Science of the Total Environment 900 (2023) 165756

Contents lists available at ScienceDirect

Science of the Total Environment

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

Artificial sound impact could put at risk hermit crabs and their symbiont anemones

Marta Solé [a,*], Steffen De Vreese [a,c], José-Manuel Fortuño [b], Mike van der Schaar [a]

[a] Laboratory of Applied Bioacoustics, Technical University of Catalonia-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

ARTICLE INFO

Editor: Rafael Mateo Soria

Keywords:
Anthropogenic sound
Calliactis parasitica
Dardanus calidus
Statocyst
Righting reflex
Scanning Electron Microscopy

ABSTRACT

The sea anemone *Calliactis parasitica,* which is found in the East Atlantic (Portugal to Senegal) and the Mediterranean Sea, forms a symbiotic relationship with the red hermit crab, *Dardanus calidus,* in which the anemone provides protection from predators such as the octopus while it gains mobility, and possibly food scraps, from the hermit crab. Acoustic pollution is recognised by the scientific community as a growing threat to ocean inhabitants. Recent findings on marine invertebrates showed that exposure to artificial sound had direct behavioural, physiological and ultrastructural consequences. In this study we assess the impact of artificial sound (received level 157 ± 5 dB re 1 μPa^2 with peak levels up to 175 dB re 1 μPa^2) on the red hermit crab and its symbiotic sea anemone. Scanning electron microscopy analyses revealed lesions in the statocyst of the red hermit crab and in the tentacle sensory epithelia of its anemone when exposed to low-intensity, low-frequency sounds. These ultrastructural changes under situations of acoustic stress in symbiotic partners belonging to different phyla is a new issue that may limit their survival capacity, and a new challenge in assessing the effects of acoustic disturbance in the oceanic ecosystem. Despite the lesions found in the red hermit crab, its righting reflex time was not as strongly affected showing only an increase in the range of righting times. Given that low-frequency sound levels in the ocean are increasing and that reliable bioacoustic data on invertebrates is very scarce, in light of the results of the present study, we argue that anthropogenic sound effects on invertebrates species may have direct consequences in the entire ecosystem.

1. Introduction

Marine invertebrates are exceptionally diverse and form part of the most abundant group in the ocean ecosystems, constituting >92 % of life (marine plants and animal species) in our oceans and inhabiting all parts of the water column. Marine invertebrates contribute to ecosystem functioning and provide services and socioeconomic development to humans. Despite their fundamental role in the marine ecosystem, the research on invertebrates lacks baseline data on the sensitivity of many species to anthropogenic pressures, as they face both direct (marine aquarium trade, deep-sea mining, physical habitat destruction) and indirect consequences (ocean acidification, biological invasions, human-mediated pollution) of human disturbance (Chen, 2021). Artificial sound is one of the most recent anthropogenic pollutions introduced into the sea and is recognised by the scientific community as a growing threat to its inhabitants, compromising the conservation of marine biodiversity (Degraer et al., 2020; Williams et al., 2015).

Invertebrates have been shown to be sensitive to artificial sound exposure (Solé et al., 2023a). Marine acoustic pollution, such as caused by marine traffic, seismic exploration, pile driving, and sonar, is changing the natural underwater soundscapes. It can mask invertebrate acoustic communication (e.g., changes in their sound production capacities Aimon et al., 2021); change behaviour regarding navigation and orientation abilities (e.g., righting reflex) (Day et al., 2019; Radford et al., 2007; Solé et al., 2023b) and foraging and antipredator behaviour (Wale et al., 2013a). Anthropogenic sound can also affect the animals' physiological condition by increasing stress levels (Vazzana et al., 2020; Wale et al., 2013b) and affect different stages of reproduction (sexual maturation, spawning success, hatching success, and larval development) (Solé et al., 2022; Nedelec et al., 2014; André et al., 2016). In addition, artificial sound exposure can cause physical alterations in the sensory structures involved in sound reception, such as statocysts (Day

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

https://doi.org/10.1016/j.scitotenv.2023.165756
Received 13 March 2023; Received in revised form 21 July 2023; Accepted 22 July 2023
Available online 25 July 2023

et al., 2016, 2019; Solé et al., 2016, 2017, 2023b). Artificial sound playbacks are used to test the effects of anthropogenic sounds on marine animals under routinely controlled experimental conditions (Solé et al., 2021a, 2023a).

In crustaceans, the statocyst (Dinh and Radford, 2021) and external cuticular hairs (Jezequel et al., 2021) are the organs responsible for acoustic and vibratory perception. Located within the basal segment of the second antenna (in decapods) and the uropod or telson of the tail (in mysids and isopods), the statocyst has a basic structure, similar in all crustacean species, consisting of complexly folded cuticular invaginations that form a canal system, which is lined with mechanosensory hairs cells overlayed by an only agglomerate (statolith) or multiple sand grains (statoconia). In some species, the sensory hairs are arranged in two to four rows (Budelmann, 1992; Cate and Roye, 1990; Rose and Stokes, 1981). When there is an external stimulus, the statolith triggers tiny deflations in the hair cells, resulting in cell body depolarization and subsequent transmission of information to the sensory nervous system where a response is elaborated. In crustaceans, statocyst input is crucial for the coordination of body positioning and movement (Newland and Neil, 1987), and the righting reflex (the capacity to recover the habitual position after falling upside down) (Their, 1968).

Among cnidarians, only the medusa stage presents marginal sensing organs (rhopalia) bearing statocysts (Werner, 1993). Scyphozoan medusas (true jellyfish) have sac-like statocysts filled with numerous small crystals, located at the distal ends of their rhopalia, and form complex sensory organs associated with pulsing, swimming, orientation, and graviception (Passano, 1982). The polyp stage of cnidarians possesses sensory organs that can detect vibrations in water associated with prey movement. Hydrozoan and Cubozoan polyps have ciliated cells in their tentacles that act as mechanoreceptors (Bouillon et al., 2006; Golz and Thurm, 1993, 1994; Tardent, 1972) and allow the animal to perceive changes in its surrounding environment.

Symbiosis refers to the biological long-term interaction between two organisms living in close association. Symbiotic relationships govern important aspects of the behaviour and relational functions of living beings that are essential for their survival (Rohde, 2002; Wohl et al., 2004); determine ecosystem stability through the regulation of trophic relations, competition, and food webs; and play a crucial role in evolution (Aanen and Eggleton, 2017; Lafferty et al., 2008; Lehtonen et al., 2013). The association between sea anemones and hermit crabs is one of the most common symbioses in temperate marine communities and represents an example of mutualism, as it has reciprocal advantages for both symbionts. Gastropod shells serve as both shelter for hermit crabs and substratum for the settlement of sea anemones (Antoniadou et al., 2012). Hermit crabs periodically need to upgrade their housing to larger shells as they grow. See a hermit crab changing its shell in (Video 1). The sea anemone, *Calliactis parasitica,* forms a symbiotic relationship with the red hermit crab, *Dardanus calidus.* This association provides protection to the hermit crab, specifically from predation by octopuses (Ross and Von Boletzky, 1979). In return, the anemone gains dispersal capability via hermit crab mobility and possibly food scraps from the hermit crab. These interactions provide benefits to the partners as well as to other organisms that form micro-communities by colonizing their association, contributing to the biodiversity of marine benthic ecosystems (Antoniadou et al., 2012). The possibility that sound exposure can alter both individual species in symbiotic relationships has not been previously studied and remains unknown. The sea anemone - hermit crab interaction constitutes a model case by which to examine the effects of low-frequency sound exposure and impact assessment on the sensory structures of the individual symbiont species.

Although statocysts have been described in different crustacean species, the morphology of this structure in the hermit crab has not been reported previously. In the current study, we describe for the first time its inner ultrastructural morphology.

and analyse the ultrastructural effects of sound exposure on the crab statocyst and anemone sensory ephitelia through scanning electron microscopy (SEM). In addition, we evaluate the hermit crab righting reflex, after sound exposure.

The exposure to low-frequency sound may impact the hermit crab and its anemone by affecting their sensory systems and their orientation abilities. The effects on the two symbiotic species could have wider-ranging impacts on the associated micro-communities, compromising the conservation of marine biodiversity in the entire ecosystem.

2. Material and methods

2.1. Animals

Adult *D. calidus* (8–10 cm length) with *C. parasitica* (2–5 cm oral disc diameter) attached to their protective snail shells were collected on the Catalan Coast (NW Mediterranean Sea) by local fishermen during May–July 2019 and kept in LAB (Laboratory of Applied Bioacoustics, 41°12′57.1"N 1°43′59.0″E) maintenance system, a closed system of recirculating sweater (at 18–20 °C, salinity 35 ‰, and natural oxygen pressure) consisting of 2 mechanically filtered fibreglass 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. Specimens were supplied with mussels (*Mytilus edulis*) and surimi ad libitum. One hundred thirty-two crabs and their attached anemones were used in this study. All the animals (exposed and controls) were kept in the same condition until the start of experimental phase Control crabs were transferred to the experimental tank and kept under the same conditions for the same duration as those that were exposed but without any sound playback.

2.2. Sound exposure protocol

Controlled exposure experiments (CEEs) were conducted on sixty-eight red hermit crabs and their attached anemone in an independent, acoustically-isolated experimental tank (2 m diameter, 1 m height, 2000 L capacity). We chose to expose them to a sweep that would cover a large range of frequencies, the components of which are commonly found to be associated with offshore operations. Following previous publications (Solé et al., 2013a, 2013b), these experiments were not designed to find threshold levels of particular frequencies that would trigger lesions, but to investigate the potential sensitivity of *D. calidus* and *C. parasitica* to artificial sound. The need to generate high-amplitude, low-frequency sounds required the use of an in-air loudspeaker, which was demonstrated in numerous previous studies to be well-suited for underwater CEEs (Solé et al., 2013b; Solé et al., 2016). The sound exposure protocol consisted of sinusoidal wave sweeps of 50–400 Hz with a 100 % duty cycle and a 1-s period for 2 h. The level received was measured by a calibrated B&K 8106 hydrophone (sound pressure levels of 157 ± 5 dB re 1 μPa^2 with peak levels up to 175 dB re 1 μPa^2) (Fig. 1). While these species may be more sensitive to the particle motion component of the received sound, such levels at frequencies in this band may be encountered in the field in the proximity of shipping traffic (for relatively duration) or construction at some distance (longer duration) (Nedelec et al., 2021; Solé et al., 2023a). The experimental design was not aimed at mapping this distribution based on the understanding that, at any location in the tank, the animals would be exposed to a contribution of both components of the sound, the characteristics of which were not within the scope of this study. Sixty-four control red hermit crabs with their attached anemone were maintained under the same conditions as the exposed crabs in the maintenance tank before and after the CEE, and were placed in the independent experimental tank under the same conditions and duration as those that were exposed but without any sound playback.

