Electronic Supplementary Material

Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise

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Study animals and husbandry

Shore crabs (*Carcinus maenas*) are native to north-western Europe and the north-western Atlantic, but have also recently invaded the North Pacific, South Australia and South Africa [S1]. They are one of the most common and well-known of all intertidal animals [S2], thus inhabiting areas that are frequented by a variety of shipping.

Animals for the single-exposure experiment were caught from Newquay harbour (50°25′N 5°5′W) on 12th and 13th October 2011 and those for the repeated-exposure experiment were caught on 2nd and 3rd May 2012. Inside Newquay harbour itself, there is sporadic traffic noise from pleasure craft, fishing and angling boat trips, and speed boats; noise from larger ships further afield is also likely, although those vessels do not enter the harbour itself. Following capture with a seine net, crabs were held for a maximum of 48 hours in salt-water tanks at the Blue Reef Aquarium, Newquay before transfer to Bristol Aquarium. The single-exposure experiment took place in November 2011; the repeated-exposure experiment in June 2012. Polystyrene boxes (to reduce noise transmission) were used to hold crabs in Bristol. The boxes (48 x 32 x 28 cm lwh) received water from one of the Aquarium display tanks, which were plumbed into advanced filtration facilities. Holding tanks were fitted with a sub-surface inflow pipe to prevent noise from water falling or collision with the tank floor; the flow was adjusted to allow complete tank flush-through every 30 min and thus ensure the maintenance of high water quality. Sound levels in holding tanks were kept as low as possible and were comparable to those for ambient-noise playback during experiments (see Fig. S1).

Holding boxes contained sand on the floor and shelters made with inverted plastic flower pots weighted down with a layer of pea gravel secured around the base with Milliput epoxy putty (The Milliput Company, Gwynedd, UK). Box lids included a mesh window to allow light to reach the animals; this light did not, however, cover all areas of the tank or reach into shelters and thus animals had a choice of light/dark conditions. Water temperature was kept at 12–14ºC, salinity at 32–35 ppt and water qualities within safe parameters (NO_2^-: <0.3 mg/l, NO_3^-: 0 mg/l, NH_3^+: ≤ 0.25 mg/l, pH: 7.4–7.9). Crabs were fed every 48 hours on a variety of previously frozen meats (cockle, mussel, shrimp, krill, sand eel, mackerel) or dry composite marine pellets (New Era Aquaculture Ltd., Thorne, UK), with any excess cleared from the holding tank during tank maintenance no more than 8 hours after feeding. Although there was a constant water change within the holding tank (see above), 25% water was removed by siphon with excess food and waste; this water was replaced through normal tank flow through. Water changes and the flow-through system ensured maintenance of constant oxygen levels and the removal of any water that had become "trapped" in the tank corners.

To ensure testing of the same individuals at the same time each day and to allow intra-individual comparisons across time, crabs in the repeated-exposure experiment were numbered using a waterproof marker (Kuretake Co, Ltd.).

Noise treatments

Original sound recordings were made at three UK ports (Gravesend: Rio de la Plata, 286-m long, 64,730 t container ship; Plymouth: Bro Distributor, 147-m long, 14,500 t LPG tanker; Portsmouth: Commodore Goodwill, 126-m long, 5,215 t ferry; recorded at ca. 200 m), using a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, High Tech Inc., Gulfport MS) and an Edirol R09-HR 24-Bit recorder (44.1 kHz sampling rate, Roland Systems Group, Bellingham WA). The recording level was calibrated for the R09-HR using pure sine wave signals, measured in line with an oscilloscope, produced by a function generator. The average noise level of each recording was assessed using Fast Fourier Transformation (FFT) analysis (units normalised to 1 Hz, Hann evaluation, 50% overlap, FFT size 1024; averaged from 2 min of recording) in Avisoft SASLab Pro v4.5.2 (Avisoft Bioacoustics, Berlin). This analysis found some variation in average noise levels between recordings: ambient tracks between 92 and 106 dB RMS re 1 µPa at 1 m, and ship-noise tracks between 126 and 136 dB RMS re 1 µPa at 1 m.

