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RESEARCH ARTICLE

Comparative sound detection abilities of four decapod crustaceans

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ABSTRACT

Sound perception and detection in decapod crustaceans is surprisingly poorly understood, even though there is mounting evidence for sound playing a critical role in many life history strategies. The suspected primary organ of sound perception is the paired statocysts at the base of the first antennal segment. To better understand the comparative sound detection of decapods, auditory evoked potentials were recorded from the statocyst nerve region of four species (*Leptograpsus variegate*, *Plagusia chabrus*, *Ovalipes catharus*, *Austrohelice crassa*) in response to two different auditory stimuli presentation methods, shaker table (particle acceleration) and underwater speaker (particle acceleration and pressure). The results showed that there was significant variation in the sound detection abilities between all four species. However, exposure to the speaker stimuli increased all four species sound detection abilities, in terms of both frequency bandwidth and sensitivity, compared with shaker table-derived sound detection abilities. This indicates that there is another sensory mechanism in play as well as the statocyst system. Overall, the present research provides comparative evidence of sound detection in decapods and indicates underwater sound detection in this animal group was even more complex than previously thought.

KEY WORDS: Auditory evoked potential, Crustacean, Sound perception, Hearing, Particle motion, Statocyst

INTRODUCTION

Sound propagates with less attenuation underwater compared with many other stimuli. Consequently, many marine animals make use of this sensory channel and regularly use acoustic signals for critical life history behaviours, such as communication [reviewed by Tyack and Miller, 2002 (marine mammals); Ladich, 2019 (fishes); Popper et al., 2001 (crustaceans)], foraging (Amorim, 2006; Radford and Montgomery, 2016), and habitat identification and orientation (reviewed by Montgomery et al., 2006). It is important to note that sound comprises two components, sound pressure and particle motion (Rogers and Cox, 1988). Far (>1–2 wavelengths) from the sound source (i.e. far field), particle motion is directly proportional to sound pressure, whereas near the source (i.e. near field), particle motion is high compared with sound pressure (Larsen and Radford, 2018). While sound pressure is the primary stimulus detected by the hearing end organs of terrestrial vertebrates (Fritzsch, 1999), particle motion is the primary stimulus detected by the hearing end organs for the large majority of marine animals (Popper and Fay, 2011; Radford et al., 2012). As such, we use a broader definition of hearing: 'the reception of vibratory stimuli of any kind and nature, provided that the sound source is not in contact with the animal's body' (Pumphrey, 1950).

Despite ample evidence that crustaceans use sound for numerous life history strategies, such as reproduction (Flood et al., 2019), social interactions (Roberts, 2021), and orientation and behaviour (Radford et al., 2007; Stanley et al., 2010, 2012), physiological data on their ability to detect and perceive sound underwater is surprisingly sparse (Breithaupt, 2002; Dinh and Radford, 2021; Edmonds et al., 2016; Hughes et al., 2014; Jézéquel et al., 2021; Lovell et al., 2005; Popper et al., 2001) compared with data for semi-terrestrial/terrestrial crustaceans (Budelmann, 1992; Popper et al., 2001; Roberts et al., 2016; Salmon et al., 1977). Decapod crustaceans have a variety of internal (chordotonal and statocyst organs) and external (superficial sensory hair fans on appendages) sensory receptors that are potentially responsive to sound and ground vibration (Popper et al., 2001; Roberts et al., 2016; Salmon et al., 1977). A number of these receptors resemble well-studied vertebrate receptors and respond to both particle motion and ground vibrations. However, there has been little research investigating whether decapod crustaceans are capable of detecting underwater sound.

Of the studies (Breithaupt and Tautz, 1988; Hughes et al., 2014; Lovell et al., 2005; Radford et al., 2016b) that have investigated the sensitivity of decapod crustaceans to underwater sounds, both single unit and auditory evoked potential (AEP) recordings have been conducted. Using a pure particle motion stimulus (mechanical shaker), it was found that crayfish (*Procambrus clarkii*) could detect sounds from 20 to 2350 Hz, with highest sensitivity at 600 Hz (Breithaupt and Tautz, 1988). Using an underwater speaker and the AEP technique, it has been shown that the prawn (*Palaemon serratus*) could detect sound between 100 and 3000 Hz, with highest sensitive at 100 Hz (Lovell et al., 2005); the mud crab (*Panopeus* spp.) detection range was from 80 to 1600 Hz (Hughes et al., 2014); the American lobster (*Homarus americanus*) range was from 80 to 250 Hz (Jézéquel et al., 2021); and the snapping shrimp (*Alpheus richardsoni*) range was from 80 to 1500 Hz (Dinh and Radford, 2021). These studies highlight that decapod crustaceans seem to be most sensitive at low frequencies (Roberts and Elliott, 2017). Furthermore, they highlight that there is a real need for standardising the type of stimulus and physiological recording technique. Also, given the definition of hearing used here, it is important to note that physiological data do not provide evidence of hearing, but rather what sounds are detected. To determine what an animal hears, behavioural experiments need to be employed, which demonstrate that the animal behaviourally reacts to the sound (Popper and Hawkins, 2021).

