

# Chapter 6

## Contribution to the Understanding of Particle Motion Perception in Marine Invertebrates

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**Abstract** Marine invertebrates potentially represent a group of species whose ecology may be influenced by artificial noise. Exposure to anthropogenic sound sources could have a direct consequence on the functionality and sensitivity of their sensory organs, the statocysts, which are responsible for their equilibrium and movements in the water column. The availability of novel laser Doppler vibrometer techniques has recently opened the possibility of measuring whole body (distance, velocity, and acceleration) vibration as a direct stimulus eliciting statocyst response, offering the scientific community a new level of understanding of the marine invertebrate hearing mechanism.

**Keywords** Particle motion • Invertebrates • Laser Doppler vibrometer

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## 1 Introduction

The extent to which sound in the sea impacts and affects marine life is a topic of considerable concern both to the scientific community and to the general public. In addition, offshore economic interests could be at risk because of a lack of a rigorous scientific approach that would lead to precaution and excessive regulation. Marine invertebrates potentially represent a group of species whose ecology may be influenced by artificial noise. Exposure to anthropogenic sound sources could have a direct consequence on the functionality and sensitivity of their sensory organs, the statocysts, which are responsible for their equilibrium and movements in the water column. Statocyst functions generally include gravity and acceleration receptors (Maturana and Sperling 1963; Budelmann and Williamson 1994). Although statocysts have received considerable scientific attention in the past, especially in cephalopods, there is a significant gap in information on sound processing by marine invertebrates; there is still a need for definitive scientific evidence of the additional role of statocysts in low-frequency sound perception. Respiratory and electrophysiological methods confirmed cephalopod sensitivity to frequencies under 400 Hz (Kaifu et al. 2008; Hu et al. 2009), supporting the scientific consensus regarding the biological significance of the particle motion component of the sound field for these species.

Invertebrate sound receptors include a wide range of statocysts (Janse 1980; Laverack 1981), which are highly sophisticated structures that are analogous to the vestibular system of the vertebrate ear (Offutt 1970; Budelmann 1988, 1992; Williamson 1995). Vibrational and directional sensitivity of the statocyst hair cells of invertebrates, particularly of cephalopods, have been reported in the literature (Williamson 1988, 1989; Packard et al. 1990; Budelmann and Williamson 1994). Because of the high degree of development of their nervous system, characterized by the large size of the neurons, cephalopods were often used for neurophysiology studies (Bleckmann et al. 1991; Budelmann et al. 1995) to determine hearing thresholds. Here, we concentrate on these species to illustrate the general sound perception of marine invertebrates.

Cephalopod statocysts show a variety of forms, but they can be grouped into three main types (Budelmann 1988) depending on species (nautiloids, octopods, and decapods). They usually include two categories of receptor systems, the macula-statolith system and the crista-cupula system. The macula-statolith system informs on changes in the position according to gravity and linear acceleration, whereas the crista-cupula system indicates changes in angular acceleration. This presents analogies with the vestibular system of the vertebrate inner ear (Colmers 1977). However, unlike ciliated cells of vertebrates, the cephalopods' statocyst sensory cells carry kinocilia. Microvilli are found surrounding the base of the kinocilium. Kinocilia and microvilli form elongated bundles. Each bundle represents a single hair cell. Every hair cell is arranged in line with an adjacent hair cell in both the crista and macula (Budelmann et al. 1973) and is unidirectional morphologically and

physiologically polarized. Adjacent accessory structures (statolith, statoconia, cupula) are responsible for the sensory perception. When there is a stimulus, tiny deflections occur in the hair bundles, resulting in cell body depolarization and subsequent transmission of the information to the sensory nervous system.

At central nervous system level, the sensory input of the statocysts is used to regulate a wide range of behaviors, including locomotion, posture, control of eye movement, and body coloration pattern. In addition, there is now evidence the statocysts are also responsible for the reception of low-frequency sound waves (Kaifu et al. 2008; Hu et al. 2009; André et al. 2011; Solé et al. 2012). The sensory epithelia of the gravity receptor system, in resemblance to the vertebrate auditory apparatus (Puel et al. 2002), have secondary sensory hair cells that are unidirectional morphologically and physiologically polarized, first-order afferent neurons, and efferent nerve fibers. The synaptic arrangements are as complex as those in the vestibular maculae (see, e.g., Sans et al. 2001); the outputs of several hair cells converge onto an afferent neuron and the output of a single hair cell diverges onto several afferent neurons. The efferent fibers of the statocyst terminate on both hair cells and the axons of afferent neurons (Budelmann et al. 1987).

