Science of the Total Environment 873 (2023) 162260

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Cross-sensory interference assessment after exposure to noise shows different effects in the blue crab olfactory and sound sensing capabilities

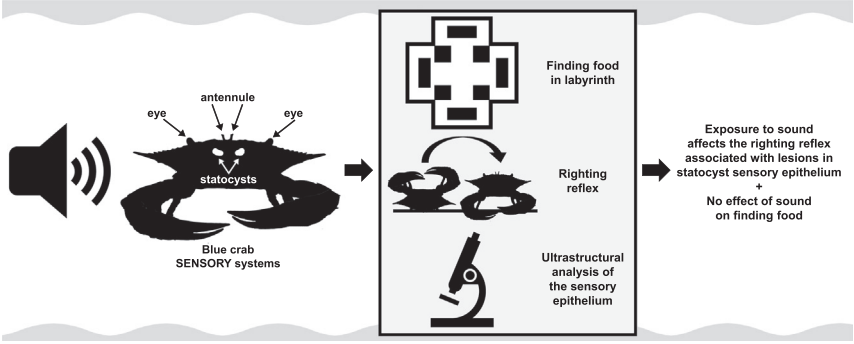
Marta Solé [a,*], Steffen De Vreese [a,c], Antonio M. Sánchez [a], José-Manuel Fortuño [b], Mike van der Schaar [a], Núria Sancho [a], Michel André [a]

[a] Laboratory of Applied Bioacoustics, Universitat Politècnica de Catalunya - BarcelonaTech (UPC), 08800 Vilanova i la Geltrú, Barcelona, Spain
[b] Institute of Marine Sciences, Spanish National Research Council (ICM-CSIC), 08003 Barcelona, Spain
[c] Department of Comparative Biomedicine and Food Science, University of Padua, 35020 Legnaro, Padua, Italy

HIGHLIGHTS

- Blue crab olfactory-mediated foraging was not negatively impacted by sound.
- There was no overall effect of natural sounds on food finding success and efficacy.
- Righting reflex effect correlated with statocyst damage was shown after artificial sound exposure.
- Statocyst presented damage but not the antenulle or eye sensory epithelia after sound exposure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Damià Barceló

Keywords:
Anthropogenic noise
Callinectes sapidus
Aquatic plus (+) maze
Foraging behaviour
Statocyst
Righting reflex

ABSTRACT

Underwater noise pollution is an increasing threat to marine ecosystems. Marine animals use sound in communication and orientation processes. The introduction of anthropogenic noise in their habitat can interfere with sound production and reception as well as with the acquisition of vital information through other sensory systems. In the blue crab (*Callinectes sapidus*), the statocyst is responsible for acoustic perception, and it is housed at the base of its first pair of antennae (antennule). The sensilla of the distal part of these antennule hosts the olfactory system, which is key for foraging. Given the anatomical proximity of the two sensory regions, we evaluated the possible interference of sound exposure with the crab ability to find food, by using an aquatic maze, and looked at the potential impairment of the righting reflex as well as at ultrastructural damages in statocysts. Although a significant effect was observed when looking at the time used by the animal to recover its habitual position ("righting reflex"), which was associated to lesions in the statocyst sensory epithelia, the time required to find food did not increase after the exposure to sound. When the crabs were exposed to natural sounds (marine background noise and sounds of their predators: *Micropogonias undulates* and *Sciaenops ocellatus*) they did not show significant differences in foraging behaviour. Although we found no unequivocal evidence of a negative impact of sound on olfactory capabilities, the study showed a clear righting reflex impairment correlated with ultrastructural damages of the statocysts. We argue that crab populations that cannot easily avoid noise sources due to their specific coastal distributions may incur in significant direct fitness costs (e.g. impairment of complex reflexes). This integrated approach to sound effect assessment could be used as a model for other invertebrate species to effectively monitor noise impact in marine environments.

* Corresponding author.
E-mail address: marta.sole@upc.edu (M. Solé).

http://dx.doi.org/10.1016/j.scitotenv.2023.162260
Received 21 November 2022; Received in revised form 8 February 2023; Accepted 11 February 2023
Available online 23 February 2023

1. Introduction

The introduction of an increasing number of anthropogenic sound sources in marine ecosystems has been a threat to their inhabitants, compromising the conservation of marine biodiversity. For marine species, the information extracted from soundscapes, which results from the combination of biological, geological, physical, and anthropogenic sounds, is vital (Lindseth and Lobel, 2018; Pijanowski et al., 2011). Thus, changing soundscapes due to the introduction of new man-made noises may alter vital marine animal sensory communication and orientation abilities, leading to detrimental consequences for the entire population (Degraer et al., 2020; Slabbekoorn et al., 2010).

Invertebrates have been shown to be sensitive to noise exposure. This sensitivity can be assessed by analysis of the morphological effects on sensory structures involved in sound perception, such as statocysts (Day et al., 2016, 2019; Solé et al., 2016, 2017); the changes in their sound production capacities (Aimon et al., 2021); the effects on their physiological condition (Vazzana et al., 2020; Wale et al., 2013a); their behavioural responses, especially communication, navigation and orientation abilities (e.g., righting reflex) (Day et al., 2019; Radford et al., 2007), and foraging and antipredator behaviours (Wale et al., 2013b). However, whether sound exposure can alter other sensory systems remains unknown.

Crustaceans are sensitive to low frequency acoustic stimuli (Goodall et al., 1990; Roberts et al., 2016; Salmon and Horch, 1972). Mechanical disturbance of water/sediment that is associated with sound waves is detected by a pair of statocysts, chordotonal organs linked to the joints of antennae or legs and internal and external sensilla (Breithaupt, 2002; Popper et al., 2001). Statocysts may detect the sound particle motion rather than to the sound pressure component. Invertebrate statocyst as linear accelerometers can detect acoustic particle motion (since the whole animal vibrates together with the water column) and are involved in underwater hearing (Budelmann, 1992). The basic structure of the statocyst is similar among all crustacean species; it is located on the basal segment of the antennule in decapods and the uropod or telson of the tail in mysids and isopods. Statocysts have inner cuticular sensory hairs polarized towards the centre and an overlying statolith, which stimulates the sensory hair cells. The sensory hairs are arranged in two to four rows (Budelmann, 1992; Cate and Roye, 1990; Rose and Stokes, 1981). Statocysts, as a part of the mechanosensory system, are associated with reflex behaviours in crustaceans. The neuronal input of a statocyst plays an essential role in coordinating body positioning and movement (Newland and Neil, 1987), including the righting response (i.e., the capacity to recover the habitual position) (Their, 1968).

Crustacean species, which are reported to be adversely impacted by high amplitude anthropogenic noise exposure (Edmonds et al., 2016), use chemical cues to regulate critical aspects of their behaviour (Hay, 2009). Crustaceans use species-specific olfactory cues to find food (Roberts and Laidre, 2019a), localize potential prey (Keller et al., 2003; Tran, 2013; Weissburg and Zimmer-Faust, 1994), avoid predators (Berger and Butler, 2001), select dens (Berger and Butler, 2001; Nevitt et al., 2000), and in odour-associative learning and odour discrimination (Steullet et al., 2002), conspecific interactions, including couple localization and mating (Giri and Dunham, 2000; Karavanich and Atema, 1998; Okamura et al., 2017; Valdes and Laidre, 2019), shell finding (hermit crabs) (Valdes and Laidre, 2018), and grooming (Daniel et al., 2001).

Animals combine information received by different sensory systems that act simultaneously. In crustaceans, the statocyst, the organ responsible for acoustic and vibration perception, is located on the base of its pair of antennules. Distally, the antennule bears olfactory sensilla, used by animals for the detection of chemical cues that allow them to find food. The antennule has a basal segment that is lodged in a fossa, as well as a flagellum (the distal part) that bears aesthetascs (olfactory sensilla used for foraging and mating behaviour by flicking) and mechanosensory sensilla (used for equilibrium and eye and righting movements) (Davie et al. Mirwan and Kevan, 2015). Given the anatomical proximity of the two sensory systems, interference with one of them could affect the neighbouring sensory modality, disturbing the processing and interpretation of the information (Halfwerk and Slabbekoorn, 2015).

Cross-sensory interference mediated by sound exposure has been described in different species of crustaceans. The blue crab (*Callinectes sapidus*) distinguishes attractive food odours from aversive odours (injured crab metabolites) (Weissburg et al., 2012). Environmental turbulence suppresses their navigation in attractive-aversive plumes (Weissburg et al., 2012). Exposure to white noise prevents common hermit crabs (*Pagurus bernhardus*) from spending much time in shell selection (Walsh et al., 2017) and results in a decrease in the number of shore crabs (*Crangon carngon*) added to a food item (Hubert et al., 2018a). When exposed to boat noise and flashing lights, Caribbean hermit crabs (*Coenobita clypeatus*) exhibit a slower antipredatory response (Chan et al., 2010). Furthermore, the number of Acadian hermit crabs (*Pagurus acadianus*) aggregated to a chemical cue source decreases during impulsive sound exposure (Roberts and Laidre, 2019b).

The blue crab (*C. sapidus*) is native to the American Atlantic coast and was introduced to Hawaii, Japan, Africa, and Europe, specifically in the waters of the Mediterranean Sea, in the last century, possibly due to the ballast water from large vessels (Mancinelli et al., 2017). In the Ebre Delta, the first blue crab specimen was caught in the waters of the Spanish Mediterranean Sea in November 2012 (Castejón and Guerao, 2013) and it has become a local plague in the last decade. It is considered one of the 100 most dangerous invasive species in the Mediterranean due to its impacts on both biodiversity and the economy (Streftaris and Zenetos, 2006). It was also included on the Spanish list of invasive exotic species in 2011 (Affairs, 2011). Prior to this study, we had proposed the use of an acoustic method to control invasive pests (André et al., 2018; Solé et al., 2021a). To evaluate the possibility of using this method in the fight against blue crab invasion, a thorough laboratory analysis of the possible effects of sound exposure on the natural behaviour of the blue crab is necessary.

Previous research was done using a complex maze to study spatial learning (Davies et al., 2019) or a two-choice set-up, such as a T maze, to assess the capability to discriminate between chemical cues (Tierney and Lee, 2008) or to study the cross-sensory interference of acoustic stressors on crustaceans (Hubert et al., 2021). A swimming plus maze test allowed assessment of the anxiety-related responses of zebrafish regardless of developmental stage (Varga et al., 2018). In the current study, we take a novel approach integrating different techniques: testing the effects on odour-mediated food-finding capacity in the blue crab (*C. sapidus*) using an aquatic plus-shaped maze (+), in combination with the righting reflex assessment (time used by the animal to recover its habitual position) after exposure to artificial sound (sinusoidal sweep), and the ultrastructural effect analysis on the statocyst, antennule, and eye sensory epithelia analysis by electron microscopy. In addition, we assessed the possible interference on olfactory-mediated food finding when exposed to natural sound (marine background and the sounds of two predators). We expected that exposure to low-frequency sound would result in significant direct fitness costs (e.g. impairment of complex reflexes) and essential abilities for their survival (e.g. foraging behaviour) due to chronic noise exposure. This multidisciplinary approach will allow us to correlate any ultrastructural damage with possible behavioural changes in the foraging behaviour and righting reflex, and determine the possible cross-modal interference between sensory systems. This allows a more complete picture of how noise affects the invertebrate biology. Our integrated approach to noise research can be used as a model for other invertebrate species and inform the development of effective methods for assessing noise impact.

