The hearing abilities of the prawn *Palaemon serratus*


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Abstract

The mechanism of sound reception and the hearing abilities of the prawn (*Palaemon serratus*) have been studied using a combination of anatomical, electron microscopic and electrophysiological approaches, revealing that *P. serratus* is responsive to sounds ranging in frequency from 100 to 3000 Hz. It is the first time that the Auditory Brainstem Response (ABR) recording technique has been used on invertebrates, and the acquisition of hearing ability data from the present study adds valuable information to the inclusion of an entire sub-phylum of animals when assessing the potential impact of anthropogenic underwater sounds on marine organisms. Auditory evoked potentials were acquired from *P. serratus*, using two subcutaneous electrodes positioned in the carapace close to the supraesophageal ganglion and the statocyst (a small gravistatic organ located below the eyestalk on the peduncle of the bilateral antennules). The morphology of the statocyst receptors and the otic nerve pathways to the brain have also been studied, and reveal that *P. serratus* possesses an array of sensory hairs projecting from the floor of the statocyst into a mass of sand granules embedded in a gelatinous substance. It is the purpose of this work to show that the statocyst is responsive to sounds propagated through water from an air mounted transducer. The fundamental measure of the hearing ability of any organism possessing the appropriate receptor mechanism is its audiogram, which presents the lowest level of sound that the species can hear as a function of frequency. The statocyst of *P. serratus* is shown here to be sensitive to the motion of water particles displaced by low-frequency sounds ranging from 100 Hz up to 3000 Hz, with a hearing acuity similar to that of a generalist fish. Also, recorded neural waveforms were found to be similar in both amplitude and shape to those acquired from fish and higher vertebrates, when stimulated with low-frequency sound, and complete ablation of the electrophysiological response was achieved by removal of the statocyst.

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1. Introduction

The oceans are virtually transparent to sound, and opaque to light and radio waves. At a wavelength of 1 m (1500 Hz), water is nearly 1,000,000 times more transparent to sound than to radio signals (Pilgrim and Lovell, 2002). This fact underlies the intense interest currently being directed toward the acoustical exploration of the ocean. Naturally produced sounds arise from a number of sources, such as breaking waves, heavy rain, volcanic activity or from marine animals (bio-acoustic sources). Vocalisations such as whale song, along with the grunts and whistles from sonic fish are especially relevant for communication purposes, and during predator prey interactions (Myrberg, 1981). There are several types of anthropogenic sources used routinely that produce intense levels of noise, such as the Low Frequency Active Sonar (LFA) used by the military in anti-submarine warfare, or from the airgun arrays used during a seismic survey of the substrate beneath the seafloor by the petroleum industry. These activities can generate noise levels in excess of 253 dB (re 1 µPa at 1 m) (Engás et al., 1996), and are comparable to the noise levels generated by a seafloor volcanic eruption, which can produce a source level of in excess of 255 dB (re 1 µPa) (Northup, 1974). Recent concerns regarding the impact of these anthropogenic...
sounds on fish and other marine animals has prompted a number of investigations into the effects of intense noise exposure on the hearing systems of marine mammals (e.g., Costa et al., 2003; Richardson et al., 1995; Whittlow et al., 1997). Additionally, several studies of the behaviour of free living fish when exposed to intense noise have been conducted (see Dalen and Knutsen, 1987; Engås et al., 1996; Pearson et al., 1992; Pickett et al., 1994), and includes the examination of log books from fishing vessels operating within 5 km of a concurrent seismic survey (Lokkeborg and Soldal, 1993).

It is known that several crustacean species produce sound; for example, the pistol shrimp (Alpheus spp.) produces a loud click by rapid closure of a specially adapted claw (Schmitz and Herberholz, 1998). The spiny lobster (Palinurus vulgaris) and the rock lobster (Palinurus longipes) make alarm sounds by drawing the base of the antenna across scale like ridges below the eyestalks; Patek, 2001; Meyer-Rochow et al., 1982). Additionally, P. longipes has been shown to take longer emerging from a hide, when feeding was preceded by a white noise (Meyer-Rochow et al., 1982). The female cricket (Gryllus bimaculatus) has the ability to localise and respond to male chirp sounds (Hedwig and Poulet, 2004; Schildberger and Hörner, 1988), using specially adapted acoustic receptors (tympanum), located in the forelegs below the knee (Huber and Thorson, 1985). On hearing the chirp, a receptive female will orientate itself toward the sound using a behavioural response known as phonotaxis (Schildberger and Hörner, 1988).

