory (Chapter 5). Due to the time-consuming nature of the threshold experiments (Chapter 4 and 5), ten to fifteen animals were used in most cases, but this is standard for such sensitivity experiments. Of course it is of note that the work of Chapter 3 did not provide sufficient replicates to draw firm conclusions but due to the ambitious nature of the work it is perhaps sufficient to have provided a detailed description of methods allowing other researchers to expand upon the study.

In the current work, the sound projector array was able to produce stimuli representative of a real pile driver and a moving vessel (Chapter 2 and 3), in terms of similar spectral range and predominant energy peaks. However, the exposure signals were, as such, representative of the stimuli in the far field rather than the near field. Furthermore it is of note that stimuli such as pile driving also produce a strong ground borne vibration produced by the contact with the seabed causing seismic waves (Markl, 1983; Aicher and Tautz, 1984; Athanasopoulos and Pelekis, 2000; Nedwell *et al.*, 2003b; Hazelwood and Macey, 2015) which are likely to affect marine organisms. The projector array did not seek to replicate this energy. However the species investigated in Chapter 2 and 3 were predominantly pelagic and therefore the benthic component of the signal may be of lesser relevance. The sound level was thought to be representive of the stimulus at 1 – 10 km of an impulsive operation, it is likely that vibration levels at this distance are low although more data are required to ascertain this for certain. Caution should also be undertaken when comparing the playback results here to exposures of similar signatures produced by ‘actual’ anthropogenic sources, since the distance between source and receiver would differ, in addition to the transmission path between the two. Therefore the sound field produced by piling playback from an array at close range, would not be directly comparable to the actual piling operation at distance from the receiver. Future work could use actual anthropogenic sources to replicate the current work to investigate these differences. In addition to this, the current fish school work only considered impulsive noise, and it would be valuable to investigate more continuous signals. Extrapolation of results between impulsive and continuous sources would be unwise due to different acoustic properties of the signals, for example it may be that habituation would occur with longer sounds.

Similarly, for the laboratory experiments, the small-scale playback of an impulsive vibration signature cannot seek to be fully representative of an actual source. However by fully describing the playback charcteristics and the sound signature, the results are still of importance to a largely unstudied field. A repetition of the work in field conditions is the next logical step, discussed later in this chapter. Moreover, the focus of the laboratory work was the use of sinusoidal signals rather than more complex stimuli.

It should also be noted that as with all behavioural experiments, once behaviour is measured it is likely to have been affected (Huber, 1988). This illustrates the need for experiments with relatively passive observation methods such as video and sonar (Chapters 2 – 5).

Chapter 1 highlighted the need for studies based in the field, as Chapters 2 and 3 were. Chapters 4 and 5 were laboratory based, however due to the lack of data on the subject, the controlled environment of the laboratory was deemed more suitable for the work. The erratic acoustic field within small laboratory tanks has been discussed within the context of the playback studies (Parvulescu, 1964b;a; Rogers, 2015). However whilst these considerations must be a factor, for the purpose of a stimulus predominantly in the substrate of the tank, the experimental set up was deemed adequate. It is of note that ideally measurements of water-borne particle motion would have been undertaken within the tank to fully rule out this as a stimulus.

6.8 Recommendations for future work

The methodology of Chapter 2 was successful in obtaining dose response data for schools of two pelagic species. The next logical step then is to repeat the method using different species, of varied hearing abilities and lifestyles, and also the same species in different contexts (for example habitat, time of year). The type of playback signature, duration and repetition could also be further investigated, for example to expose schools to shipping and airgun noise. This would allow the production of dose response curves for more species exposed to a wider range of playback signatures. A repetition of Chapter 3, as per the outlined recommendations, would ensure more successful data collection.

Future work could also extend to investigate the efficacy of mitigation measures, such as ramp-up, and also investigate whether habituation occurs after a certain period of continuous exposure, e.g. Chapman and Hawkins (1969) and Knudsen *et al.* (1992). A repetition of the above using an actual anthropogenic source such as a pile driver or airgun array would also be valuable, since the current sound projector array, whilst replicating a signature in the far field accurately, did not seek to mimic the signature in the near field or reproduce the substrate-borne energy. By reproducing the energy within the seabed in addition to the water column, benthic animals sensitive to such motion could be exposed. A more advanced 3D sonar observation system could also be used to observe the internal structure of schools in response to noise (Gerlotto *et al.*, 2004; Brehmer *et al.*, 2007; Jorgenson and Gyselman, 2009; Paramo *et al.*, 2010).

To validate the results of Chapters 4 and 5, the sensitivity and behavioural tests should be repeated in the field under free-field conditions, using a variety of substrata and environmental conditions. Since there are few data on this subject, future work must seek to understand the sensitivity of other benthic invertebrates to vibration, and couple this to field measurements of anthropogenic (and natural) vibrations. Further work could also involve the exposure of these species, and other species, to actual anthropogenic sources that create strong vibrations, for example pile driving, in a variety of situational contexts and seabed compositions.

The experiments of Chapter 5 indicate that anthropogenic vibrations may have a detrimental effect upon mussel behaviour by affecting valve closure and opening (and therefore feeding and regulatory processes). Future work could explore this further by studying mussel beds in the wild,

and in mussel farms. It is clearly of commercial importance to understand the implications of nearby vibratory sources to bivalve beds, particularly within the context of their role as bioindicators. In a similar way, it may be that vibration affects the behaviour of key commercial crustacean species and therefore should be considered.

6.9 Recommendations to the wider research field

There is a clear need for studies which expose invertebrates both to acoustic and vibratory stimuli. The current work did not aim to expose invertebrates to acoustic stimuli but it is clear from a review of the literature, and from observing new published works emerging in the field, that future work on invertebrates must seek to quantify exposure stimuli in terms of particle motion, which is likely to be the appropriate stimuli (Breithaupt and Tautz, 1990; Goodall *et al.*, 1990; Popper *et al.*, 2001). Work in laboratory tanks, whist valuable in some respects, cannot claim to be representative of natural conditions and thus un-constrained behaviour or claim to be representative of the original acoustic signature (Parvulescu, 1964b;a; Rogers, 2015), and studies such as this must be viewed with great caution. In the field, acoustic studies on invertebrates must fully measure the sound field in terms of particle motion (water and substrate), and pressure, if the effects are to ever be fully understood. These considerations also apply to experimental work involving fishes sensitive to predominantly particle motion.

Most notably there is a necessity for more behavioural studies with fishes and invertebrates such as the current work. In terms of behavioural reactions, the extent to which noise affects migratory patterns, feeding, reproduction, communication, predator-prey interactions and navigation is relatively unknown. More direct observations of animal reactions in the wild are required to examine naïve fishes which have not been affected by the trauma of capture or handling. It is of note that the current work was a logistical challenge at all stages and the difficulties of such work cannot be underestimated. The difficulties of the field are reflected in the challenge of deriving the indicators and standards for the inclusion of noise in the MSFD.

Development of an IBM approach which incorporated the results here was beyond the scope of this work, but would be valuable to the field. More long-term behavioural studies would also be advised, which could attempt to bridge the gap between individual behavioural changes and population level effects.

More generally, the need for exposure studies in field conditions, using fully quantified noise sources (real and playback), and unobtrusive observations mechanisms cannot be over emphasised. Measurements of anthropogenic sources must include substrate and water-borne particle motion, in addition to pressure. Metrics must be used appropriate to the source type. Such measurements should also be taken of the variety of methods within each source type, for example different diameter piles, different hammer types, varied substrate types to enable a full understanding of sound and vibration propagation in the marine environment. Investigations of hearing sensitivities must be undertaken in appropriate conditions as outlined by Ladich and Fay (2013). Playback studies should consider the conditions of a ‘good playback’, and consider the logistical difficulties that such work encounters (Chapter 1, as described by Chapters 2 and 3) if they are to be valuable to the field. Data from the current work, in addition to data from other

studies, may then be incorporated into conceptual models such as the one proposed in this chapter, to link individual behavioural changes to population implications.

6.10 Conclusions

With a combination of laboratory and field work in appropriate and well described vibroacoustic conditions, the current behavioural study has provided acoustic dose response curves for two key schooling pelagic fish species and vibration sensitivity curves for two key benthic invertebrate species. For example we now know that an impulsive signature of 163.2 – 163.3 dB re 1 µPa peak-to-peak is likely to elicit a 50% level of response in sprat and mackerel, mussels and hermit crabs will demonstrate behavioural changes to low frequency vibration within 0.05 – 0.55 m s^{-2}, and that the 50% level of response is 0.53 m s^{-2} for mussels. These values were previously unknown. In addition to this, the combined field-laboratory approach and the technical aspects such as the quality of the sound projector array, use of motion analysis to characterise behavioural reactions and the exposure of marine invertebrates to vibration are a new contribution to the field.

The dose response data may be now directly assembled into conceptual and population level models to allow the prediction of the effects of impulsive sound upon these two schooling fish species, for which data are currently lacking. The preliminary BRUV results provide an indication of behavioural responses to impulsive sound, and a framework for future methodologies. This will allow preliminary steps towards mitigation of such sources.

The vibration sensitivity data are an important first step towards understanding the effects of seabed vibration upon the benthic species described, something relatively unstudied. Moreover, the collation of vibration data from a range of literature allows the comparison of sensitivities to those encountered as a result of human influence. By doing so, the current work hopes to highlight the importance of substrate-borne vibration within the assessment of noise sources, allowing it to be considered as of the same importance to water-borne energy.

However, a word of caution must be included: the extrapolation of the results of this work to different species, environmental conditions and exposure sources is not yet possible. The response of a marine organism to a vibroacoustic stimulus may be a result of previous experience, the time of year, motivational state, physiology and detection ability. The results in the current work provide an indication of how particular species react within the given context, but to extrapolate beyond that would be inappropriate, more detailed studies are required before, for example, behavioural exposure criteria could be fully informed.

In conclusion, the results from the current work, together with the recommendations for future work, will be important to aid the filling of the ‘information gaps’ that exist within the underwater bioacoustics field (Hawkins *et al.*, 2014a), with the principal aim being to relate exposure levels to specific behavioural responses allowing the informing of individual-based and population level forecasts and mitigation strategies. The data may also aid the understanding about whether noise is a contaminant (i.e. - is present in the environment) or is a true pollutant (i.e. - produces a consistent biological effect) in the marine environment. This will allow us to understand, assess and control the far reaching impact of man-made sound and vibration upon the marine ecological system.

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Appendix

Additional Tables

Table A.1 Multinomial logistic regression results using type of response and depth. Density and dispersal data were grouped for mackerel due to lack of replicates for this analysis. Significance is represented by asterisks (* p<0.05, ** p<0.01).

Type of response	Group[a]	Predictor	B (SE)	95% CI for odds ratio		
				Lower	Odds ratio	Upper
Density	B	Intercept	-17.46 (6.50)			
		Depth from bottom	-0.06 (0.05)	0.86	0.95	1.04
Dispersal	B	Intercept	-25.88 (7.59)			
		Depth from bottom	-0.07 (0.06)	0.83	0.93	1.04
Density with dispersal	C	Intercept	25.14 (13.56)			
		Depth from bottom	-0.29 (0.15)*	0.56	0.75	1.01
			-14.16 (7.85)			
Depth change	B	Intercept				
		Depth from bottom	0.00 (0.05)	0.90	1.00	1.11
	C	Intercept	-27.29 (11.57)			
		Depth from bottom	-0.06 (0.07)	0.83	0.95	1.08

[a] Abbreviations as defined in Table 2.1

Table A.2 Multinomial logistic regression results using type of response and time of day. Density and dispersal data were grouped for mackerel due to lack of replicates for this analysis. Significance is represented by asterisks (* p<0.05, ** p<0.01).

Type of response	Group[a]		B (SE)	95% CI for odds ratio		
				Lower	Odds ratio	Upper
Density	B	Intercept	-17.46 (6.50)			
		Time of day	-.19 (0.16)	0.88	1.20	1.63
Dispersal	B	Intercept	-.19 (0.16)	0.88	1.20	1.63
		Time of day	-0.07 (0.15)	0.70	0.94	1.25
Density with dispersal	C	Intercept	25.14 (13.56)*			
		Time of day	0.24 (0.16)	0.93	1.27	1.75
Depth change	B	Intercept	-14.16 (7.85)			
		Time of day	-0.09 (0.17)	0.66	0.92	1.29
	C	Intercept	-27.29 (11.57)			
		Time of day	-0.18 (0.15)	0.62	0.84	1.12

[a] Abbreviations as defined in Table 2.1

Table A.3 Summary table of all notable BRUV trials undertaken during the three year period, with dates, locations, experimental equipment and logistical issues encountered.

Year	Date	Location	Setup	Problem	Results
2011	27.5 - 12.6		Prototype BRUV system	Projector array unavailable (in development)	n/a
	19.8 - 27.8	Lough Hyne		None	
2012	17.2 - 24.2		Two large speakers Small frame, two cameras with subsea central pod.	Equipment issues	n/a
	15.3 - 16.3		BRUV frame with central housing	Zero visibility	
		Bridlington	Wireless buoy Two large speakers		n/a
	16.4	Blyth		Zero visibility, no fish	
	6.8		Large frame	No fishes	9 deployments
		Blyth	Wireless buoy Two large speakers		
	4.12	South Landing	BRUV, two cameras plus umbilical to surface	Zero visibility	
	20 - 27.10	Lough Hyne	2 – 4 small speakers, BRUV and additional camera tripod	n/a	21 hours of experimental footage
2013	2.7-7.7, 9.7-13.7	Blyth (twice)		Bad weather	
	14.5	Blyth Harbour		Zero visibility	
	1.3 (Multiple)	Bridlington	Four small speakers BRUV as before	Zero visibility Equipment malfunction	n/a
	26.6 -28.6			Zero visibility Equipment malfunction	
	7.8-14.8	Plymouth Sound		Recording pod malfunction levels unknown	18 hours of experimental footage

Table A.4 Summary of experimental conditions at each station during the summer 2013 Plymouth trials. Sounds were presented at the top level, denoted by 0 dB, and then incrementally downwards in 5dB steps. Pollack (P), cuckoo wrasse (CW), goldsinny wrasse (GW), wrasse sp. (W), spotted catshark (CS), pouting (PG), squid (S), ballan wrasse (BW), conger eel (E)

Date	Station	Depth (m)	Video duration (mins)	Distance to speaker (m)	Sound (dB)	Number of playbacks	Species present
08.02.13	1	11.8	73	7-10	0	3	P
	2	13.2	103	10	0	11	P, CW, GW, W
	3	15	21	10	0	2	P, GW
09.07.13	4	19.2	70	10	0	4	CW
	4	19.2		10	-10	2	P, CW
	4	19.2		10	-30	1	P
	4	19.2		10	-25	1	CW
	5	21.4	28	10	0	4	P, CW, CS,
	5	21.4		10	-10	1	CW
	5	21.4		10	-15	1	CW
	5	21.4		10	-20	1	CW, CS
	5	21.4		10	-25	1	CS
	5	21.4		10	-30	1	CS
	6	17	9	10	0	3	CW
10.07.13	7	20	72	10	0	3	P,CW
	7	20		10	0	1	CW, PG
	7	20		10	-15	1	CW, PG
	7	20		10	-15	1	CW
	7	20		10	-20	1	CW
	7	20		10	-25	1	CW
	7	20		10	-30	1	CW
	8	21	37	10	0	3	P, CW
	8	21		10	-15	1	CW
11.07.13	9	19.3	26	7-10	0	1	S
	10	14.3	48	10	0	1	GW
	11	7	11	10	0	2	GW
	11	7		10	0	2	P, GW
	11	7		10	-10	1	GW
12.07.13	12	17	44	10	0	20	CW, BW
	13	~17	62	10	0	9	P, CW
	14	~17	110	10	0	20	P, CW
	15	~17	61	10	0	6	CW
	16	13.3	25	10	0	11	P, CW, BW
13.07.13	17	20.4	52	<10	0	5	CW
	18	20.4	60	<10	0	1	P
	19	24	51	13	0	20	P, CW, PG, E

Table A.5 Summary of behavioural responses to noise seen during the Plymouth 2013 summer trails. MON-moved out of frame, didn't return; MORI-moved out of frame with immediate return; MOR2-8, moved out of frame, returned within 2-8 minutes; PI-paused and immediately resumed behaviour; NR-no reaction; CTG – continued foraging behaviour; CN-continued normal behaviour/resumed behaviour; UR-unknown reaction. Pollack (P), cuckoo wrasse (CW), goldsinny wrasse (GW), wrasse sp. (W), spotted catshark (CS), pouting (PG), squid (S), ballan wrasse (BW), conger eel (E).

