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RESEARCH ARTICLE

Sound detection by the American lobster (*Homarus americanus*)

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ABSTRACT

Although many crustaceans produce sounds, their hearing abilities and mechanisms are poorly understood, leaving uncertainties regarding whether or how these animals use sound for acoustic communication. Marine invertebrates lack gas-filled organs required for sound pressure detection, but some of them are known to be sensitive to particle motion. Here, we examined whether the American lobster (*Homarus americanus*) could detect sound and subsequently sought to discern the auditory mechanisms. Acoustic stimuli responses were measured using auditory evoked potential (AEP) methods. Neurophysiological responses were obtained from the brain using tone pips between 80 and 250 Hz, with best sensitivity at 80–120 Hz. There were no significant differences between the auditory thresholds of males and females. Repeated controls (recordings from deceased lobsters, moving electrodes away from the brain and reducing seawater temperature) indicated the evoked potentials' neuronal origin. In addition, AEP responses were similar before and after antennules (including statocysts) were ablated, demonstrating that the statocysts, a long-proposed auditory structure in crustaceans, are not the sensory organs responsible for lobster sound detection. However, AEPs could be eliminated (or highly reduced) after immobilizing hairfans, which cover much of lobster bodies. These results suggest that these external cuticular hairs are likely to be responsible for sound detection, and imply that hearing is mechanistically possible in a wider array of invertebrates than previously considered. Because the lobsters' hearing range encompasses the fundamental frequency of their buzzing sounds, it is likely that they use sound for intraspecific communication, broadening our understanding of the sensory ecology of this commercially vital species. The lobsters' low-frequency acoustic sensitivity also underscores clear concerns about the potential impacts of anthropogenic noise.

KEY WORDS: Marine invertebrate, Crustacean, Hearing, Auditory evoked potential, Acoustic communication

INTRODUCTION

Sound is an essential and widespread sensory cue for many marine organisms. It has been known for decades that marine mammals and fish use sounds to communicate with conspecifics (Tyack and Clark, 2000; Ladich, 2015). Comparatively, for aquatic invertebrates, there are much less data on sound detection, yet there is increased understanding that they too utilize underwater sounds. For example, studies have shown that crustaceans produce sounds (Schmitz, 2002), but limited knowledge of their hearing sensitivity precludes understanding of the potential uses of sound by crustaceans for intraspecific communication (Edmonds et al., 2016).

Since the first discovery of sound production by lobsters more than 60 years ago (Moulton, 1957), the potential use of sound for intraspecific communication has been an intriguing area of study (Scrivener, 1971; Atema and Cobb, 1980; Atema and Voigt, 1995; Breithaupt, 2002). 'Buzzing' sounds are produced by lobsters through the rapid contraction of internal muscles located at the base of their second antennae, which causes their carapaces to vibrate (Mendelson, 1969). The sound features are similar in both American (*Homarus americanus*) and European (*Homarus gammarus*) lobsters, and are characterized by low frequencies (~100 Hz) with a relatively long duration (~200 ms; Fish, 1966; Henninger and Watson, 2005; Jézéquel et al., 2018). Ward et al. (2011) suggested that *H. americanus* may use these sounds primarily to deter predators such as fish. Recently, our group found that male *H. gammarus* produce repeated buzzing sounds during agonistic encounters, reviving the hypothesis for intraspecific sound communication in lobsters (Jézéquel et al. 2020a). However, we could not validate this hypothesis because there are no published data addressing whether male lobsters actually detect sounds. Accordingly, there is a clear need to address the sound sensitivity of lobsters.

Sound-detection abilities of marine invertebrates, in general, are poorly understood. Crustaceans lack air-filled spaces and compressible tissues required for sound pressure detection (Popper et al., 2001; Popper and Hawkins, 2018). However, they possess a variety of external and internal sensory receptors that have been shown to detect low-frequency particle motion (reviewed in Cohen and Dijkgraaf, 1961; Bush and Laverack, 1982; Budelmann, 1992). Superficial receptor systems include cuticular hairfan and hair-peg organs that cover their external body surface (Laverack, 1962, 1963). Chordotonal organs, which are present in the joints of body appendages, measure leg motions and are sensitive to low-frequency vibrations (Bush and Laverack, 1982). The most well-studied and potential organ for sound detection in lobsters is the internal sensory receptor called the statocyst, located in the basal segment of each antennule (Cohen, 1955). It is a fluid-filled chamber containing sand grains, together forming a statolith, which lies in contact with sensory hairs (Cohen, 1960). This receptor is primarily attributed to equilibrium and may also act as an accelerometer, responding to vibrations propagated directly through a solid medium. Historically, in lobsters, it was considered unresponsive to waterborne sounds (Cohen and Dijkgraaf, 1961). However, recent studies on crabs (*Ovalipes catharus*) and prawns (*Palaemon serratus*) indicated that their statocysts are an auditory organ (Lovell et al., 2005; Radford et al., 2016). Thus, for lobsters, it is not clear what organ (or organs), if any, are sensitive to sounds.

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Sound detection is often measured using auditory evoked potentials (AEPs), which reflect synchronous neural activity as afferent responses are conducted from the auditory end-organ to the brain (Burkhard et al., 2007). AEP recording techniques have been used extensively to construct audiograms in odontocetes and fish (e.g. Fay and Edds-Walton, 1997; Mooney et al., 2015). Audiograms represent the sound amplitudes (also termed thresholds) at certain frequencies above which the species are able to detect sounds. Recent AEP studies have also been done on invertebrates, including cephalopods (Mooney et al., 2010) and crustaceans (Lovell et al., 2005; Hughes et al., 2014; Radford et al., 2016). These invertebrates mainly detect low frequencies (below 1 kHz), with the best sensitivity around 100 Hz. Such a method could be useful to assess the frequency range of response to waterborne sounds in lobsters, as well as to explore which organs may transduce acoustic signals.

The aim of this study was to determine whether the American lobster (*H. americanus*) responds to sounds and the likely mechanism responsible for sound detection. Hearing range and sensitivity of *H. americanus* were measured using AEP techniques. We first sought to determine whether neuronal responses could be recorded, including determining recording location. We then investigated the audiograms from both male and female lobsters and compared them with the invertebrate hearing literature to place hearing in a social and comparative context. Next, we performed control experiments to validate lobster sound detection and determine the apparent sensory organ responsible for sound detection. Finally, we discussed the implications of our results for lobster ecology.

MATERIALS AND METHODS

All experiments were conducted in February and March 2020 at the research facilities of the Environmental Systems Laboratory (ESL), Woods Hole Oceanographic Institution (Woods Hole, MA, USA).

Animal collection, characteristics and care

A total of 16 individual American lobsters (*Homarus americanus* H. Milne-Edwards 1837) were used, with carapace lengths (CLs; measured from the eye socket to the posterior carapace margin) between 8.4 and 11.7 cm. Animals were bought from local fishermen several days after they were captured in traps; eight males were bought in January 2020 and eight females in February 2020. We used only intermoult individuals (as described in Aiken, 1973) with full sets of undamaged appendages. Note that preliminary experiments were performed using three animals. These three lobsters were not used for the main experiments and their results were not compiled with those of the 16 individuals with audiograms described in this paper. This sample size is standard for AEP studies in marine invertebrates (Lovell et al., 2005; Mooney et al., 2010; Hughes et al., 2014).

After collection, lobsters were immediately transferred to two large, shaded, fiberglass circular tanks (radius, 1.1 m; effective height, 0.8 m; seawater volume, 0.77 m^3) for holding in the ESL. Their claws were bound with rubber bands to avoid injury, and the rubber colours also allowed the identification of each individual lobster. The holding tanks were continuously supplied with ambient (14°C) sand-filtered seawater. One large airstone was placed in each tank to ensure high dissolved oxygen levels. Lobsters were fed with defrosted pieces of fish twice a week and were kept under the natural photoperiod. Shelters were provided in abundance using concrete blocks, and a thin layer of sand was laid on the bottom to provide a foothold for the animals. Lobsters were acclimatized for at least 2 weeks in these conditions before being used in the experiments.

Experimental set-up

The AEP recordings were performed in a dedicated rectangular opaque plastic tank (0.9×0.48×0.38 m; 0.15 m^3) placed in a quiet room in the ESL. The experimental tank was placed inside a larger plywood box lined inside with acoustic dampening open-cell foam. The foam and wood served to reduce external noise and dampen surrounding vibrations. The box rested on rubber gaskets and a dense wooden table, both of which served to further isolate the tank from surrounding vibrations. Prior to each experiment, the tank was filled with fresh, aerated, chilled seawater. The seawater temperature was measured before and after each experiment, and varied between 12±0.8°C at the start and 13.1±0.7°C at the end over a 1-h period, which is the optimal range of seawater temperature reported for the American lobster (Jury and Watson, 2013). A UW-30 underwater speaker (Electro-Voice, Fairport, NY, USA) was suspended, facing horizontally towards the lobster, 5 cm from the surface and 10 cm from the closest tank wall.