The ability of an organism to orientate itself in the 3-D marine environment requires the presence of a suitable gravity receptor. These receptors occur in many diverse organisms throughout the marine environment, and include cephalopod (Dilly et al., 1975; Bettencourt and Guerra, 2000), crustaceans (Prentiss, 1901; Schöne, 1971; Rose and Stokes, 1981; Patton and Gove, 1992) and fish (Popper and Platt, 1983; Bretschneider et al., 2001). In crustaceans, the statocyst is located either at the anterior end of the animal in the basal segment of each antennule, or posteriorly within the uropods, abdomen or telson (Farre, 1843; Cohen and Dijkgraaf, 1961; Finley and Macmillan, 2000). It has been well-established that the crustacean statocyst functions as an equilibrium organ by initiating corrective movements to maintain the animal’s position in the water column, (Cohen and Dijkgraaf, 1961; Sekiguchi and Terazawa, 1997; Finley and Macmillan, 2000; Popper et al., 2001).

In this work, we study the electrophysiological response of the statocyst in an underwater sound field, using the Auditory Brainstem Response (ABR) recording technique originally developed for use in clinical neurophysiology. Until now, this method of acquiring hearing ability has only been applied in the auditory assessments of vertebrates (Corwin et al., 1982), though the presence of afferents in the statocyst, and existence of a neural pathway terminating in the supraesophageal ganglion, indicates that the physiology of Palaemon serratus is suitable for an ABR type investigation. An ABR waveform is acquired by averaging conglomerate responses of peak potentials, arising from nuclei in the auditory pathway during acoustic stimulation (Corwin et al., 1982; Overbeck and Church, 1992). The sweep records the generation of neural waveforms over a user-defined time span termed the sweep velocity, and measures activity prior to, during and after stimulation of the receptor organ. Additional waveform generation by neural activities other than those associated with hearing, combined with muscular movements, ensure that recordings have to be repeated over 1000–2000 presentations before clear results can be obtained (Kenyon et al., 1998; Yan et al., 2000). The recorded waveforms resulting from each sweep are averaged together and a recognisable ABR waveform, which is then overlaid on the first run, to show that the evoked potentials are repeatable.

The nerves associated with the statocyst and the pathway taken to the neuropil of the antennule in the supraesophageal ganglion was examined to provide a detailed description of how acoustic signals are perceived and transmitted by the neuronal pathways. The aim of the present paper, therefore, is to examine the morphology of the statocyst receptor array of the prawn (P. serratus) using both scanning and transmission electron microscopy (SEM and TEM). Measurements of the electrophysiological response of the statocyst and Central Nervous System (CNS) to acoustic stimuli were also made, and by ablation, it was demonstrated that the evoked response was generated in the statocyst organ.

2. Materials and methods

One hundred specimens of the prawn (P. serratus) Phylum Crustacea and Class Eumalacostraca of mixed sex, and ranging in length from 27 mm (0.1 g) to 71 mm (1.9 g) were obtained from wild stock in the South West of England using a dip net. Once captured, the prawns were transferred to a marine tank divided by a fine mesh screen into four equal sized compartments of 50 L each. An Eheim type 2013 biological filter with a flow rate of 390 L/h maintained water quality and provided aeration by spraying filtered seawater back into the tank via the filter outlet pipe located 60 mm above the water surface. The ambient noise within the holding tank was measured using a hydrophone, and the sound pressure level was calculated to be 102 dB (re 1 μPa), with the Eheim pump active. In all of the experiments, and in the holding tank, the ambient water was kept at a temperature of 18 °C and a salinity of 34 psu. When not under experimental protocols, the prawns were provided with 14 h of light per day from a fluorescent tube controlled by a mains timer switch. Prior to any experimentation the prawns were divided by size into three populations, and fed on a granulated feed at a daily rate of 6 g for the large prawns, 4 g for the medium and 2.5 g for the small.
2.1. Preparation methodology for general dissection and electron microscopy

The pathway taken by the innervating nerves of the statocyst to the supraesophageal ganglion or brain was revealed by the anatomical investigation of a 54-mm *P. serratus*. The prawn was first immersed in 70% ethanol for 18 h, to “fix” the specimen prior to the investigation. Exposure of the brain and statocyst was achieved by the dissection and removal of the dorsal–rostral section of carapace, the dorsal cuticle layer of the peduncle, the left eye and the stomach.