Station	Depth (m)	Sound (db)	Species	Immediate reaction	Delayed reaction <10 mins	Behaviour attribute	Disturbance
12	17.0	0	CW	body spasm	body spasm	body spasm occurs but fish forages as normal	none
12	17.0	0	CW	body spasm	body spasm	body spasm occurs but fish forages as normal	none
12	17.0	0	CW	body spasm	body spasm	body spasm occurs but fish forages as normal	none
8	21.0	0	P	MON	UR	swam slowly out of frame	none
9	19.3	0	S	MON	UR	not enough video to ascertain	none
11	7.0	0	P	MON	UR	swam slowly out of frame	none
4	19.2	25	CW	MOR2	CN	CFG	none
5	21.4	0	CW	MOR2	CN	CFG	mackerel
8	21.0	0		MOR2	CN	CFG	none
8	21.0	0	CW	MOR2	CN	CFG	none
8	21.0	0	CW	MOR2	CN	CFG	none
12	17.0	0	CW	MOR2	CN	CFG	none
12	17.0	0	CW	MOR2	CN	CFG	none
12	17.0	0	CW	MOR2	CN	CFG	none
12	17.0	0	CW	MOR2	CN	CFG	none
12	approx 17	0	CW	MOR2	CN	CFG	none
15	approx 17	0	CW	MOR2	CN	CFG	none
15	approx 17	0	CW	MOR2	CN	CFG	none
17	20.4	0	CW	MOR2	CN	CFG	boat traffic
17	20.4	0	CW	MOR3	CN	CFG	boat traffic
2	13.2	0	CW	MOR5	CN	CFG	none
2	13.2	0	CW	MOR5	CN	CFG	none
2	13.2	0	CW	MOR5	CN	CFG	none
15	approx 17	0	CW	MOR5	CN	continued coarse and speed	none
17	20.4	0	CW	MOR7	CN	continued territorial behaviour	none
11	7.0	0	GW	MOR8	CN	CFG	none
17	20.4	0	CW	MOR8	CN	continued territorial behaviour	none
12	17.0	0	CW	MORI	CN	CFG	none
12	17.0	0	CW	MORI	CN	continued feeding on bait	none
12	17.0	0	CW	MORI	CN	continued feeding on bait	none
12	17.0	0	CW	MORI	CN	continued feeding on bait	none
12	17.0	0	CW	MORI	CN	continued feeding on bait	none
12	17.0	0	CW	MORI	CN	continued foraging behaviour	none
13	approx 17	0	CW	MORI	CN	continued feeding on bait	none
13	approx 17	0	CW	MORI	CN	continued foraging behaviour	none
5	21.4	15	CW	PI	CN	continued feeding on bait	sail boat within 20m

Table A.6 Summary details from the BRUV Blyth sea trials 2012.

Trial	Date	Time start (UTC)	Time end (UTC)	Lat	long	Depth (m)	Habitat	Notes trial	Notes playback
1	06/08/2012	12:19	13:19	55.13685	-1.48991	6.0	Kelp	no fish	na
2	06/08/2012	13:42	14:41	55.13774	-1.49235	9.0	cobble reef at kelp's edge	small gadoids	na
3	06/08/2012	15:32	16:30	55.10708	-1.48348	12.0	sand	no fish	na
4	06/08/2012	16:42	55.11352	-1.46641	18.0		sand	drop down test	na
5	07/08/2012	10:38	12:10	55.13731	-1.49202	9.5	sand	no fish	playback tests
6	07/08/2012	13:05	13:22	55.10484	-1.48838	4.8	sand next to wreck	boat repositioning	na
7	07/08/2012	13:42	14:56	55.10575	-1.48845	5.6	sand next to wreck	flat fishes, crustaceans	playback
8	07/08/2012	16:01	17:16	55.08032	-1.45508	10.8	reef	no fish, lobster	playback
9	08/08/2012	10:44	11:34	55.13033	-1.48091	10.0	reef	no fish	small projectors test

Table A.7 Claw morphology of *Pagurus bernhardus* and shell morphology of occupied *Littorina sp.* Shells related to average lower and higher threshold values (across all frequencies). Statistical significance is represented by asterisks (* p<0.05, ** p<0.01).

Variable	Indicator 1 n = 15		Indicator 2 n= 10	
SH	0.33	0.229	0.03	0.921
SW	-0.04	0.88	-0.27	0.175
SA	-0.01	0.964	0.06	0.017
CW	0.53*	0.045	0.34	0.140
CL	0.34	0.223	0.36	0.122

Table A.8 Claw morphology of *P. bernhardus* and shell morphology of occupied *Littorina* sp. shells related to average threshold values (separated by frequency, thresholds calculated from two behavioural indicators), p values shown. Shell height (SH), Aperture (SA), Cheliped length (CL), Cheliped width (CW).

	Frequency (Hz)											
	Indicator 1							Indicator 2				
Variable	5	10	20	40	90	210	410	20	40	90	210	410
SH	0.43	0.38	0.25	0.33	0.25	0.58	0.83	0.93	0.27	0.07	0.75	0.73
SW	0.78	0.67	0.81	0.83	0.24	0.27	0.21	0.33	0.75	0.77	0.21	0.16
SA	0.48	0.08	0.24	0.29	0.68	0.71	0.40	0.81	0.52	0.26	0.28	0.30
CW	0.00	0.87	0.21	0.29	0.95	0.12	0.26	0.69	0.19	0.06	0.76	0.93
CL	0.10	0.90	0.39	0.18	0.53	0.35	0.16	-0.16	0.10	0.04	0.75	0.93

Table A.9 Correlation coefficients (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 (mm)	Width (mm)	Length*width ratio
5	0.58	0.26	0.65
10	0.42	0.32	0.77
20	0.82	0.82	0.57
40	0.92	0.84	0.33
90	0.82	0.42	0.50
210	0.71	0.86	0.81
410	0.39	0.1	0.62

Published works arising from this thesis

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Responses of free-living coastal pelagic fish to impulsive sounds

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The behavior of wild, pelagic fish in response to sound playback was observed with a sonar/echo sounder. Schools of sprat *Sprattus sprattus* and mackerel *Scomber scombrus* were examined at a quiet coastal location. The fish were exposed to a short sequence of repeated impulsive sounds, simulating the strikes from a pile driver, at different sound pressure levels. The incidence of behavioral responses increased with increasing sound level. Sprat schools were more likely to disperse and mackerel schools more likely to change depth. The sound pressure levels to which the fish schools responded on 50% of presentations were 163.2 and 163.3 dB re 1 μPa peak-to-peak, and the single strike sound exposure levels were 135.0 and 142.0 dB re 1 μPa^2 s, for sprat and mackerel, respectively, estimated from dose response curves. For sounds leading to mackerel responses, particle velocity levels were also estimated. The method of observation by means of a sonar/echo sounder proved successful in examining the behavior of unrestrained fish exposed to different sound levels. The technique may allow further testing of the relationship between responsiveness, sound level, and sound characteristics for different types of man-made sound, for a variety of fish species under varied conditions.
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I. INTRODUCTION

There is increasing interest in the impact of man-made sounds in the sea upon marine organisms, including fishes (Popper and Hastings, 2009; Boyd *et al.*, 2011; Normandeau Associates, 2012). Relevant sound-generating activities include dredging for aggregates, fishing, seismic exploration for oil and gas, marine construction activities, offshore renewable energy developments, the shipping of goods and passengers, the use of low and mid frequency sonar by navies, and military blast testing (National Research Council, 2005; Popper and Hawkins, 2012; Normandeau Associates, 2012). Some of the sounds generated may affect the lives of marine animals adversely and may reduce their capacity to perform normal life functions (Slabbekoorn *et al.*, 2010). A better understanding of the effects of man-made sound on marine fishes is required for assessing the impact of sound-generating activities and mitigating any deleterious effects upon fish populations.

Close to a source, where the sound energy is greatest, the impact on an aquatic animal such as a fish may include death or injury, physiological damage and temporary threshold shift (recently reviewed by Normandeau Associates, 2012). At greater distances from the source, where the signal is weaker but still audible, fish may show changes in their behavior. Different behavioral responses may occur, depending on the level of the sound, the level of ambient noise, what the fish is doing at the time, and previous experience of the same and other sounds. Whether a fish responds or not may also depend on its condition, motivational state, and the presence of other animals including predators (De Robertis and Handegard, 2013). Significant changes in behavior might include abandonment of spawning sites, movement away from preferred habitats, disruption of feeding, increased energy consumption, and diversion or delay of migrations.

Currently, there is an urgent requirement for information to support assessments of the impact of underwater noise upon fish. There is a particular need for studies that examine variation in levels of behavioral response in parallel with detailed characterization of the sound fields, ideally using a variety of different sound measurement metrics (Southall *et al.*, 2012; Normandeau Associates, 2012).

Sound exposure experiments have previously been conducted on fish held in tanks, cages, and large enclosures (Hassel *et al.*, 2004; Boeger *et al.*, 2006; Kastelein *et al.*, 2008; Doksæter *et al.*, 2012; Thomsen *et al.*, 2012). Such experiments can be valuable in allowing detailed observation of the responses of fish under controlled conditions. However, observations made on captive fish need to be supplemented by studies in the wild. Sound fields in small tanks and enclosures are dissimilar to those prevailing in the sea, often with severe distortion of the presented sound stimuli, as Parvulescu (1964) pointed out. In addition, captive fish may not show the wide range of behavioral responses

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observed in the wild. Fish tend to behave differently when enclosed than when their movements are unrestricted (Benhaïma *et al*., 2012; Oldfield, 2011) and especially if they have been bred in captivity (Balaa and Blouin-Demers, 2011). Marine fish brought into captivity may also be damaged during capture, or their behavior affected by the circumstances under which they were reared.

In this study, the responses of free-living pelagic fish to sound playback have been examined. The fishes encountered were predominantly sprat *Sprattus sprattus* (Family *Clupeidae*), and Atlantic mackerel *Scomber scombrus* (Family *Scombridae*). The sprat, like its close relative the Atlantic herring *Clupea harengus*, is thought to be especially sensitive to sounds by virtue of its specialized auditory system (Enger, 1967; Allen *et al*., 1976; Blaxter *et al*., 1981).

Sound playback experiments were carried out in an enclosed, quiet, coastal sea lough, where fish were not accustomed to heavy disturbance from shipping and other intense sound sources. The fish were exposed to synthetic, low frequency, impulsive sounds, mimicking some of the features of sounds produced by pile drivers and seismic airguns. Their behavior was observed with a sonar/echo sounder.

II. MATERIAL AND METHODS

A. Study area

The study was carried out in Lough Hyne, County Cork, on the southwest coast of Ireland (51° 30′ N, 9° 18′ W). The lough is sheltered by land and is connected with the sea through the narrow and shallow channel of Barloge Creek. The surface area of the lough is 0.5 km^2 with a maximum depth and volume of 49 m and 9.6×10^6 m^3, respectively. The freshwater catchment is relatively small, and salinity within the lough is similar to that of local coastal waters.

Lough Hyne was designated Europe's first Marine Nature Reserve in 1981 in order to protect its rich biodiversity. More than 70 species of fish have been recorded in the lough. Acoustic surveys for pelagic fish performed along a series of transect lines across the main basins have revealed the presence of a large resident biomass of sprat (Knudsen *et al*., 2009). The sprat form dense schools during the day, which break up and disperse at night (Hawkins *et al*., 2012). Mackerel, which are primarily visual feeders, prey upon the daytime aggregations of sprat.

Sound playback experiments were undertaken from 20–27 October 2012 and 17–23 March 2013, following previous operations to develop procedures for conducting the experiments. The majority of experiments were carried out during the day, but some sound presentations were also made at night.

B. Observing fish behavior

A sonar/echo sounder system (Humminbird 998c SI) was deployed from a surface vessel to observe the behavior of fish. The transducer produced sound pulses at ultrasonic frequencies (200 and 800 kHz), and the scattering from the fish and other organisms were examined to determine the location of these organisms relative to the vessel and the seabed. The sonar/echo sounder was mounted on a vertical wooden shaft and deployed beneath a 4 m long Rigid-hull Inflatable Boat (RIB). The device consisted of a downward looking, broad-beam (20°) echo sounder placed at 0.5 m depth, operating at 200 kHz, accompanied by a side-scan sonar directed to each side of the vessel and operating at 800 kHz. The side-scan sonar was also capable of being operated in a down view mode. The system was able to detect fish directly beneath and on either side of the vessel. Figure 1 shows the experimental arrangement.

The Humminbird 998c sonar was specifically designed to find fish and incorporated a geographic positioning system (GPS) with navigational capabilities. The echo sounder and side-scan data were recorded on an internal SD card for later analysis.

Sonar systems operate by registering the echoes received from submerged objects. Because of the time taken for sound to travel through water, the time of arrival of a reflected signal provides a measure of the distance of any sound reflector from the source/receiver. Those objects that scatter or reflect sound will be referred to here as reflectors. Where reflecting organisms like fish and zooplankters were gathered together they will be referred to as aggregations, or schools where they were identified as fish. The term target will be applied to single and aggregated reflectors exposed to sound playback.