Given the absence of any obvious air-filled spaces and based on theoretical calculations (Breithaupt, 2002; Edmonds et al., 2016), it is generally assumed that crustaceans are only sensitive to the

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particle motion component of underwater sound. To shed more light on this question, the present study compared AEPs from the statocyst nerve region, the putative primary organ of sound detection in crustaceans (Breithaupt and Tautz, 1988; Lovell et al., 2005; Popper et al., 2001; Salmon et al., 1977), across four ecologically different decapod crustacean species. Importantly, the crustaceans' AEPs were compared separately with the two components of underwater sound – particle motion and pressure – by exposing the animals to either a pure particle motion stimulus via the shaker table or the combination of pressure and particle motion of a typical sound field via an underwater speaker.

MATERIALS AND METHODS

Animal care

All decapod crustaceans were captured using standard techniques and protocols. All animals were kept in flow-through holding tanks supplied with ambient seawater at the Leigh Marine Laboratory, New Zealand. Animals were a mix of males and females and held in groups, with a maximum group size of five individuals and were fed 3 times per week. Rocks and cinder blocks were placed into the tanks to provide shelter and minimise agonistic encounters. Animals were held a maximum of 2 weeks, 1 week to acclimate to tank conditions, followed 1 week to run the experiments. Species tested were: purple shore crab [*Leptograpsus variegatus* (Fabricius 1793); carapace width 39–50 mm], which is a temperate crab species commonly occurring in intertidal rocky reef habitats; red rock crab [*Plagusia chabrus* (Linnaeus 1758); carapace width 38–43 mm], a subtidal rocky reef species; the New Zealand paddle crab [*Ovalipes catharus* (White 1843); carapace width 68–79 mm], a subtidal soft sediment species; and the tunnelling mud crab [*Austrohelice crassa* (Dana 1851); carapace width 16–20 mm], an intertidal soft sediment species. Forty animals were used in these experiments: 20 (5 of each species) for *in vivo* AEP measurements in response to a speaker stimulus (delivering both pressure and particle acceleration stimuli) and 20 for *in vivo* AEP measurements in response to a shaker table stimulus (delivering particle acceleration stimulus alone). All experiments were approved by the University of Auckland Animal Ethics committee (protocol # 001404).

Auditory evoked potentials

Hearing ability of each decapod species was quantified using the AEP technique. For AEP testing, the animal was completely submerged underwater in a PVC (0.5 mm thick) tank, 1.11 m long with a diameter of 0.25 m. Animals were positioned dorso-ventrally upon a piece of clay on a Perspex slide attached perpendicular to a plastic pipette (animal holder). Rubber bands restrained the animal firmly on the holder. A micromanipulator was used to position the animal holder 8 cm from the water surface. An underwater speaker (University Sound UW-30, Columbus, OH, USA) was placed near the opposite end of the tank, approximately 0.75 m from the animal. Auditory stimuli were produced by a sound module (Tucker-Davis Technologies, TDT, Gainesville, FL, USA) operated by a computer running SigGen (version 4.4.1) and BioSig (version 4.4.1) software. The TDT apparatus linked to the underwater speaker delivered tone bursts (10 ms duration with a 2 ms rise–fall time gated through a Hanning window; 18 presentations per second) with frequencies of 80, 100, 200, 400, 600, 1000 and 2000 Hz. The presentation order of the frequencies was conducted randomly. Sound pressure level (SPL) was increased in 5 dB increments for each frequency until a stereotypical AEP was seen, and then continued for at least another 10 dB to examine suprathreshold responses. An average of 1000 responses (500 from stimuli presented at 90 deg and 500 from stimuli presented at 270 deg to cancel stimulus artefacts) was taken for each SPL at each frequency.

Stainless steel subdermal electrodes (Rochester Electromedical Inc., Tampa, FL, USA) were used to collect AEPs over a 50 ms timing window at 24,400 Hz. The recording electrode was positioned dorsally, just posterior to the right antennule, whilst the reference electrode was placed near the right first walking leg, with a ground electrode positioned under the body under a dissecting microscope. Each electrode was insulated with nail varnish, except for the tip.

Shaker evoked potential measurements

To test the effects of accelerations alone, a custom-built moving coil shaker (LDS V780 T minishaker) system was used to provide sinusoidal horizontal stimulation (see Radford et al., 2012, for shaker design), similar to tones presented in the tank. This motion stimulus was free of pressure and interference phenomena found in the tank set-up (Parvulescu, 1963), therefore primarily providing an acceleration stimulus. The animals were restrained on top of a bed of clay by rubber bands and were oriented so that the anterior end of the shrimp was in line with the moving coil system. Between each presentation frequency, individuals were dripped with 2 ml of seawater to keep the gills wet. Sinusoidal particle accelerations were generated by a sound module (TDT) operated by a computer running SigGen (version 4.4.1) and BioSig (version 4.4.1) software. The TDT apparatus linked to the shaker table delivered sinusoidal acceleration (10 ms duration with a 2 ms rise–fall time gated through a Hanning window; 18 presentations per second) with frequencies of 40, 60, 80, 100, 200, 400, 600, 1000 and 2000 Hz. An average of 1000 responses (500 from stimuli presented at 90 deg and 500 from stimuli presented at 270 deg to cancel stimulus artefacts) was taken for each SPL at each frequency in both the *x*-axis stimulus directions. AEPs were measured using the same method as the speaker stimulus (see above). Pressure wave, pressure spectral levels and particle acceleration magnitude spectra can be seen in Fig. 1.

