Preliminary Study on Bradycardia in *Scylla serrata* (Forskål, 1775) in Response to Pure Tone Stimuli

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ABSTRACT

There are growing concerns about the effects of human-generated sounds on aquatic animals. Mud crabs of the genus *Scylla*, which are economically important species, are typically farmed along coastal areas that are potentially threatened by man-made noise. We observed the heart rate of mud crabs *Scylla serrata* (Forskål, 1775) exposed to pure tones lasting 1 s (short-duration) and 30 s (long-duration). Crabs were exposed to both short- and long-duration sounds at eight frequencies (ranging from 100 Hz to 2,200 Hz). Our results revealed the following: (1) the initial sound elicited bradycardia across all experimental conditions; (2) both playing sound and the cessation of the sound elicited bradycardia in many long-duration sound tests; and (3) bradycardia disappeared following repeated exposures to sound; however (4) bradycardia was sustained in nearly all short-duration exposures at frequencies ≤1,000 Hz. We suspect that the crabs may have been more stressed than expected, as we observed sustained bradycardia frequently following short-duration exposures to lower acoustic frequencies and twice in long-duration exposure tests. The growth and health of reared crabs may be negatively impacted, which could subsequently affect the production.

Keywords: Biomarker, Crustacean, Heart beat, Pollution, Underwater noise

INTRODUCTION

Anthropogenic underwater noise is increasing globally (Duarte *et al*., 2021), raising concerns regarding its ecological impacts on aquatic animals (Di Franco *et al*., 2020; Popper *et al*., 2020). Numerous studies have investigated noise-induced effects across various biological levels, including changes in ultrastructure (Solé *et al*., 2013; Day *et al*., 2019), behavior (Solan *et al*., 2016; Jones *et al*., 2020), stress hormones and immune indicators (Slater *et al*., 2020; Staaterman *et al*., 2020), growth (Nedelec *et al*., 2016), embryonic development and larval survival (Nedelec *et al*., 2014), and heart rate (HR) (Davidsen *et al*., 2019). For example, motorboat noise has been shown to alter behavior and hormones of the anemonefish (*Amphiprion chrysopterus*) (Mills *et al*., 2020), while ship noise disrupts camouflage and anti-predator responses in juvenile shore crabs (*Carcinus maenas*) (Carter *et al*., 2020). These effects vary by sound frequency and structure, with many aquatic species being particularly sensitive to low frequency sounds (Solé *et al*., 2013; Wilson *et al*., 2022). Notably, acute stress responses such as valve closure in bivalves often diminish with repeated exposure, indicating habituation (Hubert *et al*., 2022). The use of pure tone stimuli is a valuable approach for identifying species-specific frequency sensitivities and can inform acoustic impact assessments and mitigation strategies (Smith and Rigby, 2022).

Mud crabs of the genus *Scylla* are economically important crustaceans (Jahan and Islam, 2016; Miah *et al*., 2022), with farming

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operations widely practiced from East and South Africa to Southeast Asia, and Northeast Australia (Shelley and Lovatelli, 2011). Mud crab farms are typically situated in coastal areas, which are increasingly affected by various pollutants (Vikas and Dwarakish, 2015), including noise pollution (Poole *et al.*, 2008). Heart rate (HR) is a valuable physiological biomarker in crabs (Burnovicz *et al*., 2009; McGaw and Ebrahim, 2024). It has been used to establish baseline physiological parameters in several crab species (McGaw and Nancollas, 2021). In mud crabs (*S. serrata*), HR has also been shown to strongly correlate with fluctuations in ambient temperature (Tsai *et al*., 2019). Crabs are also sensitive to sound-induced substrate vibrations (Edmonds *et al*., 2016; Hawkins *et al*., 2021; Popper *et al*., 2022). The primary structures responsible for detecting substrate vibrations are chordotonal organs (Horch, 1971; Majeed *et al*., 2013). Each of which contains 60 to over 100 sensory neurons and is associated with the joints of flexible body appendages. Chordotonal organs provide information to the central nervous system, which leads to physiological and/or behavioral responses (Wale *et al*., 2013; Tidau and Briffa, 2016; Carter *et al*., 2020; Aimon *et al*., 2021). Immediate responses to low-frequency vibrations have been observed up to 600–800 Hz (Horch, 1971). Furthermore, heartbeat regulation in crabs is neurogenic, meaning that each heartbeat is controlled by a rhythmic motor program generated by a central pattern generator known as the cardiac ganglion, which is embedded within the heart itself (Fort *et al*., 2007; Calabrese *et al*., 2016; Weineck *et al*., 2018). Neural activity in crabs is similarly controlled by a central pattern generator that exists within the central nervous system (Tang *et al*., 2012; Haley *et al*., 2018). When vibrations are detected by chordotonal organs, the neurogenic heartbeat reacts almost simultaneously.

We postulated that HR could serve as a biomarker for *S. serrata* reared under noise exposure. The current study aims to further elucidate the effects of pure tone stimuli on *S. serrata* by employing a novel approach to measure and compare their HR responses. We hypothesized that crabs would exhibit timely HR responses to acoustic stimuli; thus we measured HR in crabs exposed to pure tones of varying durations and frequencies. HR was compared before, during, and after exposures. To the best of our knowledge, this is the first study to employ such an approach in investigating the effects of anthropogenic noise on aquaculture organisms.

MATERIALS AND METHODS

The current study employed *S. serrata* in investigating HR responses to anthropogenic sounds. Specifically, an array of electrodes was implanted into *S. serrata* crabs, and electrocardiography (ECG) was recorded before, during, and after exposure to sounds at different frequencies and durations. HR was calculated as the reciprocal of the interval between the largest peaks of two adjacent ECGs. HRs obtained under different conditions were then compared.

Animal preparation and rearing

Live female *S. serrata*, identified by the dark green meshwork pattern on their swimming legs, were purchased from a local seafood market in Wushi Port (Yilan County, Taiwan). Upon arrival, each tied crab was placed in an individual tank (60×30×30 cm) filled halfway with artificial seawater (35‰ salinity) prepared using reverse osmosis water and commercially available Red Sea Salt (Red Sea Ltd.). The water initially appeared cloudy due to fine sand and fecal matter, so it was replaced hourly until it became clear. Tanks were maintained under 12 h:12 h light-dark cycle at approximately 25 °C using automated heaters, and continuously aerated using pumps to ensure adequate dissolved oxygen. Daily maintenance included removing waste and uneaten, and replacing 30% of the water. Crabs were fed once daily and reared for at least one week, during which their mobility was monitored to confirm their health for subsequent experiments.