Prior to an AEP recording experiment, one lobster was taken from the holding tank and was attached with wires to a wooden board, ventral side down. This prevented the animal from moving during the sound exposure experiment. Preliminary trials revealed that lobsters (N=3 animals that were not used for the main study) showed the strongest AEP responses when the recording electrode (diameter, 27 ga.; length, 13 mm; Rochester subdermal needle electrode, LifeSync Neuro, NY, USA) was placed near the supra-oesophageal ganglion (Cohen, 1960). The recording electrode was inserted into the basal joint of either the left or right antennule by slightly cutting the soft membrane with a scalpel. This electrode was manually inserted 3 mm beyond the carapace outer layer with the tip near the supra-oesophageal ganglion, and fixed to the rostrum using a wire to avoid any displacement. The location was confirmed using a dissection microscope. This was the standard location for all AEP audiogram recordings, except for other control experiments described below. In total, this procedure lasted less than 1 min. Considering that hairfans (and not the statocysts) covering the lobster body are actually the sensory organ responsible for sound detection (see Results), this procedure likely did not affect AEP responses. Then, the lobster was suspended horizontally in the water column of the experimental tank with its dorsal carapace located 3 cm below the surface and the anterior part of its carapace (i.e. location of the supra-oesophageal ganglion and recording electrode) facing the underwater speaker at a distance of 35 cm. Once situated, a reference electrode was inserted into the soft membrane in the telson, 20–30 cm distal from the recording electrode. A ground electrode was suspended in the water column of the tank.

All electrodes were modified by coating the entire stainless-steel portion (except the very tip 0.5–1 mm) with a thin layer of Por-15 (Morristown, NJ, USA), and their cables were coated with aluminium foil, which reduced extraneous electrical noise. The connection of the stainless-steel tip to the electrode cable was lightly coated in epoxy resin to prevent seawater from penetrating the connection. The tank was grounded using a wire connected to the outgoing seawater flow of the ESL. Prior to AEP recordings, the lobsters were acclimatized for 5 min in the experimental tank to recover from handling. After each experiment, the animals were returned to their holding tank. Each animal was used only once during the study. After each AEP experiment, the tank was drained completely, thoroughly rinsed and refilled with fresh seawater for the next experiment.

AEP recordings

The electrodes were connected to a battery-powered Grass CP-511 biological amplifier and filter (Astro-Med, West Warwick, RI,

Journal of Experimental Biology (2021) 224, jeb240747. doi:10.1242/jeb.240747

USA) that amplified the signal with a gain of 40 dB and bandpass-filtered responses from 30 to 3000 Hz. The received signal was then converted from analog to digital via a BNC-2110 data acquisition card (National Instruments, Austin, TX, USA), and saved with a custom AEP program (using National Instruments LabView software) on a laptop computer. The AEP data were sampled at 16 kHz, with a modulation rate of 1 kHz. A total of 1000 sweeps were collected and averaged for each record. These alternating stimuli were presented at 0 and 180 deg phases to remove any stimulus artefacts. The same laptop, custom program and data acquisition card were used to generate acoustic stimuli.

Preliminary trials revealed that lobsters (N=3 animals that were not used for the main study) did not respond to sound frequencies above 250 Hz (using the same set-up as in the 'Experimental set-up' section), even at the highest amplitudes the equipment could generate (~150 dB re. 1 µPa at 35 cm). Therefore, AEP recordings were performed using amplitude-modulated tone pips of 80, 100, 120, 150, 170, 200, 220 and 250 Hz, although the 80 Hz frequency was somewhat difficult to generate and was not a pure tone (see Fig. S1). The presentation order of the frequencies was random. The characteristics of the different stimuli played to the lobsters are presented in Table 1. Sound stimuli were played from the data acquisition card to a 350D attenuator (Hewlett Packard, Loveland, HP, USA), using which sound pressure levels could be manually adjusted in 1–10 dB steps, and then to an amplifier (PLA2378, Brooklyn, NY, USA), which was connected to the underwater speaker. Measurements at 35 cm started at maximum sound pressure levels (SPLs), the values of which were frequency dependent because of the characteristics of the underwater speaker (see Table 1): 110.2–134.9 dB re. 1 µPa. Associated particle acceleration levels (PALs) were between −31.9 and −17.3 dB re. 1 m s^{-2}. The ambient noise level in the experimental tank was typically below that of the acoustic stimuli used during AEP experiments (~50–80 dB re. 1 µPa^2 Hz^{-1} in the frequency range from 50 to 1000 Hz; see Fig. S1).

The SPLs were then gradually decreased with the attenuator, and the corresponding AEP responses were visually monitored. The SPLs were first decreased in 5 or 10 dB increments depending on the amplitude of the AEP response, and in 2 dB increments when close to the thresholds until the stereotypical AEP response was no longer detectable. Then, one to three additional recordings at 2–6 dB below the visually determined thresholds were made to ensure that low responses were not missed.

The theoretical minimum resonant frequency of the experimental tank was 2.7 kHz (Akamatsu et al., 2002), which was far above the highest frequency of the acoustic stimuli used during AEP experiments (250 Hz). Hence, the spectral shapes of the acoustic stimuli were not distorted (see Fig. S1). However, these low frequencies were highly attenuated because their wavelengths were larger than the tank size (e.g. a 100 Hz sound as a ~15 m wavelength; Rogers et al., 2016). In this context, after the acoustic calibration, we carefully positioned each lobster at the same distance (35 cm) from the speaker to enable comparisons between individuals.

Acoustic calibrations

We quantified sound thresholds in root-mean square particle acceleration levels (PAL_{rms}, in dB re. 1 m s^{-2}) as this is the main factor for sound detection in marine invertebrates (Popper and Hawkins, 2018). We also choose to quantify sound thresholds as root-mean square sound pressure levels (SPL_{rms}, in dB re. 1 µPa) because these units remain the most common values in the bioacoustic literature. They provide some comparison to natural ambient sound measurements. This is mainly due to the lack of recording devices available for measuring particle acceleration.

We calibrated PAL_{rms} and SPL_{rms} in the experimental tank in the absence of animals. These values were measured at the same distance as the recording electrode and lobster supra-oesophageal ganglion/statocysts were located from the underwater speaker (i.e. 35 cm). We chose to perform acoustic calibration at this location because the previous bioacoustic literature stated that the statocysts are the sensory organs for sound detection in marine crustaceans (Lovell et al., 2005; Hughes et al., 2014; Radford et al., 2016). Repeated acoustic calibrations were performed before and after AEP experiments, and showed less than 3 dB differences in SPL_{rms} values at all tested frequencies.

PALs were estimated using a tri-axial accelerometer with a custom-built waterproof housing (Model W356B11, PCB Piezotronics; sensitivity: x=1.039 mV m s^{-2}; y=1.036 mV m s^{-2}; z=1.052 mV m s^{-2}) wired through a signal conditioner (Model 480B21, Piezotronics), which multiplied the recorded voltage by a factor of 100. The accelerometer signal was input to three analog filters (one per axis; Model FMB300B, Krohn-Hite), which each applied a bandpass filter between 60 and 3000 Hz. Outputs of the filters were input to a data acquisition board (USB 6251, National Instruments), which was in turn connected to a laptop that ran a custom MATLAB (MathWorks, Natick, MA, USA) script to record the audio files. Voltage values in root-mean square for each axis (x, y and z) were calibrated to the sensitivity of the accelerometer and used to calculate the magnitude of particle acceleration (PAL_{rms}) in dB and linear scale in the same frequency range as the SPL_{rms}. The sensitivity of the accelerometer did not allow us to accurately measure the PAL thresholds (lowest acceleration levels) at the lowest frequencies (i.e. 80–120 Hz). However, PALs could be measured supra-threshold at other frequencies and calculated by verifying the attenuator steps.

Table 1. Features of the stimuli played to *Homarus americanus* by the underwater speaker in the experimental tank

Stimuli (Hz)	Duration (ms)	Number of cycles	Recording window (ms)	Presentation rate (s^{-1})	Start SPL_{rms} (dB re. 1 µPa)	Start PAL_{rms} (dB re. 1 m s^{-2})
80	30	2.4	100	8	110	−32
100		3		8	116	−31
120		3.6		8	125	−27
150		4.5		10	133	−19
170		5.1		10	135	−17
200		6		10	131	−21
220		6.6		10	126	−25
250		7.5		10	122	−21

Root-mean square sound pressure level (SPL_{rms}) and root-mean square particle acceleration level (PAL_{rms}) values were obtained through calibration measurements performed at the location of the animal's head, 35 cm away from the loudspeaker.

SPLs were determined using one pre-amplified hydrophone (HTI-96-MIN, High Tech, Long Beach, MS, USA) with a sensitivity of −165.0 dB re. 1 µPa and a flat response from 2 to 50 kHz. The hydrophone was connected to an autonomous recorder (SoundTrap ST4300, Ocean Instruments NZ) with a gain of 1 dB. SPL_{rms} was calculated as root-mean square at each tested frequency and attenuation level between 50 and 300 Hz over a 1-min period.