Sound and particle calibrations

Sound pressure and particle accelerations in the tank and particle accelerations in relation to the shaker table were calibrated in the absence of the crustacean subject. Sound pressure calibration was carried out daily using a Reson TC4013 mini hydrophone (calibration sensitivity −203 dB re. 1 V Pa^{-1}; www.teledyne-reson.com) placed in the position of the animal holder. An oscilloscope was used to measure the SPL at each frequency, which was then attenuated through BioSig software (TDT) to output the desired decibel levels. Particle accelerations for speaker-induced stimuli were calculated using a calibrated Brüel & Kjær accelerometer (Deltatron 4524 cubic triaxial accelerometer, 100 mV g^{-1}; Helsinki, Finland) that had been waterproofed and made neutrally buoyant by embedding it in a syntactic foam enclosure (Zeddies et al., 2012). The accelerometer was then connected to a three-channel conditioning amplifier (Deltatron 2693-A-OS3), with the output fed into an oscilloscope (Tektronix DPO 2014, Beaverton, OR, USA). The shaker table was calibrated using three single-axis Brüel & Kjær accelerometers (4507B-002 Deltatron accelerometer, 1000 mV g^{-1}) connected to a conditioning amplifier (Deltatron 2693- A-OS3) with the output measured on an oscilloscope (Tektronix DPO 2014). The *x*-axis was the dominant stimulus direction and was always of the order of 10–12 dB greater than the *y*- and *z*-axes. For all three particle acceleration methods, accelerations were calculated for the *x*-, *y*- and *z*-planes and the acceleration magnitude [calculated as $\sqrt{(x^2+y^2+z^2)}$] is reported.

Journal of Experimental Biology (2022) 225, jeb243314. doi:10.1242/jeb.243314

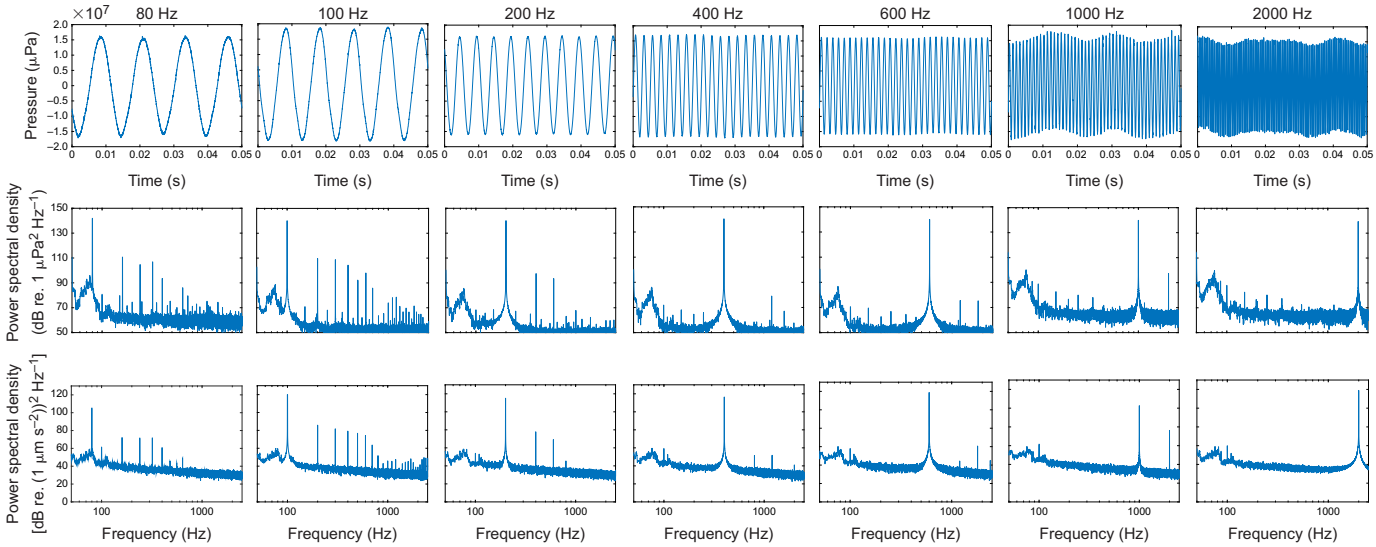

Fig. 1. Representative examples of each frequency tested with their corresponding power spectra below in both pressure (dB re. 1 µPa^2 Hz^{-1}) and particle acceleration [dB re. (1 µm s^{-2})^2 Hz^{-1}]. Measurements were made using a hydrophone in the same place, where the fish's head was positioned.