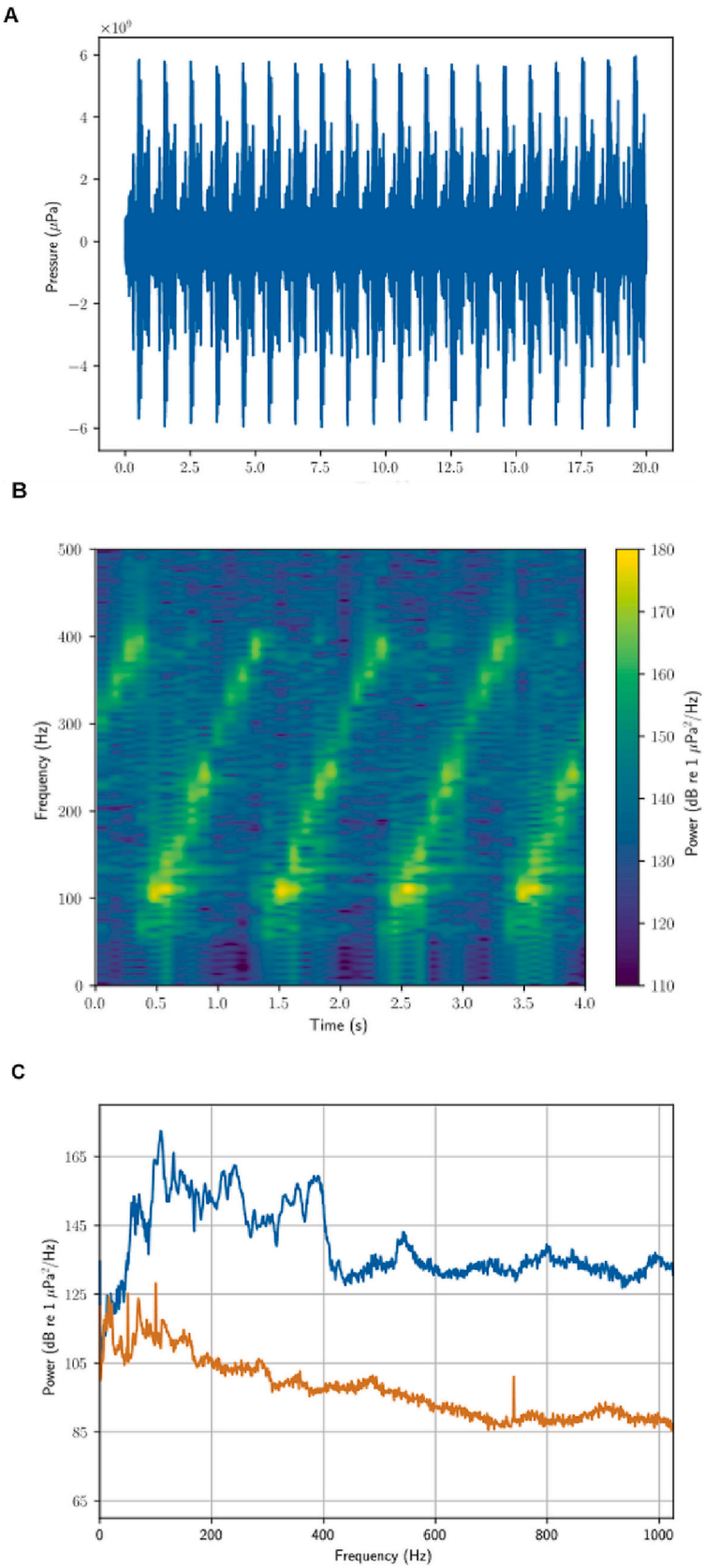

Fig. 1. A: the wave form as received inside the tank. B: a zoomed spectrogram of the waveform shown on top. C: power spectral density of the 50 to 400 Hz sweep as received by the hydrophone on top with below the background noise level recorded just before exposure.

2.3. Morphological description of the sensory epithelia. Ultrastructural analysis and quantification of lesions

For the morphological description of the hermit crab statocysts and the ultrastructural analysis of the lesions, we used 30 red hermit crabs (10 control and 20 sound-exposed crabs) and 24 anemones (8 control and 16 sound-exposed anemones). Control and exposed animals were euthanized with an overdose of anaesthesia (magnesium chloride 25 g/L) 48 h and 120 h after sound exposure (see Table 1). The hermit crab statocysts (2 statocysts/individual, $n = 60$) and arms of the anemone (2 arms/individual, $n = 48$) were dissected, fixed, and processed according to routine procedures for analysis by Scanning Electron microscopy (SEM) (e.g. Solé et al., 2013b, 2022). 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. The inner statocyst structure in the hermit crab and the surface of the anemone tentacle were analysed and described.

To evaluate the acoustic impact, lesions of the sensory epithelia in both animals were assessed.

We quantified the lesions on the hermit crab statocyst sensory epithelia. Although we observed the presence of lesions on the other inner statocyst epithelial regions, we considered the medial group, a small semi-circular field of setae overlaid by multiple statoconia, for the quantification of the statocyst. We chose this region for its central position and the presence of the statoconia associated with its setae, which plays an essential role in coordinating body positioning, movement, and orientation, including the righting response. Setae damage was quantified by classifying the setae as intact (undamaged), damaged (damage to the hairs) or missing/extruded [hair cell partially (presented as loss of the hair, leaving only the cell base) or totally extruded from the epithelium (leaving a hole)]. For all animals, lesions were assessed for both statocysts. The lesions were measured separately as the number of damaged or missing/extruded setae in the medial group divided by the number of total setae in the medial group.

The setae density was computed by summing the number of setae for each category (type of damage) in each statocyst. This number was divided over the analysed area to get the density, and then averaged over the statocysts. We tested if this averaged setae per mm^2 was similar for all test groups (control, exposed, 48, 120 h) by performing a Kruskal-Wallis test. To account for small sample sizes and possibly non-normal distributed means, we chose to use non-parametric tests for all results. If we would find that there were no significant differences in setae densities between test groups then the extruded/missing setae density could be used in comparative tests between test groups to assess the effect. We tested differences between two exposure groups with a rank sum test.

In the anemone, we considered a region 1 cm in length from the distal portion of the tentacle (2 tentacles/individual). The length of the area was determined for each sample, and 900 μm^2 (30 × 30 μm) sampling squares were placed along the sagittal axis of the tentacle at 0, 25, 50, 75, and 100 % of its length (Fig. 2). Hair cell damage was analysed by classifying the hair cells as intact (hair cell undamaged), damaged (kinocilium or surrounding stereocilia partially or entirely missing or fused), extruded (hair cell partially extruded from the epithelium), or missing (hole in the epithelium caused by total extrusion of the hair cell). Damage was quantified as the percentage of extruded and missing hair cells. The damaged category included a wide range of different types of lesions with different severities, which makes a direct comparison between animals more difficult. The categories including extruded and missing cells were well-defined and more easily compared, and the presence of extruded cells shows the limit of severe damage associated with sound exposure.

To evaluate the amount/severity of post-exposure damage we first looked at the absolute count per region (0 %, 25 %, …, 100 %) using the control specimens and excluded regions that had outlier hair counts that would complicate the combination of these areas for further tests. The regions that had a similar number of hair cells in the control samples were combined into a single total hair cell count. We compared the damage to the hair cells from the remaining zones by summing over each category and dividing by the total number of hair cells in these zones. We used Kruskal-Wallis to assess a change in the median between all test groups due to sound exposure. We used a rank sum test to test between any two test groups.

Table 1
Experiments summary table.

Righting Reflex - Crabs sacrificed after each RR experiment	N	Comments
Control crabs – 0 h	16	To test the median response time groups were finally combined into control (0,24,48,120 h) versus exposed (0,24,48,120 h).
Exposed crabs – 0 h	17	
Control crabs – 24 h	16	
Exposed crabs – 24 h	17	
Control crabs – 48 h	16	
Exposed crabs – 48 h	17	
Control crabs – 120 h	16	
Exposed crabs – 120 h	17	
SEM analysis		
Selected from control crabs - 48 h	5	1. Morphological description of the sensory epithelia
Selected from control crabs - 120 h	5	2. Measure damage to sensory epithelia – Count extruded/missing setae in two statocysts per animal – Normalize count over analysed surface area – Average the count over the statocysts to have a single value per animal.
Selected from exposed crabs - 48 h	10	
Selected from exposed crabs - 120 h	10	
Control anemones sacrificed after 48 h	4	1. Morphological description of the sensory epithelia
Exposed anemones, sacrificed after 48 h	9	2. Measure damage to sensory epithelia – Count missing/extruded hair cells in 5 regions – Average over 4 regions, excluding the 0 % region as it appeared to be much denser.
Control anemones sacrificed after 120 h	4	
Exposed anemone, sacrificed after 120 h	9	

2.4. Righting reflex

For the righting reflex experiment, we used 132 crabs (16 control and 17 sound-exposed for each test, with tests directly after exposure, and 24, 48, and 120 h after sound exposure). At each righting reflex assessment a set of tests with a control group was performed as well. We assessed the righting reflex by measuring the time required 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. The same animals were never reused in the various tests. When an animal could not right itself within 2 min the test was stopped and the animal was given a righting time of 120 s. The use of ranking statistics for comparisons between test groups made it sufficient to assign a large value in these situations. We chose the test times after exposure based on previous work (Solé et al., 2013b) in which the increase in ultrastructural damage with time was found in other species. The same researcher, blinded to the crab treatment (control or exposed), conducted this assessment for each individual using the same equipment throughout the study.