Based on particle acceleration and sound pressure data, we calculated the acoustic impedance of our experimental tank, as recommended by Popper and Fay (2011). We used the equations available in Vetter et al. (2019). The results are shown in Fig. S2.

All calculations for SPL_{rms} and PAL_{rms} were performed with custom-written MATLAB scripts (v9.1; MathWorks).

Additional experiments

After recording AEP responses for audiograms in all 16 lobsters, we performed additional experiments with the same individuals (different individuals were used for each experiment). The objectives were to perform controls to verify whether AEP recordings indeed indicated neural responses to acoustic stimuli, and to better understand the lobster sensory organs.

Controls

To confirm that the evoked potentials were neuronal in origin and in response to sound, we performed two control experiments. In the first control experiment, we performed AEP measurement experiments on dead animals (N=3). The lobsters were killed by placing them in the freezer (−40°C) for 24 h. They were then defrosted and AEP measurements were made.

In the second control experiment, we recorded AEP responses of lobsters under very low seawater temperature (N=2). First, we recorded AEP responses from the lobsters to a 100 Hz stimulus under normal (ambient) conditions (11.5°C). Then the seawater was drained, the tank was refilled with cold seawater (4.2°C), and AEP measurements were repeated. Finally, the cold seawater was drained and the tank was refilled with ambient seawater (∼11.8°C), and AEP responses were measured once more.

During these control experiments, the recording electrode was always placed at the standard location (i.e. adjacent to the supra-oesophageal ganglion), and AEP experiments were run using the protocol described in the 'AEP recordings' section.

Sensory organs

To understand the source of the AEP responses, we performed several different AEP experiments, while placing the recording electrode in locations other than the supra-oesophageal ganglion, based on the existing bioacoustics literature. We placed the recording electrode into the soft musculature of the carapace – the abdomen junction, and in the articulations of claw and leg appendages, seeking to potentially record AEPs from chordotonal organs (N=2; Bush and Laverack, 1982).

We also examined potential contributions of the antennules to AEPs. We removed both antennules of several lobsters (N=4). Removal of antennules was achieved by cutting (using a scalpel) their basal segments, which contain the statocysts (Cohen, 1955). Then, lobsters were allowed 1 week to recover. During this period, the animals behaved normally and kept feeding. This post-ablation recovery period was included to give the lobster time to settle after this procedure, as its metabolic state soon after ablation could have had a detrimental effect on AEPs (Lovell et al., 2005). We then measured AEP responses as described above (see 'AEP recordings' section).

We also assessed the potential of hairfans as sound detection organs. To do so, we sprayed the entire body surface (including legs, claws and body, except the anterior part of the carapace) of two individuals using a lacquer spray (Rust-Oleum, Vernon Hills, IL, USA). After the lacquer sealing, hairfans were completely solidified and could not be moved while touching by hand. The lobsters were allowed 3 days to recover from handling, and AEP responses were recorded.

AEP responses were finally obtained from lobsters (N=3) exposed to sounds with characteristics similar to the buzzing sounds they are known to produce (Fish, 1966; Henninger and Watson, 2005; Jézéquel et al., 2018). The acoustic stimulus had a duration of 100 ms, a fundamental frequency of 100 Hz, a SPL_{rms} of 116 dB re. 1 µPa and was presented at a rate of 3 sounds s^{-1} during a 1-min period.

Data analysis

Threshold determination

We assessed auditory thresholds using two different methods. AEP waveforms (i.e. time series) were first visually processed, a method commonly used in marine mammal, fish and invertebrate hearing investigations (Mooney et al., 2010; ANSI/ASA, 2018). We determined the attenuation levels at which responses were present and absent. The visual thresholds corresponded to the lowest attenuation levels at which responses were still present in the AEP recordings.

These analyses were complemented by fast Fourier transform power spectrum analysis (FFT; Hamming window: 321–561 points, depending on the length of the response) of the averaged waveforms using custom-written MATLAB scripts (v9.1). As with fish and squid AEPs, the FFT spectra revealed peaks at approximately twice the stimulus frequency (Egner and Mann, 2005; Mooney et al., 2010). The amplitudes of the FFT peaks also decreased as attenuation levels increased. These values were then plotted relative to the corresponding attenuation levels and a linear regression was calculated using this dataset. We collected between 4 and 10 values per tested frequency (mean, 5.9), and the points with the highest r^2 value were used to calculate the regression (Mooney et al., 2010). The point at which the linear regression crossed the y-axis corresponded to the theoretical attenuation level at which no AEP response would occur and coincided with the threshold at a given frequency (Nachtigall et al., 2007).

Statistical analysis

We first tested whether differences in means between the CLs of male (N=8) and female (N=8) lobsters were significant. As the CL data were not normally distributed in both groups (Shapiro–Wilk test, P<0.05), a non-parametric Mann–Whitney U test was used (α=0.05). Considering the small sample size used, this statistical test could have led to type II errors. The sound detection threshold data were distributed normally (Shapiro–Wilk test, P>0.05). Thus, two-way repeated measures analyses of variance (ANOVAs, α=0.05) were used to determine the effects of sex (male and female), methods (visual and regression analysis) and antennule ablation (before and after) on the sound detection thresholds (SPL_{rms}) across frequencies (Hz). When significant effects were detected, pairwise Tukey tests were used to determine whether the differences were observed among all groups (α=0.05). Statistical analyses were performed using R v3.5.1 (http://www.R-project.org/).

RESULTS

AEP waveform features

AEP responses were recorded from all 16 live lobsters tested during the main experiment. The AEP responses could be detected

Journal of Experimental Biology (2021) 224, jeb240747. doi:10.1242/jeb.240747

30–40 ms following the stimulus onset (Fig. 1). This latency accounted for the neurophysiological response latency of the animal at ∼12°C. The AEP responses were gated sine waves easily discernible above the noise level when stimulus amplitudes were high. Their durations were close to the stimulus duration (∼30 ms; Fig. 1). The response amplitudes decreased as stimulus levels decreased. Response amplitudes were higher for a given stimulus level at frequencies of best sensitivity (80–120 Hz). Indeed, at these frequencies, the peak-to-peak amplitudes often reached levels near 2 µV. All responses disappeared below the thresholds. Similar to fish and other marine invertebrates for which AEP responses have been measured, the frequency of the AEP responses corresponded to about twice the stimulus frequency (see Fig. S3).

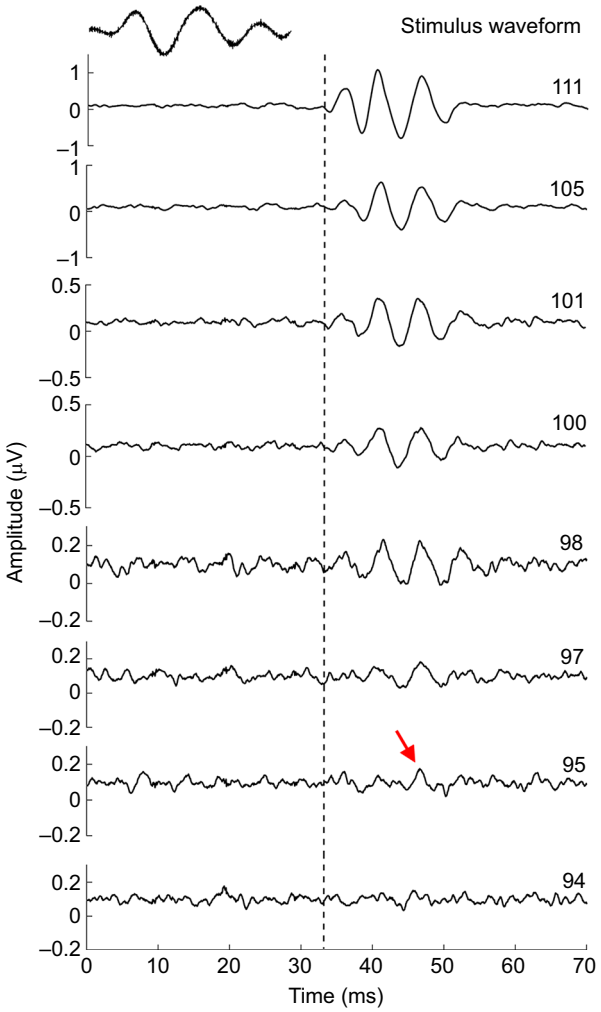

Fig. 1. Auditory evoked potential (AEP) responses from *Homarus americanus* to sound stimuli. AEP responses from a male lobster [carapace length (CL), 8.6 cm] to a 80 Hz tone-pip stimulus (waveform overlaid at the top; duration, 30 ms) with root-mean square sound pressure level (SPL_{rms}) from 111 to 94 dB re. 1 µPa. Each response was collected using 1000 sweep averages. Note that the vertical axes have different scales relative to the response amplitude. The peak frequencies of the observed responses were twice the frequency of the 80 Hz tone-pip stimulus (i.e. 160 Hz; see Fig. S3). The vertical dashed black line shows the onset of the response waveforms, which represents the latency between the stimulus exposure and the responses (here the latency is ∼33 ms). The red arrow highlights the lowest observed response from the lobster, and thus the visually determined threshold was 95 dB re. 1 µPa.

