Chemosphere 345 (2023) 140447

Contents lists available at ScienceDirect

Chemosphere

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

The impact of ocean acidification and cadmium toxicity in the marine crab *Scylla serrata*: Biological indices and oxidative stress responses

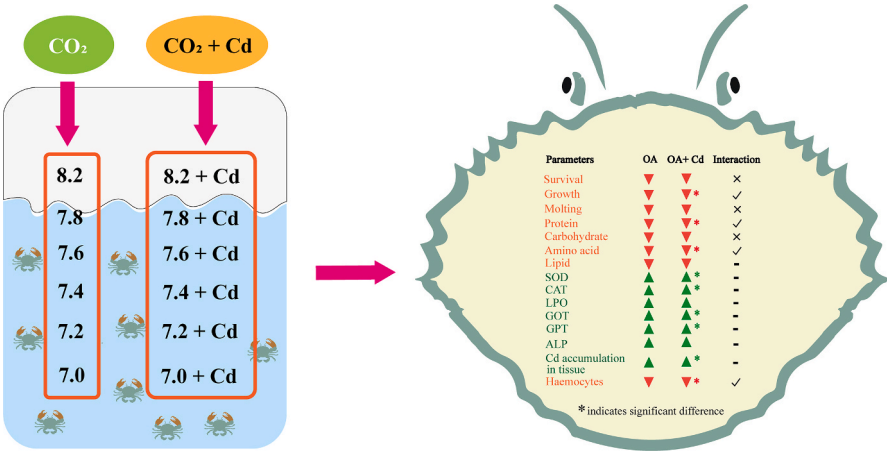
Said Hamid Thangal [a], Ramamoorthy Nandhini Priya [a], Chandrasekaran Vasuki [a], Velusamy Gayathri [a], Krishnan Anandhan [a], Arumugam Yogeshwaran [a], Thirunavukkarasu Muralisankar [a,*], Mathan Ramesh [a], Rajendran Rajaram [b], Perumal Santhanam [b], Balu Alagar Venmathi Maran [c]

[a] Department of Zoology, Bharathiar University, Coimbatore, 641046, Tamilnadu, India
[b] Department of Marine Science, Bharathidasan University, Tiruchirappalli, 620024, Tamilnadu, India
[c] Institute of Integrated Science and Technology, Nagasaki University, 1-14 Bunkyo, Nagasaki, 852-8521, Japan

HIGHLIGHTS

- Growth, food index, ALP, and heamocytes of crabs were decreased in OA + Cd exposure.
- Antioxidants and metabolic enzymes were elevated in crabs under OA + Cd treatments.
- Bioaccumulation of Cd was more in crabs subjected to OA + Cd.
- OA + Cd interaction was higher on growth, protein, amino acid, and heamocytes of crabs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Jun Huang

Keywords:
Ocean acidification
Mud crab
Cadmium
Growth
Antioxidants

ABSTRACT

Ocean acidification (OA) and heavy metal pollution in marine environments are potentially threatening marine life. The interactive effect of OA and heavy metals could be more vulnerable to marine organisms than individual exposures. In the current study, the effect of OA on the toxicity of cadmium (Cd) in the crab *Scylla serrata* was evaluated. Crab instars (0.07 cm length and 0.1 g weight) were subjected to pH 8.2, 7.8, 7.6, 7.4, 7.2, and 7.0 with and without 0.01 mg l^{-1} of Cd for 60 days. We noticed a significant decrease in growth, molting, protein, carbohydrate, amino acid, lipid, alkaline phosphatase, and haemocytes of crabs under OA + Cd compared to OA treatment. In contrast, the growth, protein, amino acid, and haemocyte levels were significantly affected by OA, Cd, and its interactions (OA + Cd). However, superoxide dismutase, catalase, lipid peroxidation, glutamic

* Corresponding author. Aquatic Ecology Laboratory, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India.
E-mail address: tsmuralisankar@buc.edu.in (T. Muralisankar).

https://doi.org/10.1016/j.chemosphere.2023.140447
Received 18 May 2023; Received in revised form 29 August 2023; Accepted 12 October 2023
Available online 17 October 2023

oxaloacetate transaminase, glutamic pyruvate transaminase, and accumulation of Cd in crabs were considerably elevated in OA + Cd treatments compared to OA alone treatments. The present investigation showed that the effect of Cd toxicity might be raised under OA on *S. serrata*. Our study demonstrated that OA significantly affects the biological indices and oxidative stress responses of *S. serrata* exposed to Cd toxicity.