For the righting reflex tests, we first looked if the test groups tested at different hours after exposure had a significant difference in median

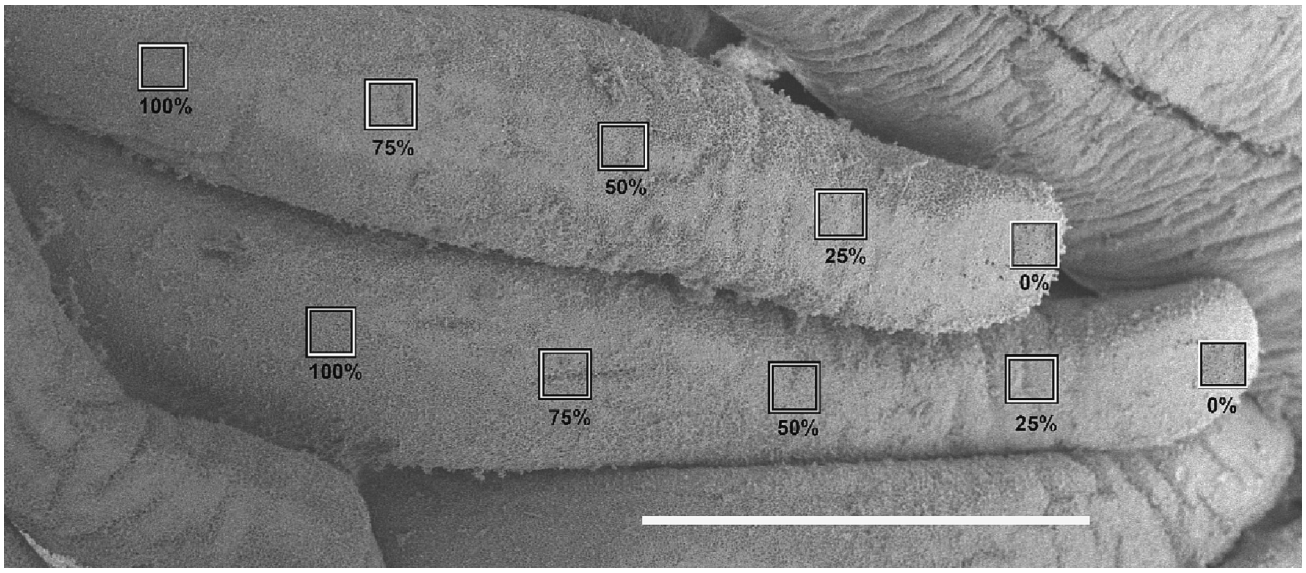

Fig. 2. Scanning electron microscopy. Hair cell count locations on the *C. parasitica* tentacles. Hair cell counts were sampled at five predetermined locations: 0, 25, 50, 75, and 100 % of the length on the axis of the tentacle. A 900 μm^2 box was placed at each sampling area and hair cells counted within each box. Scale bar: 500 μm.

response time. If this would not be the case then the test groups could be combined to obtain more samples for a test of significant shift in median response time between control and exposure. The median response times before and after exposure were tested using a Kruskal-Wallis test or Wilcoxon rank sum test depending on the number of test groups being tested. We also used a Brown-Forsythe test for equal variance, using the absolute deviation from the median.

3. Results

3.1. Morphological and ultrastructural description of the red hermit crab statocyst and anemone sensory epithelia

Here, we provide the first description of the position and morphology of the statocysts in an Anomura crab. The statocyst of *D. calidus* was a paired organ located on the dorsal side of the basal segment of the first antenna. Each statocyst comprised a cup-like invagination of the cuticle, forming a cavity with a triangular opening in the dorsal surface in contact with the external environment (Fig. 3). The cavity was covered by sensory setae organized into four groups: the medial, lateral, caudal, and ventral group (Fig. 4). All of the setae had the same external morphology but vary in size. The structure of an individual seta resembled that of a feather with its shaft rising from a bulbous base and branching outward. In the medial group, which was located on the ventral floor, these sensory setae acted as a gravity receptor when were stimulated by small particles, statoconia, which were formed by sand grains cemented together with tegmental gland secretions. The other three groups of mechanosensory setae were located in the statocyst and are stimulated for inner fluid movement (Fig. 4).

The four groups of the setae were distributed along the statocyst inner walls. A curved field made up of two rows formed a semicircle around the medial rim of the central depression. On the lateral side, this merged into the narrow end of a large semicircle of setae (lateral group, $\overline{X} = 136$; s = 0,69; $N = 30$) occupying the area lateral to the rim of the depression. Opposite this large field, on the medial side of the depression, was a smaller semi-circular field of setae (medial group, X = 30; s = 0,87; N = 30). In the centre of the cavity, a single row of setae (ventral

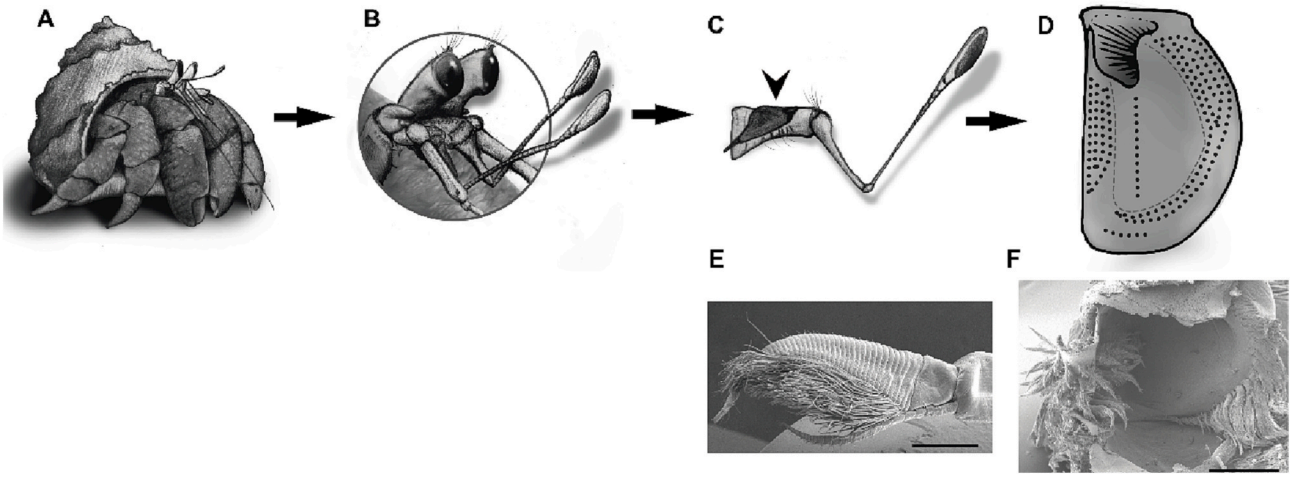

Fig. 3. Location of the hermit crab statocyst in the basal segment of the first antenna. A: Hermit crab. B: Location of the first antenna between the second pair of antennae and the eyes. C: Dorsal region of the basal segment of the first antenna. Arrowhead indicates the statocyst's triangular opening in the dorsal surface. D: Scheme of the location of the four inner setae groups. E: SEM image of the first antenna distal segment. F: SEM of the opened statocyst showing its inner cavity. Scale bars: 1 mm (E) and 500 μm (F).

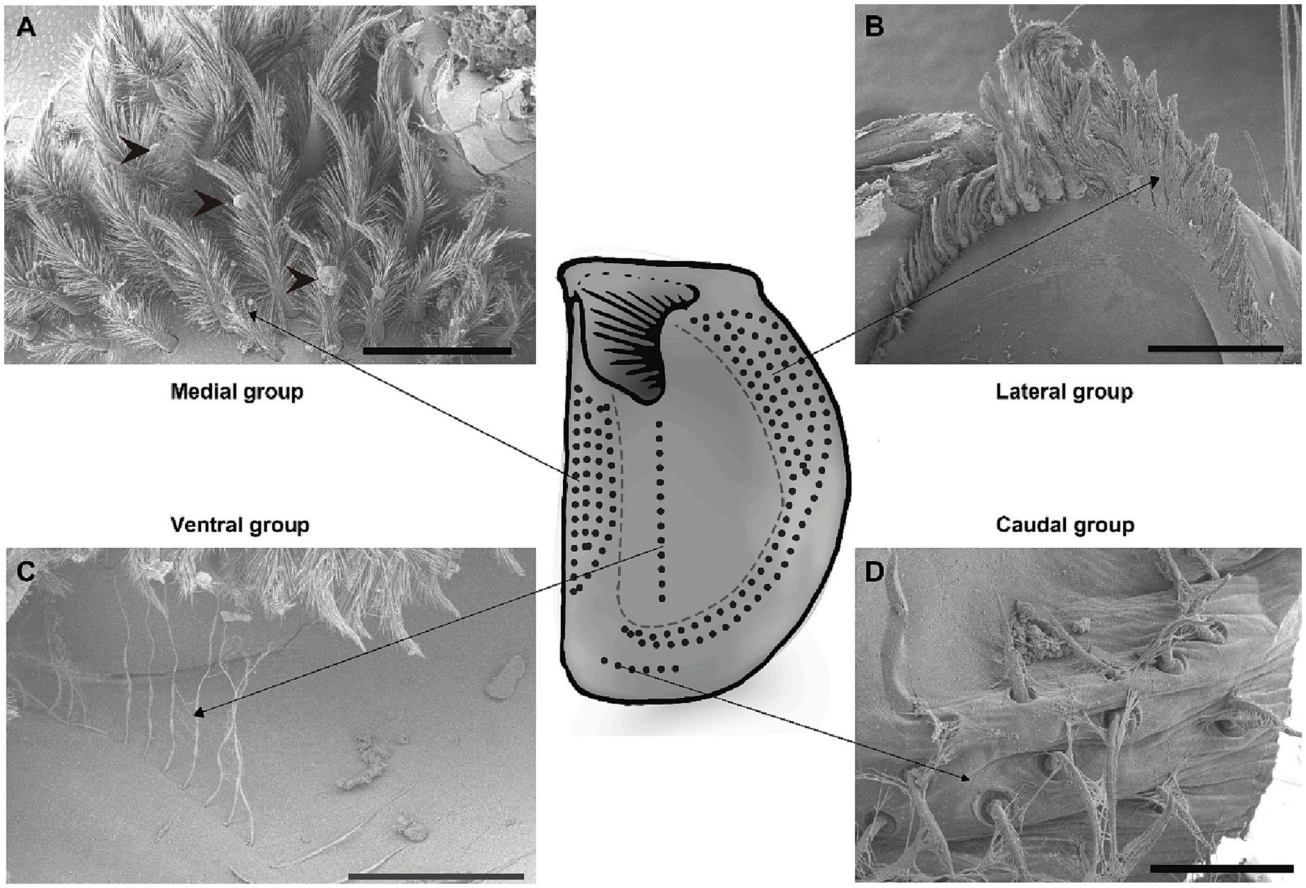

Fig. 4. Scanning electron microscopy. The location of the four setae areas in the inner cavity of the statocyst are pointed in the scheme. A: Medial group. Note the presence of statoconia (arrowheads). B: Lateral group. C: Ventral group. D: Caudal group. Scale bars: 500 μm (B), 200 μm (A, C), and 100 μm (D).