2. Material and methods

2.1. Animals

Adult *C. sapidus* (n = 108, 101 males and 7 females) were collected on Ebre Delta from the Catalan Coast (NW Mediterranean Sea) by local fishermen during July 2020 (31 crabs 11–16 cm wide and weighing 100–200 g, 29 males and 2 females) and August 2021 (77 crabs 10–16 cm wide and

weighing 90–275 g, 72 males and 5 females) and kept in the LAB's (Laboratory of Applied Bioacoustics, 41°12′57.1″N 1°43′59.0″E) maintenance system, a closed system of recirculating water (at 18–20 °C, salinity 35 ‰, and natural oxygen pressure) consisting of 2 mechanically filtered fiberglass reinforced plastic tanks of 2000 L capacity, that were connected to each other. This included a physicochemical self-filtration system with activated carbon and sand, driven by a circulation pump. Blue crabs were kept in the maintenance tank one week before the start of the experiments and supplied with mussels (*Mytilus edulis*) and surimi ad libitum until two days prior to the experiment in order to standardize hunger levels and were maintained in the tank system until the exposure. Some of these animals were used as controls and were kept under the same conditions as the experimental animals until the latter were exposed to noise (sweep exposure) in an isolated independent experimental tank located in a separate place from maintenance tanks (see Section 2.4.2). The crabs were permanently marked with waterproof paint on their carapace in order to recognize them individually.

2.2. Aquatic plus maze set-up

The behavioural interference on olfactory-mediated food finding was tested in trials that were performed using an aquatic plus maze, a cross-shaped (+) maze that contains four end compartments and one central compartment (Fig. 1). The maze consists of transparent methacrylate (5-mm-thick) with an arm length of 75 cm and arm width of 59.6 cm (total size: 153.8 cm × 153.8 cm × 20 cm). Every end compartment has an internal window of 15 cm × 10 cm. The maze walls are 20 cm high in order to avoid the crab escaping. The maze was partially submerged (10 cm) and fixed to the walls of a filled tank that was identical to the stock tank. The underwater transducer (Lubell LL9642T; frequency range: 250 Hz–20 kHz, maximum SPL: 183 dB re 1 μPa·m at 1 kHz or 193 dB re 1 μPa·m at 10 kHz supplying 100 V_{rms}) was placed 1 m below the maze on the tank floor for exposure to predator and natural marine background sounds). When the trial started, the researchers moved away from the set-up in order to prevent visual disturbance. We used a stationary hydrophone to record all trials. All trials were recorded with a GoPro video camera (GoPro HERO4® and GoPro HERO5 Session®) that was placed above the tank. The subjects were tested for their ability to associate the olfactory cue with the rewarding unconditioned stimuli while swimming/walking in the maze under different conditions.

2.3. Experimental design

Table S1 (supplementary material) summarizes the experiments.

In July 2020, we performed preliminary tests in the maze to design the protocol and standardize the research parameters, as well as experiments in the maze to evaluate the possible behaviour interference on olfactory-mediated food finding by blue crabs when exposed to natural sound (marine background and sounds of two predators).

In August 2021, we performed tests using a box to ensure the ability of crabs to detect and discriminate food signals only by odour stimulation and eliminate the possibility that animals have visual input (when compared to experiments in the maze). We also performed experiments using the maze to analyse the effects on the odour-mediated food-finding capacity and righting reflex (time used by the animal to recover its habitual position) after exposure to a sinusoidal wave sweep. The ultrastructural effects on the statocyst, antennule, and eye sensory epithelia were assessed by electron microscopy to correlate any ultrastructural damage with possible changes in the food-finding behaviour.

The preliminary tests were performed to design the protocol and to standardize the research parameters when working with the maze (see supplementary material: 2.1 Preliminary tests: protocol validation and standardization).

2.3.1. Test in a box protocol

To ensure the ability of crabs to detect and discriminate food signals and eliminate the possibility that the animals had visual input, we used a box with an internal rectangular frame (45 × 45 × 40 cm; Fig. S1). The box was filled up to 10 cm with natural salt water and food olfactory cue dispensers placed in each of the four corners. One of the dispensers was filled with fresh mussel flesh and the other three with a sponge of the same colour as the food to avoid visual discrimination. One crab was

Fig. 1. Aquatic plus maze set-up. A: Shape of the aquatic plus maze. B: The maze in the tank. C: The underwater transducer placed 1 m below the maze on the tank floor is visible.

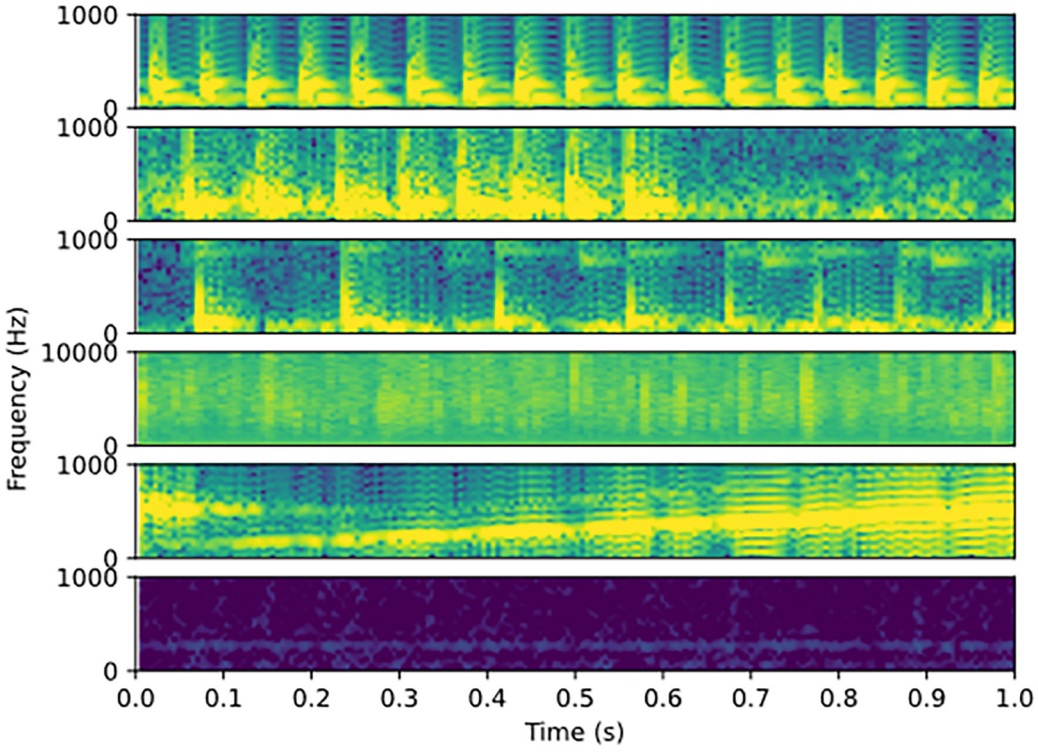


Fig. 2. Sound exposures and background noise. From top to bottom the spectrograms are: the original sounds of the Atlantic croaker, the Georgia reddrum, the reddrum knocking sound, the environmental noise, the recorded sweep in the tank, and the background noise in the tank.

located in the centre of the box in each experiment. Sixty-seven crabs were used in this experiment (32 controls and 35 crabs previously exposed to a 100–500 Hz sinusoidal wave sweep, see Section 2.4). We randomly alternated the order of the animals and the position of the dispenser. Each crab was used once in a trial. Between trials, we cleaned the box, changed the water, and placed new mussel flesh in the dispenser. The time needed to find the food was recorded with a GoPro video camera (GoPro HERO5 Session®; Video S1).

2.3.2. Maze experimental protocol

At the start of each trial, a crab was placed in the central compartment enclosed by a metallic mesh 5 min before the experiment started. One minute before the crab was released, the food cue dispenser was placed in a terminal compartment and the other three end compartment dispensers were filled with a sponge. We randomly alternated the order of the animals and the position of the dispenser and counterbalanced the order of the treatments and the position of the dispenser. Each crab was used once in

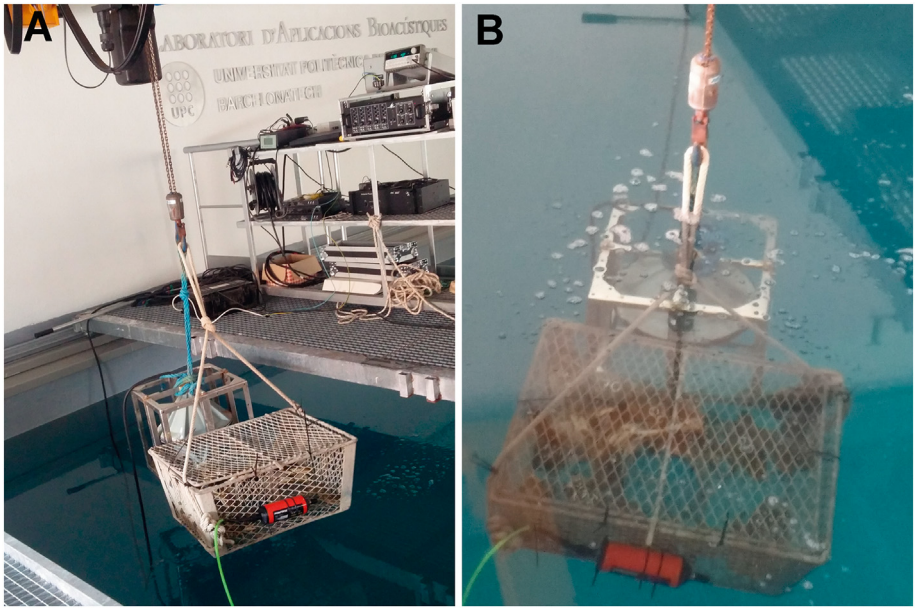


Fig. 3. Sweep exposure set-up. A: The crabs to a cage attached to the transducer are going down into the large isolated tank located in the LAB. B: The system is under the water. A calibrated hydrophone (red) is attached to the crab cage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a trial. Prior to each test, the water was changed with the help of a pump and the dispenser filled with new mussel flesh. We used a stationary hydrophone and a GoPro video camera placed above the tank to record all of the trials. In the case of behavioural trials under natural sound exposure, an underwater speaker placed 1 m below the maze on the tank floor reproduced sounds of blue crab predators and marine background sounds. We considered the trial finished when the animals found the food or after 20 min if they did not find it) (Video S2).

2.4. Sound exposure

We exposed the crabs to three different types of sounds: natural predator, marine background, and 100–500 Hz sinusoidal wave sweep.