Although little is still known about the sound perception mechanism in invertebrates (Packard et al. 1990; Bleckmann et al. 1991; Bullock and Budelmann 1991; Kaifu et al. 2008; Hu et al. 2009), recent behavioral and electrophysiological experiments conducted on cephalopods confirmed the species sensitivity to frequencies under 400 Hz (Kaifu et al. 2007, 2008; Hu et al. 2009; Mooney et al. 2010) and the important role of statocysts in sound reception (Kaifu et al. 2008; Hu et al. 2009; André et al. 2011; Solé et al. 2012). Although there is little uncertainty regarding the biological significance of particle motion sensitivity versus acoustic pressure, the question is how particle motion is detected and transmitted to the statocysts from any acoustic source.

It was suggested that the particle motion can encompass the whole body of cephalopods and cause it to move with the same phase and amplitude; the statolith organs would then be stimulated by whole body displacements (Packard et al. 1990; Kaifu et al. 2011). The statolith organs are inertial detectors in which a calcareous statocyst is attached to the sensory hair cells. When an animal accelerates, the statolith moves, bending the sensory hair cells. Thus, the statolith could serve as a receptor of kinetic sound components (Packard et al. 1990; Kaifu et al. 2011). However, to the best of our knowledge, there has been no study that showed that the whole body can vibrate when stimulated by underwater sound in aquatic invertebrates or may be neither in aquatic vertebrates such as fishes.

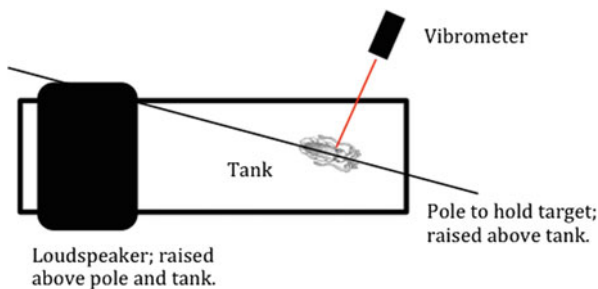
The availability of novel laser Doppler vibrometer techniques has recently opened the possibility of measuring whole body (distance, velocity, and acceleration) vibration as a direct stimulus eliciting statocyst response, offering the scientific community a new level of understanding of the marine invertebrate hearing mechanism. These techniques have already been applied to several species such as amphibians, reptiles, and crustaceans (Hetherington and Lindquist 1999; Hetherington 2001). Although some preliminary experiments have been conducted using the laser

Doppler techniques, no measurement of the whole body vibration induced by underwater sound was performed. Here we present a series of measurements conducted in controlled experimental conditions aimed at determining if cuttlefish and scallop bodies vibrate when stimulated with pure-tone sounds.

## 2 Methods

### 2.1 Controlled Exposure Experiment Conditions

Controlled exposure experiments were conducted using the experimental setup shown in Fig. 6.1. The equipment was placed inside a closed room of dimensions  $4.2 \times 3.2 \times 2.7$  m. The room contained other furniture and objects that affected the acoustic field inside the room. The water tank ( $80 \times 30 \times 40$  cm) was placed on top of a layer of foam on a table. The target (reference reflective objects, cuttlefish, and scallops) was placed on one side of the tank. The target was suspended with an elastic string from a wooden pole that was placed over the tank, supported on both sides by a tripod. The pole was not in contact with the tank. The target was positioned  $\sim 20$  cm below the water surface. The vibrometer (Polytec OFV-505 with OFV-5000 controller) was placed on an absorbing foam layer and was never moved during the experiment. The beam angle had to be adjusted with each target, together with the orientation of the pole, to ensure a proper reflection. For animal targets, a small flat piece of aluminum foil attached to the body was used as a reflector. On the other side of the tank, a loudspeaker was suspended over the water supported by two tripods; no part of the loudspeaker setup was in direct contact with the tank or pole. The pure-tone signals were generated by an Agilent wave generator and passed on to the loudspeaker through a commercial amplifier. Changing the voltage level of the wave generator, keeping the amplifier at a fixed amplification setting, varied the source sound level.