Specimens of *P. serratus* selected for EM examination were denied access to materials that could be used as otoliths, primarily by having no substrate present in the tank. Additionally, uneaten feed and other waste products were removed by ensuring that the return flow of water to the filtration system was strongest at the base of the tank. Particulate matter was drawn by the flow of water through a 5-mm gap under each of the tank divisions, through which the prawns could not pass. The denial treatment was applied to all 100 of the prawns, with the exception of Fig. 4 which was prepared for EM examination within 48 h of capture. Moulting was induced in the remaining specimens over a 24-h period using a method that involved not changing the ambient tank water for 7 days, followed by a sudden change of all the water.

The statocyst capsules were removed by dissection from 12 of the specimens, and placed in a conical dish containing 2.5 mL of 0.9% sodium chloride. The capsules were opened by making a lateral incision around the statocyst chamber using a fine scalpel. Needlepoint tweezers were used to lift the upper section of the capsule, thus exposing the sand granules and ultrastructure. The sodium chloride solution was removed using a pipette and replaced with a solution of 2.5% 3-Carboxymethyl-L-Cysteine in sodium chloride, which was used to hydrolyse the mucus surrounding the statocyst receptors. The contents of the dish were gently agitated for 2 min, after which the solution was removed and replaced with chilled fixative (2.5% glutaraldehyde in 0.1 M cacodylate buffer with 3.5% sodium chloride). The statocyst capsules were then dehydrated through a graded ethanol series ranging from 35% through 50%, 70% and 90% to absolute ethanol, prior to deiscission using the critical point drying method described by Platt (1977). Fully desiccated statocyst capsules were subsequently mounted on a specimen stub using a carbon tab, and coated with c. 8 nm of gold in an Emitech K 550 sputter coater (working at approximately $5 \times 10^{-6}$ torr). Finally, the processed specimens were investigated and photographed using a JEOL JSM 5600 scanning electron microscope operated at 15 kV, and a 15-mm working distance. Images of the ultrastructure were captured using the JEOL software, which saved the micrographs in a bitmap format. All measurements were carried out on a PC using the analySIS® (Soft Imaging System) program. The hair cell dimensions were measured using polygon length, and measurements were recorded in micrometers (µm). Measurements of hair cell dimensions (height, width, etc.) are averages taken from at least 12 observations of a feature within a similarly orientated cluster of cells, with the exception of those in Fig. 4 which were taken from five observations.

2.2. ABR methodology

In order to concisely answer the question of hearing by crustaceans, 12 prawns were stimulated with sound ranging in the frequency domain between 100 and 3000 Hz, presented at sound pressure levels from 132 dB (re 1 µPa at 1 m) to below 90 dB (re 1 µPa at 1 m). The response of the prawn to acoustic stimulation was measured using a well-established audiometry technique, with the results expressed as an audiogram or limen of sound spectral sensitivity. The ABR measurements of hearing threshold were made using a proprietary control and analysis programme, written in a LabView 7 environment. This programme both generated the stimulus signals and captured and analysed the response, and was installed onto the PC shown in Fig. 1a. The stimulus used was a sine train (sine wave pulse) which was presented to *P. serratus* at a given frequency and sound pressure level, not exceeding 130 dB (re 1 µPa at 1 m) for each of the frequencies tested. For ABR recordings to be clear, it requires that short duration tone bursts are used, especially for the low frequencies. Kenyon et al. (1998) used a two-cycle burst for frequencies between 100 and 300 Hz, a five-cycle burst with a two-cycle attack decay for frequencies between 400 and 3000 Hz. Amplification of the sound was achieved using a Pioneer type SA-420 amplifier and a 200-mm Eagle L032 loudspeaker with a frequency response range of 40–18,000 Hz. Additionally, the loudspeaker was placed inside a Faraday cage and connected to a centralised earth point located in an adjacent room where the PC, amplification and analysis equipment was set up. Connecting wires were fed through a 100-mm port in the partitioning wall.