1. Introduction

The ocean is a major saltwater source covering nearly 71% of the earth and acts as a high heat reservoir, influencing the climate patterns, hydrological and carbon cycles (Caldecott, 2008). Marine has a chief role in regulating greenhouse gases and also mitigating climate change (Dutta and Dutta, 2016). The interspace between the atmosphere and the seawater is a vibrant borderline of the earth, which controls the interchange of materials that stimulate the atmosphere's chemistry (Kim et al., 2014; Wurl et al., 2017). At present, the earth system is facing considerable deviations in biogeochemical (carbon, nitrogen, sulphur, phosphorus cycles, etc.) and physical (raining, weather, islands, rivers, oceans, etc.) processes caused by anthropogenic emission of greenhouse gases (Ciais et al., 2014). The specific effects of greenhouse gases are predictable, become more severe, and cause massive consequences on the earth (Collins et al., 2013). Currently, marine environments are being polluted by various pollutants like plastics, industrial chemicals, pesticides, heavy metals, etc. (Bashir et al., 2020; Landrigan et al., 2020).

The source for the surplus release of CO_2 **is** mainly anthropogenic activities due to the usage of fossil fuels, land-use discharges, and industrial operations (IPCC, 2014; Quéré et al., 2018), which cause adverse impacts on the ecosystem, including ocean acidification (OA). The global emission of CO_2 has increased from 34.1 GT to 37.9 GT from 2010 to 2019, respectively (Triollet and Francisco, 2020). The oceans can absorb 30% of atmospheric carbon dioxide emissions (NOAA, 2020). The atmospheric CO_2 enters the ocean by air-to-sea equilibration and is circulated by the ocean carbon cycle (Abas and Khan, 2014). Increases in dissolved CO_2 concentration lead to a decrease in the surface seawater pH (Caldeira and Wickett, 2003). Aqueous CO_2 is created when atmospheric CO_2 dissolves in seawater, resulting in the production of carbonic acid (H_2CO_3). Carbonic acid quickly breaks down into bicarbonate ions (HCO_3^-), which then breaks down into carbonate ions (CO_3^{2-}). Both of these processes result in the production of H^+ ions, which reduce the pH of the saltwater (Doney et al., 2009; Barker and Ridgwell, 2012). At the end of the 21st century, the average outward seawater pH will decrease between 0.2 and 0.4 units due to the excess emission of CO_2 to the atmosphere (Hoegh-Guldberg, 2014; Fitzer et al., 2014). In addition, OA leads to disturbances in the marine ecosystem functioning, like the production of small organisms at a low level, which can affect seafood productivity (Doney et al., 2020).

The excess H^+ ions combine with carbonate ions and leads to formation of bicarbonates and the reduction of carbonates. This scarcity of carbonates leads to the struggle of marine calcifiers to synthesize calcium carbonate in shells and other skeletal structures, which leads to severe physiological impairments. Studies have revealed that decreased seawater pH can cause poor survival, growth, and biochemical elements of the shrimp *Litopenaeus vannamei* (Muralisankar et al., 2021), elevation in antioxidants such as superoxide dismutase, catalase, and lipid peroxidase in crab *Scylla serrata* (Thangal et al., 2022). It is harder to custom calcium carbonate outer shells and skeletons of marine organisms such as pteropods, corals, and oysters due to the scarcity of carbonates under an acidified environment (Lemasson et al., 2017; Hoegh-Guldberget al., 2017; Meng et al., 2019; Rajan et al., 2023). Invertebrates like shrimps, corals, crabs, sea urchins, brine shrimp, etc., showed adverse effects on survival, growth, foraging, and reproduction subjected to OA (Dodd et al., 2015; Campbell et al., 2016; Tasoff and Johnson, 2019; Thangal et al., 2022).