**Fig. 6.1** Experimental setup for the vibration measurements

**Table 6.1** Vibration of the laser Doppler vibrometer

Vibration laser acceleration (dB re $10^{-6}$ m/s <sup>2</sup> )			
	60 Hz	120 Hz	320 Hz
x-axis	70.2	92.0	104.7
y-axis	70.9	76.8	97.7
z-axis	82.4	96.4	113.1

The x-axis was in the direction of the measurement

**Table 6.2** Acceleration of targets at different frequencies

Acceleration (Hz)													
	60	80	100	110	120	130	150	170	190	240	320	350	360
Bar	101	96	110	113	98	104	115	108	105	113	136	113	119
Scallop	100	97	109	113	96	102	109	118	95	110	136	114	115
Cuttlefish	103 <sup>a</sup>	100 <sup>a</sup>	114	114	106	106	116	112	107	115	138	108 <sup>a</sup>	115 <sup>a</sup>
Tape	106	104	116	118	109	107	114	112	108	119	140	115	117

Values are peaks in dB re  $10^{-6}$  m<sup>2</sup>/s<sup>4</sup>/Hz

<sup>a</sup>Only one measurement was used for cuttlefish at those frequencies; the other values are average measurements from two animals

## 2.2 Targets

The invertebrate representative targets that were used in this experiment were cuttlefish and scallops, and a small dense metal bar and a small piece of reflective tape were chosen as reference targets because it was expected that, due to density differences, the scallops would vibrate at a speed closer to the metallic bar while the cuttlefish, because of its neutral buoyancy, would behave more like a freely floating small tape when exposed to sound. The animals were anesthetized during the exposure to avoid movement. When long exposure times were used, the animal awaking time was monitored and the experiments were resumed after the animal was put back to sleep.

The laser configuration was calibrated using a vibration source working at 160 Hz and 9.8 m/s<sup>2</sup> (140 dB re  $a_0 = 10^{-6}$  m/s<sup>2</sup> [acceleration reference level]). It was placed at the other side of the tank and measured through both tank walls (see Table 6.1). The vibration of the laser itself was measured as well for three frequencies of interest. The vibration measurement of a target was considered reliable if it was at least 10 dB higher than the vibration of the laser.

The consistency of the measurements and the effect of the precise position of the target were tested by measuring the inanimate targets at slightly different positions. The scallop and especially the cuttlefish were much larger than the test targets and measuring their vibration only at a single spot was not expected to be representative for the whole body.

Targets were never moved between measurements of different frequencies, although sometimes the focus of the laser beam had to be adjusted to ensure good reflection. Measurements were always made in a series of two, moving from the lowest to the highest frequency, exposing the target for at least 16 s, and then repeating the sequence from the lowest frequency.

### ***2.3 Choice of Frequencies***

A first series of measurements was conducted with a wide range of frequencies (from 60 to 360 Hz; see Table 6.2), together with corresponding sound pressure level (SPL) measurement. The difficulty of working with live animals required the selection of fewer frequencies for the final round of experiments. An obvious frequency to use appeared to be 320 Hz because it induced a large response of all targets. Two other frequencies, 60 and 120 Hz, were selected for further experimentation because of their proximity to the European Union Marine Strategic Framework Directive (MSFD) indicators that are being used to evaluate noise pollution (good environmental status [GES] indicator 11.2; van der Graaf et al. 2012).

### ***2.4 Cuttlefish Body Response***

Working with a live animal is difficult, especially for long exposure, due to the need for constant monitoring and pausing the experiment to renew the anesthesia. Continuing the experiment with the exact same target position and laser orientation was often impossible. The state of the animal can normally be monitored while taking measurements. We conducted controlled exposure experiments with both dead and live animals to estimate variations and validate results obtained from the measurements.

## **3 Results**

### ***3.1 Frequency Response***

Table 6.2 shows the target accelerations at all measured frequencies.