Acoustic reception thresholds

Sound detection thresholds from the 16 tested lobsters are presented in units of PAL_{rms} and SPL_{rms} in Fig. 2. Although the mean CLs between groups of males (N=8) and females (N=8) differed significantly (Mann–Whitney U test, P<0.05; male CLs were larger), we did not find any significant differences between male and female thresholds (two-way repeated measures ANOVA, $F_{5,35}$=0.615, P=0.7). In addition, the two analysis methods (visual and FFT) provided similar thresholds (two-way repeated measures ANOVA, $F_{2,36}$=1.008, P=0.4).

Overall, lobsters significantly displayed a greater sensitivity (i.e. lower threshold) at 80–120 Hz (Tukey test, P<0.05; Table 2), with SPL_{rms} thresholds ranging between 99±2.3 and 107.5±2.7 dB re. 1 µPa (Fig. 2). Then, as the frequency of acoustic stimuli increased, the thresholds also elevated up to 120 dB re. 1 µPa (SPL_{rms}) at 220 Hz (Fig. 2). Of the 16 individuals tested, only one male and two females responded to the 250 Hz stimulus. These three individuals had the lowest thresholds amongst all tested lobsters. Thresholds in PAL_{rms} displayed the same pattern, with values ranging between −35±0.5 dB re. 1 m s^{-2} at 80 Hz and −30.2 ±1.4 dB re. 1 m s^{-2} at 220 Hz (Fig. 2). However, the PAL_{rms} thresholds were underestimated for the low-frequency band (below 150 Hz) because of the sensitivity of the accelerometer used (see 'Acoustic calibrations' section of the Materials and Methods).

Additional experiments

The AEP responses were obtained under several different control situations to confirm neural responses of the lobsters to sound, and to assess their potential hearing organs.

Controls

No responses were obtained from dead animals (N=3), nor from placing the recording electrode in locations other than adjacent to the supra-oesophageal ganglion of live animals (Fig. 3).

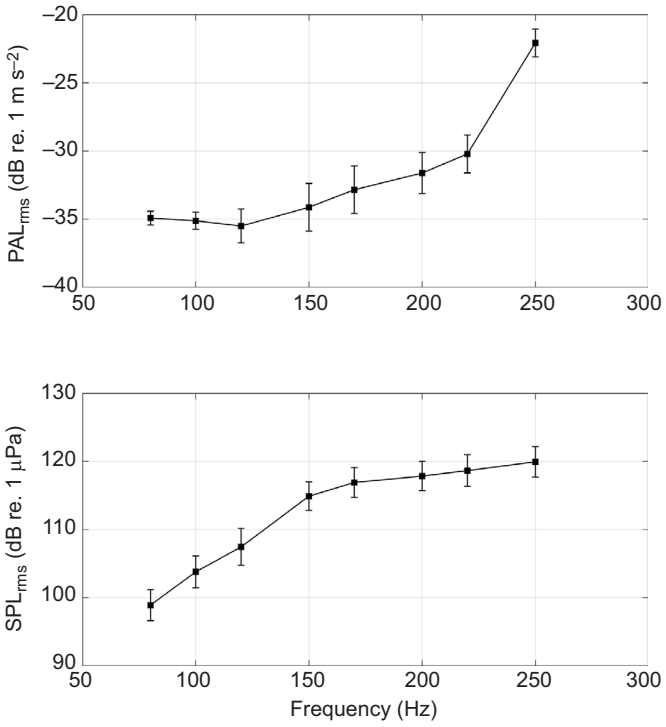

Fig. 2. Sound detection thresholds of the 16 experimental *H. americanus*. Sound detection thresholds are presented in PAL_{rms} (top) and SPL_{rms} (bottom). Error bars are s.d.

Journal of Experimental Biology (2021) 224, jeb240747. doi:10.1242/jeb.240747

Table 2. *H. americanus* SPL_{rms} (in dB re. 1 µPa) threshold shifts across frequencies

Frequency (Hz)	80	100	120	150	170	200	220
80	×	***	***	***	***	***	***
100		×	**	***	***	***	***
120			×	***	***	***	***
150				×	ns	ns	ns
170					×	ns	ns
200						×	ns
220							×

Significance levels for comparisons are indicated. Tukey test, **P<0.01, ***P<0.001; ns, not significant.

The effects of temperature on AEPs were investigated in two lobsters at 100 Hz (the frequency of maximal response amplitudes). Initial recordings were done at 11.5°C to assess baseline AEP response levels and to confirm that the response characteristics were similar to those previously established (Fig. 4). Then, lobsters were placed in cold seawater (4.2°C) and responses were measured. The recorded response waveforms were different compared with the baseline previous AEPs: the peak-to-peak amplitudes were 3- to 6-fold lower and the latencies were more than 20 ms longer. When the lobsters were returned to the baseline, acclimation seawater temperature (11.8°C), their response amplitudes and latencies returned to their initial levels (Fig. 4).

Sensory organs

Surprisingly, the four lobsters tested after ablating their antennules (including the basal segments and statocysts) presented clear AEP responses, similar to normal lobsters (Fig. 3). Indeed, we did not find any significant differences in the audiograms of these lobsters prior to and after ablating their antennules (two-way repeated measures ANOVA, $F_{6,18}$=0.779, P=0.6). This indicated that the statocysts were not the sensory organs responsible for lobster sound detection. Interestingly, when hairfans were immobilized using lacquer spray (N=2), the AEP responses from the supra-oesophageal ganglion were either extinguished or highly reduced in amplitude (Fig. 3), leading to the suggestion that these hairfans play a role in sound detection.

We also recorded AEP responses from three lobsters using acoustic stimuli similar to the buzzing sounds they produce (Fig. 5). The obtained waveform features had longer durations (~60 ms) and latencies (~50 ms), but the same frequencies (i.e. twice the stimulus frequencies), compared with previous AEPs.

DISCUSSION

This is the first study demonstrating sound detection in *H. americanus* using AEP methods. Lobsters detect sounds below 250 Hz with best sensitivity between 80 and 120 Hz, a range that encompasses the fundamental frequency of their buzzing sounds. These auditory data support the role of buzzing sounds for intraspecific communication in lobsters.

Auditory sensitivity of the American lobster and comparison with literature

Classical studies of animal audition often rely on psychophysical approaches such as behavioural responses or cardiac conditioning (Popper and Fay, 1993). To our knowledge, only one attempt of *H. americanus* sound detection has been performed using cardiac assays (Offutt, 1970). This study showed that lobsters react via bradycardia to frequencies below 150 Hz, with best sensitivity at 75 Hz. Other techniques are needed to verify and broaden these results.

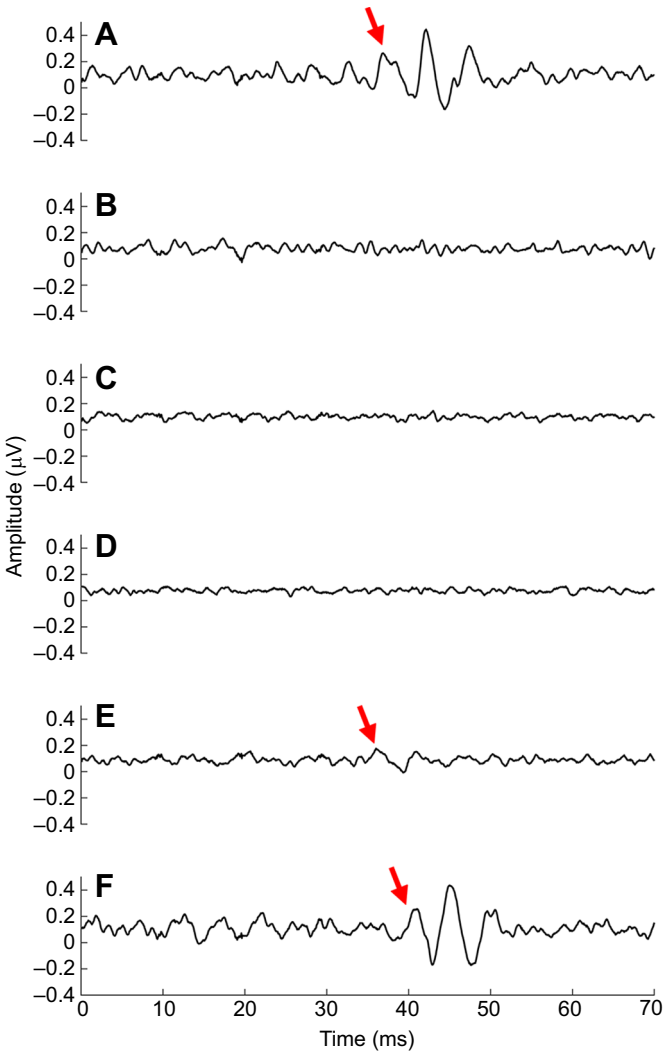

Fig. 3. AEPs from *H. americanus* to a 100 Hz tone pip at 111 dB in different control experiments. AEPs from five different animals in various conditions are shown (one individual per condition). (A) the recording electrode was in the standard recording location in the animal (near the supra-oesophageal ganglion); (B) the electrodes were suspended in the water without the animal; (C) the recording electrode was moved to the carapace–tail junction; (D) the recording electrode was in the standard recording position but the animal was dead; (E) the hairfans covering the lobster body were immobilized using lacquer (but antennules were not ablated); and (F) the antennules (including statocysts) were ablated (while the hairfans were intact). The red arrows show the AEP responses.