Acoustic impedance measurements

The sound field within the tank will be significantly affected by the tank dimensions and the material the tank is constructed from (e.g. PVC). Therefore, it is important to determine the acoustic impedance of the sound field within the tank (Popper and Fay, 2011). Acoustic impedance, defined as the particle motion to sound pressure ratio, differs in tanks compared with that in a natural free-field environment. Aquatic animals are probably detecting both sound pressure and particle motion, therefore the impedance of tanks provides essential context for understanding reported hearing abilities (Popper and Fay, 2011; Popper and Hawkins, 2019). Also, the impedance of a tank will influence the source levels required to generate the evoked potential response. Therefore, the acoustic impedance of the experimental tank setup was determined at the location of the animals' statocyst region. Tank impedance was measured against theoretical seawater in a free-field environment with a salinity of 35 ppt and 15°C (Z=1.5597 MRayl) (Bradley and Wilson, 1966; Vetter et al., 2019). Sound pressure and triaxial particle acceleration were measured simultaneously for every test frequency (80, 100, 200, 400, 600, 1500 and 2000 Hz) at three different SPLs (155, 145 and 139 re. 1 µPa). Sound pressure was measured with a TC4013 hydrophone (sensitivity 210.8 dB re. 1µPa V^{-1}; Reason, Slangerup, Denmark) and particle acceleration was measured with a neutrally buoyant and waterproofed triaxial accelerometer (Deltatron Type 4524, sensitivity: 100 mV g^{-1}; Brüel & Kjær) connected to a conditioning amplifier (Deltatron 2693-A-OS3, Brüel & Kjær). Both sensors were connected to an oscilloscope to measure peak-to-peak voltage (V_{pk-pk}) in both the pressure (dB re. 1 µPa) and particle acceleration (dB re. 1 m s^{-2}) domains. Particle acceleration was transformed into particle velocity using the formula $v=a/2\pi f$ (Nedelec et al., 2016). Then, the impedance for each frequency was calculated in MRayl, where 1 Rayl=1 (Pa s) m^{-1}. These values were then compared with the theoretical free-field impedance of seawater with a salinity of 35 ppt and 15°C, and represented on a log scale (dB re. 1.5597 MRayl) (Vetter et al., 2019). Phase of the impedance ($\Delta\Phi_{p,v}$) was calculated from the phase difference between the particle acceleration (a) waveform and the sound pressure waveform ($\Delta\Phi_{p,a}=\Phi_p-\Phi_a$). Because the phase of the particle velocity (v)

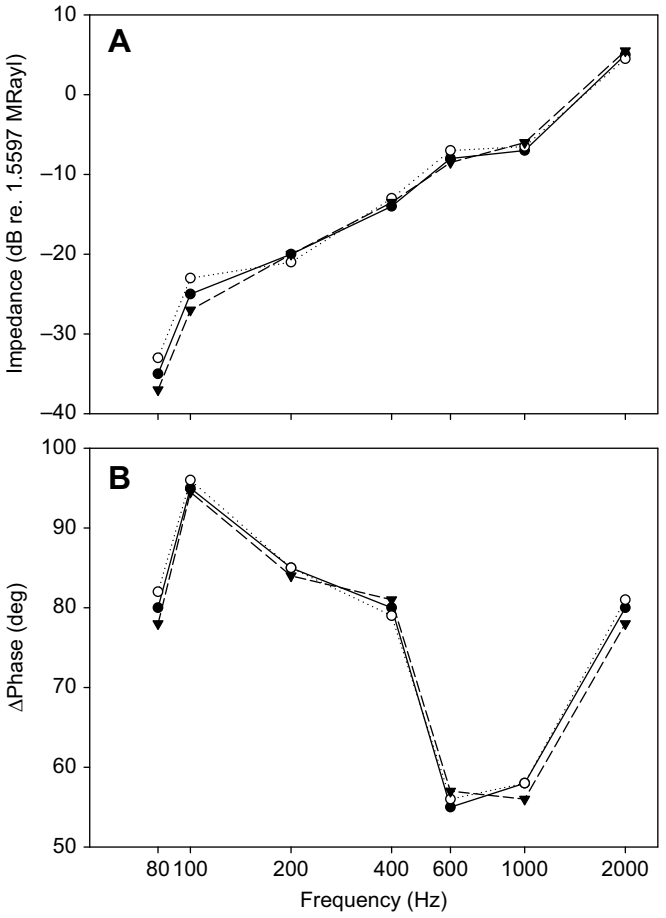

Fig. 2. Acoustic characteristics of the experimental tank and speaker. (A) Acoustic impedance [ratio of sound pressure (dB re. 1 µPa) to particle velocity (dB re. 1 m s^{-1})] in the z-axis (Z) relative to 1.5597 MRayl (the reference impedance for a free-field in 35 ppt salinity seawater at 15°C) is plotted for all the frequencies examined at three sound pressure levels (SPL): 139, 145 and 155 dB re. 1 µPa. Measurements were made using a triaxial accelerometer placed in the centre of the tank and water column. (B) Phase difference (Δ) between the pressure and particle velocity wave. Measurements were made using a triaxial accelerometer placed in the centre of the tank and water column.

Journal of Experimental Biology (2022) 225, jeb243314. doi:10.1242/jeb.243314

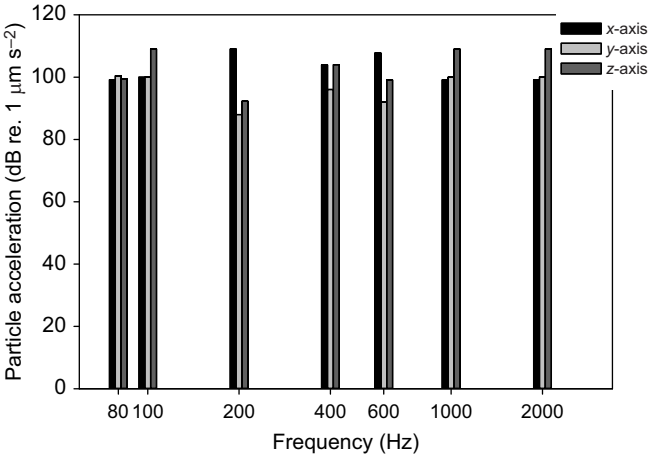

Fig. 3. Particle acceleration levels (dB re. 1 μm s^{-2}) from the underwater speaker measured in all three dimensions (x-, y- and z-axes) at all frequencies examined for three SPL: 139, 145 and 155 dB re. 1 μPa. Measurements were made using a triaxial accelerometer in the same place, where the fish's head was positioned.

waveform leads the phase of the particle acceleration waveform by 90 deg, the phase of impedance was calculated as $\Delta\Phi_{p,v}=\Delta\Phi_{p,a}+90$ deg. The results indicate that the recording system is well within the near field; also, there was not a simple relationship between velocity and pressure, because the of the confined tank environment (Fig. 2). The variation in each acceleration direction can be seen in Fig. 3.