The procedure used to acquire the acoustically evoked potentials was approved by the United Kingdom Home Office 11.03.03. The test subjects were placed into a flexible cradle formed from a soft nylon mesh rectangle saturated with seawater. Oxygenated water kept at a temperature of 18 °C was gravity fed at an adjustable flow rate of 3 mL/s and directed toward the gills. The water was held in an aerated reservoir positioned in an adjacent room, and fed to the prawn through a 4-mm-diameter plastic tube. The prawn was first placed lengthwise and centrally on an $80 \times 60$ mm rectangle of fine nylon netting, which was wrapped firmly around the cephalothorax and pleon, and the two sides of the net were held together using the clip shown in Fig. 1b.

The clip was placed in a retort stand clamp fitted with ball joint electrode manipulator arms, and the aerated water pipe (detailed in Fig. 1b). During the procedure to position the electrodes detailed in Fig. 2, the specimen and clamp were suspended over a plastic tray, and aerated water was supplied to the prawn. A retort stand and the experimental tank (L 450 mm × W 300 mm × D 200 mm) were placed on a
table with vibration inhibiting properties, located in an underground anechoic chamber L 3 m × W 2 m × H 2 m. Working under a MEIJI binocular microscope, two small holes were made in the cuticle layer using a lancet, penetrating the carapace to a maximum depth of 0.3 mm. The reference electrode was located behind the supra-orbital spine, close to the neuropil of the antennule, and the record electrode was located in the peduncle close to the statocyst, at the junction between the lateral antennular and otic ganglia. The clamp assembly with the specimen and sited electrodes were then suspended from the retort stand positioned over the experimental tank, and the prawn stationed 5 mm below the surface of the water. After the hearing assessment, the prawns were relocated to a holding tank for observation, prior to being returned to the divided aquarium.

The electrophysiological response of the prawn to acoustic stimulation was recorded using the two subcutaneous electrodes (Fig. 2), which were connected to the MS6 preamplifier by 1 m lengths of screened coaxial cable with an external diameter of 1.5 mm. The outer insulating layer of the coax was removed 15 mm from the end where the electrode tip was to be fixed, and the screening layer removed 10 mm from the cable end. The inner insulating material was then trimmed by 2 mm, and the exposed inner wire (0.5 mm diameter) was tinned with silver solder and joined to a 10-mm-long silver wire (0.25 mm diameter), tapered to a fine point. The assemblage was pushed through a 100-mm glass pipette with an internal diameter of 4 mm, until 0.4 mm of the gold wire was exposed. The remaining space inside the pipette was filled with a clear epoxy resin, and then trimmed to expose 0.3 mm of silver tip through which the AEP could be conducted. The impedance of the electrodes, both between the outer shielding and inner core, and the silver tip and MS 6, were tested using an M 205 precision digital multimeter. The impedance between the tip and pre-amplifier was found to be 0.2 V for both electrodes, and an open circuit was recorded between the outer shielding and inner core. The evoked response was amplified and digitised to 12 bits resolution and recorded. This process was repeated 2000 times and the response averaged to remove electrical interference caused by neural activities other than audition, and the myogenic noise generated by muscular activity. Each measurement was repeated twice; this aids in separating the evoked response, which is the same from trace to trace, from the myogenic noise, which varies in two successive measurements. After the averaging process, the evoked potential could be detected, following the stimulus by a short latency period of 5 ms or so. The latency is accounted for by the time it takes the sound in air to travel the 1 m to the prawn, plus 1–2 ms response latency.