The RIB was allowed to drift through areas where fish were present with the outboard engine switched off. GPS coordinates along the track of the RIB were saved and later exported to Google Earth to determine the position of each track within the lough. Echograms displayed on the echo sounder and side-scan sonar indicated depth against time (and distance traveled by the drifting vessel), with fish and other reflectors appearing above the seabed. The echograms were continuously recorded. A new echogram recording was created each time the boat moved to a new area and a new drift sequence begun. During October 2012, a thermocline

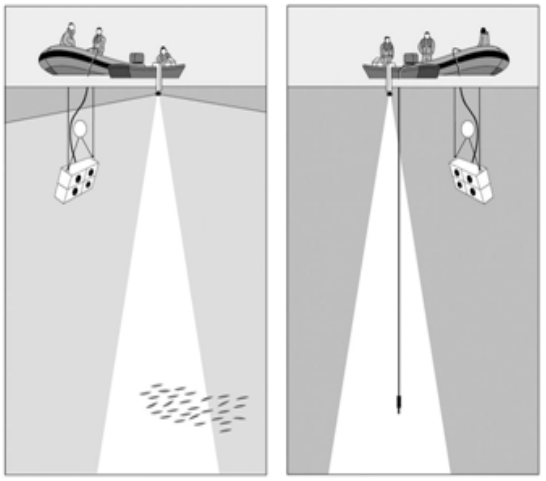

FIG. 1. A rigid-hull inflatable boat (RIB), with an outboard motor, and a small rowing boat were tethered together and allowed to drift without power. A sonar system was attached to the rowing boat to observe fish, and a sound projector array suspended from the RIB to transmit sounds.

was apparent at a depth of around 30 m, below which oxygen levels were low. Fish were not observed beneath the thermocline. Experiments were undertaken in calm sea conditions (Beaufort State Two and below) with the vessel drifting at slow speed (average speed 0.16 m/s).

C. Sound playback and monitoring

Sounds were presented from an array of four underwater sound projectors, custom made by Subacoustech Ltd. (type HPX15-100), specifically designed for broadcasting low frequency sounds to fish. The array is shown in Figs. 1 and 2. The projectors were bound together and slung beneath the RIB with the top of the projectors at a depth of 3–5 m. The projectors were connected to an InPhase IPX2400 2400 W car amplifier powered by a car battery, into which a signal was fed from either a Tascam model DR05 sound recorder, or an IBM Thinkpad laptop computer.

Each presented sound consisted of a sequence of identical low frequency pulses, repeated at regular intervals. To construct each sound pulse, white noise was filtered to give the same spectral and temporal characteristics as the impulsive sounds from impact pile driving (50 to 600 Hz with most energy at 200 Hz). A rapid onset followed by an exponential decay with a decay constant of 0.15 s was set to produce a pulse similar to that propagated in water from a single pile driver strike. A single pulse was called a "strike" and ten "strikes" were present in each sequence with a gap of 2 s between strikes. Six versions of the sound sequence were prepared to avoid pseudo replication (Hurlbert, 1984; McGregor, 1992). Six levels of each sound sequence were presented, in increments of 5 dB below the maximum level that the sound projector array could produce. At a distance of 5 m, the highest received sound pressure level was recorded as 171 dB re 1 μPa peak-to-peak. The maximum source level depended on the depth of the sound projectors and the water depth. It was estimated to be in the region of 185 dB *re*1 μPa peak-to-peak referred to 1 m, assuming spherical spreading, i.e., a transmission loss of 20log$R$. Playbacks of "silent" sound sequences (referred to as control trials) were randomly interspersed with sound exposure trials. The order of playback clips was fully randomized.

FIG. 2. The array of underwater sound projectors used for the playback of synthetic piling noise, with a 15 cm long ruler shown for scale.

Sound level measurements were made directly beneath the Humminbird sonar, in the center of its field of view, by means of a calibrated hydrophone (Reson, TC4014, sensitivity: −185 dB *re* 1 V for a sound pressure of 1 μPa, with a frequency range from 0.1 Hz to 400 kHz). The hydrophone was fully calibrated in February 2013, and the calibration checked before each series of experiments. The signal from the hydrophone was amplified by between 0 and 40 dB using a custom made battery powered amplifier, digitized using a National Instruments type 6062E data acquisition device at a sample rate of 350 000 samples per second, and stored on a laptop computer. Following sound presentations, the sound pressure levels (SPLs) were measured for each level of sound playback at different depths, and hence distances, from the sound projectors. Measurements were made over a bandwidth of 10 Hz to 20 kHz as peak-to-peak sound pressure levels (expressed as dB *re* 1 μPa). As the positive and negative peaks were of an almost identical magnitude, these measurements may be converted to peak (half peak to peak, or peak) levels by subtracting 6 dB. Sound levels were also measured as sound exposure levels (SEL), the time integral of the sound pressure squared, for a single pulse (expressed as dB re 1 μPa^2 s). Peak-to-peak particle velocity levels (expressed as dB re 1 m s$^{-1)}$ were estimated from sound pressure levels using the plane wave equation on the assumption that free field conditions prevailed. These estimates of particle velocity must be considered conservative, as close to the sea surface and to the sound source the actual values may have been rather higher.

D. Recording the presence and behavior of targets

Echograms were viewed using HUMVIEWER (version 67) software. The 200 kHz echo sounder display indicated the depth of acoustic reflectors in the water beneath the vessel, including the seabed, against time (and distance traveled) going from left to right (Fig. 3). The strength of the signal received from a reflector was indicated by its color on the echogram. The 800 kHz side-scan sonar display showed a view of the seabed and water column on either side of the vessel against time and distance, going from bottom to top. Any reflector that was judged to be of biological origin, suitable for sound playback, was called a target.

Times and locations of playbacks were marked on the recorded echograms using a waypoint tool, providing a reference point in physical space and time. Side-scan and down view displays (800 kHz) were inspected in addition to the 200 kHz echo sounder image to examine the behavior of targets. The waypoint mark was used to allow the depth of targets from the surface (m) to be measured at the time of sound playback, allowing the received sound level to be calculated from calibration measurements. The distance of the

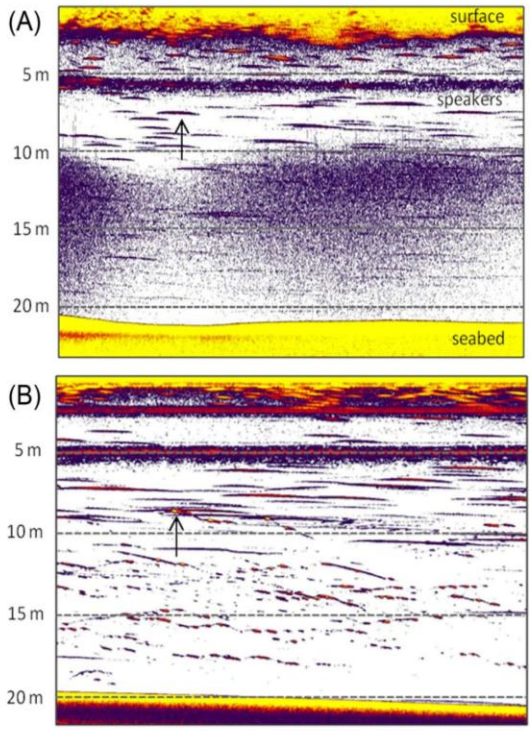

FIG. 3. (Color online) (A) Echogram from 22 October 2012 showing a Category A acoustic target identified as zooplankton. (B) Echogram from evening of 23 March showing Category D acoustic targets (individual reflectors). Some of these (the longer traces) were also encountered during daytime (see A above) and may be large zooplankton organisms. The shorter targets appeared at dusk with the breakup of sprat schools and were considered to be individual sprat. Note that there are also Category D acoustic targets (individual reflectors) above and within the diffuse layer (marked with arrows).

targets from the seabed (m) was also measured, to investigate whether responses were influence by the depth of water available for movement.

"False" trials were marked retrospectively on echograms where a target was observed but no playback had been undertaken. The timing of false trials was decided following the same criteria used for sound playback in the field. That is, a waypoint was recorded for a target at a time when sound exposure might have taken place. This procedure allowed comparison with control trials, where "silence" had been played, to investigate any influence from activation of the sound-producing equipment.

Two observers experienced in the interpretation of sonar records scored possible responses on the echograms (including control and false trials), undertaking this task separately, without knowledge of the trial conditions, and then together. A response to a trial was defined as a sudden change in depth, or echo strength of a target, or movement out of the sonar beam (cut-off) occurring during the trial. Any change in echo strength was indicated by a change in color on the echogram, and any depth change was defined as the top of the target changing in depth. Changes in echo strength could not be further quantified due to the relatively simple nature of the echo sounder being used, which did not allow raw target strength data to be accessed. Such a change was indicative of a change in density of the fish, or a change in their orientation (as target strength depends on the angle of incidence). A sharp cut off to the target indicated a dispersal of individual reflectors, which often re-appeared as a combined target at a different depth shortly afterward. Where necessary, side-scan and down view (800 kHz) echograms were also used to determine if responses had occurred.

Each response was described and later tabulated against the received sound level for different target categories. The data were scored with a zero (no response) or one (a response).

Different types of targets were observed and the data were therefore split into target categories for analysis (A–D) (Table I). Diffuse layers of very small reflectors observed in the South Basin close to the lough entrance (Category A targets) were not identified directly (Fig. 3), but Hawkins *et al.* (2012) had previously examined similar layers in the lough and reported that such layers consisted of zooplankton, including calanoid copepods, cladocerans, decapod larvae, gastropod larvae, and bivalve larvae.

Aggregations of small targets of varying density (Category B) were observed in the north and south basins of the lough. Previous studies of fish in the lough by means of sonar (Knudsen *et al.*, 2009; Hawkins *et al.*, 2012), together with sampling by rod and line during the playback studies and on other occasions, confirmed that these aggregations were schools of sprat. Examples of sprat schools are shown in Fig. 4. Under very calm weather conditions sprat schools were sometimes visible close to the sea surface, driven upward by predatory fish.

Less dense aggregations of large targets (Category C) were shown by rod and line fishing to be mackerel, with the occasional presence of other predators including Atlantic horse mackerel *Trachurus trachurus* and pollack *Pollachius pollachius*. Examples of the mackerel schools are shown in Fig. 4. The mackerel were reported by Hawkins *et al.* (2012) to be feeding on sprat, as evidenced by their close proximity to sprat schools and the presence of sprat in their stomachs.

Individual reflectors (Category D targets) that were observed at a range of depths during daytime were not identified. Oblique hauls with plankton nets in October 2012

TABLE I. Overview of the targets seen during the experiments, assigned into categories according to size, type and identification.

Target category	Description	Size classification
A	Diffuse layers of small reflectors identified as zooplankton	n/a
B	Aggregations of small reflectors identified as sprat	Small-Medium-Large
C	Aggregations of large reflectors, identified as mackerel	Small-Medium-Large
D	Individual reflectors, identified as zooplankters and fish from the breakdown of fish schools, identified as sprat	n/a

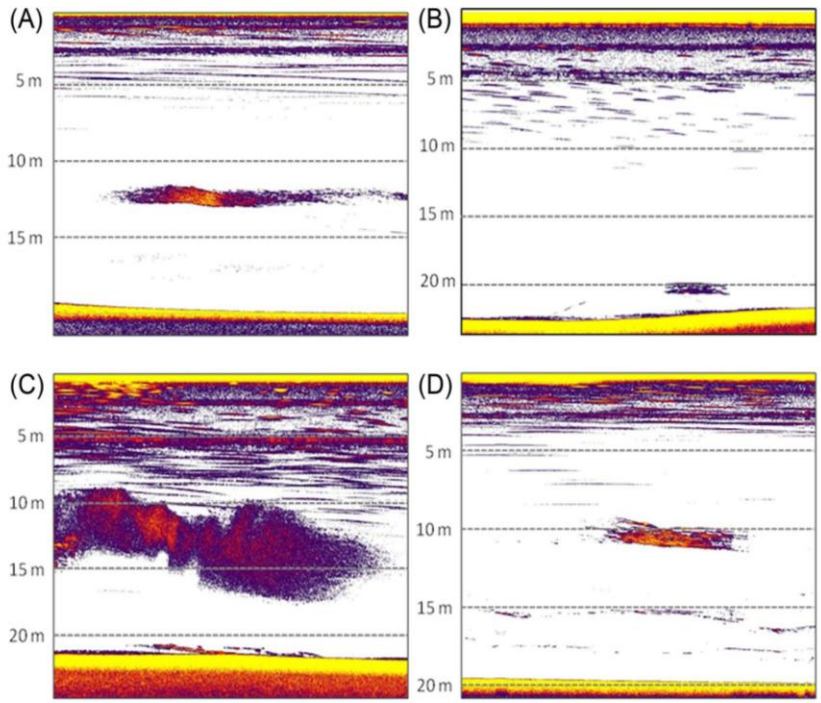

FIG. 4. (Color online) (A) Echogram from 25 October 2012 showing a [category B target (sprat)]. (B) Echogram from 19 March 2013 showing a small Category C acoustic target (mackerel). (C) Echogram from 25 October 2012 showing a large Category B aggregation. (D) Echogram from 24 October showing a medium sized Category C acoustic target (mackerel). There are Category D targets present in all the echograms.

captured many comb jellies (ctenophores), and these are the most likely source of these reflections. Other small individual reflectors, examined at night, were observed to be derived from the breakdown of Category B schools and were assumed to be predominantly sprat, although some individual mackerel and other species may also have been present. Examples of both types of single reflector are shown in Fig. 3.

E. Data analysis

Data were compiled as a single set (combining data from March and October) for the purpose of analysis. Further, different sound recordings (the six different sound files introduced to prevent pseudoreplication) were also grouped for the analysis, since their sound levels were identical. Only the two largest categories of target (categories B and C) were subjected to detailed analysis, since the other categories were either completely unresponsive (category D) or low in replicate numbers (category A). Subdivisions of the target categories made on the basis of size were not analyzed separately because the numbers of replicates were low. For similar reasons, seasonal differences in the responses were not investigated, although such differences might occur.

Initially, in order to investigate whether the equipment had an influence upon the aggregations, Fisher's exact test was used to compare responsiveness between false and control trials. There was no significant difference between the two trial types ($df = 1$, $p > 0.05$, $n = 99$) (Table II), suggesting that playback of a blank sound file through the system was having no greater effect upon targets than the presence of the drifting boat with inactive sound projectors. Control and false trials were combined for comparison with the sound exposure trials. Only 8 responses were recorded from the 99 control and false trials. Five of these responses were by type "C" aggregations. These active targets changed depth quite often even in the absence of sound playback.