Preparation of electrode

Lin *et al*. (2021) introduced a novel method for recording ECG signals in prawns using a device comprising an electrode, connecting wires, and a waterproof junction. We adapted and modified their design by constructing an electrode with three horizontally aligned leads, two signal leads and a central ground/reference lead. In this design, the middle lead operated as a ground and reference lead, and the other two leads acted as signal leads for differential signal acquisition. The leads were made from 27G stainless steel syringe needles each insulated with a PE20 polyethylene tubing leaving 1mm of the tip exposed. A microcentrifuge tube cap served as the socket base, with three holes drilled along its diameter to insert the needles. On the outer face of the socket, the exposed needle lengths were approximately 15, 13, and 15 mm (Figure 1a) between adjacent tips. Each needle tip was 45^{o} angle relative to the socket base (Figure 1b) enabling placement close to the ventral heart surface and minimizing the risk of cardiac puncture.

Electrode implantation

The specimen was secured using a clamping frame, and 12 mm × 1.5 mm incision was made with approximately 15 mm posterior to the cervical groove, corresponding to the heart margin on the carapace. The surrounding area was scraped with a knife to improve the adhesion of dental cement (Figure 2a). The three electrode leads were then inserted vertically to their full depth, and base was adjusted to ensure complete contact with the carapace. Tissue adhesion (3M Vetbond Tissue Adhesive) was applied to the wound to prevent bleeding. Once hemostasis was confirmed and the area was dried, the wound was covered with dental cement (Figure 2b).

Preparation of acoustic signals

Pure tones were employed as acoustic stimuli (Roberts *et al*., 2015; Charifi *et al*., 2017; Hubert *et al*., 2022). Sinusoidal signals were generated using Audacity 1.3.13 (Audacity Development Team) and were exported to a computer. Eight pure tones were created at the following frequencies: 100, 400, 700, 1,000, 1,300, 1,600, 1,900, and 2,200 Hz. For each frequency, both short-duration (1 s) and long-duration (30 s) sounds were prepared. These sounds were played through an underwater speaker (UWS-015, KHz Electronic Technology Co., Ltd), with amplitudes amplified using an integrated amplifier (PA-40W/DPL III, POKKA) connected to the computer.

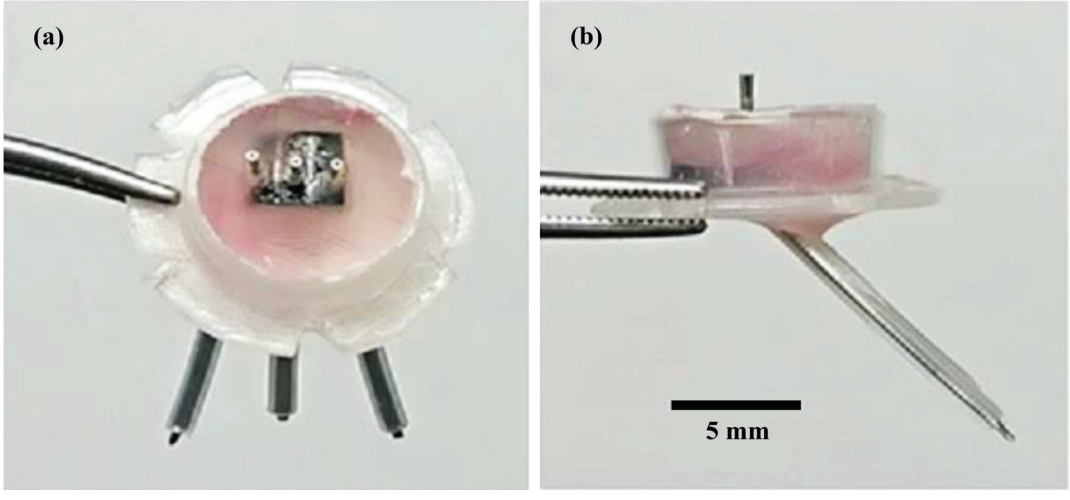

Figure 1. Electrode used for ECG recording in the specimens: (a) Top view showing the trimmed microcentrifuge tube cap with three inserted needles, (b) Side view showing 27G stainless steel needles insulated with PE20, with approximately 1 mm of the tip exposed.

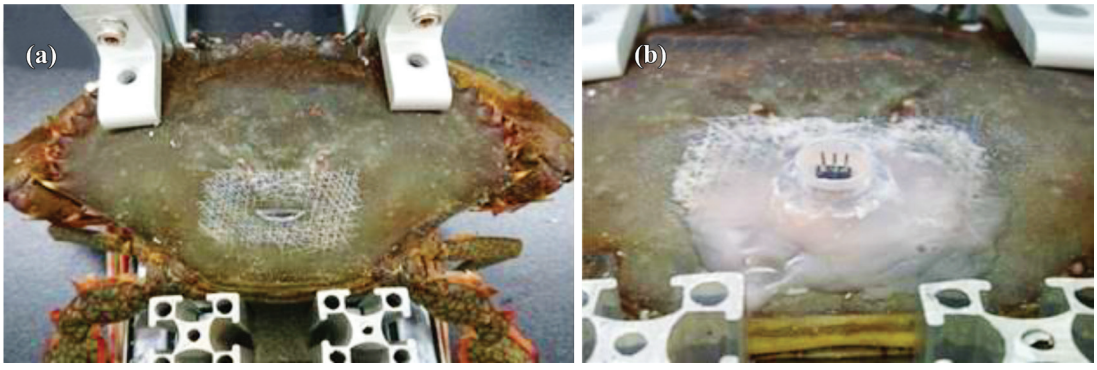

Figure 2. Implantation of the ECG electrode: (a) Incision made in the carapace using a scalpel, with the surrounding surface scraped to enhance dental cement adhesion, (b) Electrode leads inserted vertically, and the base adjusted for full contact with the carapace. Tissue adhesive was applied to stop bleeding, followed by drying and coverage with dental cement.