Although AEP methods are well established in fish, humans and other animals, they were novel for lobsters, thus the responses required some evaluation. At the most basal level, the AEP response latencies and waveform features were clearly observable at sound levels well above thresholds (see Fig. 1). Moreover, these responses did not exist when using dead animals, suggesting that the responses were not a mechanical or electrical artefact of the stimulus (Fig. 3). In addition, the response frequencies were about twice the stimulus frequencies (see Fig. S3), as seen in other invertebrates (squids, Mooney et al., 2010; crabs, Hughes et al., 2014) and fish (Egner and Mann, 2005; Rogers et al., 2020). This has been explained to be a function of hair cells that are oriented (and maximally stimulated) in-line and in the opposite phase but parallel to the direction of the acoustic waves (Fay, 1974). Thus, the current data suggest a similar mechanism in lobsters. It is

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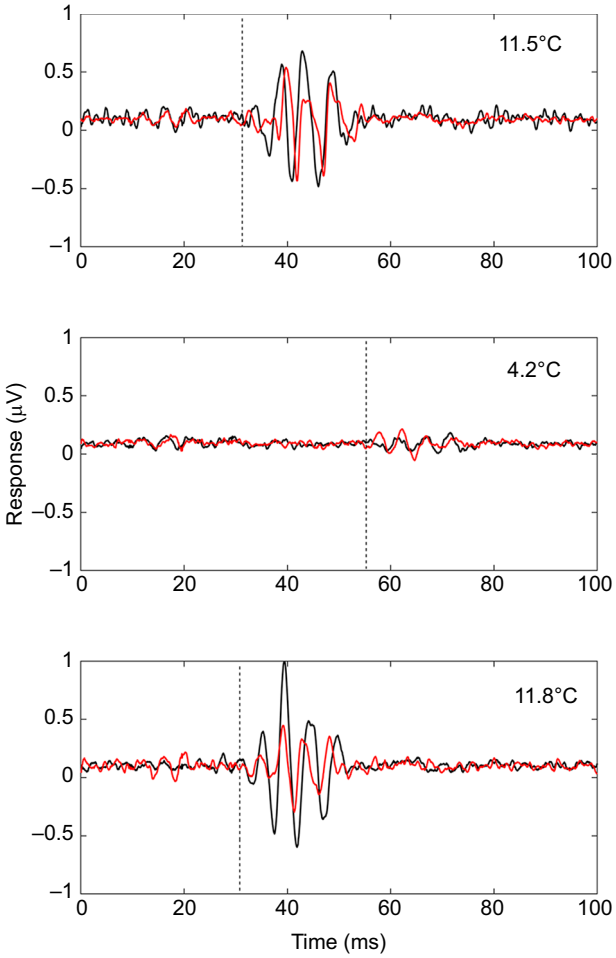

Fig. 4. AEP responses from *H. americanus* exposed to consecutive different seawater temperatures. The AEP responses from two lobsters (red and black curves) exposed to 11.5°C, then 4.2°C and then 11.8°C are shown. Responses were to a 100 Hz tone pip at 111 dB. Note that at 4.2°C, the AEP response latencies were 20 ms longer and had 3- to 6-fold lower amplitude compared with those in ambient conditions.

reasonable to consider here that hairfans may generate responses at twice the stimulus frequency, given their mechanosensory roles for particle motion detection (Laverack, 1962). Further studies will be required to validate whether the mechanosensory cells in the hairfans have alternating polarity.

Overall, the latency of AEP responses in lobsters ranged between 30 and 40 ms following the stimulus onset, a result consistent with other AEP studies (Mooney et al., 2010; Rogers et al., 2020). Interestingly, we still recorded AEP responses when placing the lobsters in cold seawater (4.2°C). However, the waveform amplitudes were highly reduced and the latencies were 20 ms longer compared with AEP recordings under warmer, ambient conditions (11.5°C; Fig. 4). These results are consistent with previous studies performed in other temperate marine crustaceans. Indeed, Young et al. (2006) showed that both neuronal conduction velocity and response amplitude of axons in the leg nerves of *Carcinus maenas* and *Ligia oceanica* decreased with temperature. Taken together, these results provide further evidence that we recorded neuronal responses from a sensory organ reacting to sounds rather than a physical artefact related to animal body vibrations to water particle motion. Overall, our results clearly demonstrate that lobsters are capable of detecting low-frequency sounds.

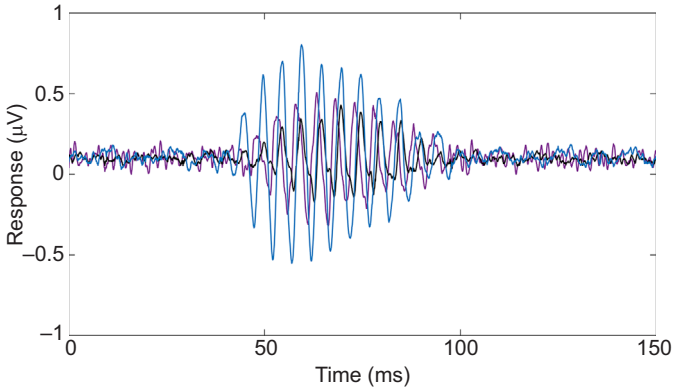

Fig. 5. AEP responses from *H. americanus* to acoustic stimuli similar to the buzzing sounds they are known to produce. AEP responses from three lobsters (blue, purple and black curves) are shown.

Studies presenting hearing abilities of marine crustaceans are scarce. Comparing hearing studies can be challenging, given the potential effects of different recording techniques, physical set-up and lack of consistency in reported threshold units (Ladich and Fay, 2013). Comparisons should be therefore considered an opening discussion. We compared our results with those of three other studies that also reported visually determined AEP hearing thresholds in three different species of marine crustaceans: prawns (*P. serratus*; Lovell et al., 2005), mud crabs (*Panopeus* spp.; Hughes et al., 2014) and paddle crabs (*O. catharus*; Radford et al., 2016). Although we found that lobsters detect sound only below 250 Hz, prawns and crabs detect sounds up to 3000 Hz (Lovel et al., 2005; Hughes et al., 2014; Radford et al., 2016), an order of magnitude difference. However, the band of best sensitivity in terms of both SPLs and PALs (i.e. the lowest thresholds) was similar for all four reported species. Lowest thresholds were found at low frequencies, below 150 Hz. Such a result is consistent with the bioacoustics literature stating that invertebrates mainly detect low-frequency sounds (Budelmann, 1992), and confirms the auditory responses obtained using cardiac assays in American lobsters (Offutt, 1970). Although lobsters have similar sensitivity in this frequency band in terms of SPL thresholds compared with prawns (Lovell et al., 2005), there is a large gap (35 dB) at 80 Hz between lobster and mud crab thresholds (Hughes et al., 2014). However, the lobster hearing thresholds in units of PALs are in the same order of magnitude as for paddle and mud crabs (Hughes et al., 2014; Radford et al., 2016). Although AEP audiograms give a reasonable estimate of auditory responses for lobsters, these results need to be complemented with behavioural thresholds for freely moving individuals (Kojima et al., 2005; Ladich and Fay, 2013; Popper et al., 2014). We also recognize the need to standardize the protocols and set-ups to make studies in crustacean hearing directly comparable (Sisneros et al., 2016).

Hearing organ

Determining which sensory organ is responsible for sound detection in marine invertebrates helps illuminate how signals are perceived. The majority of recent hearing investigations in marine crustaceans has focused on statocysts as the sensory organ (Lovell et al., 2005; Hughes et al., 2014; Radford et al., 2016). Our study has demonstrated that lobsters present no significant differences in their auditory thresholds without and with their antennules (including statocysts) ablated (see Fig. 3). Such a result indicates that the statocysts are not the sensory organs responsible for sound detection in lobsters. This corroborates a statement previously made by Cohen and Dijkgraaf (1961).

This apparent contradiction in the invertebrate bioacoustic literature may be explained by the experimental methods used in previous AEP recording experiments. For example, Hughes et al. (2014) measured AEPs in mud crabs by placing the recording electrode inside the carapace at the basal segments of the antennules (i.e. near the supra-oesophageal ganglion of the animals), as in our study. However, the authors did not perform antennule ablation and thus cannot conclude the role of statocysts for sound perception in this species. Interestingly, Radford et al. (2016) showed that paddle crabs with crushed statocysts still respond to sounds from an underwater speaker, but not to particle motion from a shaker stimulus. The authors concluded that there may be another sensory organ in *O. catharus* that could be sound sensitive.