group, X = 20; s = 0,85; N = 30) ran parallel to the medial group, and a small row of setae (caudal group, X = 12; s = 0,71; N = 30) occupied the outer area parallel to the two curved rows of the lateral group (X: Setae mean number).

The surface of the tentacle of the anemone *C. parasitica* was covered with an apical epithelium bearing multicellular complexes known as cnidocyte/supporting cell complexes (CSCCs) (Fig. 5). The CSCCs consisted of a cnidocyte surrounded by two or more supporting cells (SCs). Three different functional types of CSCCs had been identified. Two kinds of cnidocyte occurred in the tentacle: the nematocyst-containing nematocyte (CN) and the spirocyst-containing spirocyte (SP). The nematocyts exhibited a single kinocilium from its apical surface, which showed close spatial association with a bundle of stereocilia stemming from the adjacent supporting cells of the same CSCC. Together, the kinocilium and stereocilia formed vibration-sensitive hair bundles.

3.2. Ultrastructural analysis of the hermit crab statocyst and anemone sensory epithelia

SEM analysis of the hermit crab statocyst sensory epithelia showed damage after exposure in the four setae groups (Fig. 6).

We observed structural alterations of the sensory epithelium in exposed anemones, compared to healthy tissue in control animals. We observed an increase in the severity of lesions with time (see 3.3). The lesions consisted of loss of the unique kinocilium, loss of the crown of stereocilia, and hair cells partially or totally ejected from the sensory epithelium, leaving a hole (Fig. 7).

3.3. Hermit crab statocyst and anemone sensory epithelia damage quantification and data analysis

As explained above, we only considered extruded or missing setae as an indication of impact, though we counted setae in all three categories (intact, damaged, missing/extruded) to estimate setae densities. Counting was performed on two statocysts per animal and then normalized for the analysed surface area (resulting in setae/mm^2), and the results per category were then averaged to obtain a single value per category per animal. First, we tested if the averaged setae per mm^2 (counting over all categories, including damaged, to get the total setae count, Fig. 8A) was similar for all test groups (control, 48 h, 120 h) by performing a Kruskal-Wallis test. With $P = 0.36$ (Kruskal-Wallis H(2) = 2.03,), we concluded that there were no significant differences in setae densities and that the extruded/missing setae density could be used in comparative tests between test groups (Fig. 8B). Next, as the count for missing/extruded setae was 0 for both the control and 48 h groups, we compared the 120 h group to the control group using a left-tailed rank sum test to test for a difference (rank sum, $P < 0.005$, $z = -4.0$). The result indicated a significant difference in the number of damaged sensory cells.

For the anemone, to evaluate the amount/severity of postexposure damage we counted the anemone hair cells in five different regions. First, we looked at the absolute count per region using the control specimens (Fig. 9A) and concluded that the 0 % region had a hair cell count that was much higher than the other zones and too different from the other regions to allow all regions to be combined together. The other zones also had varying counts, but we decided to compare damage based on summing the counts per category over the four remaining zones and dividing by the total count. Before continuing with tests between test

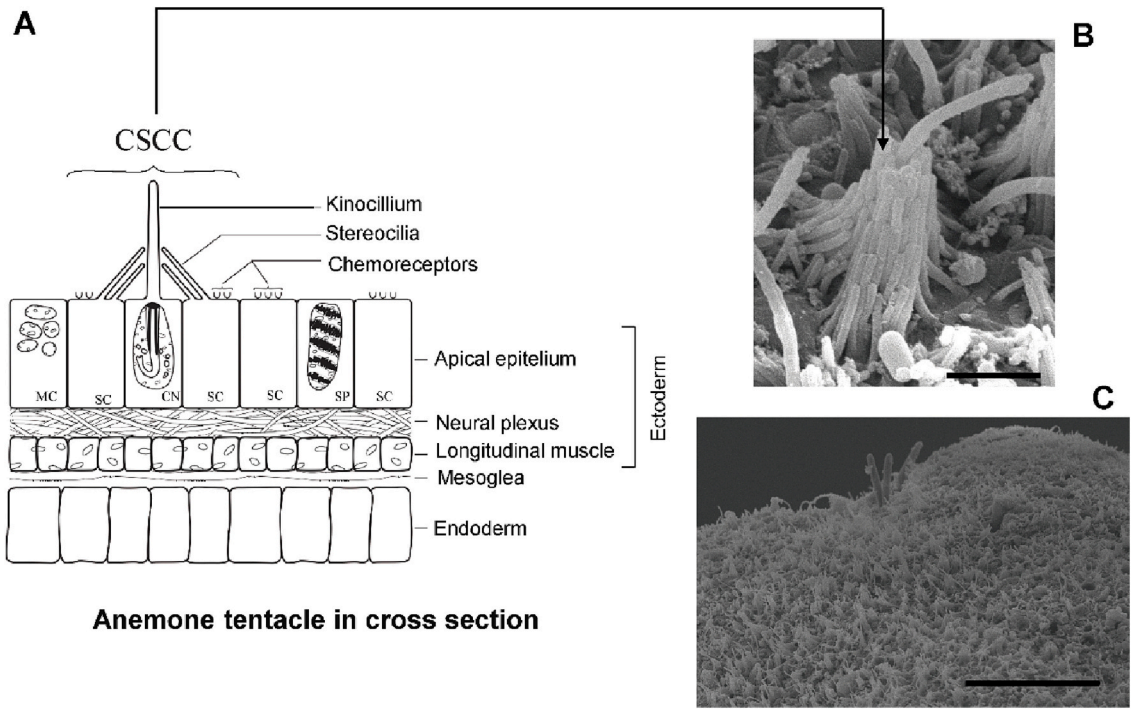

Fig. 5. Diagram (A) and ultrastructural images (B, C) of the anemone sensory epithelium. A: Diagram of an anemone tentacle in cross-section (from Ozacmak et al. (2001, reproduced with permission of: https://journals.biologists.com/jeb.). The tentacle is composed of two epithelial layers: ectoderm and endoderm separated by a mesoglea. The ectodermal layer consists of three regions: a monolayer of longitudinal muscle cells; a neural plexus; and the apical epithelial layer, which includes mucus-secreting cells (MCs), multicellular complexes known as cnidocyte/supporting cell complexes (CSCCs), and sensory cells. The CSCCs consist of a cnidocyte surrounded by two or more supporting cells (SCs). The nematocyst-containing nematocyte (CN) supports a kinocilium associated with the adjacent SCs, kinociliaTogether, the kinocilium and stereocilia form vibration-sensitive hair bundles. B: SEM image of the sensory epithelia on the anemone tentacle. C: SEM detail from the sensory epithelia. The sensory kinocilium associated with the bundle of stereocilia from the SCs is visible. Scale bars: 3 μm (B) and 30 μm (C).

groups, we looked at the total cell count between the three different test groups (Fig. 9B). We concluded that there were no differences in cell counts between the test groups of the different tests (Kruskal-Wallis, H (2) = 1.85, P = 0.40).

To assess the impact, we compared (rank sum test) the extruded/missing category between control and 48 h (Fig. 9C) and concluded that there was a significant difference (rank sum, $P < 0.005$, $z = -3.5$). To see if there was a further increase in extruded/missing cells at 120 h, we performed a left-tailed rank sum test between 48 h and 120 h and concluded that the damage was higher at 120 h (rank sum, $P < 0.005$, $z = -3.3$).

3.4. Righting reflex

We determined whether there were differences in the righting time of control individuals at each post-exposure interval (Kruskal-Wallis, H (3) = 5.4, $P = 0.14$) and concluded that individuals from all intervals could be combined into a single control group for analysis (Fig. 10A). The same test between all exposed groups (Fig. 10B; Kruskal-Wallis, H (3) = 4.03, $P = 0.26$) indicated that they could also be combined into a single test group.

A comparison between the two combined test groups (Fig. 10C; rank sum, H(1) = 0.1, $P = 0.76$) indicated that, for the median righting time, there was no overall effect. We also tested the righting time between the 0 h and 120 h groups to determine whether there was an increase over time (rank sum with left-sided tail), but no clear effect was found (rank sum, $P = 0.074$, $z = -1.4$).

There may be a visible difference in the spread of the data between the control and exposed groups, with the exposed group having a much wider range, therefore, we applied a Brown-Forsythe test. The results showed no significant difference between combined exposed and control groups (Brown-Forsythe, $P = 0.12$, F(1,130) = 2.5; Fig. 9C). However, testing the variance between the 8 individual test groups (4 control groups and 4 exposed groups sampled at 0, 24, 48, and 120 h after exposure) did suggest a difference between test groups (Brown-Forsythe, $P = 0.048$, F(7, 124) = 2.1). Testing between the 0 h and 120 h exposed groups (Brown-Forsythe, $P = 0.036$, F(1,32) = 4.8) and separately for the same control groups (Brown-Forsythe, $P = 0.090$, F(1,30) = 3.1) suggested that the spread of righting time becomes wider over time in the exposed groups, but concluding this would require a more elaborate setup to test specifically for a wider range of righting times. This was beyond the scope of the initial objectives of analysing if there might be a shift in the median righting time related to physiological damage.