Experimental tank setup

In crabs, chordotonal organs located in the walking-leg joints are essential for detecting ambient vibrations (Majeed *et al*., 2013). To maximize the efficiency of sound delivery, the sound source was positioned on the ventral side of the crab. The tank was partitioned into two compartments using a perforated acrylic sheet area 1,380 cm^2 thickness of 0.3 cm featuring straight rows of holes 0.3 cm in diameter. The upper compartment housed the specimen, while the underwater speaker was placed in the lower compartment. Artificial seawater salinity 35‰ was stored in a separate tank beneath the experimental setup, where it was continuously aerated, filtered, and maintained at 25± 1 °C. Seawater was pumped into the experimental tank to a depth of 20 cm above the acrylic sheet, with overflow returned to the storage tank. All experiments were conducted within a wooden enclosure lined with sound insulating cotton to minimize external noise interference. An LED strip was installed at the top of the enclosure, and a camera (Logitech C525) was mounted to monitor the specimens.

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Experiment protocol

After electrode implantation, crabs were allowed to acclimate in the experimental tank for at least 30 min. Following acclimatization, the pump was turned off. During ECG signal recording, crabs were allowed to move freely. Once ECG readings indicated that the heart rate (HR) had stabilized, the test was initiated. Each experimental session involved exposure to three pure tone sounds, with a minimum rest period of 30 s between sounds (Figures 3a and 3b). Between sessions, specimens were given at least 30 min of rest (Roberts *et al*., 2015; Charifi *et al*., 2017). Short-duration and long-duration sounds were administered on alternate days. No more than four sessions were conducted per day, each within a four-hour period. All experiments were completed over four consecutive days.

Pure tones frequencies were presented in random order. Each of the eight frequencies was assigned a number, and a random number generator (https://lab.25sprout.com/nrprnd/) was used to determine the sequence for each specimen. On Day 1, crabs were exposed to short-duration sounds for the first four frequencies in the random order. On Day 2, long-duration sounds of the same four frequencies were tested. On Day 3, short-duration sounds were applied for the remaining four frequencies, followed by long-duration counterparts on Day 4.

A total of 12 specimens were examined in this study.

Data acquisition and analysis

ECG signals were transmitted through a rotary connector (SRM-04U2A-125, MOUSER ELECTRONICS) to a filter amplifier (MODEL 3000, A-M Systems). The signal were amplified 5,000× and band-pass filtered between 100–300Hz before being digitalized via an analog/digital converter (PowerLab 4/25, AD INSTRUMENTS). Data were recorded using the Chart5 program (AD INSTRUMENTS) at a sampling rate of 1kHz. A stimulator (MODEL 2100 ISOLATED PULSE STIMULATOR, A-M Systems) delivered a 5-volt positive signal to mark the onset of each sound stimulus was recorded simultaneously in the ECG channel.

In short-duration sound tests, HR was calculated from ECG data collected for 10 s before (BS: baseline short) and after (AS: after short) sound exposure. HR was expressed in beats per minute (bpm) and values were compared using paired t-tests for each of three sound exposures within a session.

In long-duration sound tests, HR was estimated from 10-s ECG recording before sound exposure (BL: baseline long), during exposure (DL–1, DL–2, and DL–3: three sequential intervals during sound), and after exposure (AL: after long). HR data from these time points were analyzed using repeated measures ANOVA, followed by the least significant difference (LSD) test for multiple comparisons.

RESULTS

In experiments involving short-duration sound exposure, significant differences between heart rate before stimulation (BS) and after stimulation (AS) were observed at frequencies of 100, 700, 1,000, and 1,900 Hz across all tests (Figures 4a, 4c, 4d, and 4g). At frequencies of 400 and 1,600 Hz, significant differences were observed during the first and third tests only (Figures 4b and 4f), while at frequencies of 1,300 Hz or 2,200 Hz, significant differences occurred during the first test alone (Figures 4e and 4h). Statistical comparisons revealed consistent trends in HR responses to all short-duration sounds (Table 1). Crabs exhibited HR changes in response to the first exposure across all frequencies. However, HR responses generally decreased under repeated exposures to frequencies ≥ 1,300 Hz. In contrast, sustained bradycardia was observed under frequencies ≤ 1,000 Hz, indicating that crabs maintained sensitivity within this frequency range.