2.3. The sound field

The properties of the sound field are especially relevant when comparing the audio capabilities of both pressure-sensitive and motion-sensitive fish in the near field. In a small laboratory set-up, the complexities associated with independently measuring sound pressure and particle motion are compounded by the reflectivity of the tank sides and base. For this reason, a number of experiments have used air-mounted transducers to successfully generate sounds under-
water (e.g., Fay and Popper, 1975; Yan et al., 2000; Akamatsu et al., 2002). The principal advantage of such a system is that as the sound source is located at a distance of 1 m from the air/water interface, the moving part of the transducer does not contact the water and generates near-field displacements. In this situation, the pressure and motion of the water adjacent to the fish ear can be considered as being equal (Hawkins, 1981). The stimulus tones presented from the loudspeaker to the prawn were calibrated using an insertion calibration. A calibrated Bruel and Kjaer Type 8106 Hydrophone (Serial Number 2256725) was placed in the tank and positioned adjacent to the shrimp cephalothorax region. The signal from the hydrophone was amplified using a PE6 preamplifier and digitised using a National Instruments DAQ-6062e interface card at a sample rate of 300 kS/s. In case of non-proportionality of the response of the loudspeaker, measurements of the sound pressure were taken for each amplitude and frequency setting used. Consequently, a total of 110 individual calibration measurements were taken in the calibration process. These calibrated levels were then applied to the threshold defined by ABR measurement to provide calibrated audiograms with pressure levels traceable to International Standards.

2.4. Ablation

Specimens of *P. serratus* selected for the ablation procedure were first tested for an electrophysiological response to a 500-Hz sound presented at 110 dB (re 1 μPa at 1 m). Removal of the statocyst was achieved by making a circular cut in the cuticle layer above the chamber, and withdrawing the capsule using needle point tweezers (a procedure that took a few seconds). A sham operation was also performed, and the prawns were retested 1 h after cutting around the chamber, prior to removal of the statocyst. The prawns were then placed into an empty compartment of the holding tank and allowed to recover for 24 h, prior to being retested on the electrophysiology apparatus. The post ablation recovery period was included to give the prawn’s time to settle after the procedure, as the metabolic state of the organism can have a detrimental affect on the evoked potential (Corwin et al., 1982).

3. Results

3.1. Innervation of the statocyst

In decapod crustaceans, the lateral antennular and otic nerves extend with bi-lateral symmetry from the neuropil of the antennule; a region located centrally in the brain (Prentiss, 1901), to the statocyst and tactile bristles of the antennules. The brain of *P. serratus* lies close to the rostral extremities of the carapace, ventral to the eyestalks and posterior to the antennules. On leaving the anterior region of the brain (detailed in Fig. 3a and b), the lateral antennular (gla.) and otic ganglia (go.) project forward, and enter the antennule close to the inside edge of the peduncle. From there, the otic ganglia branches outward away from the main antennular nerve, which continues to project forward to the tactile receptors.

3.2. Scanning electron microscopy

The examination of the complete statocyst (prior to removal of the sand granules) revealed ultrastructural cell
projections extending into the mass of sand granules shown in Fig. 4a. The cells project from small apertures in the statocyst floor about 7 μm in diameter; through which the receptor connects to the peripheral fibres of the otic ganglion. At a distance of 2 μm from the base, the cell widens and forms a bulb (rb. in Fig. 4a) which has a diameter of 9 μm at its widest point, and displays a series of longitudinal ridges that run around the bulbous structure. The uppermost portion of the cell base narrows to 0.8 μm, forming a fulcrum point from where a 3.5-μm-diameter hair shaft extends 40 μm into the lumen of the statocyst, and contacts with the sand granules (sg.). The overall view of the receptor array and the tips of the cells are precluded from view by the sand and a fine structure, consisting mostly of residuals left by the desiccation process of a gelatinous mucus (mu.) that in life surrounds the sand and cell tip. The view of the statocyst ultrastructure (without sand granules attached) in Fig. 4b (taken perpendicular to the horizontal plane) shows the hair cell array from a specimen of P. serratus denied sand for 7 days post moulting.

The absence of sand granules reveals more than 70 vertical cell projections arranged in a row shaped like a crescent, covering 0.073 mm² of statocyst (Fig. 5b). Each hair cell is orientated toward a common central region (cr), and the shortest hairs (<120 μm) were found proliferating in a band running down the left side of the array, whilst the longest hairs (>170 μm) were found in the right caudal quadrant. The statocyst capsule is elliptical in shape, and the walls (Fig. 5b) symmetrically curve inward toward the base, where the receptor cells are located on a mound rising 40 μm from the floor of the capsule. From the crest of the mound, the receptor hairs project upward into the lumen of the statocyst at angles between 27° and 74° from the horizontal plane. Behind the hair cells, in the space between opposing receptors, the mound flattens and forms a plateau (pl), which is void of any ultrastructure.