Binary logistic regression was used to analyze response data (presence or absence of a response), using sound level, depth, and time of day as predictors. Multinomial logistic regression was used to analyze the type of response for the four target categories using received sound level, depth from the seabed (m) and time of day (decimal) as predictors. The reference category for the analysis was "no response." For category C targets data from two response types were grouped together due to low levels of replication. The results of both regressions were tabulated as the coefficient (B, the slope of the linear relationship), the intercept, the odds ratio (the odds of the association between exposure and outcome), and the 95% confidence limits (range) for this odds ratio.

To determine the level of sound that resulted in a specific level of response by the fish, the relationship between

TABLE II. Frequency of responses between control trials, where a clip of "silence" was played in the field, to "false" trials, which were added retrospectively.

Trial type	Response	No Response
Control	5	57
False	3	34

the received sound level and the incidence of response was examined. In toxicology, the dose response curve is used, which plots the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor. In the context of this study, the dose response curve provided a method of estimating the proportion of fish schools reacting at a particular received sound level. Dose curves were plotted using a cumulative normal distribution with nonlinear regression using ORIGINLAB (version 9.1). Although the rest of the data were analyzed using the linearity of logistic regression, the dose response curves were calculated in this way to enable comparison with data from other authors.

The dose response curves used the unweighted peak-to-peak sound pressure level (dB re 1 μPa), single strike sound exposure level (SEL) (dB re 1 μPa^2 s), and ten strike SEL (dB re 1 μPa^2 s). Additionally, peak-to-peak particle velocity (dB re: 1 m s^{-1}) and single strike particle velocity sound exposure level (dB re: 1 m^2 s^{-1}), calculated from the sound pressure measurements, were also estimated. It was assumed that the point of measurement was sufficiently distant from the source for the sound to be a plane wave and that the sound pressure was directly proportional to the particle velocity. These estimates of particle velocity must be considered conservative.

Most responses to sound playback occurred at the start of the sound, after a short period of latency, suggesting that the response was to the first strike. However, the cumulative sound exposure level for the whole sequence of ten strikes was included for completeness.

Category A and category D targets were omitted from dose response curve estimations, due to lack of responsiveness (D) and insufficient replicates (A). The lower and upper 95% confidence intervals of the response rate was calculated for both the category A and category B data sets for the SEL and peak to peak sound levels.

III. RESULTS

A. Acoustic observations

Over 13 days of observation, a total of 321 targets (either aggregations of fish or zooplankton, or individual fish) were examined, with 222 of these targets being exposed to sound playback. On average, 31 targets were encountered per day in October 2012 (sd = 12.50, n = 215) compared with 35.33 per day in March 2013 (sd = 36.35, n = 106).

Targets were present in both the North and South Basin of the lough, shown in Fig. 5. Diffuse layers of very small targets were also encountered close to the entrance to the lough, Fig. 5. Targets were found at a range of depths, from 4 to 35 m (measured to the top of the target).

B. Sound measurements

The sounds played back on the projectors were recorded and measured at a range of depths beneath the RIB following the sound exposure experiments. The time history of one such recording is shown in Fig. 6. The measurements were estimated for positions at the top of each target according to their depth in the water column,

In the first strike in the recording shown in Fig. 6, the maximum received instantaneous sound pressure was +167.6 Pa, and the minimum instantaneous sound pressure was −169.5 Pa, giving a peak-to-peak sound pressure level of 170.6 dB re 1 μPa over a bandwidth of 10 Hz to 20 kHz. The corresponding single strike SEL was 147.9 dB re 1 μPa^2 s. The received sounds from the sound projectors replicated the sharp onset and exponential decay of the pulse recorded from a typical pile strike at the appropriate distance (that is, at a distance that would yield sound pressure levels similar to those examined in this study). The rise time, defined as the time from the onset of the pulse to the time of peak pressure, was approximately 0.02 s, similar to that for seismic airgun pulses and piling strikes, again at an appropriate distance. It should be noted that much shorter rise times would be recorded very close to a seismic airgun or pile driver. The strike duration was 0.16 s longer than that for a piling strike, and 0.52 s longer than the pulse from a seismic airgun array recorded at a similar sound level to the simulated piling noise (Table III). In Fig. 7, spectra of the sounds received from the sound projectors are shown, compared to spectra from a piling strike and a seismic airgun pulse at similar sound levels. The sounds from the projector and the piling strike both have most of their sound energy concentrated in the region of 50 to 600 Hz, with energy present up to 900 Hz, although there is a slight peak in the projected sound at approximately 400 Hz. The seismic airgun pulse has a similar frequency range.

The sound levels presented to the fish were measured over the bandwidth of 10 Hz to 20 kHz. This band extends to higher frequencies than is strictly necessary for fishes, which detect sounds over a relatively narrow frequency range (recently reviewed by Popper and Fay, 2011). Very few fishes can detect frequencies above 5 kHz. However, the sounds being recorded were concentrated at frequencies below 1 kHz, and very little energy at higher frequencies contributed to the sound level measurements, despite the wide measurement bandwidth.

C. Responses to sound playback

The category A aggregations, identified as zooplankton layers, responded to the playback of sounds in a similar way on each presentation. A sudden "dent" in the top of the layer was seen at the onset of the sound sequence, although the change did not persist for the whole duration of the presentation (Fig. 8). It was evident that some components of the zooplankton responded to sound playback by changing depth, although the precise nature of this response requires closer examination.

Category B targets, the sprat schools, most commonly responded to sound exposure, following a short latency, with a complete cut-off of the acoustic target, resulting from lateral dispersal of the fish, taking them outside the sonar beam. The fish often then reappeared at a greater depth recombined into a school (Fig. 9). In some cases, there were also changes in the echo strength of the fish school, indicated

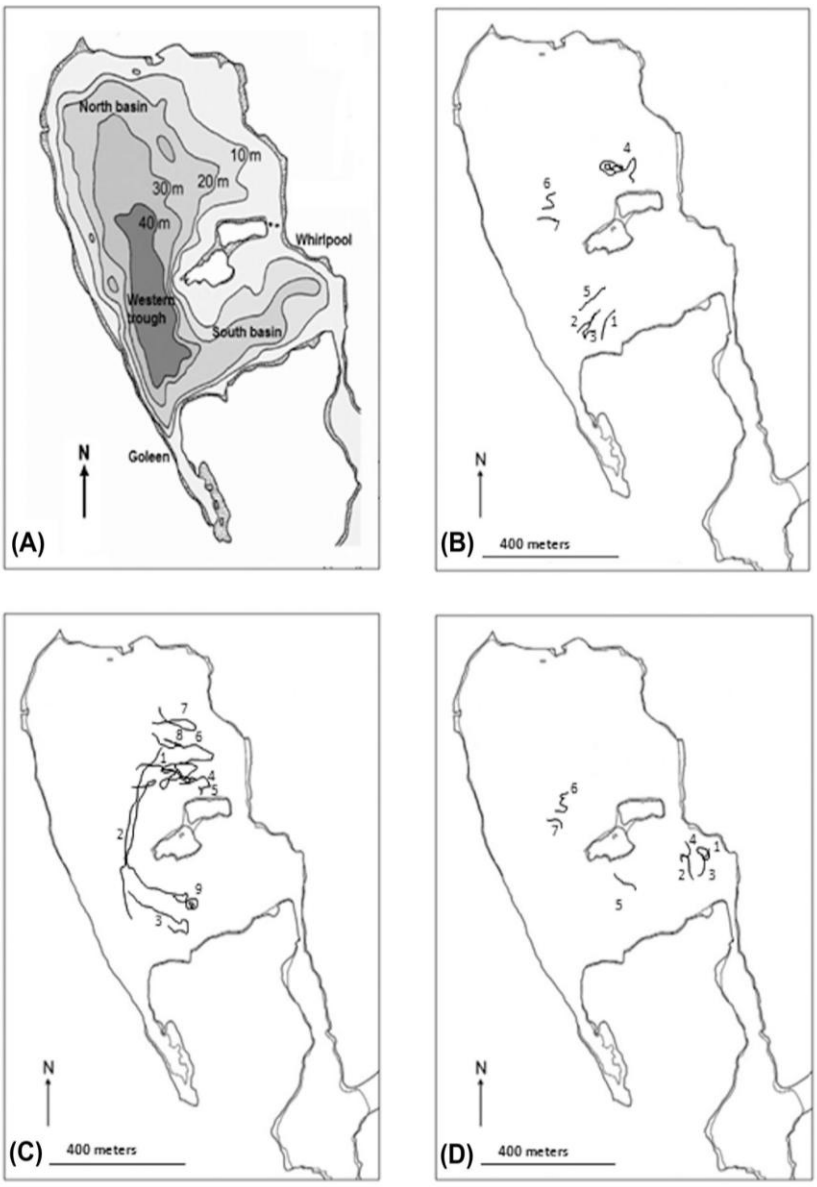

FIG. 5. (A) Depth contour map of the sampling site, Lough Hyne County Cork, on the southwest coast of Ireland. (B), (C), and (D) provide example boat tracks, marked from GPS positions recorded by the Humminbird sonar system. Boat tracks from the 22 October 2012 are labeled 1–5, and tracks from the 23 October are labeled 6–9. (D) shows the location of diffuse layers of very small targets encountered on two days of sampling (22 and 23 October 2012).

by a change in color of the target on the echogram. Some schools became less reflective and others more reflective. It is most likely that these changes represent differences in the density of fish within the schools, although they may also indicate changes in the orientation of the fish.

Category C targets, the aggregations of large reflectors identified as mackerel, responded by a complete cut off, a density change or a depth change (Fig. 10). The depth changes were often very rapid.

Individual targets, classed as category D, did not respond to sound playback (Fig. 10). Those observed at night appeared after the breakdown of sprat schools and were assumed to be predominantly sprat but perhaps also included individual mackerel and other fishes.

Responses by fish could be summarized as changes in the density of fish within a school, dispersal of the individual fish (cut-off) often followed their re-combination in a school at a different depth, or a depth change of the school without dispersal (Table IV).

Layers of zooplankton in the lough showed slight and short-lived changes in depth at the top of the layer in response to sound exposure (on seven out of nine presentations, Table IV). The latter responses occurred at received sound pressure levels ranging from 155.8 dB to 158.8 dB re 1 μPa peak-to-peak and received single strike sound exposure levels ranging from 131.3 to 140.9 dB re 1 μPa^2 s.

Logistic regression performed on response presence and absence data using sound level, depth, and time of day as

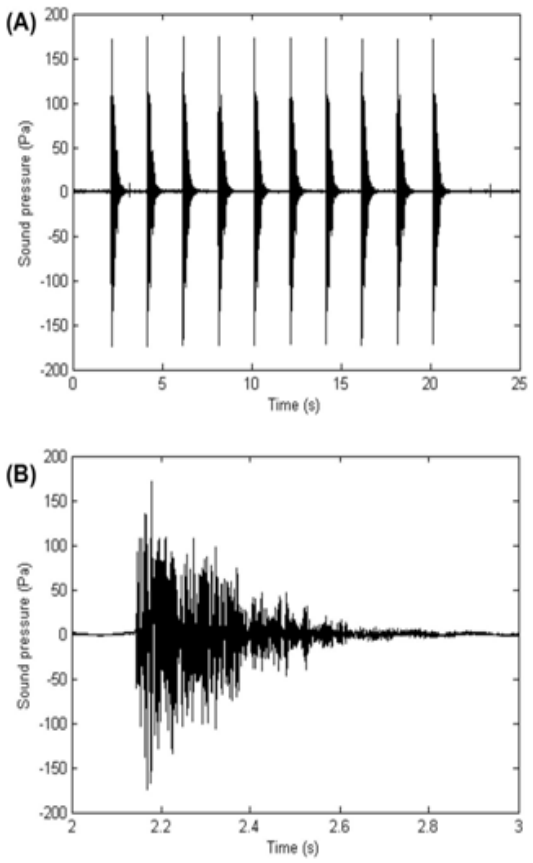

FIG. 6. Time history of sound pressure measurements for one complete 20 s playback of synthetic piling noise. (B) Expanded view of the first strike. These sound measurements were made 5 m below the sound projectors.

predictors, showed a significant ($p \leq 0.05$) increased likelihood of response with increased sound level, for both sprat and mackerel aggregations (Table V).

Multinomial logistic regression indicated that the *type* of response exhibited by both aggregation categories was significantly related to the level of sound, with the likelihood of a density change and a dispersal of the school increasing as sound level increased for sprat ($\chi^2 = 20.23$, $df = 3$, $p \leq 0.05$) and the likelihood of a depth change increasing with sound level for mackerel ($\chi^2 = 12.28$, $df = 3$, $p \leq 0.05$) (Table VI). Type of response was not significantly related to depth from the seabed (m) or time of day.

D. Dose response relationships

The 50% response levels; that is, the received sound levels, to which 50% of the category B and C schools responded, are presented in Table VII and Fig. 11. The received sound pressure levels of these response rates were estimated to be 163.2 dB re 1 μPa peak-to-peak for category B schools of sprat and 163.3 dB re 1 μPa peak-to-peak for category C schools of mackerel. In terms of received sound exposure level for a single strike, the 50% incidence level was estimated to be 135.0 dB re 1 μPa^2 s for sprat schools and 142.0 dB re 1 μPa^2 s for mackerel schools.

The lower 95% confidence interval exceeded a level of 0.5; this is the lower 95% confidence interval of the 50% response rate and is shown in Table VII. However, the upper 95% confidence interval does not reach a level of 0.5 in Fig. 11(A), 11(B), 11(C), or 11(D). This is particularly evident in Fig. 11(C), where the upper 95% confidence level only reaches a value of approximately 0.3. This means that the upper 95% confidence level of the 50% response rate could not be calculated for any set of data. Additional sound presentations at the highest sound levels would need to have been completed in order to improve confidence levels. This was not possible because the fish were typically deep in the water, distant from the speakers.

As the mackerel is likely to respond to particle motion, rather than sound pressure, the sound levels to which 50% of mackerel schools responded are also presented in terms of estimated particle velocity levels. The 50% response rate was reached at a peak-to-peak particle velocity level of -80.4 dB re 1 m s^{-1} and a single strike particle velocity sound exposure level of -101.7 dB re 1 m^2 s^{-1}.

IV. DISCUSSION

A. The responses of fish to sound playback

There have been few studies of the responses of wild free-living fish to the playback of sounds. It is difficult to detect wild fish, continue to stay in contact with them, and then observe their responses to sound playback. Maneuvering a surface vessel under power is itself likely to generate sound that will affect fish behavior. The experiments described in this paper were conducted from a silent vessel, drifting across fish targets with the engine switched off. Sounds were played back to fish at different levels and the responses observed. The very low incidence of response to control trials showed that responses by the observed targets to the presence of the boat and sound projector together with the operation of the sound playback system with a blank sound clip were minimal. The low level of response to false playback trials confirmed that the behavior described as a response by the fish rarely occurred in the absence of sound stimulation, or in response to the presence of the boat itself.