Other sensory organs, termed hairfans, are found in large numbers on the body and appendages of lobsters and other crustaceans (Budelmann, 1992). In this study, we found that lobsters with immobilized hairfans had highly reduced or extinguished AEP responses (Fig. 3). This suggests that they play a key role in sound detection. These cuticular hairs have previously been shown to be sensitive to particle motion below 300 Hz (Laverack, 1962), which encompasses the lobster hearing range found in our study. Interestingly, these structures are not present on lobster antennae and antennules (Laverack, 1963), which corroborates our results showing that AEP responses were still recorded in lobsters when their antennules were ablated. In this context, we conclude that hairfans are the sensory organs likely to be responsible for sound detection in lobsters. Although beyond the scope of this study, this could be further confirmed through direct neuronal response measurements to sounds on isolated hairfans (as in Laverack, 1962).

Note that our results are preliminary considering the small sample size used for the different experiments, although similar to other marine invertebrate AEP studies (e.g. Lovell et al., 2005, Mooney et al., 2010, Radford et al., 2016). Further studies will thus be needed to strengthen our hypothesis using a higher number of tested individuals with repeated tests (i.e. sham controls), as is commonly done in fish (e.g. Kupla et al., 2015, Vetter and Sisneros, 2020) and insects (Arthur et al., 2010).

Finally, sound detection by external hairbodies is intriguing, in part because these sensory organs are widespread in marine invertebrates (Budelmann, 1992). Although many recent studies have focused on statocysts, external hair cell sound detection greatly broadens the potential scope of marine invertebrate hearing.

Ecological implications

This study has important ecological relevance as we have demonstrated the capacities of lobsters for low-frequency sound detection. We notably found that the greatest sensitivity range encompasses the fundamental frequencies (80–120 Hz) of their buzzing sounds, and that they are also capable of detecting these buzzing sounds (Figs 2 and 5). Taken together, these results strengthen the role of buzzing sounds in intraspecific communication.

Most behavioural studies have focused on agonistic encounters in male American lobsters, and have shown that they use chemical and visual signals to communicate dominance status (Karavanich and Atema, 1998; Bruce et al., 2018). In addition, our group recently found that male European lobsters also produce many buzzing sounds during these events (Jézéquel et al. 2020a). Thus, both male *H. americanus* and *H. gammarus* may use sounds as a threat display to deter conspecifics, as shown in spiny lobsters (*Panulirus argus*; Mulligan and Fisher, 1977) and mantis shrimps (*Hemisquilla californiensis*; Staaterman et al., 2011). Furthermore, male lobsters mostly produce buzzing sounds after the first agonistic encounter between dominant and submissive individuals (i.e. when the dominant status is established; Jézéquel et al., 2020a). It has been shown that chemical signals (i.e. pheromones) released in urine are important for preserving the memory of the outcome between pairs of individuals, post-encounter (Breithaupt and Atema, 1993; Karavanich and Atema, 1998; Breithaupt et al., 1999). Thus, male lobsters could also produce buzzing sounds to recall the outcome of past encounters, in order to avoid additional fights and lower their risk of injury (Breithaupt and Atema, 2000).

We compared the differences of sound detection between males and females. This seems to be the first of such comparisons for marine invertebrates. We did not find any significant differences between the sexes, which is not surprising considering their similar anatomical morphology. Female lobsters are known to produce buzzing sounds that have similar features to those of males (Henninger and Watson, 2005). However, the natural acoustic behaviour of female lobsters has not been evaluated. Interestingly, berried (egg-carrying) females also use agonistic encounters (like males) towards conspecifics to protect their territory and eggs (Mello et al., 1999). In addition, female and male lobsters display shelter sharing and chemical communication during reproduction (Atema and Engstrom, 1971; Cowan and Atema, 1990). Both female and male lobsters could thus use buzzing sounds to communicate during these important behaviors. For example, dominant males may produce buzzing sounds to attract females in their shelters for reproduction, as shown in semi-terrestrial crabs (Popper et al., 2001). We focused on adult lobsters in this study and did not test the hearing sensitivity of juvenile lobsters; sound detection and sound production abilities of juvenile lobsters are not yet known.

Difficulties associated with tank acoustics

Although one can easily quantify the acoustic frequencies used in a hearing test, it is much more difficult to assess accurate SPLs and PALs and detection thresholds, especially for experiments in tanks (Akamatsu et al., 2002; Jézéquel et al., 2018). In the present study, the frequencies tested were below the tank resonant frequency (i.e. natural frequencies of vibration owing to the structural properties of tank walls), and thus SPLs and PALs attenuate fast when propagating away from the receiver (Jézéquel et al., 2019). To circumvent this issue, one must properly calibrate the received SPLs and PALs received by the animal (Jones et al., 2019). Here, we performed acoustic calibration at the location of the brain and statocysts because we initially assumed that statocysts were the sensory organs for sound detection, based on the bioacoustic literature (Lovell et al., 2005; Hughes et al., 2014; Radford et al., 2016). However, because lobsters are likely using hairfans along their whole bodies as acoustic receptors (see ‘Hearing organ’ section), calibrations at the sensory organ become more challenging. The SPL and PAL values vary along the body axis, and differ from those measured at the head location. Thus, the SPLs and PALs sensed by the animals may be either smaller (e.g. with hairfans on body and legs that were further away from the speaker) or higher (e.g. with hairfans present in the claws, which were closer to the speaker, as shown in Laverack, 1962). Measuring at the brain allows for an integration of these signals. However, given these considerations, it is important to remember that the thresholds presented in this study are estimates, and that they are related to the methodology we used.

Using both auditory thresholds and buzzing sound features, one may be tempted to estimate communication distances in

lobsters, i.e. the distances at which they can detect sounds. Because of the tank issues mentioned above, sound levels are difficult to assess, and associated uncertainties are likely to be important. If these results are used to infer communication distances, one must be particularly careful at properly assessing associated uncertainties.

As opposed to pressure, particle motion yields important cues, such as directionality, which is crucial for marine invertebrates to communicate and recruit (Popper and Hawkins, 2018). Hence, if lobsters can detect particle motion across their bodies through hairfans, they could estimate the distances and even the size of nearby animals. This type of communication would provide lobsters with information from a larger space around them than is possible using vision or olfaction (Atema, 2012).

In addition, particle motion might be more prevalent in the lower-frequency spectrum, facilitating communication over long distances (Mooney et al., 2016). However, Breithaupt (2002) theoretically estimated detection ranges in American lobsters of three times their body sizes (i.e. 30 cm). Such low communication distances were also found through behavioral responses in a free sound field for the Norway lobster (*Nephrops Norvegicus*; Goodall et al., 1990). This is actually explained by the high amplitude of the particle motion that prevails in the near field, whereas it dramatically decreases in the far field, as opposed to sound pressure (Popper and Hawkins, 2018). Hence, field experiments with free-moving lobsters in their habitat could enable validation of these previous results. Assessing the propagation features of their buzzing sounds underwater should also permit assessment of communication distances, as has been done recently for spiny lobsters (Jézéquel et al., 2020b).

Conclusion

This study has demonstrated low-frequency sound detection by lobsters, with the greatest sensitivity range encompassing the fundamental frequencies (80–120 Hz) and the intensity levels of their buzzing sounds. These results imply that this hearing ability could be used in intraspecific communication. This sound sensitivity also suggests that anthropogenic noise may potentially affect lobsters (NRC, 2003). Anthropogenic noise dominates low frequencies (below 1 kHz), overlapping the hearing ranges of many marine animals (Clark et al., 2009), including lobsters. A large body of literature has already shown various impacts in marine mammals, fish and cephalopods, from temporary changes in animal behaviors to lethal impacts (Madsen et al., 2006; Hawkins et al., 2015; Jones et al., 2020). In marked contrast, the potential impacts on crustaceans are still poorly understood (Edmonds et al., 2016). Thus, our results on sound detection by lobsters are a first important step that will help further studies to assess the potential impacts of anthropogenic noise on their behaviors.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: Y.J., I.T.J., L.C., T.A.M.; Methodology: Y.J., I.T.J., J.B., L.C., J.A., T.A.M.; Software: I.T.J., T.A.M.; Validation: I.T.J., J.B., L.C., J.A., T.A.M.; Formal analysis: Y.J.; Investigation: I.T.J., J.B., J.A., T.A.M.; Resources: T.A.M.; Data curation: Y.J.; Writing - original draft: Y.J.; Writing - review & editing: Y.J., I.T.J., J.B., L.C., J.A., T.A.M.; Visualization: Y.J.; Supervision: I.T.J., J.B., L.C., J.A., T.A.M.; Project administration: J.B., T.A.M.; Funding acquisition: J.B., L.C., T.A.M.