4. Discussion

The exposed sound used in these experiments was artificial, but included low frequencies that are typically found in anthropogenic noise sources at exposure levels that could be experienced close to a ship or at some range from e.g. pile driving. The duration of the exposure might be longer than what would be experienced in the field, but the primary goal was to see if this type of exposure could lead to injuries. In the sea anemone, ultrastructural analysis by SEM revealed injuries in the sensory epithelia after exposure to these low-frequency sounds. Similar results were found in the statocyst sensory epithelia of the Scyphozoan medusa species in a previous study (Solé et al., 2016). Although the polyp stage of cnidarians, including anemones, does not possess statocysts, they have sensory organs in their tentacles that can detect vibrations in water (Bouillon et al., 2006). These organs present ciliated cells that resemble those of the statocyst sensory epithelia in the medusa stages (Passano, 1982), which allow the animal to perceive changes in its surrounding environment. The comparable damage found in the two stages of cnidarians (medusa (Solé et al., 2016) and polyp (present work), with incremental effects over time, is consistent with acoustic

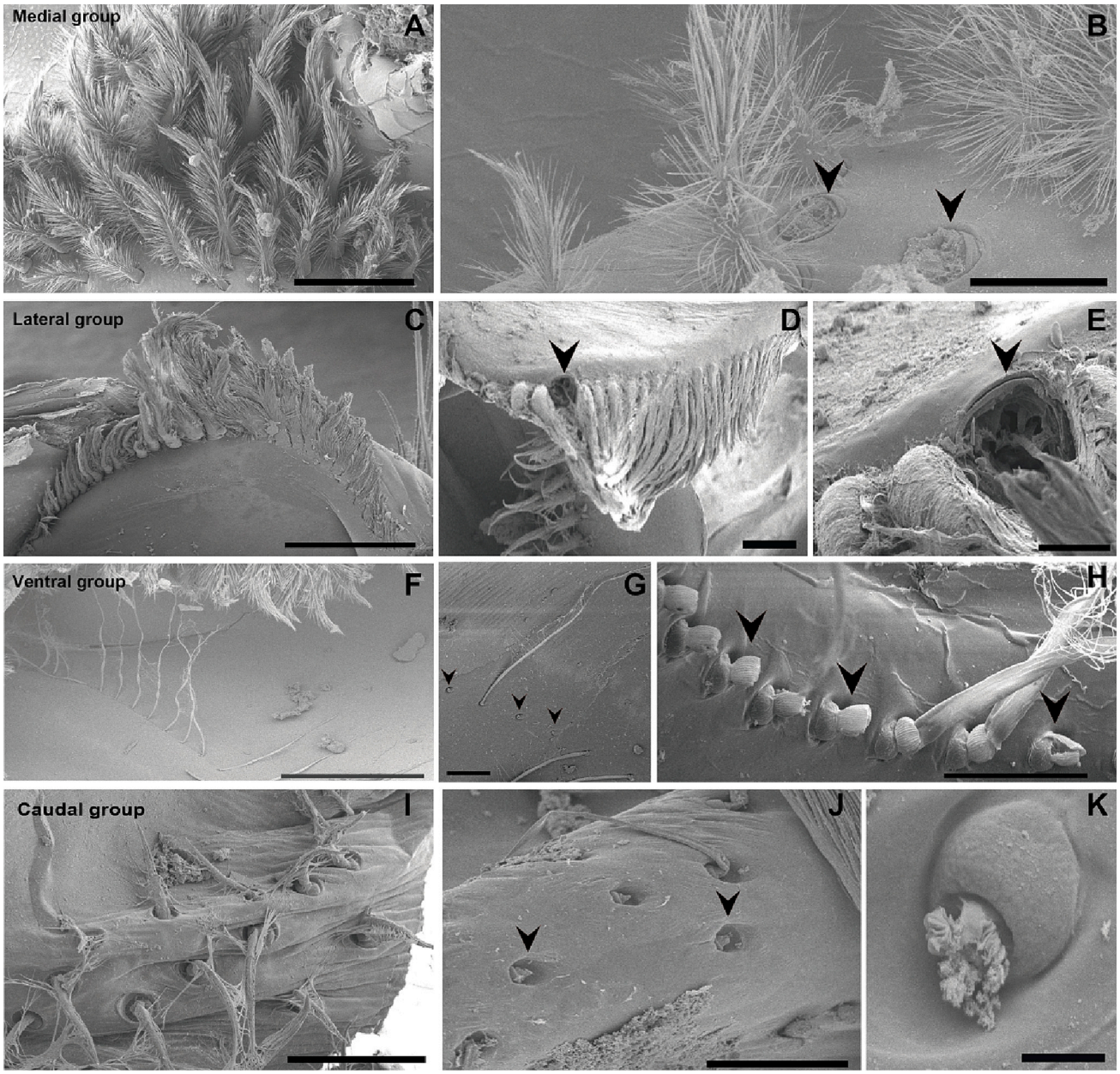

Fig. 6. SEM assessment of hermit crab statocyst setae damage after sound exposure. A, C, F, I: Control. B, D, E, G, H, J, K: Animals sacrificed 120 h after sound exposure. A: Medial group. B: Medial group setae. Arrowhead points to some setae that were completely ejected from the sensory epithelium. C: Lateral group. D: A hole (arrowhead) was left in the lateral group by setae being ejected from the sensory epithelium. E: Detail from (D) showing the hole left by the extruded setae (arrowhead). F: Ventral group. G: Some setae on the ventral group lost the hair, leaving only the cell base (arrowhead). H: Detail of the ventral group showing cell bases left by the lost hair (arrowheads). I: Caudal group. J: Arrowheads point to the cell bases that lost hairs in the caudal group. K: Detail from (J) showing the cell base of a damaged seta. Scale bars: 500 μm (C), 200 μm (A, F, D), 100 μm (I), 50 μm (B, E, G, J), 30 μm (H), and 5 μm (K).

trauma observed in other marine invertebrate species (Day et al., 2017, 2019; Solé et al., 2017, 2021a, 2021b, 2022, 2023b) and supports the idea that the detection of vibration or sound pressure waves is mediated by directionally sensitive ciliary hairs (Horridge, 1969). The damage seen in the sensory epithelia of the anemone after sound exposure could compromise its sensory capacities to detect changes in its near surroundings which could result in the animal not detecting or responding to the presence of predators or prey.

Statocysts are essential for the coordination of body positioning and movement and in reflex behaviour such as the righting reflex (Newland and Neil, 1987; Their, 1968). An impaired righting reflex was observed in the rock lobster, *Jasus edwardsii*, after field exposure to airgun signals, together with significant damage in the statocyst epithelia (Day et al., 2019), suggesting that damage to the statocyst could affect complex reflexes. In contrast, no effect on the righting time was previously observed in American lobster (*Homarus americanus*) after exposure to seismic surveys (Payne et al., 2007) and little effect in European shore crab (*Carcinus maenas*) after exposure to shipping sound (Wale et al., 2013b), although the damage on sensory epithelia was not assessed. The present study suggests a higher variation in righting time with time after exposure, but no obvious change in the median righting time which may be related to the lower severity of statocyst damage compared with previous works. Although the number of damaged cells in the crab statocyst was significantly different between control and exposed animals, and increased with the time after sound exposure, the impact in the sensory epithelia was less extensive (30 % extruded hair cells of the