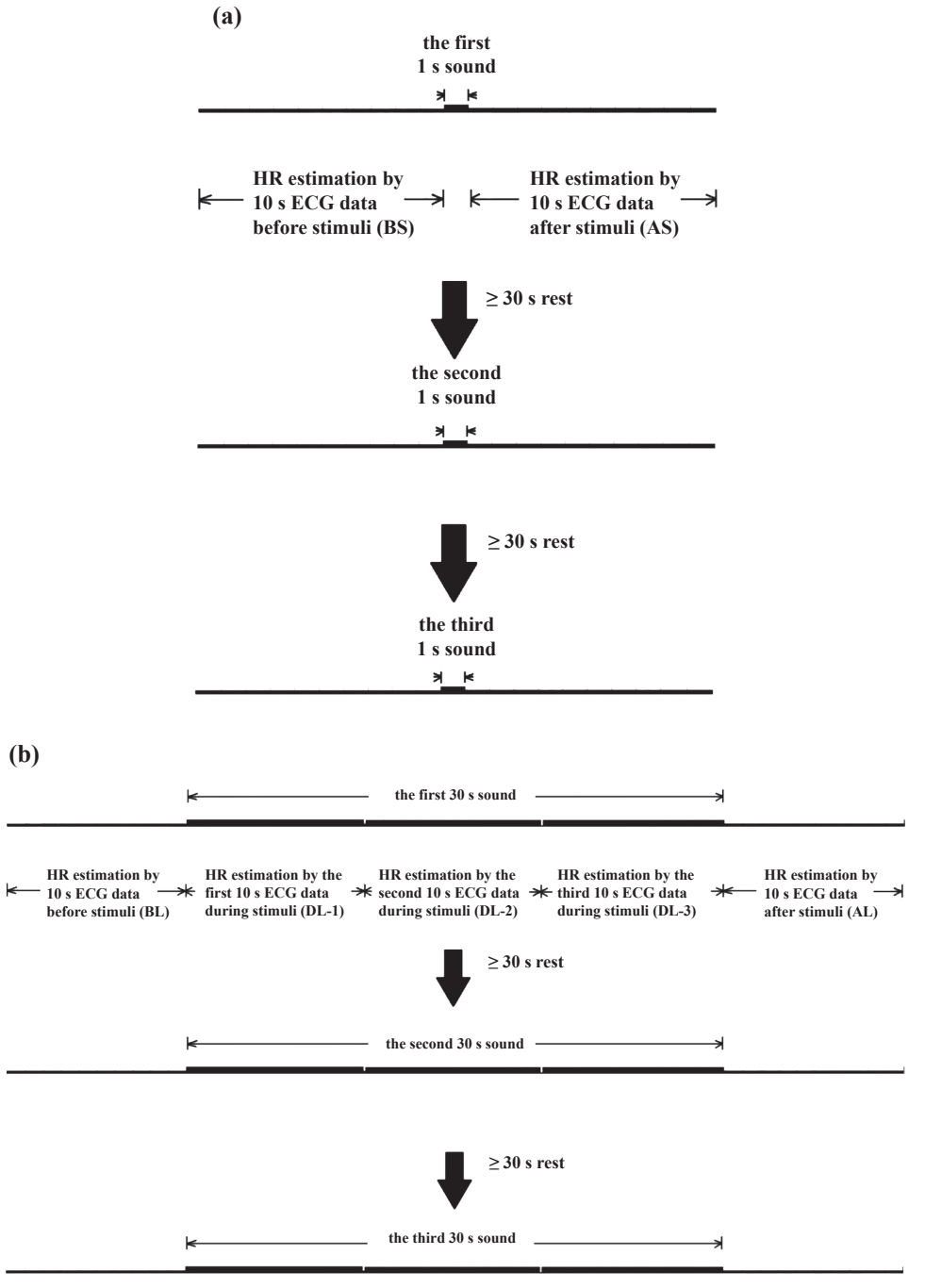

Figure 3. Schematic of the experimental protocol: (a) Short-duration sound (1 s) sessions: Each specimen was exposed to three 1-second sounds, with a rest period of at least 30 s between exposures. Heart rate (HR) was estimated by collecting 10 s of ECG data before (BS) and after (AS) the sound exposure: (b) Long-duration sound (30 s) sessions: Each specimen was exposed to three 30-second sounds, with a minimum 30 s rest between exposure. HR was estimated by collecting 10 s of ECG data before (BL) and after (AL) the sound exposure. Additionally, HR was assessed during the sound exposure by recording three 10-second ECG segments (DL–1, DL–2, and DL–3) at evenly intervals.

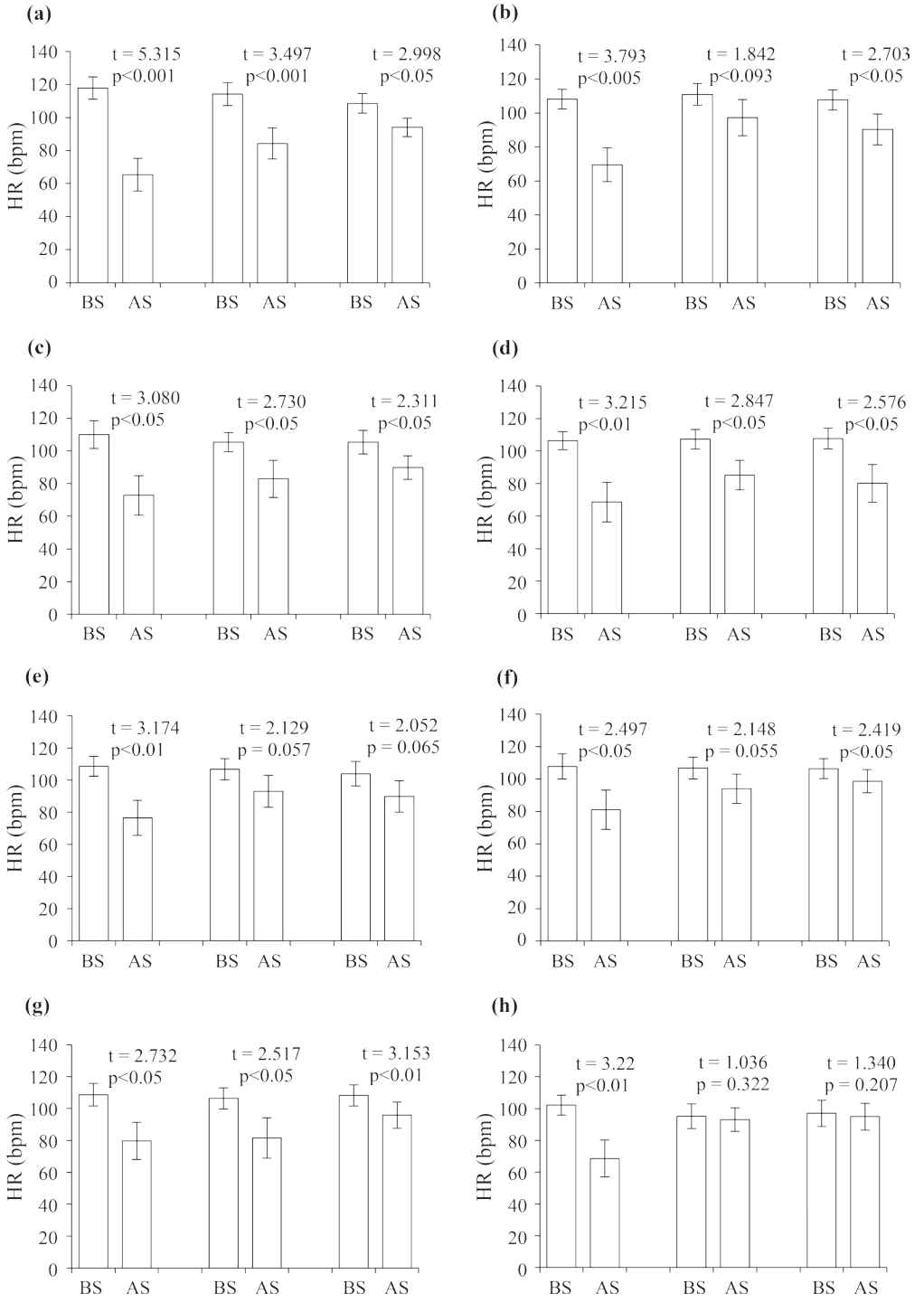

Figure 4. Comparison of heart rate (HR) before (BS) and after (AS) exposure to short-duration sounds. In each experimental session, twelve crabs were exposed to 1-second pure tones at the following frequencies: (a) 100 Hz, (b) 400 Hz, (c) 700 Hz, (d) 1,000 Hz, (e) 1,300 Hz, (f) 1,600 Hz, (g) 1,900 Hz, and (h) 2,200 Hz. HR was measured as beats per minute (bpm) from 10-second ECG recordings before and after the sound exposure.