The accumulation of heavy metals in the environment is significantly increased due to various manmade activities (Pinzón-Bedoya et al., 2020). The major sources of heavy metals are mining, tailings, industrial wastes, agricultural runoff, battery industries, paint factories, etc. Copper, lead, zinc, cadmium, mercury, and arsenic are released into the seawater from various sources. Among them, some are trace, and a few are highly toxic to the animals, which cause severe health issues due to the bioaccumulation and long-lasting (Mitra et al., 2022). Amidst metals, Cd is one of the major toxic metals that is mainly released to the environment from various industrial activities like mining, metallurgy, nickel-cadmium batteries production, fabrics dyes, etc. (Su et al., 2020; Khan et al., 2022). The toxic properties of Cd are well documented like increase of reactive oxygen species, which influences the oxidization of biological macromolecules and results in several physiological damages to tissues and organs in animals (Thévenod, 2009; Revathi et al., 2011). Cadmium exposure leads to alteration in gene regulations (HSP70, ATP6L, Prx3, and TRX) in crustaceans like the Chinese mitten crab *Eriocheir sinensis* (Tang et al., 2019) and freshwater crab *Sinopotamon henanense* (Sun et al., 2016). Besides, studies have investigated the interactive effect of OA and certain heavy metals including Cd on physiological responses, oxidative stress, and bioaccumulation of metals in marine animals like mollusks and crustaceans (Lacoue-Labarthe et al., 2009; Götze et al., 2014; Han et al., 2014; Shi et al., 2016; Cao et al., 2018a, 2018b; Zhao et al., 2021). The reduction in the oceanic pH water leads to lower OH^- and CO_3^{2-} concentrations, which cause a high fraction of free forms of metals (MillerO et al., 2009). Moreover, OA may induce the accumulation of Cd in marine animals by three different modes such as increasing Cd and the Cd^{2+}/Ca^{2+} levels in the seawater that leads to high Cd influx through the Ca channel, the decreased pH of seawater may damage the epithelial damage, which results in Cd penetration, and under acidic stress, animals may hamper Cd exclusion (Shi et al., 2016).

The crab *S. serrata* is an Indo-Pacific species having high nutrient market values (Keenan et al., 1998; Paterson and Mann, 2011). According to the report of FAO (2022), crabs are contributing about 1915.7 thousand tons to the aquaculture and fisheries economy. Among them, mud crab *S. serrata* contributes about 248.8 thousand tons to world fisheries. Nevertheless, *S. serrata* is being used as a bioindicator due to their higher sensitivity to biotic and abiotic stress (El-Kahawy et al., 2021; Flint et al., 2021). Furthermore, crabs are susceptible to changes in the ocean carbon chemistry (Whitely, 2011), hence, the modifications in the carbon chemistry in the nearyby environment can create variations in the molting, immunity, regulation of acid-base balance and calcification which reasoning to decrease in survival and effortlessly vulnerable to several biotic and abiotic elements (Rehman et al., 2021). The detrimental effect of decreased seawater pH on physiological status and bioaccumulation in marine crabs, including *S. serrata, Hyas araneus, E. sinensis,* and *Lithodes aesquispisnus* has been reported earlier (Long et al., 2021; Thangal et al., 2022). Nonetheless, the studies on the combined effect of OA with heavy metals are infancy in marine crabs. Therefore, the present study aimed to evaluate the individual effects of OA and its synergistic toxicity with the heavy metal Cd on growth (length gain, weight gain, and specific growth rate), survival, molting, feed index (feed intake and feed conversion ratio), biochemical constituents (protein, carbohydrate, amino acid, and lipid), antioxidants (superoxide dismutase and catalase), lipid peroxidation, metabolic enzymes (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase), alkaline phosphatase activity, and total haemocytes population of the economically important edible marine crab *S. serrata*, which will explore the threats towards seafood safety in future.