To give consistency between the three cases of each treatment, tracks were modified to play back in the centre of the experimental tank at received levels of 108–111 dB RMS re 1 µPa at 10 cm for ambient noise (chosen to be markedly higher than the noise floor in the tanks at low frequencies) and 148–155 dB RMS re 1 μPa at 10 cm for ship noise (representing the received acoustic pressure from a large ship of source level 170 dB RMS re 1 μPa at 1 m passing at a distance of 100 m in shallow water) (Fig. S2). All measurements were made with the same hydrophone set-up as above. All tracks were played back in experiments using: WAV/MP3 Player (Ultradisk® DVR2 560Hrs; frequency range: 20–20,000 Hz); amplifier (Kemo Electronic GmbH; 18W; frequency response: 40–20,000 Hz); potentiometer (set to minimum resistance; Omeg Ltd; 10K logarithmic); and Aqua 30 underwater speaker (DNH; effective frequency range 80–20,000 Hz). See Figs. S3 and S4 for comparative spectrograms of original sound recordings and received sounds from playbacks of both ambient and ship noise.

The noise playbacks presented a range of frequencies that are likely to fall within the hearing range of the crabs - inferred from a study on the prawn *Palaemon serratus* [S3] – but the ship-noise playbacks peaked at lower frequencies than the ambient-noise playbacks. The experimental sound levels are unlikely to be high enough to cause auditory damage to the crabs, although due to the lack of knowledge in this area the exact sound pressure level needed to cause permanent or temporary damage is currently unknown. It is likely that decapods detect the particle motion element of sound (first suggested by [S4]), but suitable equipment (e.g. a miniature accelerometer) was not available for such measurements; this is an issue that requires future research in general.

Statistical analysis

The actual amount of water in the one-litre experimental container varied depending on the size of the crab, since larger individuals displace more water. In reality, even crabs of the largest mass used in the experiments displaced only 15 ml of water and, as such, the slight variation in available water volume for different-sized crabs (985–998 ml), and the small percentage of the total water volume missing (0.2–1.5%), is unlikely to affect significantly our oxygen consumption measures. However, to make sure, measurements (mass and water displacement) from 12 crabs were used to generate the relationship between these factors: volume of water displaced (ml) = 0.20 x crab mass (g) – 3.81 (R^2 = 0.98). All oxygen consumption values from both experiments were then corrected for the actual amount of water available in the experimental chamber given the mass of the relevant crab, and these corrected values were used in the statistical analyses.

For the single-exposure experiment, a two-way ANOVA was used to assess the effect of crab mass, sound treatment (ambient or ship noise) and the interaction between the two on oxygen consumption rate. For the repeated exposure experiment, a repeated-measures ANOVA was used to assess the effect of sound treatment (ambient or ship noise), day in the sequence and the interaction between the two on mass-corrected oxygen consumption rate.

References

S1. Crothers J 1968 Biology of the Shore Crab *Carcinus maenas*. Field Studies Council, Shrewsbury.

S2. Moksnes P, Pihl L & Montfrans JV 1998 Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. *Mar. Ecol. Prog. Ser.* **166,** 211–225.

S3. Lovell JM, Findlay MM, Moate RM & Yan HY 2005 The hearing abilities of the prawn *Palaemon serratus*. *Comp. Biochem. Physiol. A* **140,** 89–100.

S4. Goodall C, Chapman C & Neil D 1990 The acoustic response threshold of the Norway lobster, *Nephrops norvegicus*. In Wiese K, Krenz WD, Tautz J, Reichert H & Mulloney B (eds.) *Frontiers in Crustacean Neurobiology*. Birkhäuser, Basel, pp. 106–113.

Figure S1. Variation in sound profiles for holding tanks and ambient-noise playbacks. Shown are illustrative power spectrographic examples from recordings made in the centre of a holding tank and 10 cm from the speaker in the experimental tank.