2.4.1. Natural sound exposure

The predator and marine background sounds were used in the maze experiments to assess the possible influence on the crab foraging time. During the maze trials, we exposed a set of crabs to the sound of two blue crab predators, the Atlantic croaker [*Micropogonias undulates* (Rhode Island University, 2001), n = 11] and red drum [*Sciaenops ocellatus* (Florida University, 2008), n = 12], and to marine background sounds (LAB-UPC recording, n = 15) (Fig. 2). The trials were randomized and the order of playback of sounds was decided in advance. The corresponding sound for each exposure was manually selected and played by an underwater transducer (Lubell LL9642T) placed 1 m below the maze on the tank floor. Sounds were within the frequency range of 250 Hz to 20 kHz as set by the device, and at a received sound pressure level (SPL) of approximately 140 dB re 1 μPa^2. Camera recordings were analysed to determine any behavioural reaction and any change in the time required to find food after natural sound exposure. An additional set of crabs (n = 11) was used as controls and their behaviour recorded without any sound playback.

2.4.2. Sweep exposure

A different experiment was performed with the sweep sound. Prior to sweep exposure, the crabs' foraging behaviour in the maze, without any sound, was recorded by the cameras. Controlled exposure experiments (CEE) were conducted on 32 crabs placed in a cage attached to the transducer (Lubell LL-1424HP; frequency range 200 Hz–9 kHz, maximum SPL 197 dB re 1 μPa·m at 600 Hz), which was placed 1 m below the cage and submerged at a depth of 3.5 m (30 cm from the floor) in a large isolated tank (150 kL) located in LAB installations (Fig. 3). The tank used for the tests is a large tank which walls are isolated (unconnected) from the infrastructure and built on vibration absorbing pads. It is a reinforced concrete vessel with free interior measurements of 9.00 × 4.00 × 4.00 m and a critical calculation resonance frequency of 41 Hz. The interior faces in contact with water are coated with several layers of TRIBAD-PVP sanitary bicomponent epoxy resin on a fiberglass mesh. It rests on a base made up of 16 mm thick waterproof MDF boards (600 kg/m^3) and a 0.3 mm PVC sheet supported on a grid of a total of 320 mechanical rubber sylomer blocks of 10 × 10 × 2.5 cm with higher support density. Along the perimeter, under the walls and maximum design supported pressure < 16.5 kN at the corners. All supported on a 30–34 cm reinforced concrete slab and micropiles foundation of Ø17.5 cm and 7.5 m deep tied with 0.60 × 0.30 m braces, in the tank support area and 0.30 × 0.30 m in the rest of the plant. The long wall facing north and the short wall facing east, are covered on the outside with 8 cm acoustic copoprene plates, DM board and 3 cm air chamber, in the first two meters concrete wall HA-30/B /20/IIa waterproof, 0.30 m thick (fc = 62.318 Hz), with AP500 S steel reinforcement in corrugated bars with a quantity of 60 kg/m^3, in contact with the ground and the following 2.3 m brick wall of 14.5 cm thick concrete sound absorber (RA = 51dBA, fc = 133.10 Hz) with 39 % voids and 1846 kg/m^3 absolute density, 6 cm XPS acoustic insulation, 1.5 COTETERM-M base layer −2 mm applied by hand, PVC mesh, coating of COTETERM mortar with a rough finish, thickness 2–3 mm, and paint. The compressive breaking stress of the concrete specimens after 14 days of being poured was between 35.5 and 45.2 N/mm^2. On the west side wall we have 8 cm acoustic copoprene plates, DM board, air chamber, same gero as before, mortar coating and paint. The south face is fully visible without coating. The south and west faces face the interior of the building. During the tests the water content was 129,600 L and without tubes or holes in the water.

Crabs were exposed to a 100–500 Hz sinusoidal wave sweep with 100 % duty cycle and 1-s sweep period for 2 h. The received SPL was 171 dB of 1 μPa^2 measured with a B&K 8106 calibrated hydrophone and a maximum level of approximately 180 dB of 1 μPa^2. Twenty-six additional crabs were maintained under the same conditions as exposed crabs before and after the CEE. These control crabs were transferred to the large tank and kept under the same conditions for the same duration as those that were exposed but without any sound playback. After sound exposure, the initial test on the foraging behaviour in the maze was repeated and recorded in order to

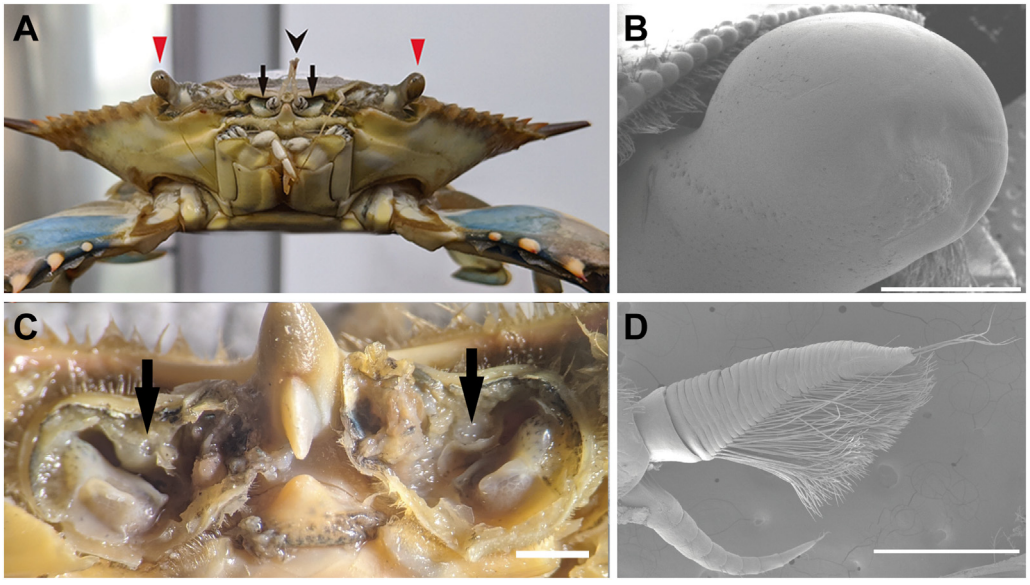


Fig. 4. Sensory systems analysed. A: Frontal view of a female blue crab. Arrows indicate the location of the statocysts, red arrowheads the eyes, and the black arrowheads the antennules. B: Scanning electron microscopy (SEM) of the eye. C: Light microscopy of the opened statocysts (arrows). D: SEM of the antennule. Scale bars: 1 mm (D), 2 mm (B), 0.5 mm (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

assess any change after exposure. In addition, the righting reflex was analysed before and after the sound exposure. After the second trials, the animals (control and exposed) were sacrificed 120 h after sound exposure and the ultrastructural effects on sensory epithelia assessed by electron microscopy to correlate any ultrastructural damage with possible changes in the food-finding behaviour.

2.5. Behavioural observation

Behaviour was assessed with or without sound reproduction through analysis of the video recordings. All experiments were recorded and behavioural reactions monitored. We manually scored whether the crab found the food and the time from emergence from its stationary position until the animal came in contact with the food. We only considered the trials in which the animal ate the food to rule out a chance encounter not mediated by the odour signal.

2.5.1. Natural sound exposure experiments

We performed 11 trials with environmental treatment (without any playback), 15 trials with marine background treatment, 11 trials with Atlantic croaker treatment, and 12 trials with red drum treatment.

2.5.2. Sweep exposure experiments

We analysed 32 control trials and 35 trials of sound-exposed crabs in the preliminary test in a box, and 26 control trials and 31 trials of sound-exposed crabs in the maze experiment.

2.6. Righting reflex

For this experiment, we used 58 crabs (26 control and 32 sound-exposed). We assessed the righting reflex by measuring the time taken for crabs to return to their habitual dorsum-up position after being placed ventrum-up in a plastic box with seawater, respecting the same sequential process for control and exposure animals (immediately, 48, and 120 h after sound exposure). We chose these times for analysis based on previous studies (Solé et al., 2013) in which the increase in ultrastructural damage with time was found in other species.

We considered that the animal had returned to the right position when the abdomen and walking legs from both sides of the body were in contact with the bottom of the box. The same researcher, blind to the crab treatment (control or exposed), conducted this assessment for each individual in each experiment and with the same equipment in throughout the study.

2.7. Ultrastructural analysis and quantification of lesions

For this experiment, we used 57 crabs (26 control and 31 sound-exposed crabs). Blue crabs were anaesthetized and sacrificed with an overdose of 2-phenoxyethanol respecting the same process for control and exposed animals 120 h after sound exposure. The statocysts, the eyes, and the antennules (Fig. 4) were fixed and processed by routine procedures for analysis by scanning electron microscopy (SEM). Fixation was performed in glutaraldehyde 2.5 % for 24–48 h at 4 °C. Samples were dehydrated in graded alcohol solutions and critical-point dried with liquid carbon dioxide in a Leica Em CPD300 unit (Leica Mycrosystems, Austria). The dried samples were mounted on specimen stubs with double-sided tape. The mounted tissues were gold coated with a Quorum Q150R S sputter coated unit (Quorum Technologies, Ltd.) and viewed with a variable pressure Hitachi S3500N scanning electron microscope (Hitachi High Technologies Co., Ltd., Japan) at an accelerating voltage of 5 kV in the Institute of Marine Sciences of the Spanish Research Council (CSIC) facilities. To evaluate the acoustic impact on these sensory structures, the possible lesions on their sensory epithelia were assessed.

The lesions on the sensory epithelia of the statocysts were quantified. To achieve this, we considered the region including the inner row setae (statolith sensilla), which are overlaid by the statolith. We chose this region because the central position and the presence of the statolith associated with its sensilla, which plays an essential role in coordinating body positioning and movement, including the righting response. Sensilla damage was quantified by classifying the sensilla as intact (undamaged) or damaged (loss of the hair, leaving only the cell base). For all animals, the lesions were assessed for both statocysts. The lesions were measured as the number of damaged sensilla in the statolith inner row divided by the number of total cells in the inner row. The two measurements per system, per animal, were then averaged for statistical tests.

For each area/sample, we had damaged cell count/area and total cell count/area values. For these two, counts were divided to compute the ratio of missing cells. We had two ratios per animal, which were averaged.