### ***3.2 Effect of Position on Measurements***

At each position, multiple measurements showed very consistent results with identical peak levels. Then, repositioning the object, which required slight focus adjustment of the vibrometer beam without moving the vibrometer itself, led to

considerable differences in some cases. At 60 and 120 Hz, some positions were quite close to the vibration levels of the vibrometer; this was not unexpected for the bar. After repositioning, the measured levels were above the self-vibration. At 320 Hz, all measurements were consistent and well above self-vibration.

### **3.3 Comparison of Dead to Alive Animals**

A slow-frequency oscillation corresponding to the breathing rate of the animal was monitored during the measurements of live individuals. The magnitude of the oscillation may be dependent on the state of anesthesia, but this breathing motion did not affect the acceleration measurement induced by the acoustic wave. The dead animal measurements were all identical between exposures, whereas the live animal measurements showed differences not only between animals but also between exposures of the same animal. The measurements of the dead animal, however, fell in the same acceleration range as those of the live animals or what was explained by a change in position.

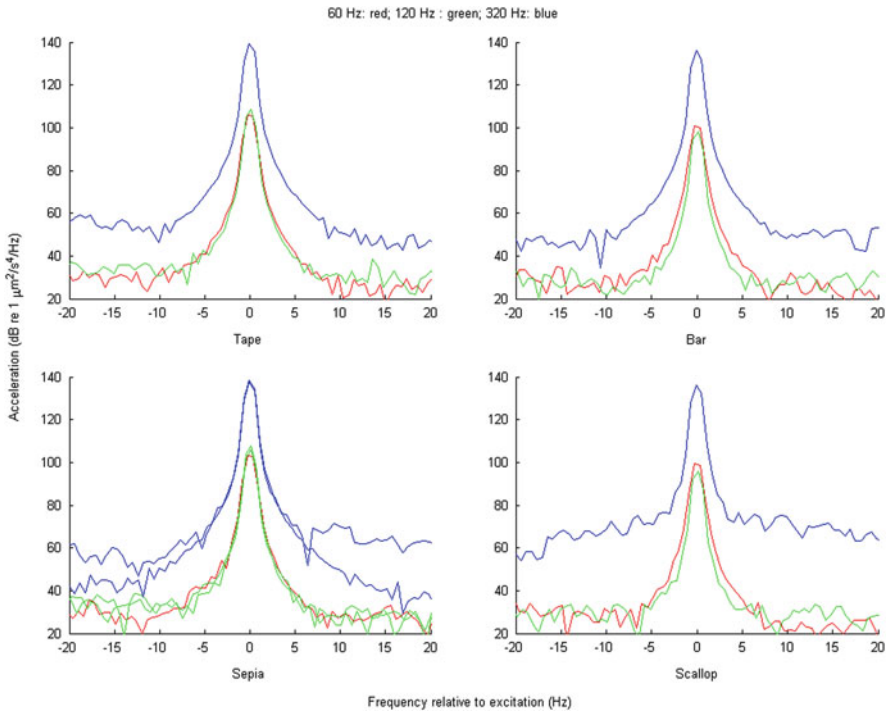
### **3.4 Body Vibration**

Figure 6.2 shows the results of the four targets (reference targets and animals) at the three chosen frequencies.

## **4 Discussion and Conclusions**

There was little doubt that marine invertebrates were sensitive to (i.e., perceive) low-frequency sounds and that this sensitivity was not directly linked to sound pressure but to particle motion. The missing component in the analysis was the demonstration that the statolith would act as an harmonic oscillator, excited when the whole animal body was vibrating when exposed to sound waves.

The differences of the laser Doppler acceleration measurements (see Table 6.1) with the target vibration measurements (Fig. 6.2) allow us to conclude that the exposure to sound has elicited the cephalopods and scallops whole body vibration, confirming the initial hypothesis that particle motion can encompass the whole body of cephalopods and cause it to move with the same phase and amplitude (Packard et al. 1990; Kaifu et al. 2011) as well as consolidating the use of laser Doppler techniques in underwater bioacoustics studies.



**Fig. 6.2** Laser Doppler target acceleration measurements at the three chosen frequencies

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