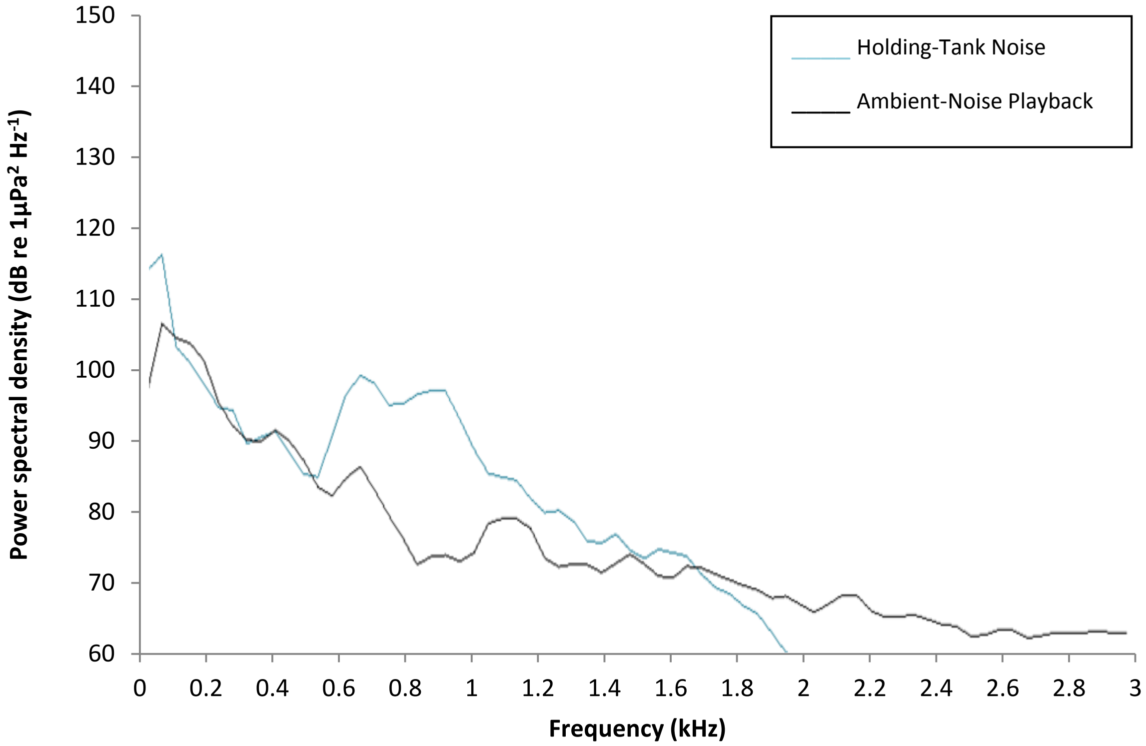

Figure S2. Variation in sound profiles for ambient conditions in the experimental tank and playbacks of both ambient and ship noise. Shown are illustrative power spectrographic examples from recordings made 10 cm from the speaker in the experimental tank.

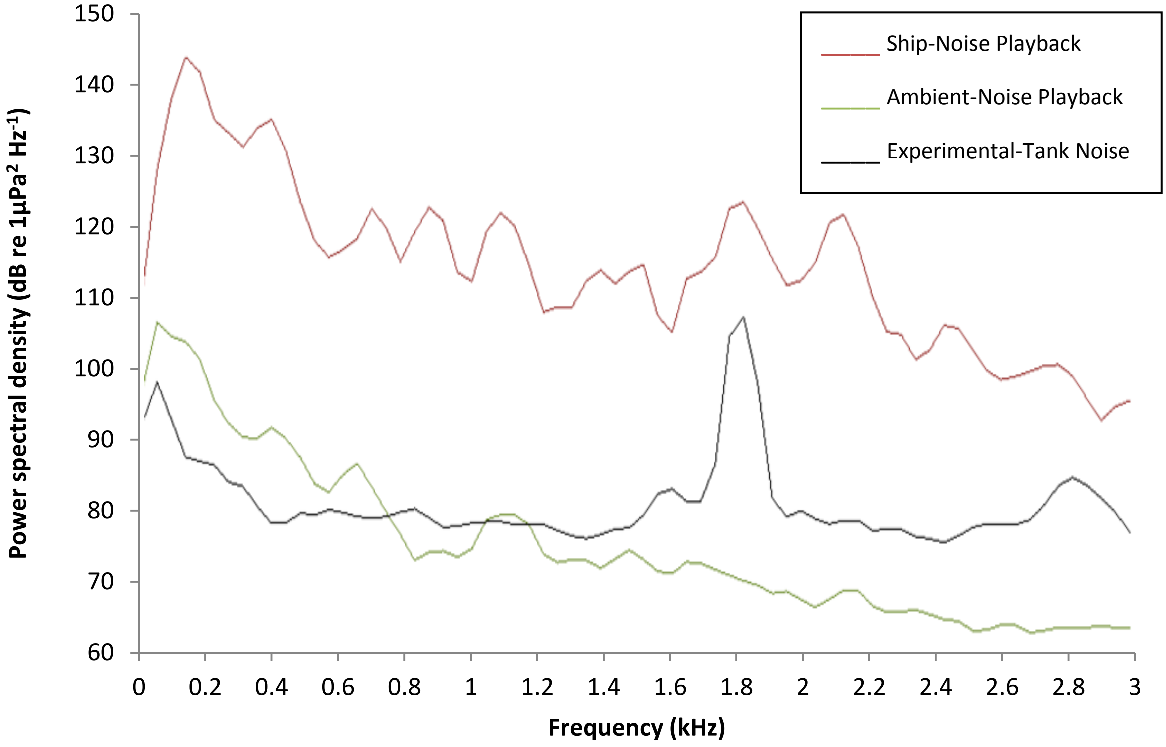

Figure S3. Illustrative spectrograms of original recording in the field and re-recording of playback of ambient noise from one of the three UK ports.