Data analyses

Threshold determination for a given frequency was undertaken both visually and through a power spectral analysis to determine the lowest possible intensity at which a response was observed. Evoked responses were averaged, and a power spectrum calculated using a 2048-point fast Fourier transform (FFT). The spectra were analysed for peaks at twice the stimulus frequency at least 3 dB above background noise. For each frequency, the lowest sound level at which such peaks were evident was defined as the auditory threshold (Mélotte et al., 2018). Homogeneity of the variance and normality were verified by using Levene tests and Shapiro–Wilk's statistics, respectively. Initial analysis (Student's *t*-test) showed that sound detection abilities between male and female crabs were similar; therefore, all crabs were pooled. To test for differences between pressure- and shaker table-derived thresholds for individual species, two-way ANOVA was used (factors: stimulus, frequency). The two lowest frequencies (40 and 60 Hz) tested on the shaker table could not be generated by the speaker, so were excluded from the analysis. To compare speaker and shaker table threshold differences between species, two-way repeated measures ANOVA (factors: stimulus and frequency) were used. Where significant differences were found, Tukey's HSD *post hoc* tests were conducted. For all tests, the significance level was α=0.05.

RESULTS

AEP traces

There were no differences in the shape or time course of the evoked responses between the different species presented with an underwater speaker (Fig. 4A) or shaker table (Fig. 4B) stimulus. In all cases,

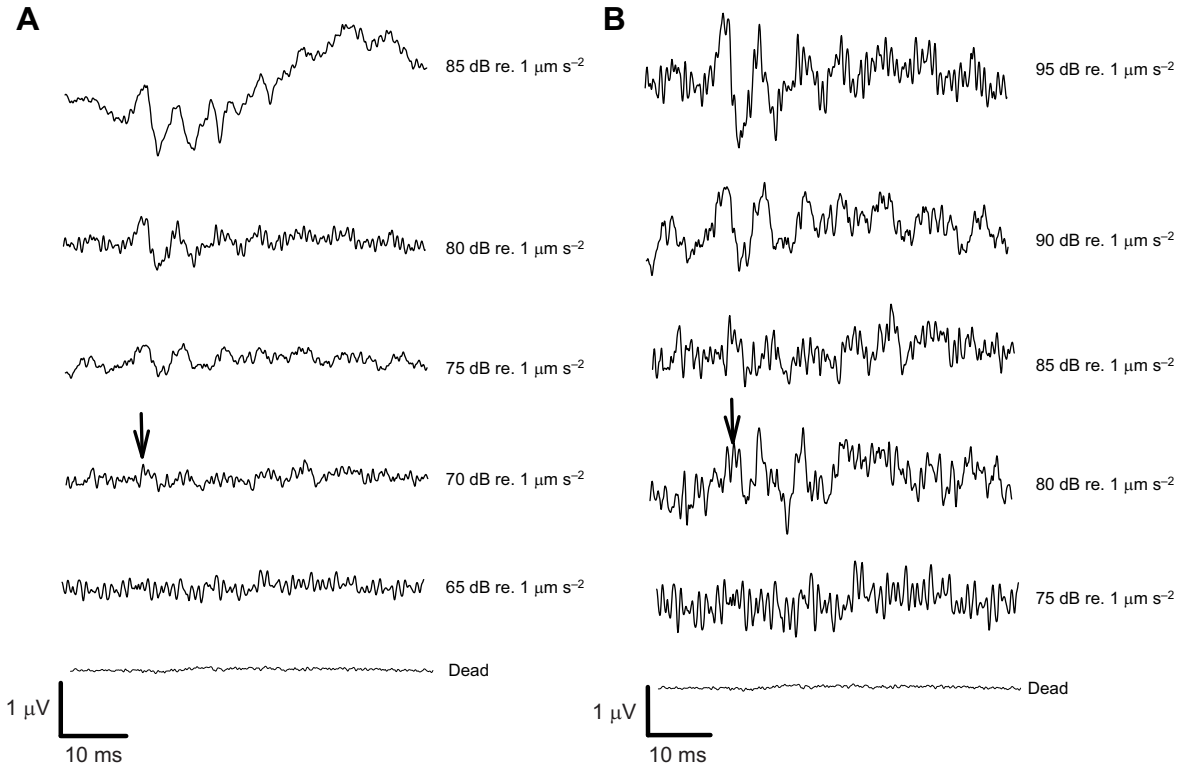

Fig. 4. Example of audio evoked potential (AEP) waveforms for the New Zealand paddle crab (*Ovalipes catharus*) in response to a particle acceleration signal generated by an underwater stimulus or shaker table. (A) A 100 Hz particle acceleration signal generated by the underwater speaker stimulus. (B) Particle accelerations generated by the shaker table stimulus. The black arrow indicates the beginning of the AEP response. The lowest particle acceleration detected can be seen at 70 dB re. 1 μm s^{-2} for the speaker stimulus and 80 dB re. 1 μm s^{-2} for shaker table stimulus.