TABLE III. Specifications and characteristics of the simulated piling noise from this study and for two actual sound sources as shown in Fig. 7.

Source	Measurement range (m)	Specification	Water depth (m)	Rise time (s)	Strike Duration (s)	Peak-to-peak level (dB re 1 μPa)
Simulated piling	5	4 projectors	10–35	0.02	0.7	170.6
Seismic airgun	3700	Bolt Model 1900LL-X[a]	10	0.02	0.2	164.2
Piling	5000	4.7 m diameter[b]	7–24	0.03	0.5	167.6

[a]The airgun listed was charged to 60 bar, data reanalyzed from Parvin (2005).
[b]Data reanalyzed from Parvin and Nedwell (2006).

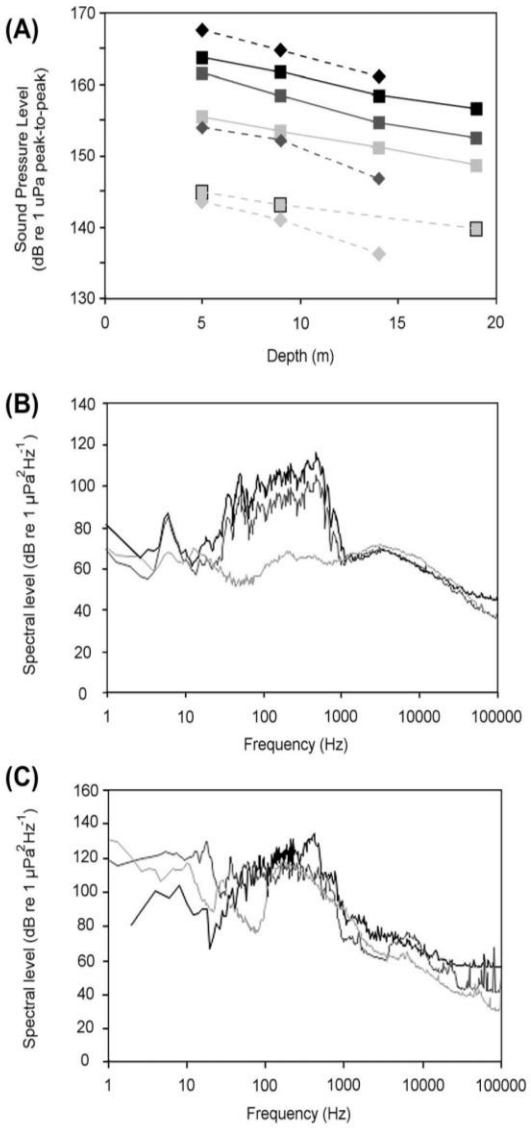

FIG. 7. Sound pressure level measurements made during sound playback. (A) Sound pressure levels at different depths below a sound projector at 2.5 m depth. Dashed lines indicate data from 23 October when the sound levels played were at maximum, −15 dB and −25 dB levels. Plain lines were from 27 October, when the sound levels used were at maximum, −5, −10, −15, and −20 dB levels. Note that the measurement of the sound at the −20 dB level on 27 October at a depth of 14 m was not distinguishable above the background noise. (B) Spectra of sounds played on 27 October during the trials. The plain black line is the maximum level of pulsed sound recorded at a depth of 5 m, the mid tone line is a level of pulsed sound 10 dB below the maximum recorded at a depth of 5 m, and the remaining line is the background noise. (C) Comparison of the simulated pile strike played during the trials (black line) with actual piling (mid-tone line) and airgun (light gray line) data, shown in Table III, reanalyzed from Parvin and Nedwell (2006) and Parvin (2005), respectively.

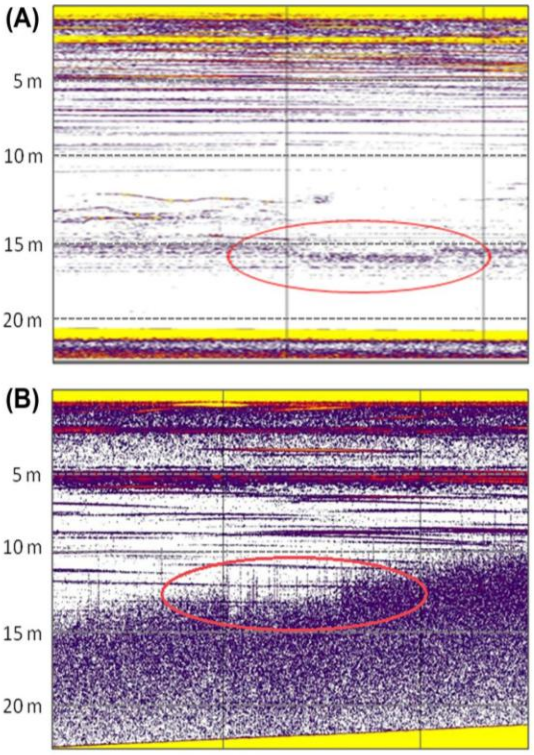

FIG. 8. (Color online) Example echograms showing responses of zooplankton layers to sound exposure. A clear "dent" is observed in the layer, circled. A vertical line indicates the beginning and end of each sound sequence.

That is, there were few false positives. The majority of false responses that were observed came from mackerel schools, which tended to show some vertical movement even in the absence of any stimulation.

Other workers have exposed marine fishes to sounds and noted their responses. Some of their experiments were performed in laboratory tanks, however, and the reported sound levels must be treated with caution. Blaxter and Hoss (1981) exposed captive Atlantic herring to 70–200 Hz sounds and obtained startle responses at received sound pressure levels of between 122 dB and 138 dB re 1 μPa. They observed that the response depended on the size of the fish. Kastelein *et al.* (2008) exposed captive marine fish of eight different species to long duration sounds at a range of frequencies in a large tank and reported behavioral response thresholds. Marked differences in response thresholds and in the frequencies that elicited reactions were obtained for the various fishes that were examined. For Atlantic herring, the 50% reaction threshold level was 30 dB above the estimated sound pressure hearing threshold level for this species (derived by Enger, 1967, from auditory evoked potentials). In controlled exposure experiments by Thomsen *et al.* (2012), Atlantic cod, *Gadus morhua*, and sole, *Solea solea*, held in large cages in the sea showed movements in response to the playback of impulsive sounds (recordings of waterborne pile driving sounds) at what were described as relatively low received sound pressure levels (sole 144 dB to 156 dB re 1 μPa peak; cod 140 dB to 161 dB re 1 μPa peak).

In the experiments at Lough Hyne, some free-living sprat schools responded to simulated pile driving sounds at a received sound pressure level as low as 140 dB re 1 μPa peak-to-peak (134 dB peak), while mackerel responded to a received sound pressure level of 143 dB re 1 μPa (137 dB peak-to-peak). The proportion of sprat and mackerel schools responding to sound playback increased at higher sound levels. For sprat, the received sound pressure level to which 50% of schools

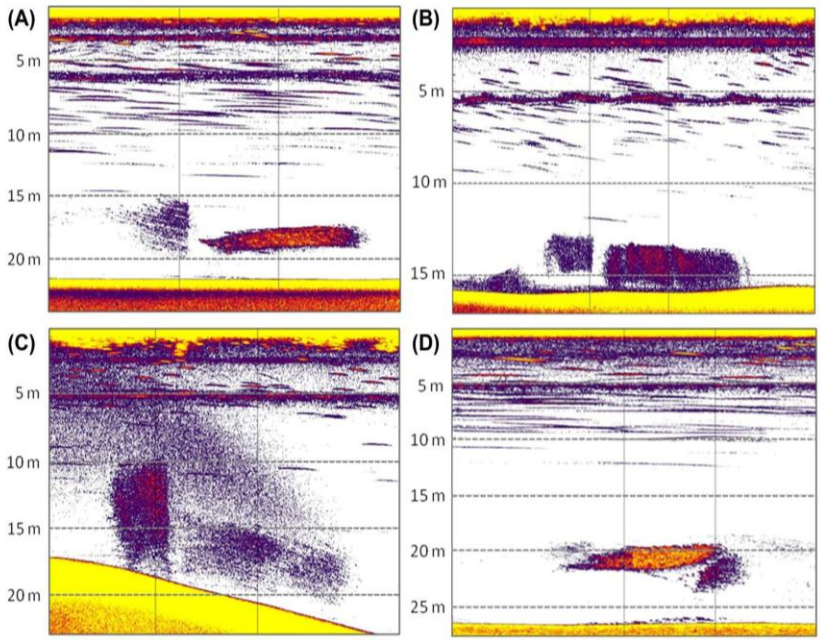

FIG. 9. (Color online) Echograms, showing typical responses of sprat schools to sound exposure. (A) Echogram of a Category B target, identified as a medium sized sprat school, cut off abruptly after the beginning of the sound, and reappearing a few seconds later as a denser school slightly closer to the seabed. (B) A medium sized sprat school cut off at the onset of the sound and reappearing seconds later slightly closer to the seabed. (C) A large sprat school cut off at the onset of the sound and reappearing at a greater depth at lower density. (D) A small sprat school increasing in density in response to sound exposure. A vertical line indicates the beginning and end of each sound sequence.

responded was 163.2 dB re 1 μPa peak-to-peak. For mackerel schools, the 50% response level was 163.3 dB re 1 μPa peak-to-peak. Zooplankton aggregations within the lough responded to received sound pressure levels ranging from 155.75 dB to 158.81 dB re 1 μPa peak-to-peak. Larger individual zooplankters (probably ctenophores and other coelenterates) observed during the day did not respond to sound playback. Moreover, individual fish from the breakdown of sprat schools observed at night did not respond to sound playback.

Slotte *et al.* (2004) exposed pelagic species of fish, including blue whiting (*Micromesistius poutassou*) and Norwegian spring-spawning Atlantic herring to sounds from

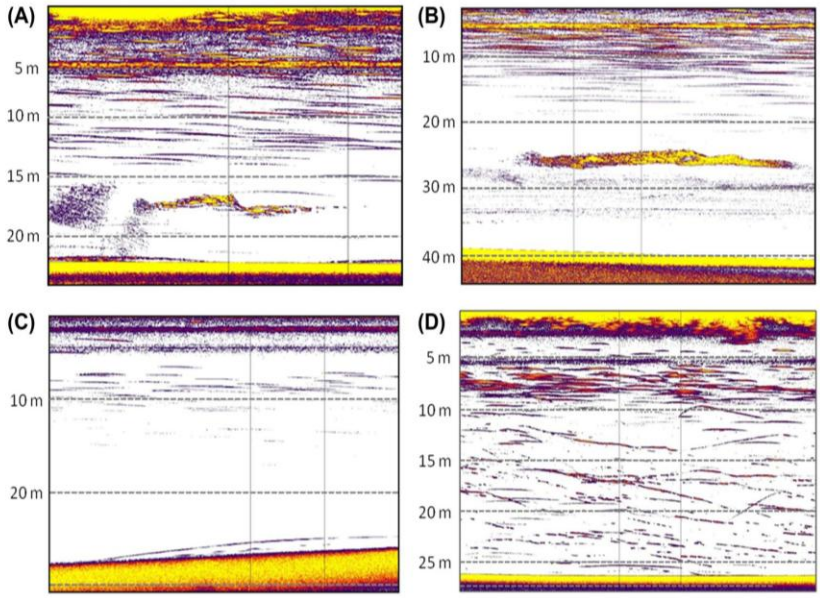

FIG. 10. (Color online) (A) Echogram showing an aggregation of large reflectors (Category C target), identified as mackerel, diving in response to sound exposure. (B) An echogram showing a Category C target, mackerel, exhibiting a change in density in response to playback. (C) Category D targets, identified as individual zooplankters, did not respond to sound playback. (D) Category D targets, identified as individual sprat, did not respond to sound playback. A vertical line indicates the beginning and end of each sound sequence.

TABLE IV. Frequency of occurrence of response type per target category, data for exposed schools only.

Category[a]	Total responses[b]	Density change	Dispersal and re-emergence	Depth change
A	7 (9)	0	0	7
B	49 (120)	21	18	10
C	16 (47)	5	2	12
D	n/a	0	0	0

[a]Abbreviations as defined in Table I.
[b]Figures in brackets give the number of presentations.

TABLE V. Binary logistic regression of response data using received SPL (re 1 μPa peak-to-peak), depth (m) and time of day (decimal) as predictors. Statistical significance is represented by asterisks (* $p \leq 0.05$, ** $p \leq 0.01$).

					95% CI for odds ratio		
Category[a]	n	Predictor	B (SE)		Lower	Odds ratio	Upper
B	164	Intercept	−18.57 **	(0.11)			
		Sound level	0.11 **	(0.03)	1.06	1.12	1.18
		Depth	0.95	(0.03)	0.89	0.95	1.01
		Time of day	0.04	(0.11)	0.85	1.04	1.28
C	74	Intercept	−25.33**	(9.11)			
		Sound level	0.16**	(0.06)	1.05	1.18	1.32
		Depth	−0.07	(0.07)	0.83	0.93	1.04
		Time of day	−0.05	(0.11)	0.77	0.95	1.18

[a]Abbreviations as defined in Table I.

seismic airguns, using echo sounders to observe the behavior of the schools. They reported that fish in the vicinity of the airguns appeared to swim to greater depths after airgun exposure. Moreover, the abundance of animals 30 to 50 km away from the area of sound exposure increased. Doksæter *et al.* (2012), using an upward looking echo sounder, reported that engine noise and impulsive sounds generated a typical avoidance response by captive herring in a sea pen, involving strong schooling, an increase in school density and a rapid downward movement of fish. In contrast, when sonar was used to observe behavioral responses in the Mackenzie River (Northwest Territories, Canada) the fishes, probably coregonids including *Coregonus nasus*, did not exhibit noticeable responses even when sound exposure levels (from a single discharge) were on the order of 175 dB re 1 μPa^2 s and sound pressure levels were over 200 dB re 1 μPa peak (Jorgensen and Gyselman, 2009). No herding or startle responses were observed from these fish.

There is some evidence that fish catches may be affected by exposure to seismic airguns. Engås *et al.* (1996) and Engås and Løkkeborg (2002) examined catch rate of haddock (*Melanogrammus aeglefinus*) and Atlantic cod exposed to a seismic survey. They found that there was a significant decline in catch rate of haddock and Atlantic cod that lasted for several days after termination of airgun use. It was concluded that the decline in catch rate resulted from fish moving away from the area as a result of sound exposure. More recently, Løkkeborg *et al.* (2012a,b) reported further experiments on catch rates and obtained data that could be interpreted as suggesting that seismic airguns may cause an increase in fish catches of some species by gill nets.

Peña *et al.* (2013) examined the real-time behavior of herring schools exposed to a full-scale seismic survey off the coast of northern Norway. No changes were observed in swimming speed, swimming direction, or school size that could be attributed to the airgun array as it approached from a distance of 27 to 2 km, over a 6 h period. The lack of a response to the seismic survey was described as "unexpected" and was interpreted as a combination of a strong motivation for feeding by the fish, a lack of suddenness of the airgun stimulus, and an increased level of tolerance to seismic shooting.