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Supplementary information

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References

Aiken, D. E. (1973). Proecdysis, setal development, and molt prediction in the American lobster (*Homarus americanus*). *J. Fish. Board Canada* **30**, 1337-1344. doi:10.1139/f73-214

Akamatsu, T., Okumura, T., Novarini, N. and Yan, H. Y. (2002). Empirical refinements applicable to the recording of fish sounds in small tanks. *J. Acoust. Soc. Am.* **112**, 3073-3082. doi:10.1121/1.1515799

ANSI/ASA (2018). ANSI/ASA **S3/SC1.6-2018**. American National Standard Procedure for Determining Audiograms in Toothed Whales through Evoked Potential Methods. Acoustical Society of America (ASA).

Arthur, B. J., Wyttenbach, R. A., Harrington, L. C. and Hoy, R. R. (2010). Neural responses to one-and two-tone stimuli in the hearing organ of the dengue vector mosquito. *J. Exp. Biol.* **213**, 1376-1385. doi:10.1242/jeb.033357

Atema, J. (2012). Aquatic odor dispersal fields: opportunities and limits of detection, communication and navigation. In *Chemical Ecology in Aquatic Systems* (ed. C. Brönmark and L. A. Hansson), pp.1-18. Oxford University Press.

Atema, J. and Cobb, J. S. (1980). Social behavior. In: *The Biology and Management of Lobsters*, Vol. 1 (ed. J. S. Cobb and B. Phillips), pp. 409-450. New York: Academic Press.

Atema, J. and Engstrom, D. G. (1971). Sex pheromone in the lobster, *Homarus americanus*. *Nature,* **232**, 261. doi:10.1038/232261a0

Atema, J. and Voigt, R. (1995). Behavior and sensory biology. In: *Biology of the Lobster, Homarus americanus* (ed. J. Factor), pp. 313-348. San Diego: Academic Press.

Breithaupt, T. (2002). Sound perception in aquatic crustaceans. In: *The crustacean nervous system* (ed. K. Wiese), pp. 548-559. Basel: Birkhäuser.

Breithaupt, T. and Atema, J. (1993). Evidence for the use of urine signals in agonistic interactions of the American lobster. *Biol. Bull.* **185**, 318. doi:10.1086/BBLv185n2p318

Breithaupt, T. and Atema, J. (2000). The timing of chemical signaling with urine in dominance fights of male lobsters (*Homarus americanus*). *Behav. Ecol. Sociobiol.* **49**, 67-78. doi:10.1007/s002650000271

Breithaupt, T., Lindstrom, D. P. and Atema, J. (1999). Urine release in freely moving catheterised lobsters (*Homarus americanus*) with reference to feeding and social activities. *J. Exp. Biol.* **202**, 837-844.

Bruce, M., Doherty, T., Kaplan, J., Sutherland, C. and Atema, J. (2018). American lobsters, *Homarus americanus*, use vision for initial opponent evaluation and subsequent memory. *Bull. Mar. Sci.* **94**, 517-532. doi:10.5343/bms.2017.1147

Budelmann, B. U. (1992). Hearing in crustacea. In: *The Evolutionary Biology of Hearing* (ed. D.B. Webster, R.R. Fay and A.N. Popper), pp. 131-140. New York: Springer-Verlag.

Burkhard, R. F., Eggermont, J. J. and Don, M. (2007). *Auditory Evoked Potentials: Basic Principles and Clinical Applications*. Philadelphia, PA: Lippincott, Williams & Wilkins.

Bush, B. M. H. and Laverack, M. S. (1982). Mechanoreception. In: *The biology of Crustacea*, Vol. 3 (ed. S.L. Atwood and D.V. Sandeman), pp. 399-468. New York: Academic Press.

Clark, C. W., Ellison, W. T., Southall, B. L., Hatch, L., Van Parijs, S. M., Frankel, A. and Ponirakis, D. (2009). Acoustic masking in marine ecosystems: intuitions, analysis, and implication. *Mar. Ecol. Prog. Ser.* **395**, 201-222. doi:10.3354/meps08402

Cohen, M. J. (1955). The function of receptors in the statocyst of the lobster *Homarus americanus*. *J. Physiol.* **130**, 9-34. doi:10.1113/jphysiol.1955.sp005389

Cohen, M. J. (1960). The response patterns of single receptors in the crustacean statocyst. *Proc. R. Soc. Lond.B Biol. Sci.* **152**, 30-49. doi:10.1098/rspb.1960.0020

Cohen, M. J. and Dijkgraaf, S. (1961). Mechanoreception. In *The physiology of Crustacea*, Vol. 2, (ed. T. H. Waterman), pp. 65-108. New York: Academic Press.

Cowan, D. F. and Atema, J. (1990). Moult staggering and serial monogamy in American lobsters, *Homarus americanus*. *Animal Behaviour,* **39**, 1199-1206. doi:10.1016/S0003-3472(05)80792-4

Edmonds, N. J., Firmin, C. J., Goldsmith, D., Faulkner, R. C. and Wood, D. T. (2016). A review of crustacean sensitivity to high amplitude underwater noise: data needs for effective risk assessment in relation to UK commercial species. *Mar. Pollut. Bull.* **108**, 5-11. doi:10.1016/j.marpolbul.2016.05.006

Egner, S. A. and Mann, D. A. (2005). Auditory sensitivity of sergeant major damselfish *Abudefduf saxatilis* from post-settlement juvenile to adult. *Mar. Ecol. Prog. Ser.* **285**, 213-222. doi:10.3354/meps285213