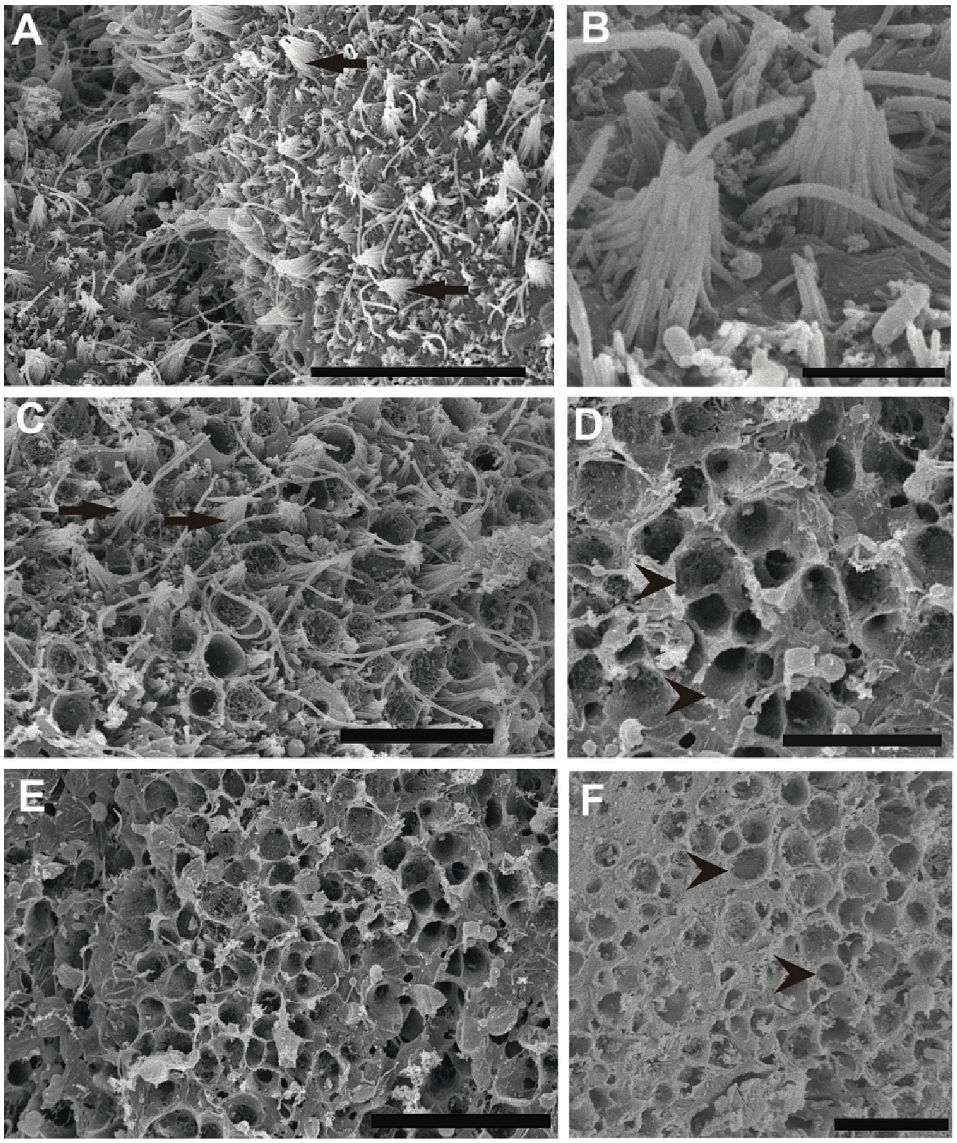

Fig. 7. Ultrastructural assessment (SEM) of anemone sensory epithelia damage. A, B: Control, C, D: Animals sacrificed 48 h after sound exposure. E, F: Animals sacrificed 120 h after sound exposure. A: The sensory epithelium on the tentacle surface of the anemone is characterized by long kinocilia surrounded by a sort of "folded crater" of stereocilia (arrows). B: Detail of (A) showing the hair cell sensory structure (long kinocilia surrounded by a bundle of stereocilia). C, D: 48 h after sound exposure: some remaining hair cells on the damaged sensory epithelia are visible (arrows) near holes left by extruded hair cells. D: Detail from (C). The loss of the kinocilia and the crown of stereocilia left a hole in the supporting epithelium (arrowheads). E, F: 120 h after sound exposure: completely ejected hair cells left holes in the surface of the epithelium (arrowheads). Scale bars: 20 μm (A, F, E), 10 μm (C, D), and 3 μm (B).

sensory epithelia) than the massive acoustic trauma shown in other species (e.g. 80 % in *Sepia officinalis, Lepeophtheirus salmonis*, *Cotylorrizha tuberculata* or *Jasus edwardsii)* (Day et al., 2017, 2019; Solé et al., 2017, 2021a, 2021b). This apparent variability in damage intensity could be explained by differences in the interspecific sensitivities and experimental designs, and confirms the need for studies by species.

Damage to the sensory epithelium of the hermit crab statocyst may compromise its active behaviour in perceiving and reacting to the presence of predators. In addition, a dysfunction of the hermit crab statocyst as a consequence of the effects of sound exposure on its sensory epithelia (kinocilium or surrounding stereocilia partially or entirely missing or fused, hair cell partially extruded from the epithelium or missing) could affect a wide range of behaviours driven by its mechanosensory input, including graviception, movement of the eyes, movement of the antennae, and coordination of the tail (Davie et al., 2015). This behavioural effects will be studied in future works.

Damage to the sensory epithelium of anemone hair cells may compromise their ability to detect predators and, consequently, their ability to transfer autonomously or through a crab to a new shell, compromising its survival. In addition, impairment of the hermit crab sensory system after sound exposure could affect their predator perception capacity, gravitational perception, eye and antennae movement, tail coordination, body position, movement, and righting response. These effects has not been assessed in the present work. These systems play a fundamental role in the survival of the crab. For the hermit crab, the symbiosis represents increased protection from predators, as cephalopods are the main predators of hermit crabs that are not resistant to the nematocyst toxins of cnidarians (Brooks, 1991; Vafeiadou et al., 2012). For the sea anemone, it represents protection from predators (Brooks and Gwaltney, 1993), increased substrate availability (Riemann-Zórneck and Anemones, 1994), increased feeding capacity (Stachowitsch, 1980), increased mobility, and the possibility of direct feeding by its host (Fautin, 1992).

Hermit crabs and their sea anemones also play a fundamental function in the conservation of the biodiversity of benthic ecosystems where hermit crabs play an essential role as predators, scavengers, detritivores, and filter-feeders (Schembri, 1982). Any external pressure, such as artificial sound pollution, can compromise these fundamental tasks for the food webs integrating the marine ecosystem. In soft-bottom benthic communities, the empty shells are likely to be buried in the substrate unless they are used by hermit crabs as protection (Creed, 2000); thus, hermit crabs influence the abundance and distribution of other invertebrates by using these gastropod shells (Gutiérrez et al., 2003; Williams and McDermott, 2004). The gastropod shells inhabited by

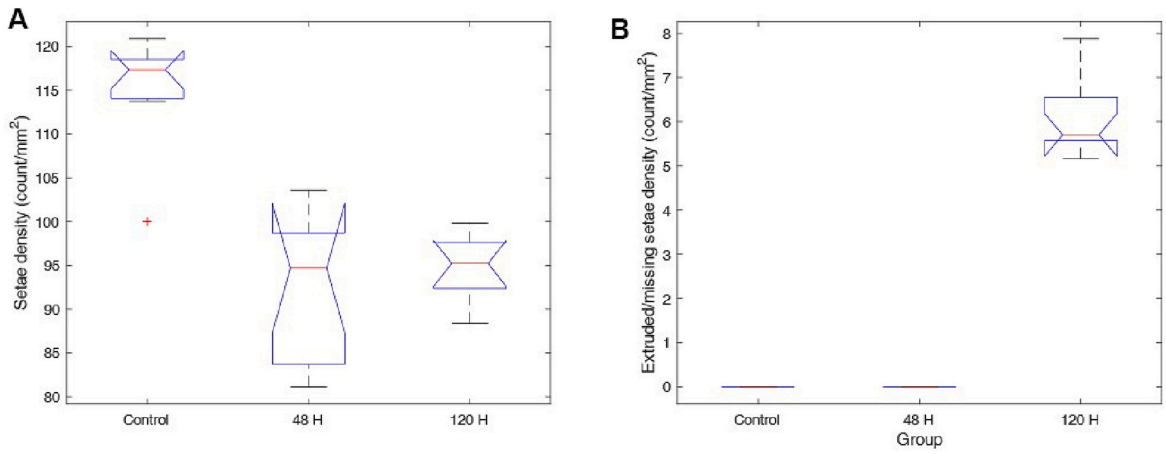

Fig 8. Box plots showing the setae density in the hermit crab medial group The boxes are delimited by the 25th and 75th percentiles, the median is the red line; whiskers extend to at most 1.5 times the interquartile range, the distance between the bounding percentiles (or to the furthest sample within that distance) with red + markers showing outliers beyond that range. The notched area shows the 95% confidence interval around the median with non-overlapping notch zones showing significantly different medians at 5% significance level. A wrapped-around box border indicates that that percentile fell within the 95% confidence interval of the median. A: Setae density in the hermit crab statocyst sensory epithelia in the three treatment groups. Kruskal-Wallis, P = 0.36. B: Density of extruded/missing setae in the three different treatment groups. Rank sum test control vs 120 h P < 0.005.