At Lough Hyne, the responses exhibited by both sprat and mackerel schools were dependent on the sound level. With increasing sound levels sprat schools were more likely to change density and/or disperse, whereas mackerel were more likely to exhibit depth change. It was also evident, however, that the responsiveness of sprat changed from day to night. Single sprat during the night did not respond at all to sound playback.

Hawkins *et al.* (2012) reported that sprat schools formed in Lough Hyne during the day, when mackerel preyed upon them. The schools rapidly depleted their surroundings of zooplankton at this time, suggesting that individual fish

TABLE VI. Multinomial logistic regression of response data using received SPL (re 1 μPa peak-to-peak) as a predictor. Density and dispersal data were grouped for mackerel due to low numbers of replicates. Statistical significance is represented by asterisks (* $p \leq 0.05$, ** $p \leq 0.01$).

						95% CI for odds ratio		
Response type	Category[a]	n	Predictor	B (SE)		Lower	Odds ratio	Upper
Density	B	164	Intercept	−17.46	(6.50)			
			Sound level	0.09*	(0.04)	1.01	1.09	1.18
Dispersal	B		Intercept	−25.88	(7.59)			
			Sound level	0.16**	(0.05)	1.08	1.18	1.29
Density with dispersal	C	74	Intercept	−25.14	(13.56)			
			Sound level	0.14	(0.09)	0.96	1.15	1.37
Depth change	B	164	Intercept	−14.16	(7.85)			
			Sound level	0.08	(0.05)	0.99	1.09	1.19
	C	74	Intercept	−27.29	(11.58)			
			Sound level	0.18**	(0.08)	1.04	1.20	1.39

[a]Abbreviations as defined in Table I.

TABLE VII. 50% response levels for sprat and mackerel, as taken from FIG 11. Particle velocity levels were calculated using the plane wave equation. The acoustic impedance of seawater was calculated using a density of 1.03×10^3 kg m^{-3} and a speed of sound at 10 °C and 35‰ salinity of 1489.82 m s^{-1} (Chen and Millero, 1977).

Species	Measure	95% CI lower	50% response level
Sprat	Peak-to-peak SPL[a]	159.1	163.2
	Single strike SEL[b]	123.2	135.0
	Cumulative SEL[b]	133.2	145.0
Mackerel	Peak-to-peak SPL	160.3	163.3
	Single strike SEL	130.7	142.0
	Cumulative SEL	140.7	152.0
	Peak-to-peak particle velocity level[c]	−83.4	−80.4
	Single strike particle velocity exposure level[d]	−113.0	−101.7

[a]dB re 1 μPa.
[b]dB re 1 μPa^{2}s.
[c]dB re 1 m s^{-1}.
[d]dB re 1 m^{2}s^{-1}.

within the schools were food-limited. At night, when predation by visual predators such as mackerel was greatly reduced, the sprat schools broke up and the individual sprat dispersed, perhaps allowing them to forage and feed more effectively. It would seem that during the day, when sprat are aggregated in schools as a defense against predation, they are especially sensitive to sound. At night, when they are pre-occupied with feeding, they no longer respond to extraneous sounds. As Peña *et al*. (2013) have suggested, there may be a trade-off by fish between the cost of lost feeding opportunity and the assessed risk of predation. These findings give emphasis to the importance of motivational state in determining whether fish respond to sounds. De Robertis and Handegard (2013) have remarked that reactions of fish to the sounds of approaching survey vessels are variable and difficult to predict. They have emphasized the importance of understanding how fish perceive the risk associated with particular sound stimuli and have suggested that many factors related to environmental conditions or the internal state of the organism, such as physiological state, parasite load, or exposure to predators, may affect decision-making.

Another factor that may influence the response of fish is the possibility that they may become accustomed to sound through repeated exposure. Chapman and Hawkins (1969) observed that repeated stimulation of a fish school with an airgun gradually resulted in habituation of the response. Although the fish initially dived to a greater depth they later ascended steadily through the water column despite continued stimulation. Peña *et al*. (2013) suggested that repeated exposure of fish to sounds in their study might have led to a weaker response or even a lack of response. Habituation to

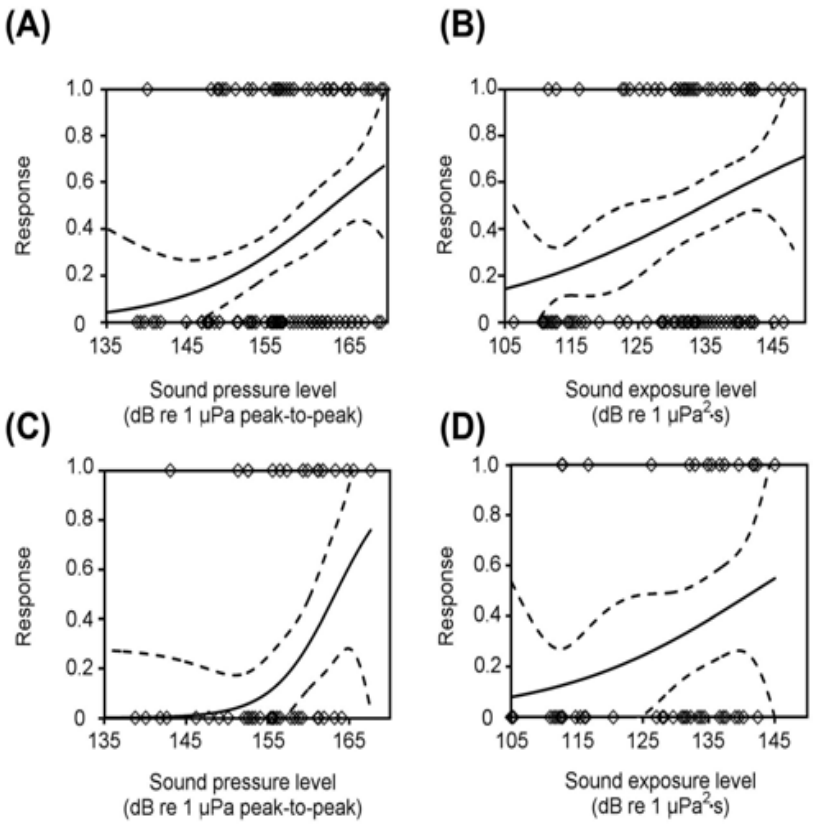

FIG. 11. Responses of targets to sound exposure. In each figure the solid line represents the non-linear regression fit to the data, and the dashed lines are the 95% confidence intervals. (A) Response of Category B targets (sprat) to received sound pressure measured as peak-to-peak levels. (B) Response of sprat to received sound measured as the single strike sound exposure level (SEL). (C) Response of Category C targets (mackerel) to received sound pressure measured as peak-to-peak levels. (D) Response of mackerel to the received sound measured as the single strike sound exposure level (SEL).

repeated stimulation by man-made sounds is not without consequences: It may result in the fish becoming less responsive to sounds from other sound sources, including approaching predators, posing real risk to their survival.

The experiments reported here were carried out in an enclosed area where the use of powered vessels was restricted. Care was also taken to avoid exposing the same schools to repeated stimulation by moving the vessel to a new position once a drift path had been completed. Neither the sprat schools nor the mackerel schools showed any evidence of a reduction in responsiveness with time. There is the possibility, however, that habituation might occur if the same school of fish was subjected to repeated stimulation with sound.

B. The significance of the responses observed

It was evident that both sprat and mackerel schools consistently showed strong responses to the playback of man-made sounds. It is important, however, to evaluate whether exposure of organisms to man-made sounds has any detrimental effects upon their populations, or upon the natural communities to which they belong (Francis and Barber, 2013).

In the case of sprat schools, responses involved an initial dispersal of what was previously a stable school, with the individual fish showing a greater degree of separation as they moved to greater depths. However, it was apparent on some occasions that the fish then re-assembled in a school. Similar dispersal responses by sprat schools were reported by Knudsen *et al*. (2009) and were accompanied by a release of gas bubbles from the swim bladders of the fish, which would affect their subsequent buoyancy. Such responses undoubtedly have a metabolic cost in terms of increased levels of activity and energy consumption. They may also reduce foraging and provisioning efficiency or induce physiological stress. Such disturbance has the potential to reduce body condition and affect reproductive success. In addition the response may expose the fish to higher levels of predation, affecting their survival. If we follow the suggestion from Frid and Dill (2002) that responses by animals to man-made disturbance follow the same economic principles used by prey encountering predators, then dispersal of a school incurs a cost, affecting other activities. Although a response to sound on a single occasion might not have a significant cost, repeated responses would be expected to have deleterious effects.

It has been suggested that school formation provides protection against predation through a variety of mechanisms including reducing encounter rates with predators, increased collective vigilance, and creation of predator confusion (Williams, 1966; Hamner and Hamner, 2000; Jeschke and Tollrian, 2007). When fish disperse or scatter they lose the benefit of being part of an aggregation. The observation that the fish may later re-aggregate suggests that there is a strong tendency for sprat to gather together, at least during the day, to preserve the protection that schooling conveys.

In the case of mackerel, the responses were less pronounced and generally consisted of a change in depth of the fish school, without significant scattering of individuals. Examination of the echo traces of mackerel not exposed to sound playback suggests that they are very mobile fish that frequently change depth as they move about in their search for prey. Changes in their behavior following sound exposure appear to be more transient and perhaps less likely to result in long-term consequences for the individual fish or their populations.

Closer study of the effects of disturbance of fish, in terms of the details of the actual behavior being exhibited and the bio-economic consequences of that behavior, is required if we are to understand fully the effects of exposure to man-made noise. Fish in different areas, at different times, with differing experience of previous sound exposure, may behave differently.

C. The relevant sound stimulus

The sounds transmitted in the experiments were intended to mimic the water-borne components of the sound propagating away from a pile driver. It is impossible in practice to fully simulate the sounds generated by a pile driver using an underwater sound projector. A real pile is driven into the substrate with considerable force, generating a seismic wave traveling outward through the seabed and capable of leaking back into the water for a short distance from the seabed (Hazelwood, 2012). However, unlike demersal fishes like the sole and cod, which may live close to the seabed, pelagic fishes like the sprat and mackerel generally live in mid water and will be exposed mainly to water-borne sound.

Comparison of the received sounds from the sound projectors with actual water-borne impulsive sounds from pile drivers showed that they had very similar rise times, although the pulse duration was slightly longer than that for real piling sounds. The sound spectrum was also similar to samples of piling and seismic airguns. Overall, the simulated impulsive sounds closely resembled the water-borne impulsive sounds from both pile drivers and seismic airguns at tens of kilometers distance from the source.

The water-borne sound pressure level from individual pile strikes may exceed 210 dB re 1 μPa peak-to-peak at 100 m from the source, and may, depending on propagation conditions, exceed 160 dB re 1 μPa peak-to-peak at a distance of 10 km. The response levels reported here suggest that sprats, mackerel, and zooplankton would show changes in their behavior at considerable distances—many kilometers—from a pile driving operation. The seismic airguns used in exploration for oil and gas also produce rather similar pulses of sound to those transmitted in this study. It is likely that these too will be detected, leading to changes in behavior by fish at considerable distances from the source.

An important factor in determining whether a particular species responds to a sound will be its hearing ability. Early experiments recording auditory evoked potential potentials (AEPs) from the ears of Atlantic herring in the laboratory suggested that hearing sensitivity in this species is acute (Enger, 1967). The clupeid fishes are thought to be especially sensitive to sounds by virtue of specialized gas-filled bullae in the head, associated with the ear, that enables them

to detect sound pressure (Enger, 1967; Allen *et al.*, 1976; Blaxter *et al.*, 1981). It has recently been pointed out that hearing thresholds based on AEP measurements often result in higher thresholds than those obtained from behavioral conditioning experiments (Ladich and Fay, 2013); behavioral conditioning studies of hearing have a face validity that AEP measurements lack. However, so far behavioral conditioning thresholds are lacking for this important group of fishes. It is difficult to determine hearing thresholds in sprat and herring using behavioral conditioning techniques because of their fragility in captivity. Data from other clupeids (Mann *et al.*, 2001; Mann *et al.*, 2005) have confirmed that their hearing bandwidth is wide, extending up to 5 kHz, compared with many other species of fish. It seems, however, that the herrings, sardines, and anchovies (Clupeinae) do not detect ultrasonic frequencies, unlike the closely related shads (Alosinae) (Mann *et al.*, 2005).

The hearing of Atlantic mackerel has not been studied, again because of the difficulties in maintaining this very active fish in captivity. The Atlantic mackerel lacks a swim bladder, an organ which serves as an accessory hearing organ in many other fishes and which enables them to detect sound pressure (Popper and Fay, 2011). Without a swim bladder, it is likely that the mackerel is sensitive to particle motion, as shown for the other species lacking swim bladders. Iversen (1969) examined hearing in a closely related scombrid fish lacking a swim bladder, the mackerel tuna *Euthynnus affinis*, and found that it was much less sensitive to sound than the yellowfin tuna *Thunnus albacares*, a species with a swim bladder.

Sprat and mackerel may represent two extremes in terms of their hearing abilities, although in the absence of carefully measured behavioral conditioning thresholds to sounds for either species, we can only speculate about the details of those differences. The sprat, like the Atlantic herring, may be especially sensitive to sounds over a wide frequency range compared with the mackerel. The latter is expected to follow the mackerel tuna (Iversen, 1969) in being less sensitive to sound and likely to respond only over a narrow frequency band. Remarkably, however, despite likely differences in their hearing abilities the sprat and mackerel responded in the playback experiments to impulsive sounds at rather similar sound levels. This may be the result of mackerel being more ready to respond to any stimulus—the results from Lough Hyne suggest that they are perhaps more "flighty" than sprat. It is also interesting that aggregations of zooplankton responded to similar sound levels, although they showed only limited short-lived changes in depth. The zooplankton aggregations in the lough are being preyed upon by the sprat (Hawkins *et al.*, 2012), and the sprat preyed upon by the mackerel, in what Herman Melville described as the "universal cannibalism of the sea." All three categories of aggregation responded to the playback of impulsive sounds at similar levels.

It was evident in observing the responses to sound by zooplankton layers and by sprat and mackerel schools that the responses occurred soon after onset of the sound, after a brief latent period. Essentially the animals were responding to receipt of the initial sound pulses. The received sound levels have been described in terms of the peak-to-peak sound pressure level and the single strike sound exposure level (Table VII), where the latter is a measure of the total sound energy within the pulse, calculated by integrating the square of the pressure waveform over the duration. It is not yet clear, however, which of the characteristics of impulsive sounds are especially important in evoking behavioral responses from animals. The peak excursion in sound pressure, the rise time, and the total energy in the pulse may all play a role. Further experiments are required to elucidate the relative importance of these different parameters in evoking a response. In the case of the mackerel, which may be sensitive to particle motion rather than sound pressure, the results have also been presented in terms of the peak-to-peak particle velocity level and the single strike particle velocity exposure level. Although the particle velocity is proportional to the sound pressure in a plane acoustic wave, under other circumstances the magnitude of particle velocity would be more difficult to predict. Thus, close to a sound source, within the near field, the magnitude of particle motion is higher for a given sound pressure. Close to the sea surface, which provides pressure release, the magnitude is also greater.