Fay, R. R. (1974). Sound reception and processing in the carp: saccular potentials. *Comp. Biochemi. Physiol. A Physiol.* **49**, 29-42. doi:10.1016/0300-9629(74)90539-8
Fay, R. R. and Edds-Walton, P. L. (1997). Diversity in frequency response properties of saccular afferents of the toadfish, *Opsanus tau*. *Hear. Res.* **113**, 235-246. doi:10.1016/S0378-5955(97)00148-2
Fish, J. F. (1966). Sound production in the American lobster, *Homarus americanus* H. Milne Edwards (Decapoda Reptantia). *Crustaceana* **11**, 105-106. doi:10.1163/156854066X00504
Goodall, C., Chapman, C. and Neil, D. (1990). The acoustic response threshold of the Norway lobster, *Nephrops norvegicus* (L.) in a free sound field. In *Frontiers in Crustacean Neurobiology* (ed. K. Wiese, W.-D. Krenz, J. Tautz, H. Reichert and B. Mulloney), pp. 106-113. Birkhäuser: Basel.
Hawkins, A. D., Pembroke, A. E. and Popper, A. N. (2015). Information gaps in understanding the effects of noise on fishes and invertebrates. *Rev. Fish Biol. Fish.* **25**, 39-64. doi:10.1007/s11160-014-9369-3
Henninger, H. P. and Watson, W. H., III (2005). Mechanisms underlying the production of carapace vibrations and associated waterborne sounds in the American lobster, *Homarus americanus*. *J. Exp. Biol.* **208**, 3421-3429. doi:10.1242/jeb.01771
Hughes, A. R., Mann, D. A. and Kimbro, D. L. (2014). Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. *Proc. R. Soc. B* **281**, 20140715. doi:10.1098/rspb.2014.0715
Jézéquel, Y., Bonnel, J., Coston-Guarini, J., Guarini, J. M. and Chauvaud, L. (2018). Sound characterization of the European lobster *Homarus gammarus* in tanks. *Aquatic Biology* **27**, 13-23. doi:10.3354/ab00692
Jézéquel, Y., Bonnel, J., Coston-Guarini, J. and Chauvaud, L. (2019). Revisiting the bioacoustics of European spiny lobsters *Palinurus elephas*: comparison of antennal rasps in tanks and *in situ*. *Mar. Ecol. Prog. Ser.* **615**, 143-157. doi:10.3354/meps12935
Jézéquel, Y., Coston-Guarini, J., Chauvaud, L. and Bonnel, J. (2020a). Acoustic behaviour of male European lobsters (*Homarus gammarus*) during agonistic encounters. *J. Exp. Biol.* **223**, jeb211276. doi:10.1242/jeb.211276
Jézéquel, Y., Chauvaud, L. and Bonnel, J. (2020b). Sounds from spiny lobsters can be detectable over kilometers underwater. *Sci. Rep.* **10**, 7943. doi:10.1038/s41598-020-64830-7
Jones, I. T., Stanley, J. A., Bonnel, J. and Mooney, T. A. (2019). Complexities of tank acoustics warrant direct, careful measurement of particle motion and pressure for bioacoustic studies. *Proc. Meetings Acoustics* **37**, 010005. doi:10.1121/2.0001073
Jones, I. T., Stanley, J. A. and Mooney, T. A. (2020). Impulsive pile driving noise elicits alarm responses in squid (*Doryteuthis pealeii*). *Mar. Pollut. Bull.* **150**, 110792. doi:10.1016/j.marpolbul.2019.110792
Jury, S. H. and Watson, W. H., III (2013). Seasonal and sexual differences in the thermal preferences and movements of American lobsters. *Can. J. Fish. Aquat. Sci.* **70**, 1650-1657. doi:10.1139/cjfas-2013-0061
Karavanich, C. and Atema, J. (1998). Individual recognition and memory in lobster dominance. *Anim. Behav.* **56**, 1553-1560. doi:10.1006/anbe.1998.0914
Kojima, T., Ito, H., Komada, T., Taniuchi, T. and Akamatsu, T. (2005). Measurements of auditory sensitivity in common carp *Cyprinus carpio* by the auditory brainstem response technique and cardiac conditioning method. *Fish. Sci.* **71**, 95-100. doi:10.1111/j.1444-2906.2005.00935.x
Kulpa, M., Bak-Coleman, J. and Coombs, S. (2015). The lateral line is necessary for blind cavefish rheotaxis in non-uniform flow. *J. Exp. Biol.* **218**, 1603-1612. doi:10.1242/jeb.119537
Ladich, F. (2015). *Sound Communication in Fishes*. Vienna: Springer.
Ladich, F. and Fay, R. R. (2013). Auditory evoked potential audiometry in fish. *Rev. Fish Biol. Fish.* **23**, 317-364. doi:10.1007/s11160-012-9297-z
Laverack, M. S. (1962). Responses of cuticular sense organs of the lobster, *Homarus vulgaris* (Crustacea)-II. Hair-fan organs as pressure receptors. *Comp. Biochem. Physiol.* **6**, 137-145. doi:10.1016/0010-406X(62)90160-3
Laverack, M. S. (1963). Responses of cuticular sense organs of the lobster, *Homarus vulgaris* (Crustacea)—III. Activity invoked in sense organs of the carapace. *Comp. Biochem. Physiol.* **10**, 261-272. doi:10.1016/0010-406X(63)90227-5
Lovell, J. M., Findlay, M. M., Moate, R. M. and Yan, H. Y. (2005). The hearing abilities of the prawn *Palaemon serratus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **140**, 89-100. doi:10.1016/j.cbpb.2004.11.003
Madsen, P. T., Wahlberg, M., Tougaard, J., Lucke, K. and Tyack, A. P. (2006). Wind turbine underwater noise and marine mammals: implications of current knowledge and data needs. *Mar. Ecol. Prog. Ser.* **309**, 279-295. doi:10.3354/meps309279
Mello, J. J., Cromarty, S. I. and Kass–Simon, G. (1999). Increased aggressiveness in gravid American lobsters, *Homarus americanus*. *Aggr. Behav.* **25**, 451-472. doi:10.1002/(SICI)1098-2337(1999)25:6<451::AID-AB6>3.0.CO;2-I
Mendelson, M. (1969). Electrical and mechanical characteristics of a very fast lobster muscle. *J. Cell Biol.,* **42**, 548-563. doi:10.1083/jcb.42.2.548
Mooney, T. A., Hanlon, R. T., Christensen-Dalsgaard, J., Madsen, P. T., Ketten, D. R. and Nachtigall, P. E. (2010). Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. *J. Exp. Biol.* **213**, 3748-3759. doi:10.1242/jeb.048348
Mooney, T. A., Kaplan, M. B. and Lammers, M. O. (2016). Singing whales generate high levels of particle motion: implications for acoustic communication and hearing? *Biol. Lett.* **12**, 20160381. doi:10.1098/rsbl.2016.0381
Mooney, T. A., Yang, W.-C., Yu, H. Y., Ketten, D. R. and Jen, I.-F. (2015). Hearing abilities and sound reception of broadband sounds in an adult Risso's dolphin (*Grampus griseus*). *J. Comp. Physiol. A* **201**, 751-761. doi:10.1007/s00359-015-1011-x
Moulton, J. M. (1957). Sound production in the spiny lobster *Panulirus argus* (Latreille). *Biol. Bull.* **113**, 286-295. doi:10.2307/1539086
Mulligan, B. E. and Fischer, R. B. (1977). Sounds and behavior of the spiny lobster *Panulirus argus* (Latreille, 1804) (Decapoda, Palinuridae). *Crustaceana* **32**, 185-199. doi:10.1163/156854077X00575
Nachtigall, P. E., Mooney, T. A., Taylor, K. A. and Yuen, M. M. (2007). Hearing and auditory evoked potential methods applied to odontocete cetaceans. *Aquat. Mamm.* **33**, 6-13. doi:10.1578/AM.33.1.2007.6
NRC (2003). *Ocean Noise and Marine Mammals*. Washington, DC: The National Academies Press.
Offutt, G. C. (1970). Acoustic stimulus perception by the American lobster *Homarus americanus* (Decapoda). *Experientia* **26**, 1276-1278. doi:10.1007/BF01898016
Popper, A. N. and Fay, R. R. (1993). Sound detection and processing by fish: critical review and major research questions. *Brain Behav. Evol.* **41**, 14-25. doi:10.1159/000113821
Popper, A. N. and Fay, R. R. (2011). Rethinking sound detection by fishes. *Hear. Res.* **273**, 25-36. doi:10.1016/j.heares.2009.12.023
Popper, A. N. and Hawkins, A. D. (2018). The importance of particle motion to fishes and invertebrates. *J. Acoust. Soc. Am.* **143**, 470-488. doi:10.1121/1.5021594
Popper, A. N., Salmon, M. and Horch, K. W. (2001). Acoustic detection and communication by decapod crustaceans. *J. Comp. Physiol. A* **187**, 83-89. doi:10.1007/s003590100184
Popper, A. N., Hawkins, A. D., Fay, R. R., Mann, D. A., Bartol, S., Carlson, T. J., Coombs, S., Ellison, W. T., Gentry, R. L., Halvorsen, M. B. et al. (2014). Sound exposure guidelines for fishes and sea turtles: a technical report prepared by ANSI-accredited standards committee S3/SC1 and registered with ANSI. *Spinger Briefs in Oceanography* **2**, 23-32. doi:10.1007/978-3-319-06659-2_6
Radford, C. A., Tay, K., Goeritz, M. L. (2016). Hearing in the paddle crab, *Ovalipes catharus*. *Proc. Meetings Acoustics* **27**, 010013. doi:10.1121/2.0000259
Rogers, P. H., Hawkins, A. D., Popper, A. N., Fay, R. D. and Gray, M. D. (2016). Parvulescu revisited: small tank acoustics for bioascousticians. In *The Effects of Noise on Aquatic Life II* (ed. A. N. Popper and A. D. Hawkins), pp. 933-941. New York: Springer.
Rogers, L. S., Putland, R. L. and Mensinger, A. F. (2020). The effect of biological and anthropogenic sound on the auditory sensitivity of oyster toadfish, *Opsanus tau*. *Journal of Comparative Physiology A,* **206**, 1-14. doi:10.1007/s00359-019-01381-x
Schmitz, B. (2002). Sound production in Crustacea with special reference to the Alpheidae. In: *The Crustacean Nervous System* (ed. K. Wiese), pp. 536-547. Berlin: Springer.
Scrivener, J. C. E. (1971). Agonistic behavior in the American lobster, *Homarus americanus*. *Fish. Res. Board Canada. Tech. Rep.* **235**, 1-115.
Sisneros, J. A., Popper, A. N., Hawkins, A. D. and Fay, R. R. (2016). Auditory evoked potential audiograms compared with behavioral audiograms in aquatic animals. In *The Effects of Noise on Aquatic Life II* (ed. A.N. Popper and A.D. Hawkins), pp. 1049-1056. New York: Springer.
Staaterman, E. R., Clark, C. W., Gallagher, A. J., de Vries, M. S., Claverie, T. and Patek, S. N. (2011). Rumbling in the benthos: acoustic ecology of the California mantis shrimp *Hemisquilla californiensis*. *Aquat. Biol.* **13**, 97-10. doi:10.3354/ab00361
Tyack, P. L. and Clark, C. W. (2000). Communication and acoustic behavior of dolphins and whales. In: *Hearing by Whales and Dolphins* (ed. W.W.L. Au, A.N. Popper and R.R. Fay), pp. 156-224. New York: Springer.
Vetter, B. J. and Sisneros, J. A. (2020). Swim bladder enhances lagenar sensitivity to sound pressure and higher frequencies in female plainfin midshipman (*Porichthys notatus*). *J. Exp. Biol.* **223**, jeb225177. doi:10.1242/jeb.225177
Vetter, B. J., Seeley, L. H. and Sisneros, J. A. (2019). Lagenar potentials of the vocal plainfin midshipman fish, *Porichthys notatus*. *J. Comp. Physiol. A* **205**, 163-175. doi:10.1007/s00359-018-01314-0
Ward, D., Morison, F., Morrissey, E., Jenks, K. and Watson, W. H., III (2011). Evidence that potential fish predators elicit the production of carapace vibrations by the American lobster. *J. Exp. Biol.* **214**, 2641-2648. doi:10.1242/jeb.057976
Young, J. S., Peck, L. S. and Matheson, T. (2006). The effects of temperature on peripheral neuronal function in eurythermal and stenothermal crustaceans. *J. Exp. Biol.* **209**, 1976-1987. doi:10.1242/jeb.02224