2.8. Statistical analysis

Some of the experimental results provided proportions, such as the proportion of control and exposed animals that found the food in the maze. The

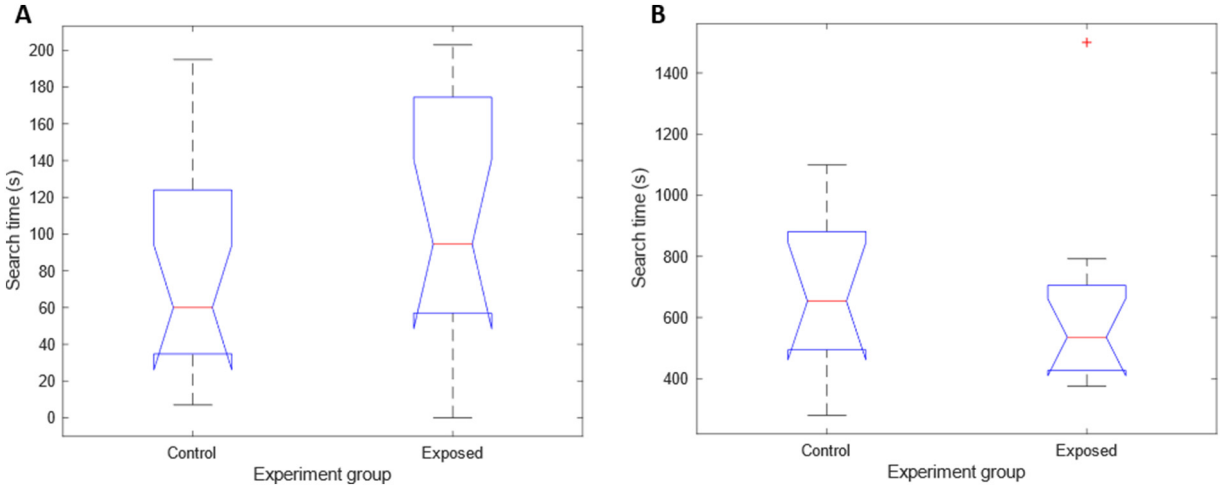


Fig. 5. Effects of sound exposure (sweep) on food search time. Food encounter times for control and exposed crabs. A: Box experiment (Control: N = 17; Exposed: N = 16). B: Maze experiment (Control: N = 10; Exposed: N = 12). The box marks the 25th and 75th percentiles, with the red line the median. The notches indicate the 95 % confidence interval around the median. The whiskers extend to the most extreme point not considered an outlier, with maximum distance 1.5 times the interquartile range. Outliers which fall outside that range are shown as a '+'. Note that the search time cannot be negative. The 25th percentile falls within the confidence interval, resulting in the folded shape. All notch zones overlap, indicating no difference at a 5 % significance level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

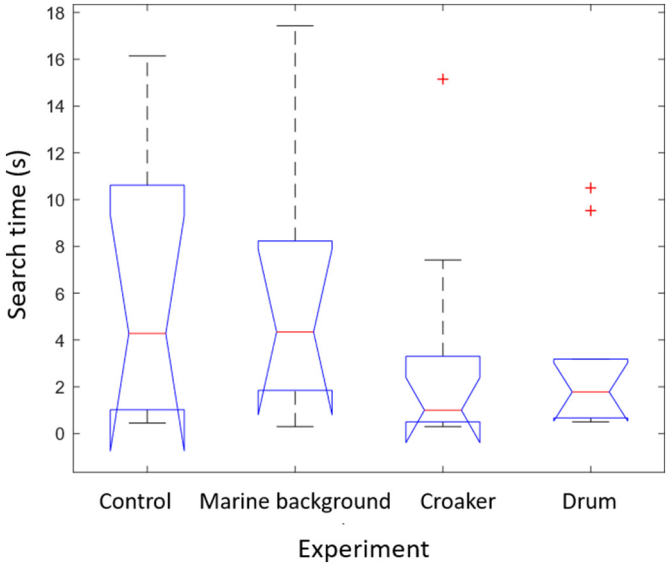


Fig. 6. Effects of natural sound exposure on food search time. No playback (control, N = 9), marine background (N = 8), croaker sound (N = 10), and drum sound (N = 10). Data are shown as the median and 95 % confidence interval. There was no significant difference in median search times (P = 0.24, Kruskal-Wallis). The box marks the 25th and 75th percentiles, with the red line the median. The notches indicate the 95 % confidence interval around the median. The whiskers extend to the most extreme point not considered an outlier, with maximum distance 1.5 times the interquartile range. Outliers which fall outside that range are shown as a '+'. Note that the search time cannot be negative, but the 95 % confidence interval around the median does extend below 0. The 25th percentile falls within the confidence interval, resulting in the folded shape. All notch zones overlap, indicating no difference at a 5 % significance level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significance of the difference between these proportions was tested through a proportional chi-squared test (Fleiss et al., 2003).

The righting reflex tests were performed using the same animal before and after exposure. To find significant differences in response times, we used a Wilcoxon paired signed rank test (Gibbons and Chakraborti, 2011). For experiments to investigate the difference between exposed groups under different conditions, we used a Wilcoxon rank sum test (Hollander and Wolfe, 1999). For experiments with multiple groups (>2) to determine whether they had different medians, we used a Kruskal-Wallis test (Daniel, 1990).

3. Results

The results of the preliminary test (supplementary material/2. Material and Methods) were used for validation and standardization of the final protocol (supplementary material/3.Results).

3.1. Behavioural observation in a box

The effect of the sweep sound exposure on foraging behaviour was assessed. In the control treatment, 17 of 32 animals reached the food. Of 35 exposed crabs, 16 crabs reached the food. The chi square test between these proportions resulted in a P = 0.5 or no difference in the proportion of crabs that reached the food within the set time. Comparing the time required to find the food between control and exposed with a rank sum test resulted in P = 0.3 and we concluded that the sweep exposure had no influence (Fig. 5A).

3.2. Behavioural observations in the maze

3.2.1. Sweep exposure

The effect of the sweep sound exposure on foraging behaviour was assessed. The proportion of animals eating was 10/26 for controls and 12/31 for exposed crabs, which is not significantly different (P = 1.0, chi-squared or P = 0.5, rank sum; Fig. 5B).

3.2.2. Natural sound exposure

The effect of exposure to natural sounds on foraging behaviour was assessed. There was no clear effect of the playback of different sounds on the search time (P = 0.24, Kruskal-Wallis; Fig. 6).

3.3. Righting reflex

The righting reflex after sound exposure was assessed at various times after exposure and compared to control animals. First, we wanted to see if these controls could be grouped together, as there should be no difference in the response time of the same animal between the test at 0 h and the last test at 120 h. A low P-value here would indicate rejection of H0 that all data came from the same distribution and indicate that the response of

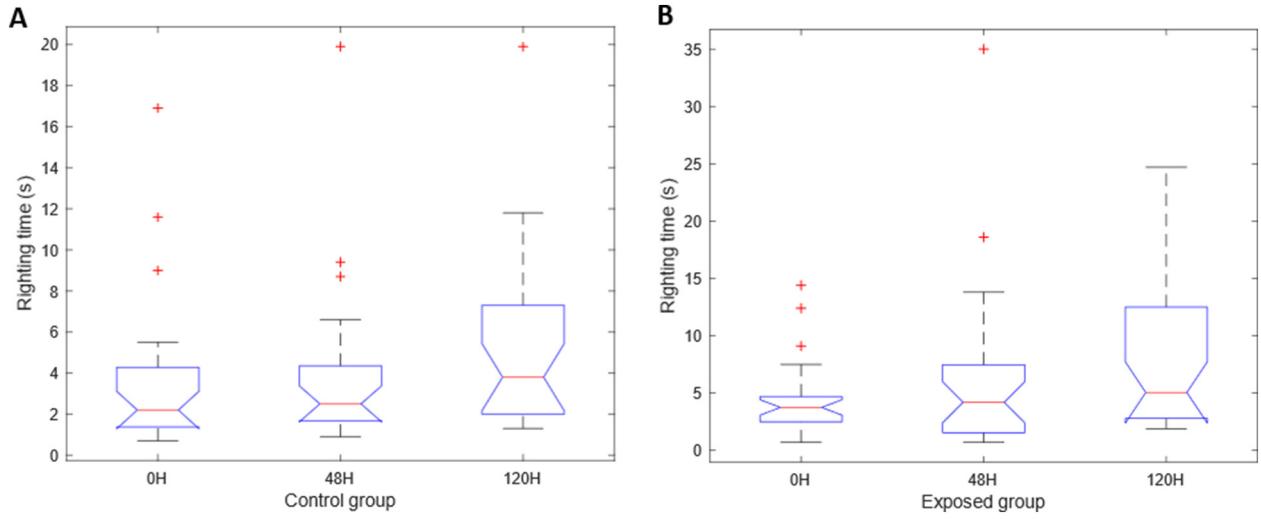


Fig. 7. Effects of sound exposure (sweep) on righting reflex. A: Control groups at 0 h (N = 25), 48 h (N = 23), and 120 h (N = 26). P = 0.10, Kruskal-Wallis. B: Exposed groups at 0 h (N = 26), 48 h (N = 27), and 120 h (N = 32). P = 0.08, Kruskal-Wallis. The box marks the 25th and 75th percentiles, with the red line the median. The notches indicate the 95 % confidence interval around the median. The whiskers extend to the most extreme point not considered an outlier, with maximum distance 1.5 times the interquartile range. Outliers which fall outside that range are shown as a '+'. All notch zones overlap, indicating no difference at a 5 % significance level. A rank sum test between 120H control and exposed resulted in P = 0.07. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

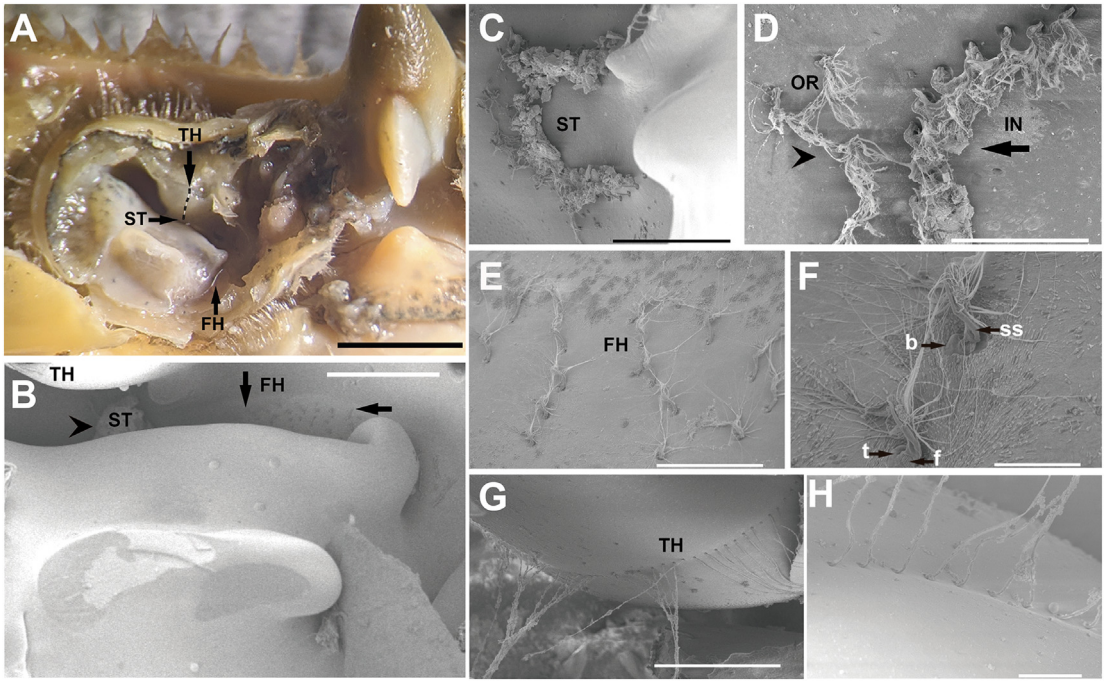


Fig. 8. Structure of the statocyst in the blue crab. A: Light microscopy of the opened left statocyst. Arrows show the distribution of the three groups of mechanosensory setae: thread (TH), free hook (FH), and statolith (ST). (B–H: SEM) B: Closest view of the area including the three setae groups on scanning electron microscopy (SEM). Arrows indicate the free hook area and arrowhead the statolith position. C: Statolith sensilla with attached statoconia (grains of statolith). D: Detail of the statolith sensilla. Arrow indicates the inner row (IN) and arrowhead the outer row (OR). E: Free hook setae area. F: Detail from (E) shows the different parts of the setae (bulb (b), fulcrum (f), setal shaft (ss), tooth (t)). G, H: Thread setae. Scale bars: 0.5 mm (A, B), 200 μm (C), 100 μm (D, E), 20 μm (F), 300 μm (G), 50 μm (H). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the captive animals was changing over time. Using the paired signed rank test, the P-value between these two control groups was 0.06, which is somewhat low. Thus, we concluded that there may be a temporal relationship with the response time. The Kruskal-Wallis test had a P-value of 0.10, which is also low. Therefore, we did not combine all control animals into a single group for further tests and expected to see a slight increase in response time for the exposed animals (Fig. 7A).