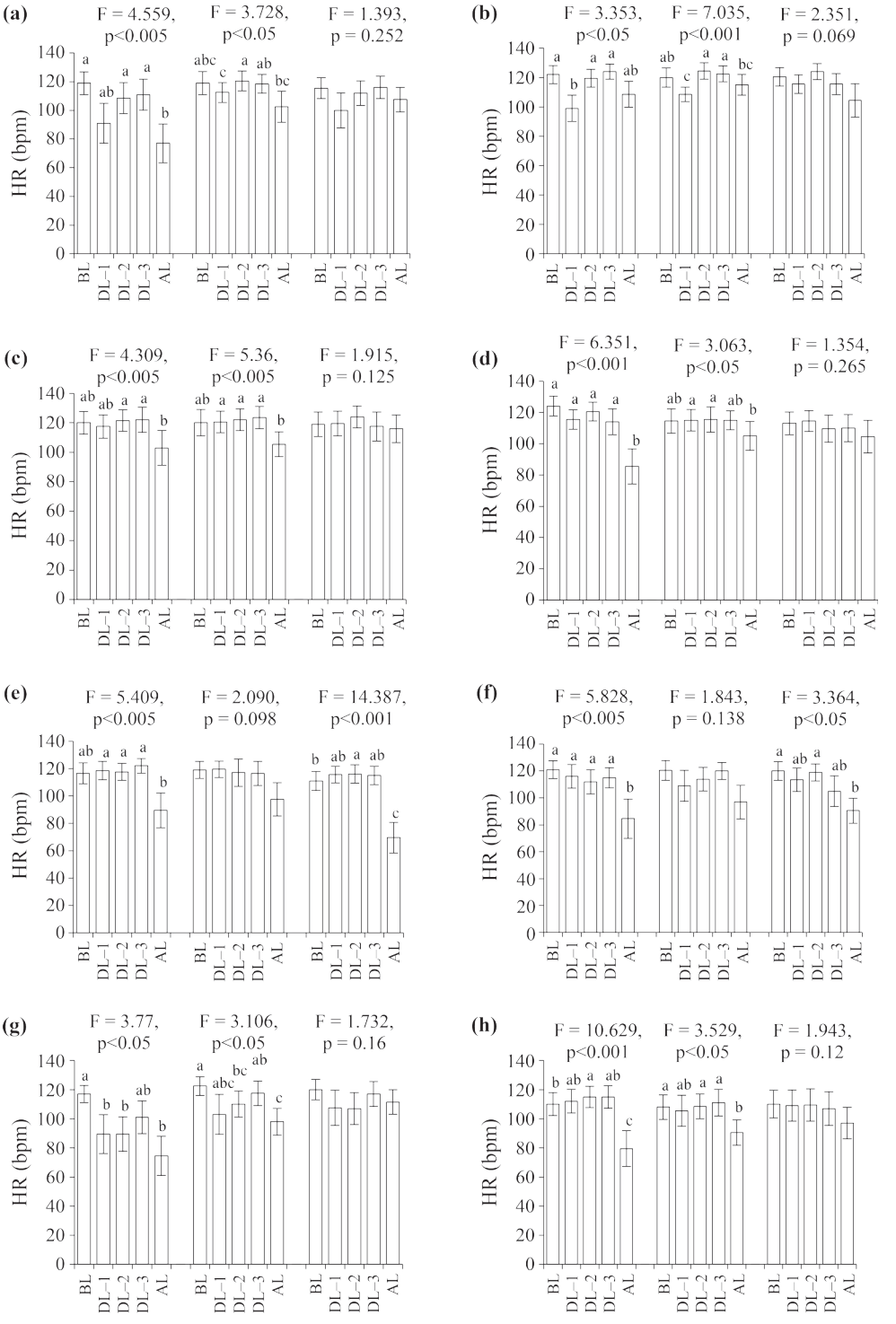

Figure 5. Comparisons of HR before (BL), during (DL–1, DL–2, and DL–3), and after (AL) long-duration sounds. In each experimental session, twelve mud crabs were exposed to long-duration sounds at frequencies of (a) 100 Hz, (b) 400 Hz, (c) 700 Hz, (d) 1,000 Hz, (e) 1,300 Hz, (f) 1,600 Hz, (g) 1,900 Hz, and (h) 2,200 Hz.

In experiment long-duration sound exposure, significant differences were observed during the first and second tests at frequencies of 100, 400, 700, 1,000, 1,900, and 2,200 Hz (Figures 5a, 5b, 5c, 5d, 5g and 5h). At frequencies of 1,300 and 1,600 Hz, significant differences were observed during the first and third tests (Figures 5e and 5f). HR readings were consistently lower at AL and occasionally lower at DL–1 compared to HR at BL, DL–2, and DL–3. These findings demonstrate that significant bradycardia occurred during sound exposures and immediately after the sounds ceased. Statistical comparisons revealed general trends in HR responses to all long-duration sounds (Table 2). Crabs exhibited HR changes to the first exposure across frequencies; however, repeated exposures led to reduced HR responses.

Table 1. Summary of heart rate (HR) responses to short-duration sounds across different frequencies. Grey-shaded rows indicate the frequency range where bradycardia was consistently most tests, indicating a maintained sensitivity to acoustic stimuli.

	100 Hz	400 Hz	700 Hz	1,000 Hz	1,300 Hz	1,600 Hz	1,900 Hz	2,200 Hz
First test	***	**	*	**	**	*	*	*
Second test	**	ns	*	*	ns	ns	*	ns
Third test	*	*	*	*	ns	*	**	ns

Note: ***p < 0.001; ** p < 0.01; * p < 0.05; ns = non−significant

Table 2. Summary of heart rate (HR) responses to long-duration sounds with various across different frequencies. Grey-shaded rows indicate the frequency range where bradycardia was consistently in the first and second tests, however, repeated exposures led to reduced HR responses.