In setting sound exposure criteria for fish and other aquatic animals, it has become commonplace to present sounds in terms of their cumulative sound exposure level (see for example Southall *et al.*, 2007). The SEL for each pulse or sound event is aggregated to calculate the total SEL (or cumulative SEL) for the entire exposure duration. Values for the cumulative SEL have been presented in this paper for the sound stimuli presented to sprat and mackerel. But they may be of limited significance, given that the fish responded at the start of the sequence of pulses, before the sounds had accumulated. Moreover, as the response of the fish involved movement, the estimation of cumulative SEL should ideally take account of the changing distances from the source. Handegard *et al.* (2013) have recently considered the efficacy of different exposure metrics to explain the disturbance of fish by seismic surveys and have demonstrated the difficulties in applying cumulative sound energy metrics to disturbances in behavior. Further experiments are required to elucidate the relative importance of the different acoustical characteristics of impulsive sounds in evoking behavioral responses.

V. CONCLUSIONS

The use of sonar to observe fish in Lough Hyne proved very successful in enabling the responses of unrestrained fish schools to the playback of synthetic piling noise to be examined. The deployment of the Humminbird sonar/echo sounder system from a drifting boat provided a useful means for observing the behavior of fish in response to impulsive sounds. The responses of sprat and mackerel schools to sound playback were clear and varied according to the sound level.

Responses to impulsive sounds by both sprat and mackerel occurred at relatively low sound levels, similar to those recorded at several kilometers distance from an operating pile driver or seismic airgun. Currently, with the prospect of

increasing levels of noise being generated in the sea, a search is under way for clear sound exposure criteria to be applied in assessing the impact of man-made sounds upon fish (reviewed by Normandeau Associates, 2012). It is critical for regulatory agencies to gain knowledge of the levels of sounds that may be of potential harm to fish, as well as levels that are of little consequence. Developing these criteria poses problems, since they may have to be set for species that differ in their behavior and/or physiology, their location, the time of year, and their previous experience of sound exposure. Currently, there are almost no data on those sound levels that result in behavioral effects.

In this paper, data have been presented on the levels of impulsive sound to which sprat and mackerel respond. However, these data cannot yet be used to define the sound exposure criteria. More detailed studies of the behavior of these species are required to establish whether the responses observed are likely to result in adverse effects upon the survival of individuals. Examination of the effects of repeated exposure of the same fish to sound is also important. Does the behavior of the fish change, with responses less likely when sounds are repeated?

There is a need for further studies describing the behavioral responses of free-living fish to man-made sounds, since neither the short nor long term effects are well understood. The experiments described in this paper provide a first step toward investigating such responses. They have allowed examination of the relationship between the response and the sound level, expressed in different metrics. Such studies would be greatly facilitated by the use of a more sophisticated sonar system that is capable of examining the behavior of individual fish within an aggregation in three dimensions and that is able to measure changes in the target strength of the fish.

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Sensitivity of Crustaceans to Substrate Borne Vibration

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Key words: substrate vibration, sensitivity threshold, crustaceans, anthropogenic noise, vibration reception

Abstract

There is increasing interest in the responsiveness of crustaceans to vibrations, especially in the context of marine developments, where techniques such as pile driving create strong vibrations that are readily transmitted through the seabed. Experiments were undertaken under controlled conditions to investigate the sensitivity of unconditioned crustaceans to substrate borne vibration. Subjects were exposed to a range of frequencies and amplitudes using the staircase method of presentation to determine thresholds of response. Behavior varied according to the strength of the stimuli and included bursts of movement and rapid bouts of movement.

Introduction

In addition to the sound pressure variations accompanying transmission of a sound, there is also a back and forth motion of the component particles of the medium, the particle motion. It has been conjectured that crustaceans are responsive to particle motion rather than sound pressure (Budelmann, 1991, Goodall *et al.,* 1988, Breithaupt & Tautz, 1990, Popper *et al.*, 2001). Sound is widely produced by crustaceans (Schmitz, 2002) however the biological relevance of production is unclear, and their sensitivity to signals is relatively unknown compared to that of fish.

The particle motion component of a signal can propagate away from a source via the water column or the seabed (Nedwell *et al.*, 2003), or as a combination of both. In the sea bed, this energy can

be transmitted as compressional, shear, or surface waves (for example Rayleigh waves) (Aicher & Tautz, 1990), with the signal changing in terms of frequency and amplitude with attenuation (see Markl, 1983 for a comprehensive review of this topic). There is very little information on the ability of UK coastal crustaceans to detect these waves. For the purposes of this paper, the term vibration will refer to substrate borne particle motion (Hill, 2009).

Detection Mechanisms

There is evidence that detection of particle motion utilizes mechanoreceptors located in the joints, antennal flagellae, statocysts and appendages (Breithaupt & Tautz, 1990, Goodall *et al.*, 1988, Breithaupt & Tautz, 1988, Monteclaro *et al.*, 2010, Tautz & Sandeman, 1980). Particle motion is higher for a given sound pressure in the near field of a sound source, and it has been shown that *Nephrops norvegicus* (norway lobster) only responds to sound stimuli less than one meter away (Goodall *et al.,* 1988, Breithaupt & Tautz, 1990).

Methods for studying sensitivity involve the isolation of particular sensory detectors, for example the statocysts, thorax hairs, campaniform sensilla, antennules or chelae mechanoreceptive hairs (Barth, 1980, Tautz & Sandeman, 1980, Breithaupt & Tautz, 1988, Monteclaro *et al.*, 2010). However a full understanding of the sensitivity of the whole organisms requires observations on the behavioral responses to vibration. For example Goodall *et al.* (1988) observed that *N. norvegicus* responded to stimuli with clear postural changes (abdominal extension and claw waving). These were clear enough to test the threshold of response to water borne particle motion in the laboratory and the field. Heinisch and Wiese (1987) and Bergahahn *et al.* (1995) reported clear flicking of the second antennae of *Crangon crangon* (brown shrimp) in response to vibration. Antennal movements in crayfish *Orconectes limosus* (Spinycheek crayfish) have also been reported in response to a water borne stimulus (Tautz, 1987). Another cue utilised has been displacement of the walking legs (Breithaupt, 2002).

Sensitivity to Vibration

Other studies have focussed upon the semi-terrestrial fiddler crabs (*Uca sp.)* rather than marine species, as they use substrate vibrations to communicate during reproductive behavior (Aicher & Tautz, 1990). Thresholds of sensitivity have been determined using electrophysiological techniques (Salmon, 1973, Salmon & Hyatt, 1977, Aicher & Tautz, 1984) and behavioral observations (Salmon & Atsaides, 1969) or a combination of both (Salmon, 1971, Salmon & Hyatt, 1977). The sensitivity of the lobster *Homarus americanus* (American lobster) and *C. crangon* to vibrations has also been investigated (Offut, 1970, Heinisch & Wiese, 1987).

Data Collation

Response thresholds of *Pagurus bernhardus* (common hermit crab) to vibration were investigated in the current study. Hermit crabs were chosen due to the clear anti-predator mechanism (withdrawal) they exhibit under stressful conditions (Chan *et al.*, 2010). Thresholds were determined to substrate vibration at several frequencies using the staircase method of threshold determination described by Cornsweet (1962).

Thresholds to vibration by other crustacean species have been summarised from the literature and are compared with those for hermit crabs. Units of measurement are given as they were originally stated (in terms of particle acceleration or displacement) and are also converted into particle acceleration for comparison (marked with asterisks). Care must be taken when interpreting the results of these studies, since a wide range of techniques have been used. Values of sensitivity to water borne particle motion are not provided here, since this is not the focus of this paper.

Experimental Methods

Hermit crabs were kept in holding tanks at low densities (water temperature on average 11-12°) in an isolated cold room under a 12 hr light, 12 hr dark regime prior to experiments. The crabs were fed on a diet of mixed shellfish every two days, and starved for 48 hr prior to use. Experiments were carried out on individual crabs, in a plastic tank (51 cm x 41 cm), with water depth 31 cm and a sand substrate (depth 1.5 cm). The tank sat on a custom made base, built to minimize vibrations entering the tank from the ground. Each crab was acclimatized in the experimental tank overnight prior to threshold determination. The experimental tank incorporated a small custom made 'arena', within which the subject could freely move during the presentations.

Subjects were presented with sine waves in a range of 5- 400 Hz, at each of 11 different amplitudes. A Roland R-09HR MP3 recorder was used to play back the signals, and was connected to a car amplifier (JL Audio XD 200/2 200 W 2 channel) and an LDS v101 electromagnetic shaker. The shaker was mounted above the experimental tank on a separate frame from the base, with a custom made carbon fiber stinger rod descending vertically to the substrate.

Substrate vibrations in the vertical axis were recorded continuously with a waterproofed Bruel and Kjaer piezo-electric accelerometer (Type 4333, sensitivity 20.6 mV/g), connected to a battery powered Bruel and Kjaer charge amplifier type 2635, an ADInstrument Powerlab module and a laptop computer with Chart 5 software (v 5.5.6) installed. In later experiments a three dimensional waterproof geophone (SM-7 370 ohm, IO) was used to determine the vibrations in all three axis, connected to the same ADInstrument Powerlab module. Calibration measurements were taken at the end of experiments to measure the vibration inside the arena. This enabled the calculation of a correction factor for received vibrations inside the arena from the measurements taken next to the arena.

Thresholds were determined at each frequency by the staircase method (Cornsweet, 1962). The threshold was estimated as the amplitude of the stimulus which the animals reacted to on only 50% of the presentations, taken as an average of ten iterations. A TV camera was situated above the tank, connected to a small LCD screen which was situated on a table away from the experimental tank. The experimenter could then sit at distance without influencing the subjects behavior, and adjust the signal accordingly.

One crab was tested per day, with the order of frequency presentation randomized. Amplitudes of each frequency were presented two minutes apart, after preliminary tests indicated that reactions

lasted for only a few seconds after each stimuli ended. Between frequencies, a gap of 20 to 30 minutes was used to allow the subject to recover.

Results

Clear behavioral changes could be seen in response to vibrations. These ranged from a full or partial retraction into the shell at the highest signal amplitudes, down to a clear 'sweep' of the antennular flagellum at the lowest amplitudes, with other postures in between.

The highest sensitivity to vibration was measured at 10 Hz, with an average sensitivity value of 0.10 m s^{-2} (n = 10) in the vertical direction. A flat response curve was obtained overall, with sensitivities ranging between 0.1- 0.5 m s^{-2}, with the values at 100 and 200 Hz being slightly higher (thought to be due to a slight variation in the input signal). Background vibration levels on the vertical axis were in the region of 0.001 m s^{-2}. The stimulus itself was sinusoidal with typically greater than 85% of the energy at the desired frequency, and was strongest in the vertical axis.

Table 1 Thresholds of highest sensitivity to vibration for a variety of crustacean species. Units of measurement are given as originally stated (acceleration or displacement), those marked (astericks) have been converted.

Reference	Threshold (m s^{-2})	Threshold (µm)	Frequency (Hz)	Species	Method of determination
Aicher & Tautz (1984)	0.005		20	*Uca pugilator*	Electrophysiology
Bergahahn et al. (1995)	0.4*		20 – 200	*Crangon crangon*	Behavioral
Heinisch & Wiese (1987)	0.81	0.7	170	*Crangon crangon*	Behavioral
Salmon & Atsaides (1969)	0.067*	0.03	400	*Uca pugilator*	Behavioral
Salmon (1973)	0.0175*		50	*Uca minax*	Behavioral
Salmon (1971)	0.04 0.06		30 60	*Uca pugilator* *Uca rapax*	Behavioral and electrophysiology
Barth (1980)	0.0002	0.4	20-20 100-130	*Carcinus maenus*	Electrophysiology

The highest sensitivity of 0.1 m s^{-2} at 10 Hz is in the region of previously reported sensitivities to vibration, for example Heinisch and Wiese reported a threshold of 0.81 m s^{-2} for *C. crangon* (Heinisch & Wiese, 1987). Other threshold values from the literature are shown in table 1.

Discussion

The data presented shows that the majority of the thresholds fall below 200 Hz, that is the crustaceans examined appear to be most sensitive to low frequencies, which are likely to be within the range of biological signals (Hill, 2009). Detection of low frequency vibrations may be useful for prey location, predator detection, reproductive display, communication and advertisement as seen

in terrestrial organisms such as insects and scorpions (Hill, 2009). However evidence for these uses has only been seen in the semi terrestrial *Uca sp.* This degree of sensitivity raises the question of whether manmade vibrations, generated by pile-drivers, seismic air guns or operating wind turbines, may also be detected. Unfortunately, despite their importance for evaluating effects, few data are available on the levels of substrate vibration produced by anthropogenic activities. There have been recent attempts to measure and model vibrations such as pile driving (Hazelwood, 2012; Hazelwood and Macey- this volume), but field measurements are required before the effects of these vibrations upon crustaceans can be fully understood and predicted.

In this study the vibratory stimulus was presented using a stinger rod connected to the substrate of a tank. This may not be representative of a typical anthropogenic source of vibration, such as an impact pile driver, since the vibration may propagate through the substrate in a number of different ways. Whilst the tank setup is far from perfect, in light of the paucity of data on this subject, these experiments provide an important first step in investigation the effects of man-made sources of vibration upon bottom-living crustaceans. Ideally a special tank, able to recreate the full range of substrate waves should be used for such experiments. Moreover, it is important to consider the effects on behavior of different waveforms, including the impulsive waves produced by sources such as pile drivers. if possible, such experiments should also be carried out under field conditions, on naïve animals; preliminary tests for this approach are currently in progress.

The initial results suggest that the sensitivity of crustaceans to substrate vibrations is sufficient to enable them to detect anthropogenic disturbances propagated through the seabed. Although detection of particle motion through the water borne pathway may only be possible close to the source (Goodall *et al.*, 1988, Popper *et al.*, 2001), crustaceans may be able to detect substrate vibrations at greater distances from the source.

Conclusions

The experimental method described was successful in establishing behavioral thresholds for the hermit crab *P. berhardus* to substrate vibration. The thresholds obtained begin to provide an understanding of the levels of vibration that could potentially cause behavioral changes in the natural environment, an area of research that has been neglected in recent years.