To determine whether response times changed for exposed animals over time, we compared 0 h to 48 h and to 120 h (these are the same animals measured at different moments after exposure). The paired signed rank test resulted in $P = 0.4$ and $P = 0.01$, respectively. This was considered a strong indication that the response time was changing over time. The Kruskal-Wallis test resulted in $P = 0.08$. The pattern found here was similar to that found for the control animals, though possibly more apparent (Fig. 7B).

Next, we tested controls and crabs exposed at 120 h to determine whether exposure had an effect on the righting reflex. A rank sum test between these groups resulted in $P = 0.07$. We concluded that the righting reflex slowed down over time, and that there may be an additional effect due to sound exposure.

3.4. Ultrastructural analysis

3.4.1. Morphological and ultrastructural description of the blue crab statocyst, antennule, and eye

As a Portunid crab, the blue crab possesses a complex decapod statocyst located in the basal segment of the antennule (Fig. 8). The compression of the statocyst walls forms two circular canals (one on the horizontal plane and one on the vertical plane). Three groups of mechanosensory setae (thread, free hook, and statolith) are located in the statocyst canals and are stimulated for inner fluid movement (thread and free hook setae) or the sensilla (statolith). The sensilla are located in the ventral floor and are overlaid by the statolith that are formed by sand grains cemented together by tegumental gland secretions (Fig. 8C, D). Statolith sensilla are lined up in two concentric rows: inner row ($\overline{X} = 27$; $N = 57$) and outer row ($\overline{X} = 8$; $N = 57$). Both rows are overlaid by the statolith and hook-shaped, curving to the centre. The structure of the three groups of statocyst setae is similar, with a bulb (the proximal portion of the sensillum), a setal shaft, a tooth (the smooth portion of the bulb), and a fulcrum (a transverse fold; Fig. 8F) and filamentous hairs, which in the case of statolith sensilla have attached statoconia (Fig. 8C, D).

The antennules have a basal segment that is lodged in a fossa, and a flagellum (the distal part) that bears a bundle of numerous aesthetascs (olfactory sensilla; Fig. S2).

The eye comprises a greatly elongated eyestalk supporting a compound eye that has a pigmented and rounded cornea bearing hexagonal facets in hexagonal packing. Some setae are visible on different areas around the eye (Fig. S3).

3.4.2. Ultrastructural analysis of statocyst sensory epithelia, eye and antennule

No lesions compatible with acoustic impact were found on any of the antennules or in the eyes of the exposed crabs (Figs. S2, S3). Damage was observed in the statolith sensilla, free hook setae, and thread setae by SEM (Fig. 9). Damage was identified by classifying the sensilla as intact or damaged on SEM images.

3.4.3. Statocyst sensory epithelium quantification and data analysis

To quantify damage, we used the statolith inner row sensilla. We quantified the damage by classifying the statolith inner row sensilla as intact or damaged (Fig. S4). The percentage of damaged sensilla was determined for 2 statoliths per animal and the average per animal taken as a single measurement. The median damage in the control group was significantly less than the damage in the exposed group 120 h after exposure ($P = 0.0$, rank sum).

4. Discussion

Mazes are very useful instruments for obtaining a quantitative measure of the efficiency of an animal. Mazes of complex design have been used in

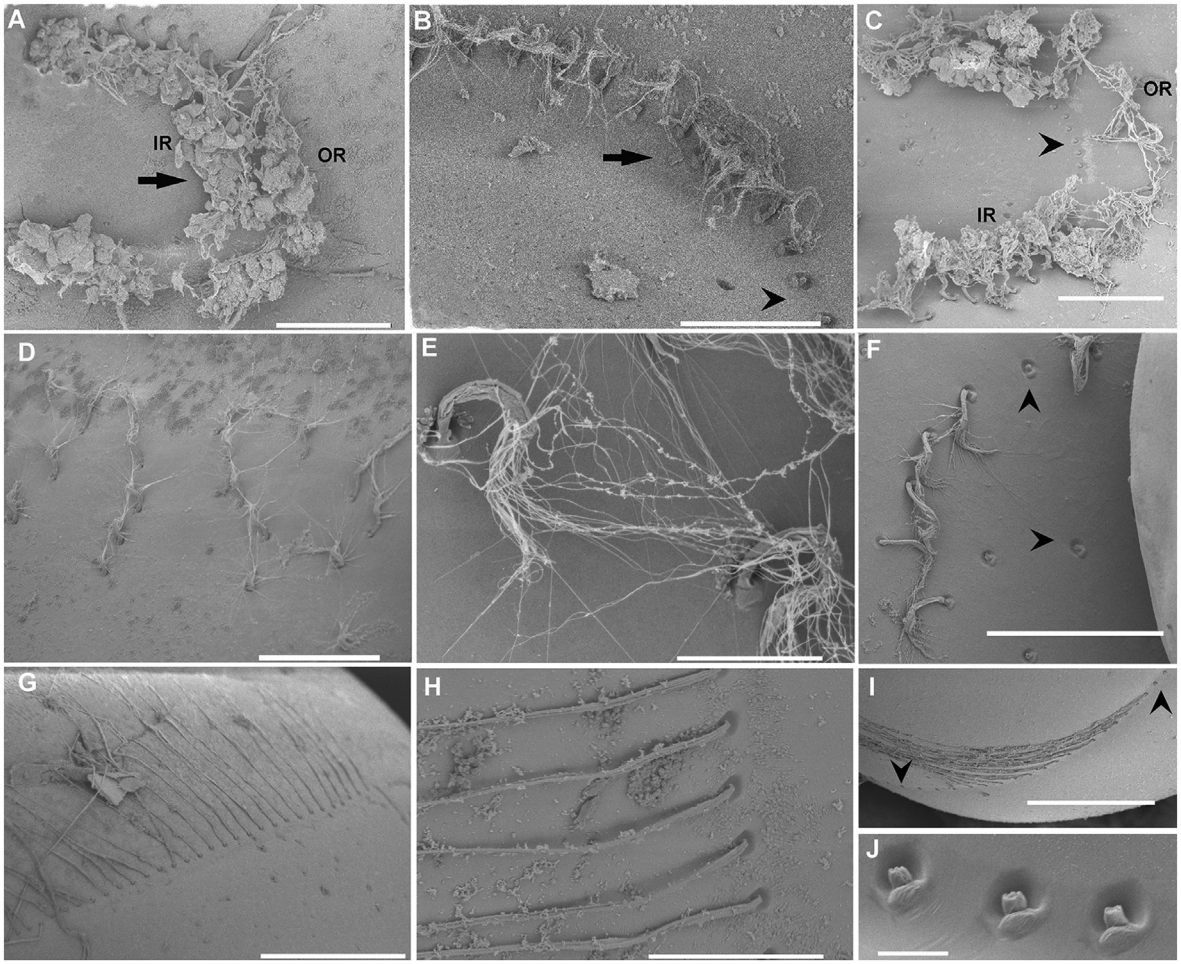


Fig. 9. Assessment of statocyst setae damage by scanning electron microscopy. A, B, D, E, G, H: Control; C, F, I, J: Exposed animals. A: Statolith sensilla overlaid by the statolith grains. Arrow points to the inner row (IR). OR, outer row. B: Inner row (arrow) of statolith sensilla clean of statolith grains. Arrowhead points to some damaged sensilla. C: The two rows of statolith sensilla are visible. The inner row shows damaged sensilla (arrowhead). D–F: Free hook setae area. E: Detail from D showing healthy setae. F: Arrowheads point to some damaged setae. G–J: Thread setae. G, H: Healthy setae. I: Arrowheads point to some damaged setae. J: Detail from I showing damaged setae. Scale bars: 100 μm (A, C, D, F), 50 μm (B, H), 30 μm (E), 200 μm (G, I), 10 μm (J).

the study of spatial learning in insects (Mirwan and Kevan, 2015; Zhang et al., 2000) and fishes. A recent study used a complex swimming plus maze test paradigm to assess anxiety-related avoidance of shallow water by larval stages of zebrafish (*Danio rerio*) (Varga et al., 2018). The design of this maze has similarities to our labyrinth. Much simpler designs (cross, Y, or T) have been used for crustaceans (Fossat et al., 2014; Hubert et al., 2021; Shuranova et al., 2005; Tierney and Lee, 2008). Only one recent study used a more complex multi-turn maze, similar to those used in classical mouse studies, to investigate learning in the European shore crab, *Carcinus maenas* (Davies et al., 2019). Our labyrinth is a complex aquatic plus-shaped maze that was used for first time on crustaceans and allowed us to assess the foraging behaviour of the blue crab.

Snitman et al. (2022) reported that key crab species (*Neohelice granulata*) locomotion activity diminished when exposed to diverse sound. On the contrary, our results showed that the food-finding success and foraging efficiency of blue crabs in an aquatic plus maze were not affected by different natural sounds (marine background, croaker sound, and drum sound). There was no clear effect from the playback of these different sounds on the foraging time. Although some previous experiments demonstrated that different species of crab exhibit changes in their anti-predator reaction (Wale et al., 2013b) or suppress their resource consumption in the presence of experimental acoustic stimuli from multiple predatory fish species (Chan et al., 2010; Hughes et al., 2012, 2014), exposure to the playback sounds of two blue crab predators in the present study did not lead to increased foraging duration. This finding suggests that olfactory-mediated food finding was not negatively affected by these sounds. Variable crab responses to individual predator species may reflect the differences in risk that each of these predators pose to crabs.