	100 Hz	400 Hz	700 Hz	1,000 Hz	1,300 Hz	1,600 Hz	1,900 Hz	2,200 Hz
First test	**	*	**	***	**	**	*	***
Second test	*	***	**	*	ns	ns	*	*
Third test	ns	ns	ns	ns	***	*	ns	ns

Note: : *** p < 0.001; ** p < 0.01; * p < 0.05; ns = non−significant

DISCUSSION

Exposure to noise can induce stress in crabs, as evidenced by a range of molecular, physiological, and behavioral responses (Pati *et al*., 2023). One of the earliest and most consistent indicators of this stress is bradycardia - a transient reduction in heart rate - which occurs as part of the natural, automatic, and adaptive "fight-or-flight" response. This phenomenon has been documented in various crustaceans (Burnovicz *et al*., 2009; Canero and Hermitte, 2014). For example, Cuadras (1981) observed bradycardia in crayfish in response to ambient, non-startling stimuli, while Burnovicz *et al*. (2009) found that sustained bradycardia was triggered by more threatening inputs.

In the present study, short-duration sounds with frequencies ≤1,000 Hz consistently elicited bradycardia in mud crabs across all three trials (Table 1). These findings highlight the particular sensitivity of crabs to low-frequency noise and suggest that such sounds may be interpreted as potentially threatening even when brief. According to Barrios *et al*. (2021), bradycardia may function as an internal regulatory mechanism in response to threats, modulating the allocation of energy and nutrients within the hemolymph. However, the long-term physiological consequences of recurrent bradycardia in crabs remain largely unexplored.

Importantly, our data also reveal evidence of habituation in heart rate (HR) responses following repeated exposure to the same acoustic stimulus. In most sessions, the magnitude of bradycardia declined in the second and/or third trials, indicating that crabs may recognize certain sounds as non-threatening upon repeated exposure. Nonetheless, in some cases, bradycardia persisted even after rest periods longer than 30 minutes, suggesting partial or incomplete habituation. Two key characteristics were observed: (1) reduced HR responses following repeated exposures to the same stimulus, and (2) heightened responses upon introduction of a novel stimulus. These are consistent with established criteria for habituation (Thompson and Spencer, 1966; Groves and Thompson, 1970; del Rosal *et al*., 2006; Bejder *et al*., 2009; Dehaudt *et al*., 2019).

Similar habituation effects have been observed in other invertebrates. For example, mussels showed reduced valve closure responses to repeated pure tones (Hubert *et al*., 2022), and crabs exhibited lower oxygen consumption during playback of ship noise (Wale *et al*., 2013). In our study, HR proved to be a fast and sensitive biomarker of acoustic sensitivity, with recovery occurring within 10 seconds after brief exposures (DL–1). Notably, this is the first study to report recurrent bradycardia at stimulus cessation, a newly observed phenomenon that may reflect residual or delayed stress processing (Figure 5).

While previous research has explored general effects of anthropogenic noise on aquatic organisms (Day *et al*., 2019; Di Franco *et al*., 2020; Popper *et al*., 2022), our results suggest that stimulus offset may play a previously underappreciated role in physiological stress responses. This nuance has significant implications for aquaculture and environmental noise management.

Anthropogenic noise is not only a research tool but also a persistent environmental factor. Unlike controlled pure tones, real-world noise sources such as small boats emit complex, broadband sounds with variable amplitudes, typically ranging from 10 Hz to 10 kHz and peaking between 1–5 kHz (Hildebrand, 2009). These sounds originate from multiple sources - engines, propellers, and collapsing bubbles (Smith and Rigby, 2022) - each contributing different frequency components. Noise source identification (Fragasso *et al*., 2024) and the controlled use of pure tones, as implemented in this study, are essential for assessing frequency-specific responses and guiding the development of quieter marine equipment.

Previous studies have shown that bradycardia can be triggered by various external cues, including visual, tactile, and vibrational stimuli, or even by the presence of an observer (McGaw and Ebrahim, 2024). These responses are rapid and may be regulated by the cardiac ganglion via direct neural control. In coastal mud crab aquaculture environments, animals are repeatedly exposed to unpredictable noise, which could trigger bradycardia and ventilatory reversals. Repeated activation of these physiological responses could impair juvenile growth (Imtiaz *et al*., 2024) and potentially affect meat quality in market-size individuals (Santos *et al*., 2019). Nevertheless, the effects of chronic exposure to anthropogenic noise in aquaculture settings remain underexplored.

In summary, this study demonstrates that short-duration, low-frequency acoustic stimuli reliably elicit bradycardia in crabs and that HR can serve as a rapid, non-invasive indicator of physiological stress. Repeated exposure to certain sounds resulted in habituation, while novel stimuli or abrupt offsets could still elicit strong cardiac responses. The discovery of recurrent bradycardia at stimulus cessation represents a novel contribution to the field and raises new questions about invertebrate stress physiology. Future research should investigate the long-term effects of repeated bradycardia, the thresholds for habituation versus sensitization, and whether recovery patterns differ between natural and anthropogenic sounds. The real-time HR monitoring method developed in this study provides a foundation for future studies and noise mitigation strategies in aquaculture. Ultimately, understanding how aquatic animals respond to specific acoustic parameters will be critical for managing environmental soundscapes and improving the welfare of farmed species.

CONCLUSIONS

We concluded that HR can be used as a timely and sensitive biomarker for detecting responses to acoustic stimuli in crabs. This conclusion is supported by previous findings on sound-evoked vibrations, the role of chordotonal organs, and the neurogenic nature of heartbeat regulation in crabs. In this study, we compared the HR of *S. serrata* exposed to different sound frequencies and observed the following: (1) HR responses to sound were manifested as bradycardia; (2) Bradycardia was consistently and immediately triggered by sound exposure, regardless of duration and frequency; (3) Sudden changes in the presence or absence of sound elicited bradycardia, particularly in many long−duration tests; (4) HR returned to baseline levels following repeated exposures in some cases, suggesting a degree of habituation; (5) However, bradycardia persisted in response to nearly all short-duration sounds with frequencies lower than or equal to 1,000 Hz.

These findings demonstrate that HR is a valuable physiological indicator that can rapidly reflect auditory stress in crabs, independent of ultrastructural, biochemical, behavioral, or survival-related changes.

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LITERATURE CITED

Aimon, C., S.D. Simpson, R.A. Hazelwood, R. Bruintjes and M.A. Urbina. 2021. Anthropogenic underwater vibrations are sensed and stressful for the shore crab *Carcinus maenas*. **Environmental Pollution** 285: 11748. DOI: 10.1016/j.envpol.2021.117148.

Barrios, N., M. Farias and M.A. Moita. 2021. Threat induces cardiac and metabolic changes that negatively impact survival in flies. **Current Biology** 31(24): 5462–5472.