Journal of Experimental Biology (2022) 225, jeb243314. doi:10.1242/jeb.243314

the response traces showed repeated peaks and troughs in the response waveform that persisted throughout the recording duration. However, there were very slight differences observed in the shape of the AEP traces between the different presentation stimuli (Fig. 4).

Comparison between different species

There were significant differences in sound detection ability between the four species tested for each of the measured stimuli: sound pressure- ($F_{3,99}$=9.17; P<0.001), speaker particle acceleration- ($F_{3,99}$=9.02; P<0.001) and shaker table-determined ($F_{3,154}$=12.67; P<0.001) sound detection ability (Fig. 5). The purple shore crab was the least sensitive to sound pressure compared with the other three species, which had similar sensitivities (Fig. 5A). Speaker particle acceleration-determined sound detection ability (Fig. 5B) exhibited significant variation in both frequency bandwidth and sensitivity between the four different species. The purple shore crab was the least sensitive and had the narrowest frequency bandwidth (80–400 Hz), while the New Zealand paddle crab had the widest frequency bandwidth (80–2000 Hz). Shaker table-determined sound detection ability (Fig. 5C) showed the least variation between species, with the red rock crab, the New Zealand paddle crab and the tunnelling mud crab exhibiting a similar ability to detect sound in both sensitivity and bandwidth. The purple shore crab was the least sensitive and had the narrowest frequency bandwidth.

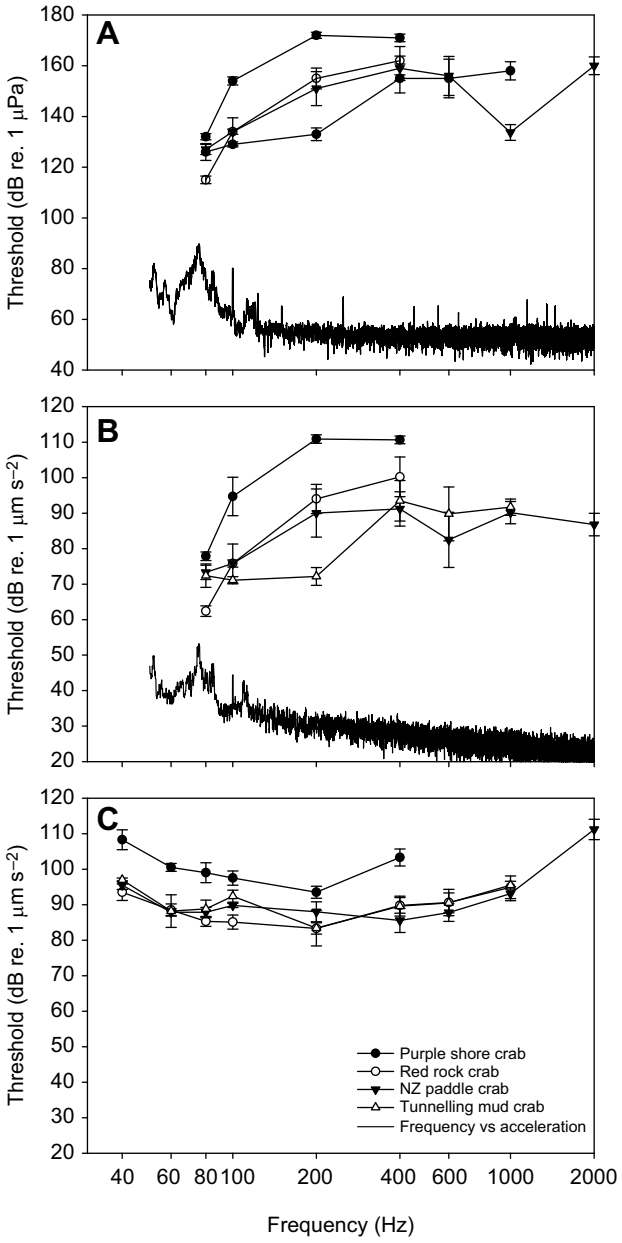

Fig. 5. Mean (±s.e.m.) sound detection thresholds of the four crustaceans examined. Sound detection thresholds were determined using (A) pressure (dB re. 1 µPa; n=5), (B) particle acceleration (dB re. 1 µm s^{-2}) generated with an underwater speaker (n=5) and (C) particle acceleration (dB re. 1 µm s^{-2}) generated with a shaker table (n=5). Ambient background noise of the tank in both pressure (A) and particle acceleration (B) is shown.

Comparison between different stimuli

Below 200 Hz, speaker-derived sound detection ability for the tunnelling mud crab was significantly more sensitive ($F_{5,59}$=5.35; P<0.001) than shaker table-derived sound detection ability. Above 200 Hz, sound detection ability was similar between the two auditory generation stimuli (Fig. 6A). In contrast, the red rock crab ($F_{3,39}$=11.79; P<0.001) and New Zealand paddle crab ($F_{6,69}$=7.64; P<0.001) were significantly more sensitive below 100 Hz for speaker-derived sound detection ability and showed similar sensitivity between the two auditory generation stimuli above 100 Hz (Fig. 6B,C). The purple shore crab was somewhat unusual as the speaker-derived particle acceleration detection ability was only more sensitive at 80 Hz, and less sensitive at 200 Hz ($F_{3,39}$=10.53; P<0.001; Fig. 6D).