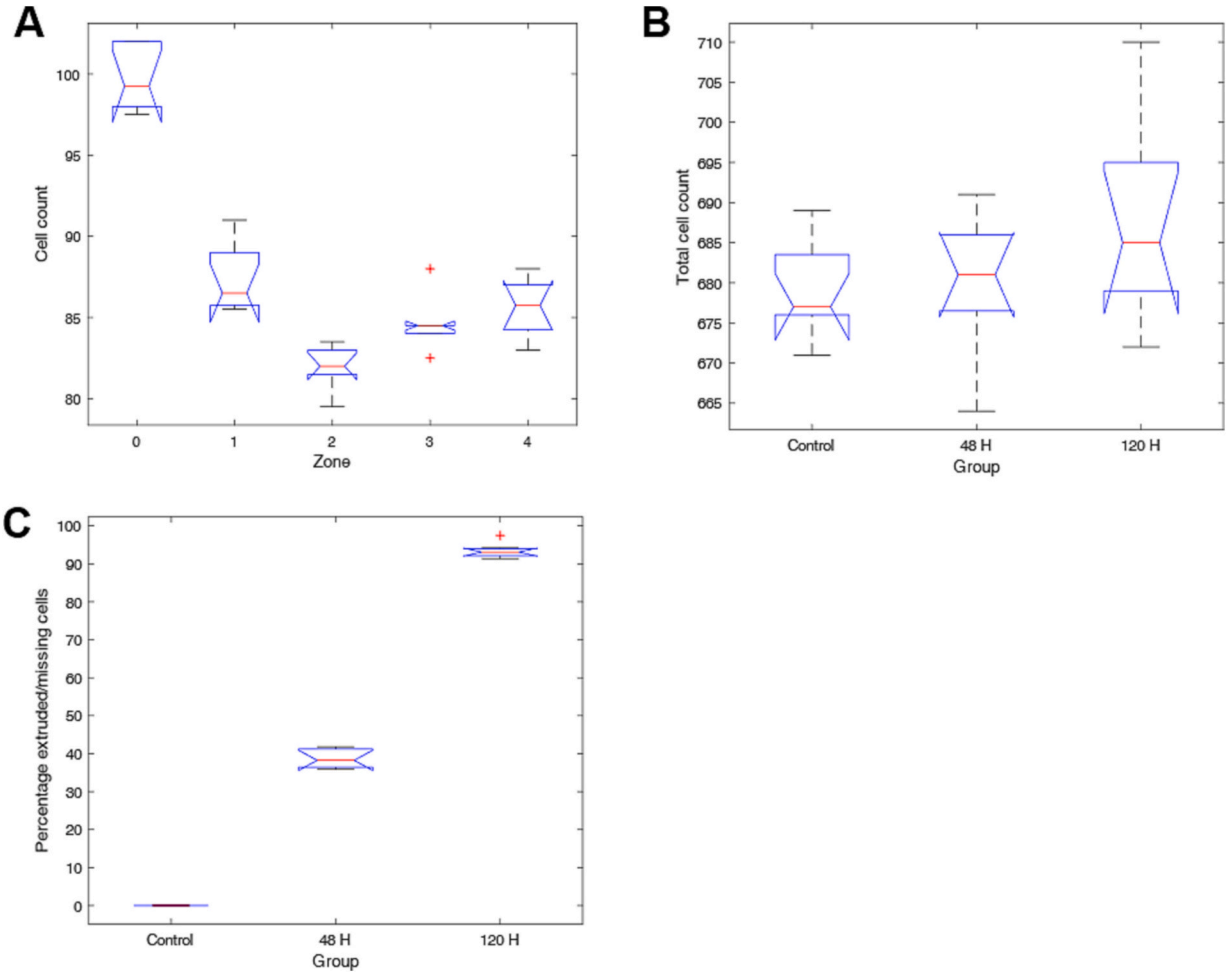

Fig. 9. Box plots showing the number of anemone tentacle sensory epithelia hair cells. In these figures a box is delimited by the 25th and 75th percentiles, the median is the red line; whiskers extend to at most 1.5 times the interquartile range, the distance between the bounding percentiles (or to the furthest sample within that distance) with red + markers showing outliers beyond that range. The notched area shows the 95 % confidence interval around the median with non-overlapping notch zones showing significantly different medians at 5 % significance level. A wrapped-around box border indicates that percentile fell within the 95 % confidence interval of the median. A: Hair cell count per region in anemone sensory epithelia (0 %, 25 %, 50 %, 75 %, 100 %) from control animals, showing that the 0 % area has a much higher density than the other zones. B: Total hair cell count per test group (Kruskal-Wallis, $P = 0.40$).C: Percentage of extruded/missing hair cells.

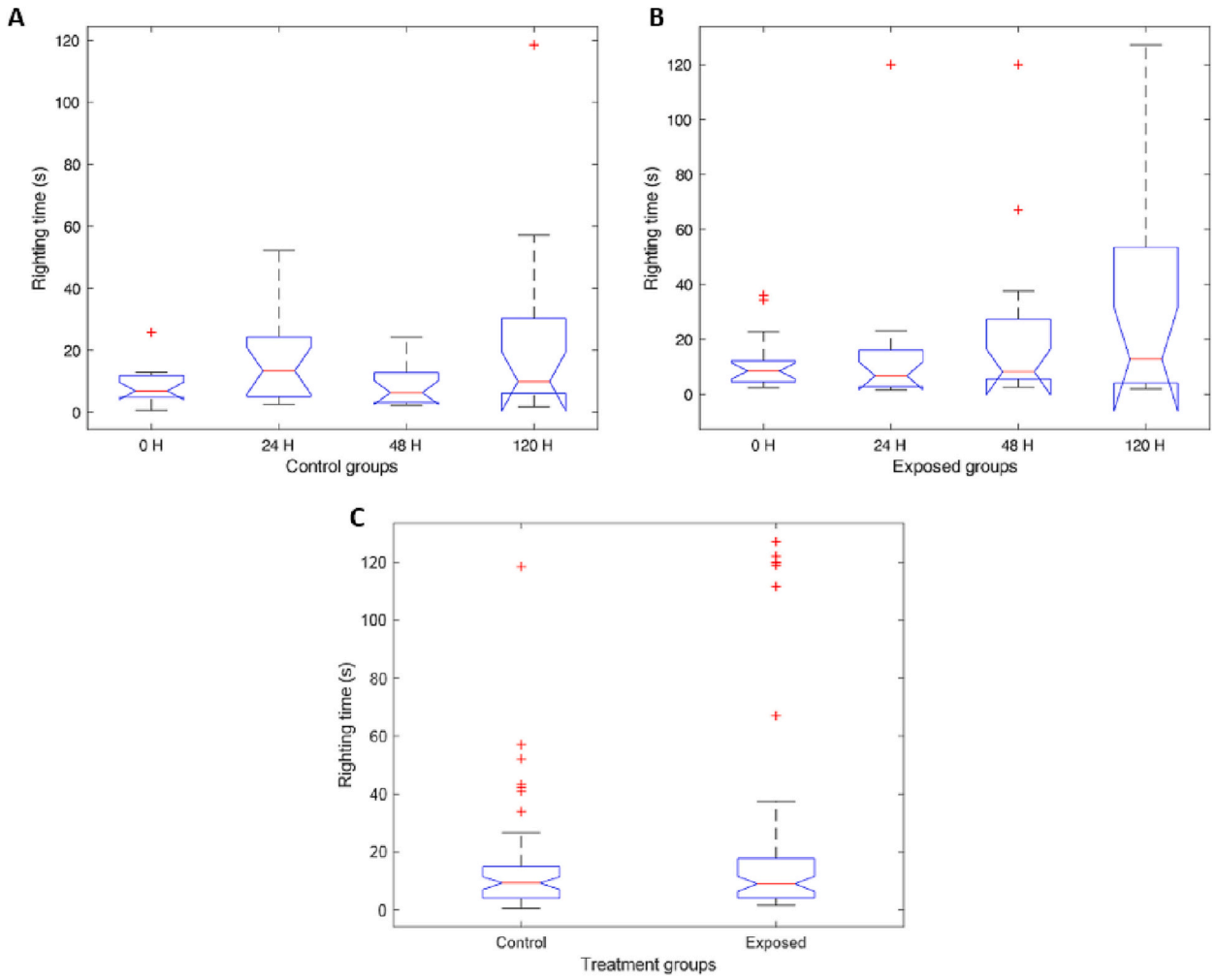

Fig. 10. Effects of sound exposure on the righting reflex of the hermit crab. In these figures a box is delimited by the 25th and 75th percentiles, the median is the red line; whiskers extend to at most 1.5 times the interquartile range, the distance between the bounding percentiles (or to the furthest sample within that distance) with red + markers showing outliers beyond that range. The notched area shows the 95 % confidence interval around the median with non-overlapping notch zones showing significantly different medians at 5 % significance level. A wrapped-around box border indicates that percentile fell within the 95 % confidence interval of the median. Distribution of righting times is shown. A: Control group at different experiment times (Kruskal-Wallis, $P = 0.14$). B: The exposed group after each exposure duration (Kruskal-Wallis, $P = 0.26$). C: Comparison between control and exposed groups (Kruskal-Wallis, $P = 0.76$).

hermit crabs are colonized by a wide variety of other epibiont and endolithic organisms, forming marine micro-communities (Caruso et al., 2003; Turra, 2003; Williams and McDermott, 2004). Hermit crabs help prolong the presence of empty gastropod shells on the seabed, allowing them to be colonized by these small organisms (Williams and McDermott, 2004). Therefore, the distribution and abundance of hermit crabs and their selection of gastropod shells influences the presence of shell microinhabitants (Jones and Gutiérrez, 2007). Coastal shallow water suffers anthropogenic threats due to the impacts of human activities e.g., noise pollution) (Jenkins, 2003). Acoustic stress in symbiotic partners could limit the survival of hermit crabs and its anemone, compromising their essential role in the oceans.

5. Conclusion

Our study, although based on a single replicate, revealed injuries in the statocyst sensory epithelium of the red hermit crab *D. calidus* and the sensory epithelia of the sea anemone *C. parasitica* after exposure to low-intensity, low-frequency sounds. Both species showed incremental damage with time after exposure, consistent with acoustic trauma observed in other taxa, including the medusa stage of cnidarians. Crab statocysts are involved in a wide range of behaviours, including graviperception, movement of the eyes, movement of the antennae, and coordination of the tail. The sensory bundles in the anemone apical epithelium allow predator detection. Although we have not determined a strong effect on the righting reflex, further research is needed to determine if a possible statocyst dysfunction in hermit crabs could lead to changes in behaviours vital to their survival as has been observed in other species. Sea anemones and hermit crabs provide benefits to other organisms that colonize their biotic formation, contributing to preserving the biodiversity of marine benthic ecosystems by supporting diverse micro-communities. How the ultrastructural changes under situations of acoustic stress could limit their survival capacity, challenging their essential role in the oceanic ecosystem is a new topic that has to be studied.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165756.

Ethical statement

Although there are no legal requirements for studies involving crabs and cnidarians 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 for animal experiments). 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. José-Manuel Fortuño: Formal analysis, Writing- reviewing and editing. Mike van der Schaar: Investigation, Data curation, Visualization, Writing- reviewing and editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

We acknowledge the Journal of Experimental Biology (https://journals.biologists.com/jeb) to allow the reproduction of the scheme from Ozacmak et al. (2001) (doi:https://doi.org/10.1242/jeb.204.11.2011) in Fig. 5A.

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

References

Aanen, D.K., Eggleton, P., 2017. Symbiogenesis: beyond the endosymbiosis theory? J. Theor. Biol. 434, 99–103.

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., Kaifu, K., Solé, M., Van Der Schaar, M., Akamatsu, T., Balastegui, A., Sánchez, A.M., Castell, J.V., 2016. Contribution to the understanding of particle motion perception in marine invertebrates. Adv. Exp. Med. Biol. https://doi.org/10.1007/978-1-4939-2981-8_6.

Antoniadou, C., Vafeiadou, A.M., Chintiroglou, C., 2012. Symbiosis of sea anemones and hermit crabs in temperate seas. In: Pedroso, A.F., Camisão, C.C. (Eds.), Symbiosis: Evolution, Biology and Ecological Effects. NOVA Science Publishers, Inc., New York, pp. 95–117.

Bouillon, J., Philippe, G., Betsch, J., Bouchet, P., Érard, C., Ohler, A., Gottini-charles, B., 2006. An Intruction to Hydrozoa. MÉmoires du Muséum national d'Histoire naturelle. Publications Scientifiques du Muséu, Paris.

Brooks, W.R., 1991. Chemical recognition by hermit crabs of their symbiotic sea anemones and a predatory octopus. Hydrobiologia 291–295.

Brooks, W.R., Gwaltney, C.L., 1993. Protection of symbiotic cnidarians by their hermit crab hosts: evidence for mutualism. Symbiosis 1–13.