3.3. Transmission electron microscopy

The TEM section in Fig. 6a shows a cross section through the hair cell base and structures present in the
Fig. 6. (a) TEM micrograph of the hair cell base from the statocyst of *P. serratus*, fs. fibrous strands, cl. cuticle layer, cno. cuticular notch, n. nuclei, pnf. peripheral nerve fibre, sv. synaptic vesicles. (b) Saccular hair cell and innervating nerve fibres from the ear of *D. labrax* (from Lovell et al., in preparation), cb. cell body, cp. cuticular plate, k. kinocilia, n. nucleus, pnf. peripheral nerve fibres, s. stereocilia. (c) SEM micrograph of the statocyst hair cell from *P. serratus*, cb. cell base ts. tapering section. (d) SEM micrograph of the ciliary bundles projecting from the epithelial surface of *D. labrax* (From Lovell et al., in preparation), k. kinocilia, s. stereocilia. Bars=5 µm.

Fig. 7. (a) TEM section through the cell base from *P. serratus*, showing the cell nucleus (n.), and the beginning of the angled cell tip (ct.), and vesicles (v.) which appear to be associated with the fibrous strands (hatched area). (b) Fibrous strands (fs.) of the cell root, and the synaptic vesicles (sv.) located in the peripheral nerve bed.
peripheral nerve bed, from the statocyst of *P. serratus*.

Fig. 6b shows a cross section through a hair cell from the saccule of the European sea bass (*Dicentrarchus labrax*), which has been included in this section along with the SEM of the hair cell (Fig. 6d) for comparative purposes. The two hatched lines drawn on the prawn hair cell SEM micrograph presented in Fig. 6c shows the locations from where the statocyst TEM sections in Fig. 6a was taken.

The “root” of the statocyst hair cell is buttressed by supporting cells with large nuclei (n.), and fibrous strands (fs.) resembling actin filaments, which can be seen extending into the peripheral nerve bed through the cuticular plate. The filaments may help anchor the hair cell into position, and work in conjunction with a small notch in the cuticle layer (cno.) containing part of the lower cell body. The filament strands terminate 15 µm below the cuticle layer, in a region containing rounded structures less than 0.75 µm in diameter, which are thought to be the synaptic vesicles between the hair cell and the peripheral otic nerve fibres (pnf.). Close examination of the TEM section through the statocyst hair cell body (Fig. 7a) reveals that it contains a single nucleus (n.) positioned at the top of the cell. The hatched line in the basal region of the cell marks the perimeter of two vesicles, which appear to be associated with the fibres in the cell root. Fig. 7b shows the fibrous strands as they terminate in the synaptic vesicles (sv.) located 15 µm below the cell base.

3.4. Electrophysiological response to auditory stimuli

In order to concisely answer the question of hearing by crustaceans, 12 prawns of mixed sex were stimulated with sound ranging in the frequency domain between 100 and 3000 Hz, presented at sound pressure levels from 130 dB (re 1 µPa at 1 m) to below 90 dB (re 1 µPa at 1 m). The ABR recording technique has been successfully applied in the auditory assessments of both mammalian and non-mammalian vertebrates. An ABR waveform is acquired by averaging conglomerate responses of peak potentials, arising from nuclei in the auditory pathway during acoustic stimulation. The AEPs presented in Fig. 7 were recorded using the Medelec MS 6 biological amplifier with subcutaneous electrodes positioned using a jointed clamp assembly, and the prawn held in place using a fine mesh nylon cradle. The reference electrode was located in proximity to the neuropil of the antennule, and the record electrode located at the junction between the lateral antennular and otic ganglia. The acoustically evoked neural waveforms presented in Fig. 7, were recorded from *P. serratus* in response to tone bursts ranging in frequency from 500 to 3000 Hz, and averaged over 2000 stimulus presentations (100 and 300 Hz have not been included for scaling reasons). The waveforms show a series of peaks contiguous with the stimulus sound.