Bejder, L., A. Samuels, H. Whitehead, H. Finn and S. Allen. 2009. Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. **Marine Ecology Progress Series** 395: 177–185.

Burnovicz, A., D. Oliva and G. Hermitte. 2009. The cardiac response of the crab *Chasmagnathus granulatus* as an index of sensory perception. **Journal of Experimental Biology** 212(2): 313–324.

Calabrese, R.L., B.J. Norris and A. Wenning. 2016. The neural control of heartbeat in invertebrates. **Current Opinion in Neurobiology** 41: 68–77.

Canero, E.M. and G. Hermitte. 2014. New evidence on an old question: is the "fight or flight" stage present in the cardiac and respiratory regulation of decapod crustaceans? **Journal of Physiology-Paris** 108(2–3): 174–186.

Carter, E.E., T. Tregenza and M. Stevens. 2020. Ship noise inhibits colour change, camouflage, and anti-predator behaviour in shore crabs. **Current Biology** 30(5): R211–R212.

Charifi, M., M. Sow, P. Ciret, S. Benomar and J.-C. Massabuau. 2017. The sense of hearing in the Pacific oyster, *Magallana gigas*. **PLoS ONE** 12(10): e0185353. DOI: 10.1371/journal.pone.0185353.

Cuadras, J. 1981. Behavioral determinants of severe cardiac inhibition. **Physiological Psychology** 9(4): 384–392.

Davidsen, J.G., H. Dong, M. Linné, *et al*. 2019. Effects of sound exposure from a seismic airgun on heart rate, acceleration and depth use in free-swimming Atlantic cod and saithe. **Conservation Physiology** 7(1): coz020. DOI:10.1093/conphys/coz020.

Day, R.D., R.D. McCauley, Q.P. Fitzgibbon, K. Hartmann and J.M. Semmens. 2019. Seismic air guns damage rock lobster mechanosensory organs and impair righting reflex. **Proceedings of the Royal Society B: Biological Sciences** 286 (1907): 20191424. DOI: 10.1098/rspb.2019.1424.

Dehaudt, B., M. Nguyen, A. Vadlamudi and D.T. Blumstein. 2019. Giant clams discriminate threats along a risk gradient and display varying habituation rates to different stimuli. **Ethology** 125(6): 392–398.

del Rosal, E., L. Alonso, R. Moreno, M. Vázquez and J. Santacreu. 2006. Simulation of habituation to simple and multiple stimuli. **Behavioural Processes** 73(3): 272–277.

Di Franco, E., P. Pierson, L. Di Iorio, *et al*. 2020. Effects of marine noise pollution on Mediterranean fishes and invertebrates: A review. **Marine Pollution Bulletin** 159: 111450. DOI: 10.1016/j.marpolbul.2020.111450.

Duarte, C.M., L. Chapuis, S.P. Collin, *et al*. 2021. The soundscape of the Anthropocene ocean. **Science** 371: 6529. DOI: 10.1126/science.aba4658.

Edmonds, N.J., C.J. Firmin, D. Goldsmith, R.C. Faulkner and D.T. Wood. 2016. A review of crustacean sensitivity to high amplitude underwater noise: Data needs for effective risk assessment in relation to UK commercial species. **Marine Pollution Bulletin** 108: 5–11.

Fragasso, J., K.M. Helal and L. Moro. 2024. Transfer-path analysis to estimate underwater radiated noise from onboard structure-borne sources. **Applied Ocean Research** 147: 103979. DOI: 10.1016/j.apor.2024.103979.

Fort, T.J., V. Brezina and M.W. Miller. 2007. Regulation of the crab heartbeat by FMRFamide-like peptides: multiple interacting effects on center and periphery. **Journal of Neurophysiology** 98: 2887–2902.

Groves, P.M. and R.F. Thompson. 1970. Habituation: a dual-process theory. **Psychological Review** 77: 419–450.

Haley, J.A., D. Hampton and E. Marder. 2018. Two central pattern generators from the crab, *Cancer borealis*, respond robustly and differentially to extreme extracellular pH. **eLife** 7: e41877. DOI: 10.7554/eLife.41877.

Hawkins, A.D., R.A. Hazelwood, A.N. Popper and P.C. Macey. 2021. Substrate vibrations and their potential effects upon fishes and invertebrates. **The Journal of the Acoustical Society of America** 149(4): 2782. DOI: 10.1121/10.0004773.

Hildebrand, J.A. 2009. Anthropogenic and natural sources of ambient noise in the ocean. **Marine Ecology Progress Series** 395: 5–20.

Horch, K. 1971. An organ for hearing and vibration sense in the ghost crab *Ocypode*. **Zeitschrift für vergleichende Physiologie** 73: 1–21.

Hubert, J., E. Booms, R. Witbaard and H. Slabbekoorn. 2022. Responsiveness and habituation to repeated sound exposures and pulse trains in blue mussels. **Journal of Experimental Marine Biology and Ecology** 547: 151668. DOI: 10.1016/j.jembe.2021.151668.

Imtiaz, N., J. Wang, K. Waiho, R. Li, C. Wang and Q. Wu. 2024. The impact of noise on the stress response of *Portunus trituberculatus* in a land-based monoculture system. **Canadian Journal of Zoology** 102(8): 635–644.

Jahan, H. and M.S. Islam. 2016. Economic performance of live crab (*Scylla serrata*) business in the southwest coastal region of Bangladesh. Int. **International Journal of Fisheries and Aquatic Studies** 4(1): 453–457.

Jones, I.T., J.A. Stanley and T.A. Mooney. 2020. Impulsive pile driving noise elicits alarm responses in squid (*Doryteuthis pealeii*). **Marine Pollution Bulletin** 150: 110792. DOI: 10.1016/j.marpolbul.2019.110792.

Lin, H.-T., T.-C. Chen, J.-P. Change, Z.-Y. Chou, S.-Y. Chiou and M.-L. Tsai. 2021. Simple method for long-term recording of electrocardiogram signals in prawns. **Aquatic Biology** 30: 56–68.

Majeed, Z.R., J. Titlow, H.B. Hartman and R. Cooper. 2013. Proprioception and tension receptors in crab limbs: Student laboratory exercises. **Journal of Visualized Experiments** 80: 51050. DOI: 10.3791/51050.

McGaw, I.J. and S.J. Nancollas. 2021. Patterns of heart rate and cardiac pausing in unrestrained resting decapod crustaceans. **Journal of Experimental Zoology Part A: Ecological and Integrative Physiology** 335(8): 678–690.