Although some studies have shown increased activity among blue crabs and American lobsters (*Homarus americanus*) when exposed to simulated low-frequency vessel noise and mid-frequency sonar (Hudson et al., 2022), or reduced food aggregation in shore crabs (*C. maenas*) in a field study (Hubert et al., 2018b), recent work on a T-maze (Hubert et al., 2021) demonstrated that the foraging efficiency of shore crabs is not affected by exposing the crabs to a boat sound playback or an ambient control. Our results are in line with this T-maze experiment on shore crabs. In our set-up, after low frequency sound exposure, the foraging duration of the animals was not affected. Similar results were found regarding the foraging behaviour of shore crabs in a small tank (Wale et al., 2013b). The apparent disparity in results could be explained by the differences in the intraspecific sensitivities and experimental designs of the studies, especially between field and tank studies, where particle motion features are different, and confirms the need for species-specific studies by species, avoiding generalization within taxonomic groups. Although invertebrates are more sensitive to the sound particle motion rather than to the sound pressure component of the sound, the small dimensions of our setup did not allow to measure the particle motion component in the present work.

Crustaceans use multiple sensory systems (visual, chemical, acoustic, tactile, electrical, thermal) to obtain the necessary information for communication in a multimodal signaling process (Hebets and Rundus, 2011).

Chemical communication is considered the oldest and most widespread channel for communication in crustaceans, which incorporates chemical signals into multimodal displays. In addition, some aquatic crustaceans possess bimodal chemo-mechanosensory sensilla (e.g. spiny lobster *Panulirus argus*; Cate and Derby, 2002). Some species combine chemical and hydrodynamic as well as chemical and visual cues (Hebets and Rundus, 2011). Aquatic crustaceans couple chemical signal production with the generation of water currents in order to transmit these signals (Atema, 1985; Breithaupt, 2001; Breithaupt and Eger, 2002) during social interactions. Another example of use of combinate sensory signals is the red swamp crayfish *Procambarus clarkia* mating process, in which a female requires a combination of visual and chemical stimuli to select the larger male. In mate assessment by *P. clarkii*, visual and chemical information seem to act as 'non-redundant signals' (Aquiloni and Gherardi, 2007).

The ghost crab *Ocypode platytarsus* is another example of an animal that uses a communication process presenting cross-sensory interference of the visual/acoustical and vibrational cues in crustaceans (Clayton, 2008). Several studies have suggested "cross-modal" influences of anthropogenic sound, eliciting changes in behaviours facilitated by other senses (Roberts and Laidre, 2019b). For example, shell selection by the European hermit crab (Walsh et al., 2017) and Acadian hermit crab (Roberts and Laidre, 2019b) is probably mediated by cross-modal influences. For selection of the shell, hermit crabs use a combination of chemical, visual, and tactile cues. Sound could have an indirect influence on this behaviour mediated by other sensory channels. These types of changes caused by sound exposure could be harmful in many ways. They could originate a fast use of energy reserves normally utilized by growth, reproduction, and anti-predator behaviour due to increased movement (Wale et al., 2013a; Zhou et al., 2018). In a similar way, the stress due to sound exposure could lead to a reduction in appetite and foraging behaviours (Wale et al., 2013b), limiting the energy stocks. In our study, sound exposure did not seem to affect foraging behaviour. In addition, these changes in foraging could lead to disruptions in the natural competition for food in natural conditions (Hubert et al., 2018b). Although we did not observe changes when exposed to the sounds of predators, other studies have shown that antipredator behaviours can be disrupted and the risk of predation increased Chan et al., 2010; Day et al., 2019; Wale et al., 2013b). We wanted to study whether cross-sensory interference in foraging behaviour occurs when blue crabs are exposed to sound playback. Although this process has been described in some previous experiments on other species of crustaceans (Hubert et al., 2018a; Walsh et al., 2017; Weissburg et al., 2012), the olfactory-mediated foraging behaviour of the blue crab was not negative affected when exposed to sound in our set-up. Similarly, Hubert et al. (2021) reported no evidence of cross-modal interference in European shore crabs.

Ultrastructural analysis of the statocyst, antennule, and eyes of exposed and control crabs was performed parallel with the behavioural analysis in order to provide a morphological explanation for eventual cross-sensory interference. Eye movements in crabs are driven by mechanosensory input from statocysts (Zeil and Hemmi, 2010). Antennulae are responsible for detecting odours (chemical stimuli) and giving direction to foraging behaviour, in addition to their essential role in mediating the response to pheromones in courtship and mating (Gleeson, 1982) through their aesthetascs (olfactory sensilla) (Davie et al., 2015). Other mechanosensory roles mediated by the antennula are the maintenance of equilibrium by triggering righting movements (Budelmann, 1992), controlling eye movement and position (Sandeman and Okajima, 1972), and providing a putative auditory function (Davie et al., 2015). Statocysts enable the equilibrium control, whereas equilibrium responses include the activation of eye movements (Davie et al., 2015). This intricate network of interconnections between vital functions mediated by the different sensory systems made us anticipate cross-sensory interference. Ultrastructural analyses did not show an effect of sound exposure in the sensory epithelia of the eyes and antennae, which may be related to the absence of noise effects on the olfactory and visual abilities of the blue crab. However, we observed damage in the statolith inner row sensilla, though the lesions were significantly fewer than what has been reported in other species in previous studies (Day et al., 2019; Solé et al., 2013, 2016, 2021a). Furthermore, the low level of lesions in the statocysts consequently explains the absence of cross-sensory interference in foraging behaviour. Although we found these effects to be relatively modest, they can be related to the increasing time required for the righting reflex after sound exposure.

Sensory epithelia sound overstimulation cause alterations on stereocilia of hair cells (losing, buckling, bending, breaking) (Hamernik et al., 1984; Raphael, 2002) or sensory hair cell loss (Hawkins and Schacht, 2005). Typical processes causing this hair cell degeneration are necrosis (swelling of the cell body and rupture of the plasma membrane) and apoptosis (chromatin compaction and fragmentation of the cell body) (Li et al., 1995). Dead hair cells after sound exposure can be removed by extrusion from the epithelium. In the present work damage to the hairs were shown as loss of the hair, leaving only the cell base. This partial cell extrusion is consistent with degenerating processes and posterior cell extrusion after sound exposure described in other species (e.g. in the basilar papilla of the avian inner ear (Cotanche, 1987) and in the mammalian organ of Corti (Spoendlin, 1971). Two mechanisms could be involved in noise-induced hearing loss as a consequence of hair cell degeneration in mammals: direct mechanical damage induced by excessive movement of the cochlear partition (after short exposures at high intensities) and metabolically induced damage resulting in distortion of the homeostasis of the organ of Corti (after long exposures with moderate intensities). It is probable that both mechanisms contribute to the process. However, this process can also be observed at the periphery of a violent acoustic trauma where open holes are left following the expulsion of the cell apex. Mechanical damage (partial or total loss of sensory cells) and metabolically induced damage (swollen sensory cells, vacuolization of cytoplasm, mitochondrial degeneration, damage to dendrites) was indeed observed in cephalopod statocysts sensory epithelia (Solé et al., 2013). The partial cell extrusion of the blue crab setae could be explained by similar processes of mechanical induced damage where the excessive movement of the hair would induce their loss. Analysis by Electron Transmission Microscopy could determine the mechanism of a possible metabolically induced damage origin of these lesions in future works. In addition, the statocyst sensory epithelium is responsible of its endolymph secretion, which is composed of protein and calcium. Dysfunction in the damaged epithelium leads to inaccurate release of the endolymph components resulting in abnormal physiological functioning of the statocyst and the vital capacities that it regulates (Solé et al., 2019). Morphological and physiological deterioration of sensory epithelia could lead to temporary deafness, which could result in the crab's inability to respond to the presence of predators and locate prey and mates, compromising its survival.

The righting reflex plays a relevant role on anti-predation, as the animals move from a vulnerable position to a position where anti-predatory behaviours are possible. Previous studies have investigated the righting reflex in the European shore crab with regard to shipping noise, showing a scarce effect on the righting time (Wale et al., 2013b). In contrast, this reflex was unaffected in American lobsters after exposure to seismic surveys (Payne et al., 2007). A more recent study showed impaired righting and significant damage to the statocyst of the rock lobster, *Jasus edwardsii*, using field-based exposure to air gun signals (Day et al., 2019), indicating that damage to the statocyst can impair complex reflexes. Our results in blue crab reinforce this hypothesis, and the relatively low level of lesions on the statocyst correlates with the reduction in righting reflex over time. The variance in the damage level could be explained again by differences in intraspecific sensitivities and the experimental designs.

Summarizing, we have not found any impact of sound exposure on the blue crab behaviour similar to those previously described in other crustacean species (e.g. in hermit crab shell selection (Roberts and Laidre, 2019a, 2019b; Walsh et al., 2017) the consumption of energy reserved for growth, reproduction and anti-predator behaviour (Wale et al., 2013a, 2013b), competition for food under natural conditions (Hubert et al., 2018b), cross-sensory interference in foraging behaviour (Hubert et al., 2018a) in shore crab). Unlike the present study, in these previous works, an ultrastructural assessment of the statocyst, antennulae and eyes to

evaluate a possible cross-sensory interference, was not carried out. This analysis has allowed us to determine lesions at the level of the sensory epithelium of the statocyst, not previously described in other species of portunoid crustaceans. In addition, it has allowed to determine a possible relationship between these ultrastructural lesions and a dysfunction in the equilibrium maintenance, controlled by the statocyst, and which in turn would affect the time required for the righting reflex after exposure to sound. These lesions were significantly smaller than those reported in previous studies on other species including cephalopods (Solé et al., 2013, 2017, 2022), cnidarians (Solé et al., 2016), gastropods (Solé et al., 2021a) and plants (Solé et al., 2021b). In these invertebrate species, the acoustic trauma was massive, affecting almost all of the statocyst sensory epithelia hair cells, and inhibiting vital functions such as feeding, mating or reproduction. This study contributes to the knowledge on the underwater sound exposure sensitivity interspecific differences among invertebrates.

5. Conclusion

Our study assessed the eventual cross-sensory interference between information mediated by the statocyst, eye, and antennulae after sound exposure. The morphological and ultrastructural effects of noise on these sensory organs were studied and correlated with the righting reflex. In addition, effect of natural sound (marine background and predators) on olfactory-mediated foraging behaviour in blue crabs was examined. Our results do not provide evidence of a negative effect of natural or low-frequency sweep sounds on food-finding success and foraging duration. However, an increase in the righting time after exposure to low-frequency sound exposure was correlated with damage to statocyst sensory epithelia, confirming the idea that damage to the statocyst can impair complex reflexes. These results contribute to the knowledge of the threat that anthropogenic noises could potentially represent when introduced in coastal ecosystems, altering vital marine animal sensory communication and orientation abilities, leading to detrimental consequences on entire populations.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162260.

Ethical statement

Although there are no legal requirements for studies involving crabs and bivalves in Spain, the experimental protocol strictly followed the necessary precautions to comply with current ethical and welfare considerations when dealing with vertebrates and cephalopods in scientific experimentation (Royal Decree 1386/2018, of November 19; Directive 2010/63/EU). This process was also carefully analysed and approved by the Ethical Committee for Scientific Research of the Technical University of Catalonia, BarcelonaTech (UPC) (approval code B9900085). This process limited the number of animals used in the experiments.