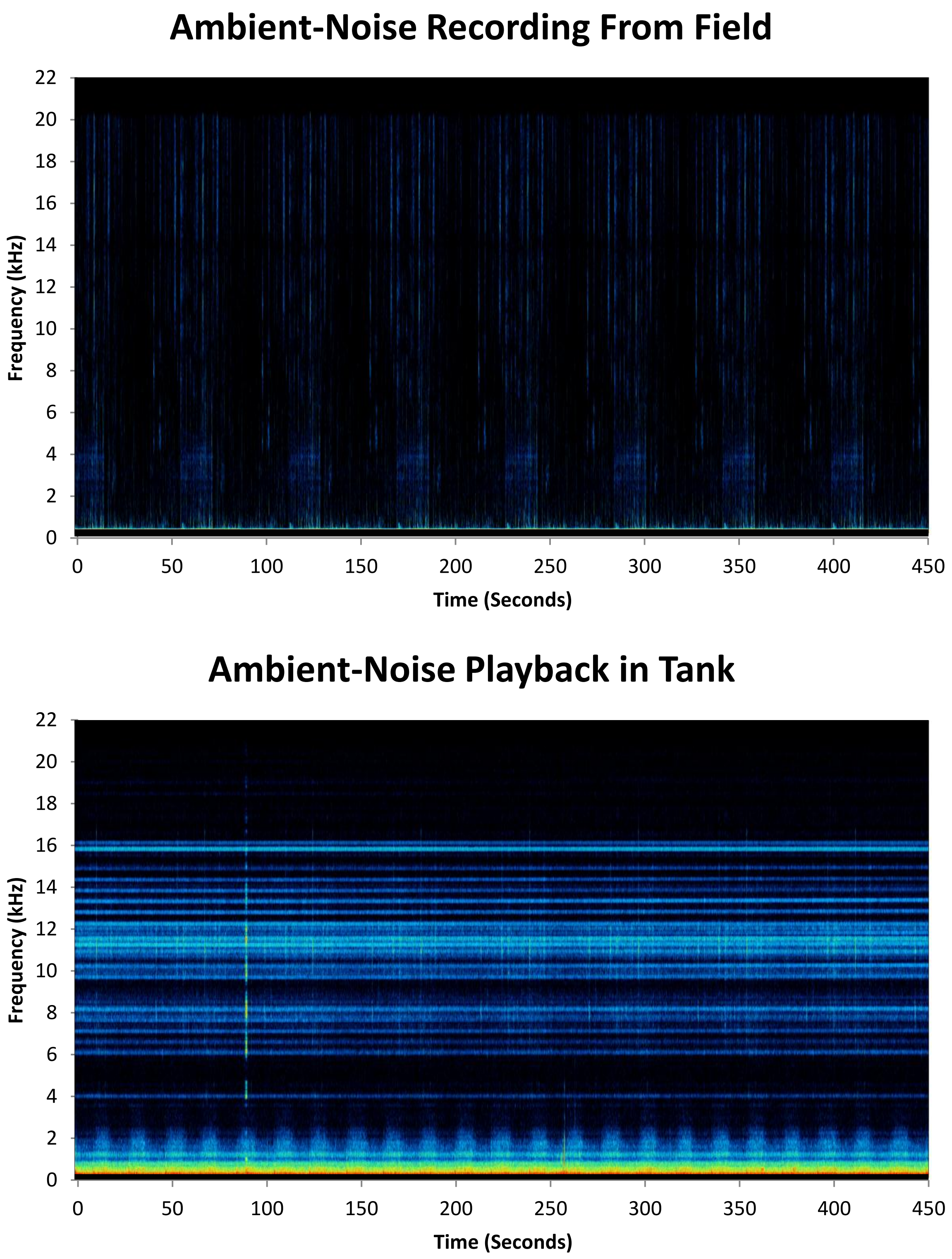

Figure S4. Illustrative spectrograms of original recording in the field and re-recording of playback of ship noise from one of the three UK ports