2. Materials and method

2.1. Green crab instars maintenance

Totally 800 green crabs (*S. serrata*) instars (carapace length 0.6 cm) were brought from Rajiv Gandhi Center for Aquaculture, Thirumullaivasal, Tamilnadu, India. Instars (0.6 cm length and 0.09 g weight) were transported in two six-litter transparent plastic covers packed with oxygenated seawater and seagrasses to avoid fights and cannibalism. Instars were lodged in a 500 L fiber tank at research laboratory condition for ten days, providing continuous aeration. The crabs were fed 100% of their body mass two times (40% during the day and 60% at night) daily with the brine shrimp (Alava et al., 2017). About ¼ of seawater was renewed daily from the stock tank, and unfed feed and other metabolic wastes were removed without disturbing the crabs.

2.2. Acidification of seawater and experimental setup

The pH manipulation of the seawater setup was planned as per the earlier report of Riebesell et al. (2010) with slight modifications as reported by our previous study (Thangal et al., 2022). In short, the acidification set-up consists of a CO_2 cylinder, cylinder valve, pressure meter, flow meter, CO_2 gas tube, CO_2 valve, CO_2 diffuser, pH meter connected with the probe, aerator with controller, air vent, air stone, aquaria (50 L) and pre-holed polyvinyl chloride (PVC) pipe cages. Seawater pH was manipulated manually by mild releasing of 99.9% pure CO_2 using a CO_2 valve through an insulated rubber tube that connected with an air diffuser in the seawater of each aquarium till the anticipated pH was reached. The pH of seawater in each aquarium was observed continuously by a separate pH meter which was connected with separate probes that were always immersed in seawater until the end of the experiment. The fluctuating pH was balanced at the desired level by manually releasing the CO_2 at an average time interval of 4 h per day.

After accommodation, exactly 120 crabs instar (carapace length 0.7 ± 0.0 cm) were reared in six aquaria (20 × 6) which served as acidification with pH 8.2 (control), 7.8 (estimated ocean pH by IPCC in 2100), and other four randomly selected pH treatments (pH 7.6, 7.4, 7.2, and 7.0) to know the extreme pH effect on the marine crabs for OA alone experiment. For OA with Cd experiment, 120 crabs were reared in six other aquaria (20 × 6) served as acidification with 0.01 mg L^{-1} of $CdCl_2$ (pH 8.2 + 0.01 mg L^{-1}, 7.8 + 0.01 mg L^{-1}, 7.6 + 0.01 mg L^{-1}, 7.4 + 0.01 mg L^{-1}, 7.2 + 0.01 mg L^{-1}, and 7.0 + 0.01 mg L^{-1}) for sixty days. The concentration of Cd was randomly selected as per the earlier studies of Cao et al. (2018b). Crabs were individually housed in a multi-cell-based cage system and seawater pH was manipulated as per our earlier study (Thangal et al., 2022). In short, each aquarium was partitioned using 20 polyvinyl chloride pipe cages (9 cm diameter and 20 cm height) with 32 holes in each cage for water current, and individual crab was placed in each polyvinyl chloride pipe cage. Mild aeration was also provided to the crabs to prevent the re-equilibrating using an air controller. Both experiments were done in triplicate. After reaching each desired pH in the respective experimental aquarium, the water quality parameters such as pH, salinity, and temperature were assessed daily throughout the experiment and every 10 days interval the dissolved oxygen (DO), and alkalinity were assessed by the standard method (APHA, 2005), and total CO_2 (TCO_2), partial carbon dioxide (pCO_2), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), a saturation of calcite (ΩCa), and aragonite (ΩAr) were computed by CO_2 calculator designed by Robbins et al. (2010) throughout the experiment. Further, 0.01 mg L^{-1} Cd level was maintained in each aquarium meant for OA + Cd treatments by adding the appropriate level of $CdCl_2$ solution while renewing half of the aquarium water daily throughout the experiment. Moreover, the level of Cd in seawater was measured in all aquaria after adding Cd. During the experimental period, the crab instars were fed with brine shrimp for the first week and chopped squid (*Uroteuthis edulis*) and shrimp (*Penaeus monodon*) muscle meat for the remaining days twice per day (Ganesh et al., 2015).