McGaw, I.J. and R.A. Ebrahim. 2024. Cardiovascular physiology of decapod crustaceans: from scientific inquiry to practical applications. **Journal Experimental Biology** 227(20): jeb247456. DOI: 10.1242/jeb.247456.

Miah, M.R., M.M. Hossain and M.M. Islam. 2022. Assessing sustainability aspects of mud crab (*Scylla* sp.) fishery and its link to social-ecological traps in the Bangladesh Sundarbans. **Coastal Management** 50(4): 346–371.

Mills, S.C., R. Beldade, L. Henry, D. Laverty, S.L. Nedelec, S.D. Simpson and A.N. Radford. 2020. Hormonal and behavioural effects of motorboat noise on wild coral reef fish. **Environmental Pollution** 262: 114250. DOI: 10.1016/j.envpol.2020.114250.

Nedelec, S.L., A.N. Radford, S.D. Simpson, B. Nedelec, D. Lecchini and S.C. Mills. 2014. Anthropogenic noise playback impairs embryonic development and increases mortality in a marine invertebrate. **Scientific Reports** 4: 5891. DOI: 10.1038/srep05891.

Nedelec, S.L., S.C. Mills, D. Lecchini, B. Nedelec, S.D. Simpson and A.N. Radford. 2016. Repeated exposure to noise increases tolerance in a coral reef fish. **Environmental Pollution** 216: 428–436.

Pati, S.G., B. Paital, B.F. Panda, S. Jena and. K. Sahoo. 2023. Impacts of habitat quality on the physiology, ecology, and economical value of mud crab *Scylla* sp.: A comprehensive review. **Water** 15(11): 2029. DOI: 10.3390/w15112029.

Poole, S., J. Mayze, P. Exley and C. Paulo. 2008. Maximising revenue within the NT mud crab fishery by enhancing post-harvest survival of mud crabs. Department of Primary Industries and Fisheries, Brisbane. https://www.frdc.com.au/project/2003-240. Cited 1 Mar 2025.

Popper, A.N., A.D. Hawkins and F. Thomsen. 2020. Taking the animals' perspective regarding anthropogenic underwater sound. **Trends in Ecology and Evolution** 35(9): 787–794.

Popper, A.N., L. Hice-Dunton, E. Jenkins, *et al*. 2022. Offshore wind energy development: Research priorities for sound and vibration effects on fishes and aquatic invertebrates. **The Journal of the Acoustical Society of America** 151(1): 205. DOI: 10.1121/10.0009237.

Roberts, L., S. Cheesman, T. Breithaupt and M. Elliott. 2015. Sensitivity of the mussel *Mytilus edulis* to substrate-borne vibration in relation to anthropogenically generated noise. **Marine Ecology Progress Series** 538: 185–195.

Santos, T.C., R.S. Gates, C.F. Souza, I.F.F. Tinôco, M.G.L. Cândido and L.C.S.R. Freitas. 2019. Meat quality parameters and the effects of stress: A review. **Journal of Agricultural Science and Technology B** 9: 305–315.

Shelley, C. and A. Lovatelli. 2011. **Mud Crab Aquaculture: A Practical Manual.** FAO., Rome, Italy. 78 pp.

Slater, M., E. Fricke, M. Weiss, A. Rebelein, M. Bögner, M. Preece and C. Radford. 2020. The impact of aquaculture soundscapes on whiteleg shrimp *Litopenaeus vannamei* and Atlantic salmon *Salmo salar*. **Aquaculture Environment Interactions** 12: 167–177.

Smith, T.A. and J. Rigby. 2022. Underwater radiated noise from marine vessels: A review of noise reduction methods and technology. **Ocean Engineering** 266: 112863. DOI: 10.1016/j.oceaneng.2022.112863.

Solan, M., C. Hauton, J.A. Godbold, C.L. Wood, T.G. Leighton and P. White. 2016. Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. **Scientific Reports** 6: 20540. DOI: 10.1038/srep20540.

Solé, M., M. Lenoir, M. Durfort, M. López-Bejar, A. Lombarte and M. André. 2013. Ultrastructural damage of *Loligo vulgaris* and *Illex coindetii* statocysts after low frequency sound exposure. **PLoS ONE** 8(10): e78825. DOI: 10.1371/journal.pone.0078825.

Staaterman, E., A.J. Gallagher, P.E. Holder, C.H. Reid, A.H. Altieri, M.B. Ogburn, J.L. Rummer and S.J. Cooke. 2020. Exposure to boat noise in the field yields minimal stress response in wild reef fish. **Aquatic Biology** 29: 93–103.

Tang, L.S., A.L. Taylor, A. Rinberg and E. Marder. 2012. Robustness of a rhythmic circuit to short- and long-term temperature changes. **Journal of Neuroscience** 32: 10075–10085.

Tidau, S. and M. Briffa. 2016. Review on behavioral impacts of aquatic noise on crustaceans. **Proceedings of Meetings on Acoustics** 27(1): 010028. DOI: 10.1121/2.0000302.

Tsai, M.-L., W.-P. Hsu and T.-C. Chen. 2019. Evaluation of suitable temperature range for post-harvest processing of mud crabs through cardiac performance. **Aquaculture Research** 50: 3711−3719.

Thompson, R.F. and W.A. Spencer. 1966. Habituation: a model phenomenon for the study of neuronal substrates of behavior. **Psychological Review** 73: 16–43.

Vikas, M. and G.S. Dwarakish. 2015. Coastal pollution: A review. **Aquatic Procedia** 4: 381–388.

Wale, M.A., S.D. Simpson and A.N. Radford. 2013. Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. **Biology Letters** 9(2): 20121194. DOI: 10.1098/rsbl.2012.1194.

Weineck, K., A.J. Ray, L.J. Fleckenstein, M. Medley, N. Dzubuk, E. Piana and R.L. Cooper. 2018. Physiological changes as a measure of crustacean welfare under different standardized stunning techniques: Cooling and electroshock. **Animal (Basel)** 8(9): 158. DOI: 10.3390/ani8090158.

Wilson, L., M.K. Pine and C.A. Radford. 2022. Small recreational boats: a ubiquitous source of sound pollution in shallow coastal habitats. **Marine Pollution Bulletin** 174: 113295. DOI: 10.1016/j.marpolbul.2021.113295.