Sensitivity to vibration is particularly important in light of increasing marine developments around the coast. Many of these activities are likely to generate substrate vibrations, in addition to producing water borne sounds. There are also other natural sources of substrate vibration that may be of interest to animals living on the seabed. The effects of substrate transmission should not be overlooked when investigating the effects of noise pollution on the marine environment.

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Effects of Sound on the Behavior of Wild, Unrestrained Fish Schools

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Abstract

To assess and manage the impact of man-made sounds upon fish we need information on how behavior is affected. Here wild, unrestrained pelagic fish schools were observed under quiet conditions using sonar. Fish were exposed to synthetic piling sounds at different levels using custom-built sound projectors, and behavioral changes examined. In some cases the depth of schools changed after noise playback: full dispersal of schools was also evident. The methods we have developed for examining the behavior of unrestrained fish to sound exposure have proved successful and may allow further testing of the relationship between responsiveness and sound level.

Introduction

A number of sound playback experiments have been undertaken in recent years, but the majority of these have been undertaken on captive fish. Laboratory studies have shown a behavioral response by fish to sound stimuli (Blaxter *et al.*, 1981, Kastelein *et al.*, 2007, Kastelein *et al.*, 2008), however the acoustic conditions within small tanks are not directly comparable to the natural environment (Griffin, 1950, Parvulescu, 1964), making the results of such studies difficult to interpret.

To address this problem, a number of field-based studies have been undertaken (Engås *et al.*, 1995, Boeger *et al.*, 2006, Thomsen *et al.*, 2012, for example), but have used large cages or netting pens. Fish tend to behave differently when enclosed than when they are free and

unrestricted (Benhaïma *et al.*, 2012), especially if they have been bred in captivity or damaged during capture and handling (Balaa & Blouin-Demers, 2011), putting the results of these studies into question.

With this in mind, in order to fully evaluate the responses of wild fish to man-made sounds, experiments are preferred with free-swimming fish in their natural habitat with a passive observation technique. The logistics of such experiments are not simple, because of the difficulties of monitoring unrestrained fish without influencing their behavior and the issues of accurately reproducing sounds on demand. However the study outlined here is a successful example of such a field experiment. The methods developed here may be the first step in investigating the responsiveness of free living schools of fish to various sounds at different levels.

Material and Methods

Experiments were undertaken at Lough Hyne, County Cork, Ireland (51◦ 30′ N, 9◦ 18′ W). This site has been a marine nature reserve since 1981 and has a low level of boat activity. Human influence is minimal, providing quiet conditions for sound experiments. Previous studies have shown large numbers of *Sprattus sprattus* (sprat) in the Lough, appearing as large schools during the day, when the fish are preyed upon by *Scomber scombrus* (mackerel). These schools break up at night and the individual fish disperse over a wide area (Hawkins *et al.,* 2012).

The experiments were undertaken in two trips, 20- 27th October 2012 and March 17 – 23rd 2013, with four previous trips to develop the experimental setup and methodology, and to map the locations of fish schools.

Observations of Fish Behavior

A rigid inflatable boat (RIB), with an outboard motor, and a small rowing boat were tethered together, and allowed to drift without power for the experiments. A Humminbird 998c SI sonar was mounted on a wooden beam and suspended from the side of the rowing boat, at a depth of 0.5 m. The transducer produced a downward beam (20° width) at 200 kHz and side scan beams at 800 kHz, both operating well above the hearing range of the species investigated. Sonar recordings were saved onto an SD memory card together with GPS data and later viewed with Humviewer software.

Sound Playback and Monitoring

A custom made sound projector array (Subacoustech Ltd.) was used to playback clips of synthetic pile driving sound. The system consisted of four underwater projectors specifically made to produce low frequency sounds. This unit was suspended from the RIB, as far away from the sonar beam as possible to avoid it appearing as a strong target on the sonar system. An InPhase

IPX2400 car amplifier (2400 watts) powered by a car battery was connected to the speakers, with the input signal played from a Tascam model DR05 recorder or an IBM thinkpad laptop computer.

The twenty second long synthetic sound in the playback experiments consisted of ten, sharp-onset, low frequency pulses intended to mimic the signal from a pile driver. Each 'strike' was two seconds apart and was constructed from white noise of 50 to 600 Hz with most power at 200 Hz to mimic the spectral characteristics of piling. To avoid pseudoreplication six versions of the sound were used, each created with the same characteristics (ie onset time and filtered frequency ranges) but with a different white noise used in each case. Six levels were played, in increments of 5 dB below the maximum volume. The order of the versions and levels of signatures was fully randomized, with 'silences' interspersed to check that the equipment itself did not have an influence upon the fish.

A series of calibration measurements were taken to enable calculation of received levels at the top of the acoustic targets. The calibrations were made using a Reson TC4014 hydrophone with a sensitivity of -186 dB re 1V for a sound pressure of 1µPa, with a frequency range from 0.1 Hz to 400 kHz. A custom made (Subacoustech Ltd.) amplifier was used to amplify the signal by between 0 and 40 dB and a National Instruments type 606E data acquisition device (sampling rate 350 kHz) was used to digitize the signal before storage on a laptop. Sound level measurements were taken each day at a number of depths from 4 m to 19 m, enabling the levels received by the fish to be estimated.

Experimental Procedure

The two boats were allowed to drift, without power, until the sonar system displayed characteristic acoustic targets from fish. Sound playback then commenced and the resulting responses from the targets were recorded on the Humminbird sonar. The coupled boats drifted across the Lough under the action of the wind and tide, with sound playback being undertaken when targets were encountered. Often multiple schools were encountered on each track, but suitable gaps (5 to 10 minutes whilst the boat continuously drifted) were left between presentations to avoid exposing the same school on multiple occasions. Recordings from the Humminbird were made continuously, with a new recording track for each location within the Lough. The positions and timing of each sound playback were noted, recorded using the waypoint facility on the Humminbird, and subsequently displayed on the sonar trace. Playback of blank sound files was carried out at random intervals as controls, interspersed between full experimental sound presentations.

Playback experiments were typically undertaken on schools at less than 25 meters depth. In the October experiments fish schools were only found at depths shallower than 30 m because of the presence of a strong thermocline, below which oxygen levels were greatly reduced. In the March experiments the thermocline was absent. Sampling was generally undertaken under calm sea conditions (Beaufort Sea state two and below) to ensure that the vessel drifted at a suitable slow speed (average speed 0.16 m s^{-2}). Sampling of the acoustic targets detected on the Humminbird was undertaken by rod and line fishing and plankton net tows.

Data Analysis

Data was pooled together for the purposes of analysis. Echograms were viewed in Humviewer (v 67) software with the precise times of playback marked using the waypoint tool. 'False' playbacks were randomly added to display some schools that were not exposed to sound playback, so that normal behavior could be observed. Echograms were scored as a response '1' or no response '0', by two experienced observers, the binary data was then analysed in SPSS (v19) to investigate whether sound level had an effect on response. For analysis the targets were grouped into categories according to density, size and overall appearance.

Results

A total of 236 targets (aggregations and individual targets) were exposed to sound playback at received levels ranging from 148.6 to 103.9 dB re 1µPa^{2}s (SEL) per strike, 158.6 to 113.9 dB re 1µPa^{2}s (SEL) over the 20 s duration of a playback, or 171.3 to 127.9 dB re. 1µPa peak to peak with energy predominantly in the range 50 to 800 Hz. The sound pressure level at 5 m from the speakers was recorded as being between 164 and 168 dB re. 1µPa (peak to peak) and 170 to 172 re. 1µPa (peak to peak) for October and March respectively. Targets were recorded in a range of depths 4 – 35 m in similar regions of the Lough on both trips.

The aggregations of small targets were confirmed to be sprat, and looser aggregations of larger targets as mackerel. Very small diffuse targets, seen in one region of the Lough, were thought to be zooplankton as previously described by Hawkins *et al.* (2012).

Responses were seen to sound playback at a variety of sound levels. These responses involved density changes within aggregations, dispersal (complete cut-off and re-emergence at a different depth) and depth changes. Two examples of the behavior of sprat schools in response to sound playback are shown in Figures 1 and 2. Both of these were classed as responses. The beginning and end of the playback period is marked with a line. In addition to the downward pointing sonar beam, the side scan beam also proved a useful additional tool to aid determination of responses. The use of 'silent' playbacks showed that the equipment had little effect upon the targets, and 'false' playbacks gave an indication of the behaviour of the targets in the absence of noise.

Discussion

The data obtained from these experiments confirms that the responses of free living fish to the playback of sound can readily be observed and used as a basis for determining those sound levels and sound characteristics that produce a clear-cut response. The methodology and equipment was reliable and easily reproduced. As the boat was drifting in the water throughout the experiments it was possible to monitor a school for long enough to playback sounds and determine the response. Concerns that a 'response' may actually be a school just leaving the sonar beam can be addressed by large numbers of replicate experiments and the insertion of controls and false playbacks, where

no sounds are presented. The possibility that the equipment itself had an influence upon the fish (for example shadow or other visual effects) was removed.

Fish did move in and out of the beam of the sonar (and moved within the beam) on some occasions even when sounds were not presented, simply as part of their normal behavior. However, by describing particular criteria for a positive response any confusion was generally avoided. There were very few occasions when positive responses were recorded in the absence of sound playback.

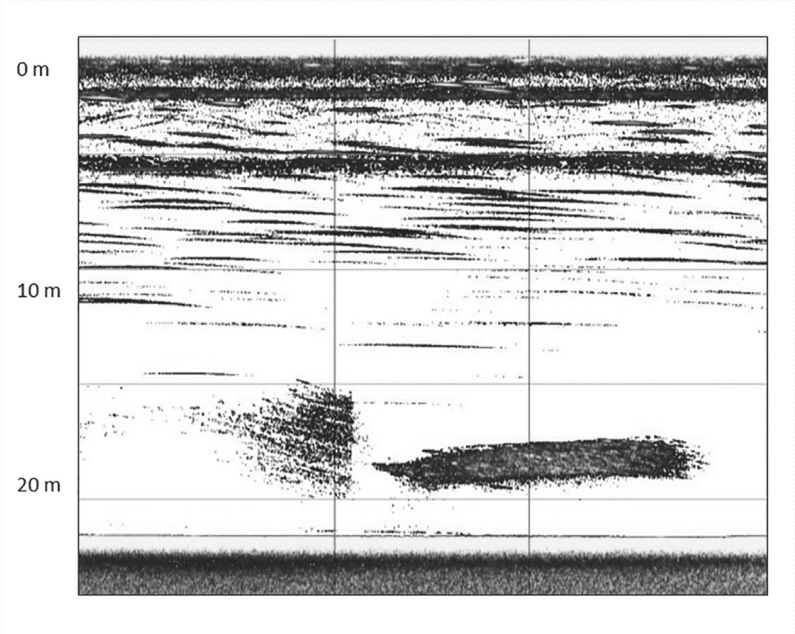

Figure 1 Example echograms of responses of sprat schools to sound playback. Echogram from 25th October, a sprat school showed an abrupt cut off a the beginning of the playback and reappeared a few seconds later lower in the water column and more densely packed.

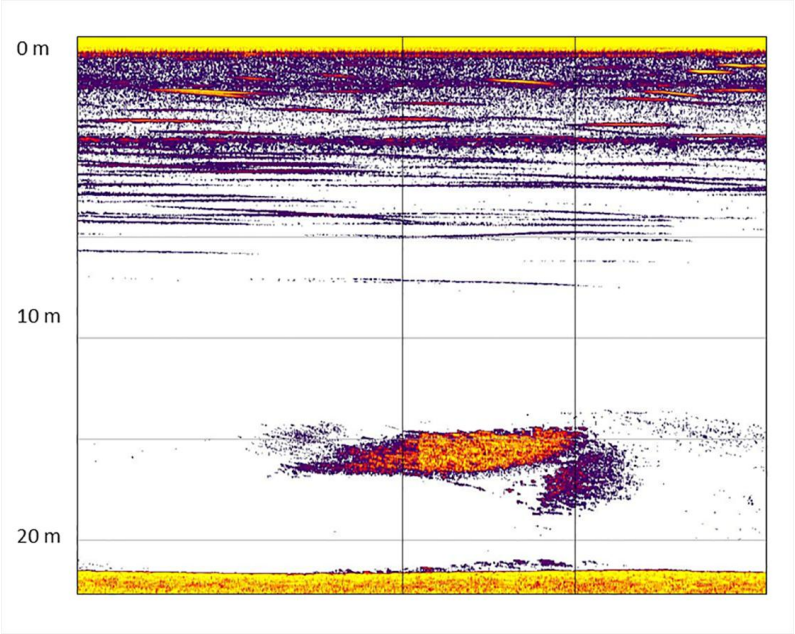

Figure 2 Echogram from 24th October, the school appeared to become more dense after the onset of playback, shown by the brightening of the colour.

The experiments were conducted in an enclosed area, under very quiet sea noise conditions, with fish that were not being exposed to sounds from other man-made sources. Whether fish that are repeatedly exposed to a variety of man-made sounds react, remains to be determined through further experiments.

The sounds produced in this study mimicked the water borne sounds produced by a pile driver in the water column. However it should be noted that a pile driver also produces a strong ground borne vibration through the impact of the pile with the seabed. This vibration travels outwards from the source along the seabed (via compressional, shear and Rayleigh waves, see paper by Hazelwood & Macey- this volume) and the energy is also passed back into the water column. The sound projector array used in this study cannot reproduce these ground borne vibrations, which may be especially important for fish and crustaceans close to the seabed. The fish investigated here were pelagic, however, and ground borne vibration may not be important to these species. In addition to this, the noise level in this study is roughly representative of the level at 1 km from a pile driver, ground borne vibrations at this distance may be minimal.

Sonar is commonly used to observe the behavior of fish schools, and has been used in the past to investigate reactions to research vessels (see De Robertis & Handegard, 2013 for a comprehensive review). There have been fewer studies involving sonar observations with playback systems, but behavioral changes such as the ones described here, have been shown before. Doksæter *et al.* (2012) used upward pointing sonar and found that captive *Clupea harengus* (herring) showed an increase in schools density and a depth change in response to engine noise and impulsive sounds. Furthermore, Slotte *et al.* (2004) used sonar to observe fish schools, and found that the pelagic species showed depth changes when exposed to seismic air guns. Diving of schools and the scattering of individual fish is likely to be the standard response of pelagic fishes in reaction to a threat, such as a predator, for example Wilson & Dill (2002) found that herring dropped in the water column and increased in speed when exposed to predator (simulated odontocete) sounds.

Conclusions

The methods described in this study enabled the reactions of free-living, unrestrained fish to be observed in response to the playback of sounds. The responses were clear, but there were indications that the type of reaction may vary depending upon the received sound level, the type of fish school, and perhaps the nature of the sound stimulus. There is a clear need to describe those behavioral responses to man-made sounds that may have harmful effects upon fish populations, and to distinguish these responses from incidental responses that have little impact. Further experiments of this kind on free-living fish will undoubtedly yield useful results.

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