DISCUSSION

To our knowledge, this is the first study that provides a comparative examination of the sound detection abilities of decapods to both a pure particle motion stimulus (delivered through a shaker table) and a mixed particle motion and sound pressure stimulus (delivered through a traditional underwater speaker). This allowed a direct comparison and evaluation of different stimuli presentation mechanisms to sound detection capabilities in decapods. Furthermore, comparisons were made between four species that occupy two distinct broad habitat types: rocky reef versus soft sediment. It was shown that decapod sound detection was both species and habitat specific, with the soft sediment species appearing to be the most sensitive (i.e. lowest hearing thresholds) and having a wider frequency bandwidth. More importantly, the present study provides some much-needed comparative sound detection data on a range of decapod crustacean species.

The present study found that all examined species displayed a similar AEP response to both types of stimuli. Qualitatively, the AEP responses from the statocyst nerve region were similar for both stimuli and across species, with virtually no differences in the shape or time course of the AEP waveforms. The frequency doubling effect observed in the waveform spectrum has been seen previously in crustaceans, squid and fish (Dinh and Radford, 2021; Hughes et al., 2014; Jézéquel et al., 2021; Mooney et al., 2010; Popper and Fay, 1993), and is indicative of an auditory system with directionally sensitive sensory hair cells. Without further anatomical and physiological experiments, one can only speculate about the mechanism for the differences in hearing abilities observed across species. Aside from the underlying neural correlate, the complexity of the statocyst structure itself might be related to the sensitivity of sound detection. The anatomy of the statocyst is very intricate in Portunid crabs (Cate and Roye, 1997), where a large number of

Journal of Experimental Biology (2022) 225, jeb243314. doi:10.1242/jeb.243314

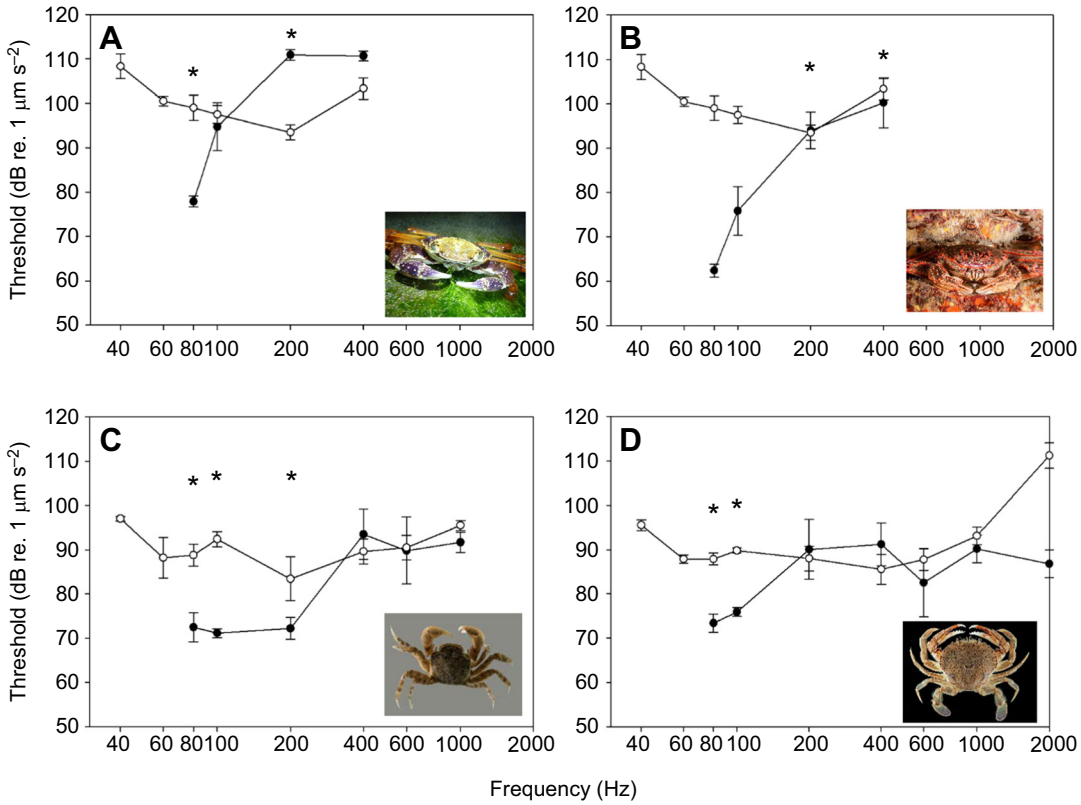

Fig. 6. Mean (±s.e.m.) particle acceleration thresholds (dB re. 1 µm s^{-2}) determined for both speaker (filled circles) and shaker table (open circles) stimulus for the four crustaceans examined. (A) Purple shore crab (*n*=5); (B) red rock crab (*n*=5); (C) tunnelling mud crab (*n*=5); and (D) New Zealand paddle crab (*n*=5). Photo credit (all photos): Richard Taylor.

sensory receptors or sensilla located in the canal system play a role in inducing compensatory eye movement (Sandeman and Okajima, 1972). This level of complexity could also be the basis for the New Zealand paddle crab's ability to detect sounds at low intensities, compared with the purple shore crab and red rock crab.