Credit authorship contribution statement

Marta Solé: Conceptualization, Methodology, Investigation, Data curation, Visualization, Formal analysis, writing - original draft preparation. Steffen De Vreese: Investigation, Data curation, Visualization, Writing - reviewing and editing. Antonio Sánchez: Visualization, Writing - reviewing and editing. José-Manuel Fortuño: Formal analysis, Writing - reviewing and editing. Mike van der Schaar: Investigation, Data curation, Visualization, Writing - reviewing and editing. Núria Sancho: Investigation, Data curation, Writing - reviewing and editing. Michel André: Conceptualization, Methodology, Investigation, Funding acquisition, Writing - reviewing and editing.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Author J.-M. Fortuño acknowledges the institutional support of the "Severo Ochoa Centre of Excellence" accreditation (CEX 2019-000928-S).

References

Affairs, S.M., 2011. Real Decreto 1628/2011, de 14 de noviembre, por el que se regula el listado y catálogo español de especies exóticas invasoras.
Aimon, C., Simpson, S.D., Hazelwood, R.A., Bruintjes, R., Urbina, M.A., 2021. Anthropogenic underwater vibrations are sensed and stressful for the shore crab *Carcinus maenas*. Environ. Pollut. 285. https://doi.org/10.1016/j.envpol.2021.117148.
André, M., Solé, M., Van der Schaar, M., de Vreese, S., 2018. A method for inducing lethal lesions in sensory organs of undesirable aquatic organisms by use of sound. Licensed to Seasel Solutions AS [NO/NO]; P.O. Box 93 N-6282 BRATTVÅG (NO). WO 2018/167003 A1.
Aquiloni, L., Gherardi, F., 2007. Assessing mate size in the red swamp crayfish *Procambarus clarkii*: effects of visual versus chemical stimuli. Freshw. Biol. https://doi.org/10.1111/j.1365-2427.2007.01911.x.
Atema, J., 1985. Chemoreception in the sea: adaptations of chemoreceptors and behaviour to aquatic stimulus conditions. Soc. Ex. Biol. Symp. 39, 386–423.
Berger, D.K., Butler, M.J., 2001. Octopuses influence den selection by juvenile Caribbean spiny lobster. Mar. Freshw. Res. 52, 1049–1053.
Breithaupt, T., 2001. Fan organs of crayfish enhance chemical information flow. Biol. Bull. 200, 150–154.
Breithaupt, T., 2002. Sound perception in aquatic crustaceans. The Crustacean Nervous System, pp. 548–558 https://doi.org/10.1007/978-3-662-04843-6_41.
Breithaupt, T., Eger, P., 2002. Urine makes the difference: chemical communication in fighting crayfish made visible. J. Exp. Biol. 205, 1221–1231.
Budelmann, B.U., 1992. Hearing in Crustacea. In: Webster, D.B., Fay, R.R., P.A.N. (Eds.), The Evolutionary Biology of Hearing. Springer-Verlag, pp. 131–139 https://doi.org/10.1007/978-1-4612-2784-7_9.
Castejón, D., Guerao, G., 2013. A new record of the American blue crab, Callinectes sapidus Rathbun, 1896 (Decapoda: Brachyura: Portunidae), from the Mediterranean coast of the Iberian Peninsula. Bioinvas. Rec. 2, 141–143.
Cate, H.S., Derby, C.D., 2002. Hooded sensilla homologues: structural variations of a widely distributed bimodal chemomechanosensillum. J. Comp. Neurol. 444, 345–357.
Cate, H.S., Roye, D.B., 1990. Ultrastructure and physiology of the outer row statolith sensilla of the blue crab *Callinectes sapidus*. Crustaceans 17 (3), 398–411.. https://academic.oup.com/jcb/article-abstract/17/3/398/2418896.
Chan, A.A.Y.H., Giraldo-Perez, P., Smith, S., Blumstein, D.T., 2010. Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol. Lett. 6, 458–461. https://doi.org/10.1098/rsbl.2009.1081.
Clayton, D., 2008. Singing and dancing in the ghost crab *Ocypode platytarsus* (*Crustacea*, *Decapoda*, *Ocypodidae*). J. Nat. Hist. 42, 141–155.
Daniel, P.C., Shineman, M., Fischetti, M., 2001. Comparison of species, chemosensory activation of antennular grooming behaviour in five of decapods. Mar. Freshw. Res. 52, 1333–1337.
Cotanche, D.A., 1987. Regeneration of hair cell stereociliary bundles in the chick cochlea following severe acoustic trauma. Hear. Res. 30 (2–3), 181–195.
Daniel, W., 1990. Kruskal–Wallis one-way analysis of variance by ranks. Applied Nonparametric Statistics, 2nd ed. PWS-Kent, Boston, pp. 226–234.
Davie, P.J.F., Guinot, D., Ng, P., 2015. Anatomy and functional morphology of Brachyura. Crustacea. Koninklijke Brill NV, Leiden, pp. 11–163.
Davies, R., Gagen, M.H., Bull, J.C., Pope, E.C., 2019. Maze learning and memory in a decapod crustacean. Biol. Lett. 15. https://doi.org/10.1098/rsbl.2019.0407.
Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Hartmann, K., Semmens, J.M., 2016. Assessing the Impact of Marine Seismic Surveys on Southeast Australian Scallop and Lobster Fisheries.
Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Hartmann, K., Semmens, J.M., 2019. Seismic air guns damage rock lobster mechanosensory organs and impair righting reflex. Proc. R. Soc. B Biol. Sci. 286. https://doi.org/10.1098/rspb.2019.1424.
Degraer, S., Carey, D.A., Coolen, J.W.P., Hutchison, Z.L., Kerckhof, F., Rumes, B., Vanaverbeke, J., 2020. Offshore wind farm artificial reefs affect ecosystem structure and functioning: a synthesis. Oceanography 33, 48–57. https://doi.org/10.5670/oceanog.2020.405.
Edmonds, N.J., Firmin, C.J., Goldsmith, D., Faulkner, R.C., 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. https://doi.org/10.1016/j.marpolbul.2016.05.006.

Fleiss, J.L., Levin, B., Paik, M.C., 2003. In: Shewart, W.A., Wilks, S.S., Fleiss, J.L., Levin, B., Paik, M.C. (Eds.), Statistical Methods for Rates and Proportions https://doi.org/10.1002/0471445428.

Florida University, 2008. Red drum sounds (*Sciaenops ocellatus*). [WWW document]Dosits URL https://dosits.org/galleries/audio-gallery/fishes/red-drum/.

Fossat, P., Bacque-Cazenave, J., Du Deurwaerdere, P., Delbecque, J.-P., Cattaert, D., 2014. Anxiety-like behavior in crayfish is controlled by serotonin. Science 344, 1293–1298. https://doi.org/10.1126/science. 1248811.

Gibbons, J.D., Chakraborti, S., 2011. Nonparametric Statistical Inference. 5th ed. Chapman & Hall/CRC Press, Taylor & Francis Group, Boca Raton, FL.

Giri, T., Dunham, D.W., 2000. Female crayfish (*Procambarus clarkii* (Girard, 1852)) use both antennular rami in the localization of male odour. Crustaceana 73, 447–458.

Gleeson, R.A., 1982. Morphological and behavioral identification of the sensory structures, mediating pheromone reception in the blue crab *Callinectes sapidus*. Biol. Bull. 163, 162–171.

Goodall, C., Chapman, C., Neil, D., 1990. The acoustic response threshold of the Norway lobster, *Nephrops norvegicus* (L.) in a free sound field. In: Wiese, K., Krenz, W.D., Tautz, J., Reichert, H., Mulloney, B. (Eds.), Frontiers in Crustacean Neurobiology. Birkhäuser Verlag, Basel, pp. 106–113 https://doi.org/10.1007/978-3-0348-5689-8_11.

Halfwerk, W., Slabbekoorn, H., 2015. Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. Biol. Lett. 11. https://doi.org/10.1098/rsbl.2014.1051.

Hamernik, R.P., Turrentine, G., Roberto, M., Salvi, R., Henderson, D., 1984. Anatomical correlates of impulse noise-induced mechanical damage in the Cochlea. Hear Res. 13, 229–247.

Hawkins, J.E., Schacht, J., 2005. Sketches of otohistory — Part 10: noise-induced hearing loss. Audio. Neurootol. 10 (6), 305–309.

Hay, M.E., 2009. Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. Annu. Rev. Mar. Sci. 1, 193–212.

Hebets, E.A., Rundus, A.S., 2011. Chemical Communication in a Multimodal Context. 41. Eileen Hebets Publications.. https://digitalcommons.unl.edu/bioscihebets/41.

Hollander, M., Wolfe, D.A., 1999. Nonparametric Statistical Methods. John Wiley & Sons, Inc., Hoboken, NJ.

Hubert, J., Campbell, J., van der Beek, J.G., den Haan, M.F., Verhave, R., Verkade, L.S., Slabbekoorn, H., 2018a. Effects of broadband sound exposure on the interaction between foraging crab and shrimp — a field study. Environ. Pollut. 243, 1923–1929. https://doi.org/10.1016/j.envpol.2018.09.076.

Hubert, J., Campbell, J., van der Beek, J.G., den Haan, M.F., Verhave, R., Verkade, L.S., Slabbekoorn, H., 2018b. Effects of broadband sound exposure on the interaction between foraging crab and shrimp — a field study. Environ. Pollut. 243, 1923–1929. https://doi.org/10.1016/j.envpol.2018.09.076.

Hubert, J., van Bemmelen, J.J., Slabbekoorn, H., 2021. No negative effects of boat sound playbacks on olfactory-mediated food finding behaviour of shore crabs in a T-maze. Environ. Pollut. 270, 116184. https://doi.org/10.1016/j.envpol.2020.116184.

Hudson, D.M., Krumholz, J.S., Pochtar, D.L., Dickenson, N.C., Dossot, G., Phillips, G., Baker, E.P., Moll, T.E., 2022. Potential impacts from simulated vessel noise and sonar on commercially important invertebrates. PeerJ 10, 1–19. https://doi.org/10.7717/peerj.12841.

Hughes, A.R., Rooker, K., Murdock, M., Kimbro, D.L., 2012. Predator cue and prey density interactively influence indirect effects on basal resources in intertidal oyster reefs. PLoS One 7. https://doi.org/10.1371/journal.pone.0044839.

Hughes, A.R., Mann, D.A., Kimbro, D.L., 2014. Predatory fish sounds can alter crab foraging behaviour and influence bivalve abundance. Proc. R. Soc. B Biol. Sci. 281. https://doi.org/10.1098/rspb.2014.0715.

Karavanich, C., Atema, J., 1998. Olfactory recognition of urine signals in dominance fights between male lobster, *Homarus americanus*. Behaviour 135, 719–730.

Keller, T.A., Powell, I., Weissburg, M.J., 2003. Role of olfactory appendages in chemically mediated orientation of blue crabs. Mar. Ecol. Prog. Ser. 261, 217–231.

Li, L., Nevill, G., Forge, A., 1995. Two modes of hair cell loss from the vestibular sensory epithelia of the guinea pig inner ear. J. Comp. Neurol. 355, 405–417.

Lindseth, A.V., Lobel, P.S., 2018. Underwater soundscape monitoring and fish bioacoustics: a review. Fishes 3. https://doi.org/10.3390/fishes3030036.