3.4.1. Threshold determination

Threshold responses from twelve 50–55 mm (medium) prawns were determined visually from the sequentially arranged waveforms for each frequency tested, in accordance with Kenyon et al. (1998). Fig. 6 shows ABR waveforms evoked from *P. serratus* in response to a 500-Hz tone burst, presented initially at between 120 and 132 dB (re 1 µPa at 1 m), and attenuated in steps of 4 dB (re 1 µPa at 1 m) ordinarily, and 2 dB (re 1 µPa at 1 m) as the hearing threshold was approached. When two replicates of waveforms showed opposite polarities (see 110 dB traces in Fig. 6), the response was considered as being below threshold (cf. Kenyon et al., 1998).

Fig. 8. Audiogram for *P. serratus*, determined visually from the sequential ABR waveform data, and by calculating the RMS of threshold SPL values of the stimulus sounds, presented at 100, 300, 500 750, 1000, 1500, 2000 and 3000 Hz tone bursts.

![Fig. 8. Audiogram for *P. serratus*, determined visually from the sequential ABR waveform data, and by calculating the RMS of threshold SPL values of the stimulus sounds, presented at 100, 300, 500 750, 1000, 1500, 2000 and 3000 Hz tone bursts.](image-url)

![Fig. 9. ABR waveforms from *P. serratus* in response to a 500-Hz tone burst attenuated in 2-dB steps. Averaged traces of two runs (2000 sweeps each), for each intensity are overlaid and arranged sequentially. Bar=1 µv.](image-url)
3.4.2. Audiogram for *P. serratus*

The audiogram shown in Fig. 8 was produced using sequential ABR waveform threshold data, acquired from frequencies of 100–3000 Hz, presented in steps between 200 and 500 Hz. The hearing thresholds of 12 mixed-sex *P. serratus* was measured, and follows a ramp like profile, determined by calculating the lowest intensity stimulus sounds (recorded underwater using the hydrophone located

![Audiogram](image)

Fig. 10. ABR waveforms in response to a 500-Hz sound presented 10 dB (re 1 µPa at 1 m) above threshold. Run A was recorded prior to the sham operation, and run B was recorded 1 h after cutting the cuticle layer covering the statocyst capsule as a sham operation. Y axis scale=µV×100.

![Evoked potentials](image)

Fig. 11. Evoked potentials from *P. serratus* to a 500-Hz tone presented 10 dB above threshold and averaged over 2000 sweeps. Runs a and b were recorded with the statocyst present, after cutting round the cuticle layer; whilst c and d were recorded 24 h after removal of the organ (the electrodes were removed and replaced between each run). Y axis scale=µV×100.
adjacent to the antennule) that evoked a repeatable ABR response (112 dB in Fig. 9). The profile follows a steady downward gradient to 100 Hz (the lowest frequency tested), and indicates that the “best” frequency in terms of threshold could be below this frequency.

3.5. Ablation

Removal of the statocyst was achieved by making a circular cut in the cuticle layer above the chamber, and withdrawing the capsule using needle point tweezers (a procedure that took a few seconds). Prior to removal of the statocyst, the prawn was retested with the cuticle layer cut as a sham operation. This procedure revealed that the AEP was no longer present, and was probably due to an imbalance in the hydrostatic pressure inside the antennule. The sham operation data presented in Fig. 10 were acquired by retesting the prawns 1 h after cutting around the chamber, prior to removal of the statocyst, and shows that the AEP eventually returns.

Fig. 12. Auditory evoked potentials from *P. serratus* to tone bursts of 500, 750, 1000, 1500, 2000 and 3000 Hz, and averaged over 2000 sweeps. The AEP at each amplitude tested has been overlaid, and shows a reduction in the response latency with increasing frequency. Scale=μV×100.
On removal of the statocyst, the prawns were placed in the empty fourth compartment of the holding tank, and allowed to recover for 24 h, prior to being retested on the electro-physiology apparatus. The post ablation recovery period was included to give the prawn’s time to settle after the procedure, as the metabolic state of the organism can have a detrimental affect on the evoked potential (Corwin et al., 1982). Additionally, the recovery period was included to allow for the equalisation of the hydrostatic pressure within the antennule. The evoked potentials shown in Fig. 11 were recorded from a 45-mm prawn, in response to a 300-Hz tone, presented at an intensity 10 dB above threshold. The first two runs (A and B, with two replicates of each run) were acquired from the prawn prior to the ablation procedure, and the subsequent two runs (C and D, with two replicates of each run) were recorded 24 h later. The electrodes were removed and replaced between each run, to confirm that the response was consistently repeatable, and to ensure that the absent responses in runs C and D was due to the ablation experiment, and not an extraneous factor associated with electrode placement.