Contrary to predictions based on our current understanding of crustacean hearing, the underwater speaker-determined auditory detection thresholds were more sensitive than the pure particle motion-determined auditory thresholds, especially at low frequencies (≤200 Hz). This result was also reported for the snapping shrimp (Dinh and Radford, 2021). Analogous to otolithic hearing in fish, the statocyst is directly sensitive to the particle movement of an acoustic field because of whole-body accelerations (Budelmann, 1992; Montgomery et al., 2006; Rogers and Cox, 1988). The shaker table is purely an acceleration stimulus that would directly stimulate a loaded hair cell-based system (hair cells with a mass), such as the statocyst. In contrast, the speaker generates both particle motion and pressure and would stimulate both loaded and unloaded sensory systems (hair cells without a mass). Therefore, the differences observed in sound detection ability (in both bandwidth and sensitivity) between the different stimulus mechanisms (speaker versus shaker table) strongly suggest that the statocyst system in decapods is not the only sensory system capable of detecting underwater sound. This notion is further supported by studies (Jézéquel et al., 2021; Radford et al., 2016b) showing that ablating the statocyst in the American lobster and New Zealand paddle crab did not reduce the AEP sound detection response. However, ablating the sensory hair fans that cover the body of American lobsters reduced the AEP sound detection response (Jézéquel et al., 2021). Hence, other sensory systems might contribute to underwater particle motion detection in crustaceans. Candidate systems that might contribute are the plethora of proprioceptors and sensory hairs that can be found in crustaceans (Budelmann, 1992; Montgomery et al., 2006; Popper et al., 2001).

There is growing evidence that aquatic brachyurans, such as the New Zealand paddle crab (Flood et al., 2019) and the Italian paddle crab (Buscaino et al., 2015), can produce sounds and vibrations; however the biological function of these sounds is still poorly understood. Flood et al. (2019) have shown that the New Zealand paddle crab produces two types of low frequency sounds, the zip (peak frequency 660 Hz) and bass (peak frequency 45 Hz), that were thought to be used by males competing for reproductive females. Both sounds fall within the frequency bandwidth to which the New Zealand paddle crab was most sensitive. Furthermore, Hughes et al. (2014) found that the ecologically important crab (*Panopeus* spp.) reduced feeding rates in response to vocalisations of their main predator, toadfish (*Opsanus tau*), hardhead catfish (*Ariopsis felis*) and the black drum (*Pogonias cromis*). One of the main fish predators of the New Zealand paddle crab, the bluefin gurnard (*Chelidonichthys kumu*), produces sounds (Radford et al., 2016a) that also fall within the frequency bandwidth to which the New Zealand paddle crab was most sensitive. This highlights the importance of sound reception and detection for this particular species for the successful implementation of sound-mediated life history strategies.

An interesting point to note was that the two soft sediment species (New Zealand paddle crab and tunnelling mud crab) were more sensitive (lower thresholds) and had wider frequency bandwidths than the two species from a rocky shore habitat (purple shore crab and red rock crab). This result might be an adaption to habitat sound

levels and the reliable information that can be gathered at different frequencies in the two habitats, with less reliable information available in the presence of wave action at the rocky shoreline. A similar effect has been observed in several groups of freshwater fish, where fish in quieter habitats have evolved enhanced hearing sensitivity and expanded frequency bandwidth than those in louder habitats (Amoser and Ladich, 2005).

Alternatively, these differences could be attributed to an enhanced sensitivity to substrate-borne vibration. There is mounting evidence that substrate-borne vibrations are important to invertebrates in general (see Roberts and Elliott, 2017, for review). With respect to crustaceans, it has been shown that a range of decapods (crabs, lobsters and shrimp) can seismically communicate (see Taylor and Patek, 2010, for review). For example, the land hermit crab, *Coenobita compressus* (Roberts, 2021), and ghost crabs (Taylor and Patek, 2010) can communicate using substrate-borne vibrations during social interactions. Also, a behavioural study (Roberts et al., 2016) has shown that the marine hermit crab, *Pagurus bernhardus*, is sensitive to ground vibrations between 5 and 410 Hz. Substrate-borne vibrations may be important for soft sediment species – in particular, the tunnelling mud crabs live in borrows and this could be a means of communication between animals living in different borrows. The low frequency bass signal (45 Hz) produced by the New Zealand paddle crab (Flood et al., 2019) could potentially be a seismic signal used for communication. Although there were only two species from each habitat examined here, it provides two interesting concepts for further research.

Ideally, experiments should be conducted in the free-field environment (for example, Hawkins and Chapman, 1975; Hawkins and Johnstone, 1978; Hawkins and Sand, 1977) because of the complicated acoustics associated with producing sound fields within tanks (Parvulescu, 1963). As a result, it has now become standard practice to present acoustic impedance measurements of the tank environment (Popper and Fay, 2011; Popper and Hawkins, 2019). Here, the acoustic impedance results from where the statocyst region of the animals was located within the tank show that these experiments were conducted in the near-field environment. Furthermore, physiological experiments do not examine true hearing responses, but rather what the animals can detect (Popper and Hawkins, 2021; Popper et al., 2020). Therefore, this study provides valuable data about the frequency bandwidth that several brachyuran species could detect, providing a framework within which free-field behavioural studies can be conducted; furthermore, it highlights the differences between animals from different habitats and indicates that the statocyst was probably not the only sensory organ involved in sound detection.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
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