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., Ribeiro, F., 2017. The Atlantic blue crab *Callinectes sapidus* in southern European coastal waters: distribution, impact and prospective invasion management strategies. Mar. Pollut. Bull. 5–11.

Mirwan, H.B., Kevan, P.G., 2015. Maze navigation and Bumblebees, route memorization by worker (Hymenoptera:, (*Bombus impatiens* (Cresson) Apidae)). J. Insect Behav. 28, 345–357. https://doi.org/10.1007/s10905-015-9507-3.

Nevitt, G., Pentcheff, N.D., Lohmann, K.J., Zimmer, R.K., 2000. Den selection by the spiny lobster *Panulirus argus*: testing attraction conspecific odors in the field. Mar. Ecol. Prog. Ser. 203, 225–231.

Newland, P.L., Neil, D.M., 1987. Statocyst control of body, uropod righting reactions in different planes of tilt in the Norway lobster, *Nephrops norvegicus*. J. Exp. Biol. 131.

Okamura, S., Kawaminami, T., Matsuura, H., Fusetani, N., Goshima, S., 2017. Behavioral assay and chemical characters of female sex pheromones in the hermit crab *Pagurus filholi*. J. Ethol. 35, 169–176. https://doi.org/10.1007/s10164-017-0507-y.

Payne, J.F., Andrews, C.A., Cook, A.L., Christian, J.R., Branch, S., Canada, O., 2007. Pilot study on the effects of seismic air gun noise on lobster (*Homarus americanus*). Canadian Technical Report of Fisheries and Aquatic Sciences. vol. no. 2712.

Pijanowski, B.C., Villanueva-Rivera, L.J., Dumyahn, S.L., Farina, A., Krause, B.L., Napoletano, B.M., Gage, S.H., Pieretti, N., 2011. Soundscape ecology: the science of sound in the landscape. Bioscience 61, 203–216. https://doi.org/10.1525/bio.2011.61.3.6.

Popper, A.N., Salmon, M., Horch, K.W., 2001. Acoustic detection and communication by decapod crustaceans. J. Comp. Physiol. A. 187, 83–89. https://doi.org/10.1007/s003590100184.

Radford, C.A., Jeffs, A.G., Montgomery, J.C., 2007. Directional swimming behavior by five species of crab postlarvae in response to reef sound. Bull. Mar. Sci. 80, 369–378.

Raphael, Y., 2002. Cochlear pathology, sensory cell death and regeneration. Br. Med. Bull. 63, 25–38.

Rhode Island University, 2001. Atlantic croaker sounds (*Micropogon undulatus*). [WWW document]Dosits URL https://dosits.org/galleries/audio-gallery/fishes/atlantic-croaker/.

Roberts, L., Laidre, M.E., 2019a. Noise alters chemically-mediated search behavior in a marine hermit crab: studying cross-modal effects on behavior. 5th International Conference on the Effects of Noise on Aquatic Life. 37, p. 070001. https://doi.org/10.1121/2.0001023.

Roberts, L., Laidre, M.E., 2019b. Finding a home in the noise: cross-modal impact of anthropogenic vibration on animal search behaviour. Biol. Open 8. https://doi.org/10.1242/bio.041988.

Roberts, L., Cheesman, S., Elliott, M., Breithaupt, T., 2016. Sensitivity of *Pagurus bernhardus* (L.) to substrate-borne vibration and anthropogenic noise. J. Exp. Mar. Biol. Ecol. 474, 185–194.

Rose, R.D., Stokes, D.R., 1981. A crustacean statocyst with only three hairs: light and scanning electron microscopy. J. Morphol. 169, 21–28. https://doi.org/10.1002/jmor.1051690103.

Salmon, M., Horch, K., 1972. Sound production and acoustic detection by Ocypodid crabs. In: Winn, H., Olla, B. (Eds.), Recent Advances in the Behavior of Marine Organisms. Plenum Press, New York, New York, pp. 60–96.

Sandeman, D.C., Okajima, A., 1972. Statocyst induced eye movements in the crab *Scylla serrata*. I. The sensory input from the statocyst. J. Exp. Biol. 57, 187–204.

Shuranova, Z., Burmistrov, Y., Abramson, C.I., 2005. Crayfish, habituation to a novel environment in the *Procambrus cubensis*. J. Crustac. Anim. Behav. 135, 131–137. https://doi.org/10.1016/j.Anbehav.2017.11.010).

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., Popper, A.N., 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427. https://doi.org/10.1016/j.tree.2010.04.005.

Snitman, S.M., Mitton, F.M., Marina, P., Maria, C., Giuseppa, B., Gavio, M.A., Sal Moyano, M.P., 2022. Effect of biological and anthropogenic habitat sounds on oxidative stress biomarkers and behavior in a key crab species. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 257, 1–42. https://doi.org/10.1016/j.cbpc.2022.109344.

Solé, M., Monge, M., Andrè, M., Quero, C., 2019. A proteomic analysis of the statocyst endolymph in common cuttlefish (*Sepia officinalis*): an assessment of acoustic trauma after exposure to sound. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-45646-6.

Solé, M., Lenoir, M., Durfort, M., López-Bejar, M., Lombarte, A., Van Der Schaar, M., André, M., 2013. Does exposure to noise from human activities compromise sensory information from cephalopod statocysts? Deep Sea Res. 2 Top Stud. Oceanogr. 95. https://doi.org/10.1016/j.dsr2.2012.10.006.

Solé, M., Lenoir, M., Fontuño, J.M., Durfort, M., Van Der Schaar, M., André, M., 2016. Evidence of Cnidarians sensitivity to sound after exposure to low frequency noise underwater sources. Sci. Rep. 6. https://doi.org/10.1038/srep37979.

Solé, M., Sigray, P., Lenoir, M., Van Der Schaar, M., Lalander, E., André, M., 2017. Offshore exposure experiments on cuttlefish indicate received sound pressure and particle motion levels associated with acoustic trauma. Sci. Rep. 7. https://doi.org/10.1038/srep45899.

Solé, M., Fortuño, J.-M., van der Schaar, M., André, M., 2021a. An Acoustic Treatment to Mitigate the Effects of the Apple Snail on Agriculture and Natural Ecosystems. J. Mar. Sci. Eng. 9, 969. https://doi.org/10.3390/jmse9090969.

Solé, M., Lenoir, M., Durfort, M., Fortuño, J.-M., van der Schaar, M., de Vreese, S., André, M., 2021b. Seagrass *Posidonia* is impaired by human-generated noise. Commun. Biol. 4, 743. https://doi.org/10.1038/s42003-021-02165-3.

Solé, M., de Vreese, S., Fortuno, J.-M., van der Schaar, M., Sánchez, A.M., André, M., 2022. Commercial cuttlefish exposed to noise from offshore windmill construction show short-range acoustic trauma. Environ. Pollut. 119853. https://doi.org/10.1016/j.envpol.2022.119853.

Spoendlin, H.H., 1971. Primary structural changes in the organ of Corti after acoustic overstimulation. Acta Otolaryngol. 71, 166–176.

Steullet, P., Krützfeldt, D.R., Hamidani, G., Flavus, T., Ngo, V., Derby, C.D., 2002. Dual antennular chemosensory pathways in lobsters. J. Exp. Biol. 205, 851–867.

Streftaris, N., Zenetos, A., 2006. Alien marine species in the Mediterranean — the 100 'WorstInvasives' and their impact. Mediterr. Mar. Sci. 7, 87–118.

Their, D.W., 1968. Lobster righting responses and their neural control. Proc. R. Soc. B 170, 435–456. https://doi.org/10.1098/rspb.1968.0049.

Tierney, A.J., Lee, J., 2008. Spatial learning in a T-maze by the crayfish *Orconectes* rusticus. J. Comp. Psychol. 125, 31–39. https://doi.org/10.1037/a0020866.

Tran, M.V., 2013. Divergent reactions to olfactory foraging cues between two ecologically similar, sympatric hermit crab species. J. Crustac. Biol. 33, 512–518. https://doi.org/10.1163/1937240x-00002154.

Valdes, L., Laidre, M.E., 2018. Resolving spatio-temporal uncertainty in rare resource acquisition: smell the shell. Evol. Ecol. 32, 247–263. https://doi.org/10.1007/s10682-018-9937-4.

Valdes, L., Laidre, M.E., 2019. Scent of death: evolution from sea to land of an extreme collective attraction to conspecific death. Ecol. Evol. 9, 2171–2179. https://doi.org/10.1002/ece3.4912.

Varga, Z.K., Zsigmond, Á., Pejtsik, D., Varga, M., Demeter, K., Mikics, É., Haller, J., Aliczki, M., 2018. The swimming plus-maze test: a novel high-throughput model for assessment of anxiety-related behaviour in larval and juvenile zebrafish (*Danio rerio*). Sci. Rep. 8, 1–11. https://doi.org/10.1038/s41598-018-34989-1.

Vazzana, M., Ceraulo, M., Mauro, M., Papale, E., Dioguardi, M., Mazzola, S., Arizza, V., Chiaramonte, M., Buscaino, G., 2020. Effects of acoustic stimulation on biochemical parameters in the digestive gland of Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819). J. Acoust. Soc. Am. 147, 2414–2422. https://doi.org/10.1121/10.0001034.

Wale, M.A., Simpson, S.D., Radford, A.N., 2013a. Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. Biol. Lett. 9, 20121194. https://doi.org/10.1098/rsbl.2012.1194.

Wale, M.A., Simpson, S.D., Radford, A.N., 2013b. Noise negatively affects foraging and anti-predator behaviour in shore crabs. Anim. Behav. 86, 111–118. https://doi.org/10.1016/j.anbehav.2013.05.001.

Walsh, E.P., Arnott, G., Kunc, H.P., 2017. Noise affects resource assessment in an invertebrate. Biol. Lett. 13. https://doi.org/10.1098/rsbl.2017.0098.

Weissburg, M., Atkins, L., Berkenkamp, K., Mankin, D., 2012. Dine or dash? Turbulence inhibits blue crab navigation in attractive-aversive odor plumes by altering signal structure encoded by the olfactory pathway. J. Exp. Biol. 215, 4175–4182. https://doi.org/10.1242/jeb.077255.

Weissburg, M.J., Zimmer-Faust, R.K., 1994. Odor plumes and how blue crabs use them in finding prey. J. Exp. Biol. 197, 349–375. https://doi.org/10.1242/jeb.197.1.349.

Zeil, J., Hemmi, J.M., 2010. Crabs and their visual world. In: Breed, M.D., Moore, J. (Eds.), Encyclopedia of Animal Behavior. Academic Press, London, pp. 411–420.

Zhang, S., Mizutani, A., Srinivasan, M.V., 2000. Maze navigation by honeybees: learning path regularity. Learn. Mem. 7, 363–374. https://doi.org/10.1101/lm.32900.

Zhou, W., Huang, X., Xu, X., 2018. Changes of movement behavior and HSP70 gene expression in the hemocytes of the mud crab (*Scylla paramamosain*) in response to acoustic stimulation. Mar. Freshw. Behav. Physiol. 51, 3–14.