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.

Caruso, T., Falciai, L., Zupo, V., 2003. Decapoda Anomura Paguridea: morpho-functional relationships and influence of epibiotic anemones on shell use along a bathymetric cline. Crustaceana 76, 149–166.

Cate, H.S., Roye, D.B., 1990. Ultrastructure and physiology of the outer rowstatolith sensilla of the blue crab *Callinectes sapidus*. Downloaded from. https://academic.oup.com/jcb/article-abstract/17/3/398/2418896. by Universitat Politecnica de Catalunya user on 10 June 2019 Crustaceans.

Chen, E.Y.S., 2021. Often overlooked: understanding and meeting the current challenges of marine invertebrate conservation. Front. Mar. Sci. 8, 1–20. https://doi.org/10.3389/fmars.2021.690704.

Creed, J.C., 2000. Epibiosis on cerith shells in a seagrass bed: correlation of shell occupant with epizoite distribution and abundance. Mar. Biol. 137, 775–782. https://doi.org/10.1007/s002270000429.

Davie, P.J.F., Guinot, D., Ng, P., 2015. Anatomy and functional morphology of brachyura. In: Koninklijke Brill, N.V. (Ed.), Crustacea. Leiden, pp. 11–163.

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., 2017. Exposure to seismic air gun signals causes physiological harm and alters behavior in the scallop *Pecten fumatus*. Proc. Natl. Acad. Sci. U. S. A. 114, E8537–E8546. https://doi.org/10.1073/pnas.1700564114.

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.

Dinh, J.P., Radford, C., 2021. Acoustic particle motion detection in the snapping shrimp (*Alpheus richardsoni*). J. Comp. Physiol. A. 207, 641–655. https://doi.org/10.1007/s00359-021-01503-4.

Fautin, D.G., 1992. A shell with a new twist. Nat. Hist. 50–57.

Golz, R., Thurm, U., 1993. Ultrastructural evidence for the occurrence of three types of mechanosensitive cells in the tentacles of the cubozoan polyp *Carybdea marsupialis*. Protoplasma 173, 13–22. https://doi.org/10.1007/BF01378858.

Golz, R., Thurm, U., 1994. The ciliated sensory cell of *Stauridiosarsia producta* (Cnidaria, Hydrozoa) - a nematocyst-free nematocyte? Zoomorphology 114, 185–194. https://doi.org/10.1007/BF00403266.

Gutiérrez, J.L., Jones, C.G., Strayer, D.L., Iribarne, O.O., 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101, 79–90.

Horridge, G.A., 1969. Statocysts of medusa and evolution of stereocilia. Tissue Cell 1, 341–353.

Jenkins, M., 2003. Prospects for biodiversity. Science (1979) 302, 1175–1177.

Jezequel, Y., Jones, I.T., Bonnel, J., Chauvaud, L., Atema, J., Mooney, T.A., 2021. Sound detection by the American lobster (*Homarus americanus*). J. Exp. Biol. 224 (6), jeb240747. https://doi.org/10.1242/jeb.240747.

Jones, C.G., Gutiérrez, J.J., 2007. On the purpose, meaning, and usage of the physical ecosystem engineering concept. In: Cuddington, K., Byers, J.E., Wilson, W.C., Hastings, A. (Eds.), Ecosystem Engineers. Plant to Protists, Theoretical Ecology Series. Academic Press, Elsevier, pp. 3–24.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., 2008. Parasites in food webs: the ultimate missing links. Ecol. Lett. 11, 533–546.

Lehtonen, J., Schmidt, D.J., Heubel, K., Kokko, H., 2013. Evolutionary and ecological implications of sexual parasitism. Trends Ecol. Evol. 28, 297–306.

Nedelec, S.L., Radford, A.N., Simpson, S.D., Nedelec, B., Lecchini, D., Mills, S.C., 2014. Anthropogenic noise playback impairs embryonic development and increases mortality in a marine invertebrate. Sci. Rep. 4, 13–16. https://doi.org/10.1038/srep05891.

Nedelec, S.L., Ainslie, M.A., Andersson, M.H., Cheong, S.-H., Halvorsen, M.B., Linné, M., et al., 2021. Best practice guide for measurement of underwater particle motion for biological applications. In: Exeter, UK: University of Exeter for the IOGP Marine Sound and Life Joint Industry Programme. https://doi.org/10.25607/OBP-1726 (89pp. & Appendices).

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.

Ozacmak, V.H., Thorington, G.U., Fletcher, W.H., Hessinger, D.A., 2001. N-acetylneuraminic acid (NANA) stimulates in situ cyclic AMP production in tentacles of sea anemone (*Aiptasia pallida*): possible role in chemosensitization of nematocyst discharge. J. Exp. Biol. 204, 2011–2020. https://doi.org/10.1242/jeb.204.11.2011.

Passano, L.M., 1982. Scyphozoa and Cubozoa. In: Shelton, G.A.B. (Ed.), Electrical Conduction and Behaviour in "Simple" Invertebrates. Oxford University Press, Oxford, pp. 149–202.

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*) (St. John's Canadian Technical Report of Fisheries and Aquatic Sciences No. 2712).

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.

Riemann-Zórneck, K., Anemones, 1994. Taxonomy and ecological aspects of the Subarctic Sea anemones *Hormathia digitata*, *Hormathia nodosa* and *Allantactis parasitica* (Coelenterata, Actiniaria). Ophelia 39, 197–224.

Rohde, K., 2002. Ecology and biogeography of marine parasites. Adv. Mar. Biol. 43, 1–86.

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.

Ross, D.M., Von Boletzky, S., 1979. The association between the pagurid *Dardanus arrosor* and the actinian *Calliactis parasitica*. Recovery of activity in "inactive" *D. arrosor* in the presence of cephalopods. Mar. Behav. Physiol. 6, 175–184.

Schembri, P.J., 1982. Feeding behaviour of fifteen species of hermit crabs (Crustacea: Decapoda: Anomura) from the Otago region, southeastern New Zealand. J. Nat. Hist. 16, 859–878. https://doi.org/10.1080/00222938200770691.

Solé, M., Lenoir, M., Durfort, M., López-Bejar, M., Lombarte, A., André, M., 2013a. Ultrastructural damage of *Loligo vulgaris* and *Illex coindetii* statocysts after low frequency sound exposure. PLoS One 8, 1–12. https://doi.org/10.1371/journal.pone.0078825.

Solé, M., Lenoir, M., Durfort, M., López-Bejar, M., Lombarte, A., Van Der Schaar, M., André, M., 2013b. 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.

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., Fortuño, J.M., De Vreese, S., van der Schaar, M., André, M., 2021b. Sea lice are sensitive to low frequency sounds. J. Mar. Sci. Eng. 9, 765.

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.

Solé, M., Kaifu, K., Mooney, T.A., Nedelec, S.L., Olivier, F., Radford, A.N., Vazzana, M., Wale, M.A., Semmens, J.M., Simpson, S.D., Buscaino, G., Hawkins, A., Aguilar de Soto, N., Akamatsu, T., Chauvaud, L., Day, R.D., Fitzgibbon, Q., McCauley, R.D., André, M., 2023a. Marine invertebrates and noise. Front. Mar. Sci. 10, 1129057. https://doi.org/10.3389/fmars.2023.1129057.

Solé, M., De Vreese, S., Sánchez, A.M., Fortuño, J.-M., van der Schaar, M., Sancho, N., et al., 2023b. Cross-sensory interference assessment after exposure to noise shows different effects in the blue crab olfactory and sound sensing capabilities. Sci. Total Environ. 873, 162260 https://doi.org/10.1016/j.scitotenv.2023.162260.

Stachowitsch, M., 1980. The epibiotic and endolithic species associated with the gastropod shells ecology, inhabited by the hermit crabs *Paguristes oculatus* and *Pagurus cuanensis*. Mar. Ecol. 1, 73–101.

Tardent .S.V., P, 1972. Ultrastructure of mechano- receptors of the polyp *Coryne pintneri* (Hydrozoa, Athecata). Exp. Cell Res. 72, 265–275.

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.

Turra, A., 2003. Shell condition and adequacy of three sympatric intertidal hermit crab populations. J. Nat. Hist. 37, 1781–1795.

Vafeiadou, A.M., Antoniadou, C., Chintiroglou, C., 2012. Symbiosis of sea anemones and hermit crabs: different resource utilization patterns in the Aegean Sea. Helgol. Mar. Res. 66, 385–392. https://doi.org/10.1007/s10152-011-0279-7.

Vazzana, M., Ceraulo, M., Mauro, M., Papale, E., Dioguardi, M., Mazzola, S., Arizza, V., Chiaramonte, M., Buscaino, G., 2020. Ef0fects 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. Noise negatively affects foraging and antipredator behaviour in shore crabs. Anim. Behav. 86, 111–118. https://doi.org/10.1016/j.anbehav.2013.05.001.

Wale, M.A., Simpson, S.D., Radford, A.N., 2013b. 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.

Werner, B.S., 1993. Cnidaria, Nesseltiere. In: Kaestner, A. (Ed.), Lehrbuch Der Speziellen Zoologie. Fischer, Stuttgart, Stuttgart, pp. 11–305.

Williams, J.D., McDermott, J.J., 2004. Hermit crab biocoenoses: a worldwide review of the diversity and natural history of hermit crab associates. J. Exp. Mar. Biol. Ecol. 305, 1–128.

Williams, R., Wright, A.J., Ashe, E., Blight, L.K., Bruintjes, R., Canessa, R., Clark, C.W., Cullis-Suzuki, S., Dakin, D.T., Erbe, C., Hammond, P.S., Merchant, N.D., O'Hara, P. D., Purser, J., Radford, A.N., Simpson, S.D., Thomas, L., Wale, M.A., 2015. Impacts of anthropogenic noise on marine life: publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24. https://doi.org/10.1016/j.ocecoaman.2015.05.021.

Wohl, D.L., Arora, S., Gladstone, J.R., 2004. Functional redundancy supports biodiversity environment, and ecosystem function in a closed and constant. Ecology 85, 1534–1540.