4. Discussion

The hearing ability of the prawn (P. serratus) has been clearly demonstrated by this work using ABR audiometry, and offers conclusive evidence of low-frequency sound detection of frequencies ranging from 100 to 3000 Hz by an invertebrate from the sub-phylum crustacea. For hearing in the strictest sense to be attributed to an organism, the physiological response sound should be initiated by a specialised receptor mechanism (Myrberg, 1981), shown by this work to be generated in the statocyst. Current literature states that this organ is purely responsive to angular rotations and strong vibrations propagated directly through a solid medium, and is not responsive to sounds propagated in either air or water (Cohen and Dijkgraaf, 1961). It is highly probable that Cohen and Dijkgraaf did not find evidence of hearing due to masking of the AEP by neural activities other than audition; and from myogenic noise generated by muscular activity. To produce clear waveforms of an auditory response, it is recommended that AEP recordings be averaged for at least 1000–2000 stimulus presentations (Kenyon et al., 1998; Yan et al., 2000). The amplitude and shape of the electrophysiological response from P. serratus shown in Figs. 9 and 12 bear a remarkable similarity to AEPs generated by fish and higher vertebrates (see Corwin et al., 1982; Kenyon et al., 1998; Yan, 2002).

The two statocyst organs found in P. serratus lie adjacent to one another with medial symmetry, in the basal peduncle segment of the antennule. As can be seen in Figs. 4 and 5a, the statocyst is innervated by the otic ganglion, which emanates from a bed of peripheral nerve fibres lying under the mound directly beneath the receptor array (see Fig. 6a). The otic nerve terminates in the neuropil of the antennule, which is located in the ventral/anterior region of the brain.

The dissection of the 54-mm prawn in Fig. 3a shows that the total length of the neuronal pathway taken by the otic nerve, from the centre of the statocyst organ to the centre of the supraesophageal ganglion, is approximately 600 μm. However, the direct distance between the neuropil and the peripheral nerve fibres located below the statocyst, was found to be 500 μm. This is due to the curved pathway taken by the otic nerve, which first projects forward with the lateral antennular ganglion along the inside edge of the peduncle for 300 μm. From here, the otic ganglion branches away from the antennular ganglion at angles approaching 45° either side of the midline, from where it extends for a further 300 μm to the centre of the peripheral otic nerve bed. The schematic in Fig. 13 summarises the physiological work and shows the hair cells and the sand granule otoliths, along with the pathway taken by the otic ganglion, to the neuropil of the antennule and supraesophageal ganglion.

It is clear by the evidence presented in this work that the perception of sound in the frequency domain by P. serratus is similar in range to hearing in generalist fish, which is capable of both hearing and responding to sounds within a frequency bandwidth of 30 Hz to around 2000 Hz (Bretschneider et al., 2001), and is reliant on the phase variance between the three otolithic organs and the surrounding flesh to stimulate the sensory hairs of the inner ear (Lu, 2004). The audiogram presented in Fig. 8 follows a similar ramp like profile to those obtained from the cichlid A. ocellatus, which is considered to detect a best frequency of 100 Hz (Kenyon et al., 1998); however, lower frequencies were not tested. We therefore conclude that at least one species from the invertebrate sub-phylum of crustacea, is sensitive to the motion of water particles displaced by low-frequency sounds ranging from 100 Hz up to 3000 Hz. Although a number of physiological and behavioural experiments have been conducted on fish to assess the impact of noise on the auditory system none have, so far,
been directed toward the crustaceans, a major link in the oceanic food chain. The long-term effects of intense low-frequency sounds on the shrimp hearing ability and ecology is not known, but the data presented here shows that there is a need to include crustaceans in such an assessment, in order to gain a more insightful perspective of the effect of intense noise in the marine ecosystem.

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References