2.3. Analysis of Cd in the seawater and crabs

The Cd level was measured in both the OA and OA + Cd experimental waters as well as dried crab tissues. A Millipore filtration unit was used to filter the seawater samples (0.45 μm mesh filter paper), and 1000 mL of the filtered seawater was then put into a 2000 mL separating funnel. Then, 10 mL of 1% ammonium pyrrolidine dithiocarbamate was added, and the mixture was vigorously agitated. Exactly 25 mL of isobutyl methyl ketone solution was added and agitated for 15 min, and the upper organic phase was collected following the development of distinct layers. The resultant organic stage was extracted using 50% HNO_3 and diluted up to 25 mL using double-distilled water (Vinothkannan et al., 2022). One gram of dried crab tissue was taken in a clean, acid-washed beaker, which was then subjected to triple acid digestion using 10 mL of triple acid (HNO_3, $HClO_4$, and H_2SO_4) in a ratio of 9:2:1 on a hot plate at 60 °C. The resultant extract was filtered through an ashless Whatman Grade 1 filter paper and diluted with double-distilled water to a concentration of 25 mL. A flame atomic absorption spectrophotometer (AAS) (Shimadzu AA-7000, Japan) supplied with a mixture of air and acetylene was used to analyze the Cd level in processed samples of water and crab tissue (Arumugam et al., 2018).

The minimal detection limit for Cd and working wavelength in the flame mode was <0.01 ppm and 228.80 nm respectively, and triplicate readings were taken for each sample for QA/QC. To calibrate the equipment, control blanks, and standardized reference materials were employed throughout the analysis. Ultrapure deionized water was utilized to prepare all of the chemicals for metal analysis. The standard curve was plotted using known standards of various strengths to fit within the ranges of absorbance of the samples and the regression coefficient (R2) value higher than 0.99 was achieved for all the standard curves. After the completion of every 10 samples, the AAS instrument was cleaned with deionized water, and a blank reading was taken to rule out any contamination from the sampling process.

2.4. Survival, development, molting, and food index

On the 60th day of the trial, the survival, morphometry [weight gain (WG), length gain (LG), and specific growth rate (SGR)], molting and food indices [feed conversion ratio (FCR)], and feed intake (FI) were evaluated by following the calculations.

Survival (%) = no. of live crab/no. of crab introduced × 100.

Weight gain (g) = final weight (g)-initial weight (g).

Length gain (cm) = final length (cm)-initial length (cm).

Specific growth rate (% d^{-1}) = ln (final weight) - ln (initial weight)/total days × 100).

Molting rate (no. of molt d^{-1}) = total no. of molt/total days.

Feed conversion ratio = feed intake/weight gain.

Feed intake (g $crab^{-1}$ d^{-1}) = feed consumption/crab number/days.

2.5. Estimation of biochemical components

Estimation of total tissue protein, amino acid, carbohydrate, and lipid was done in *S. serrata* using the procedures of Lowry et al. (1951), Moore and Stein (1948), Roe (1955), and Barnes and Blackstock (1973) respectively. To determine the protein content, 100 mg of crab muscle tissue was homogenized in 80% ethanol, centrifuged at 1960×*g* for 15 min, and the precipitate was then dissolved in 1 mL of 1 N NaOH, 5 mL of an alkaline copper solution, and incubated for 20 min at room temperature. The reaction was combined with precisely 0.5 mL of Folin-Ciocalteu phenol reagent, which was then incubated for 20 min. Finally, the color intensity was measured at 650 nm against a blank solution using a UV–visible spectrophotometer. The reference solution was freshly made with bovine serum albumin. For the measurement of

amino acid, 500 mg of crab tissue was homogenized with 2 mL of a 1:1 mixture of sodium tungstate and 0.66 N H_2SO_4, centrifuged at 1960×*g* for 10 min. Precisely, 0.5 mL of supernatant was mixed with 4.5 mL of distilled water and 0.5 mL of 4% ninhydrin reagent. Cotton plugs were placed on the test tubes and then placed in an 80 °C water bath. A UV–visible spectrophotometer was employed to determine the color intensity at 540 nm against a blank solution after the test tubes were cooled to room temperature. The amino acid leucine was used as a reference solution.

The total amount of carbohydrates in the crab tissues was determined by homogenizing 500 mg of crab muscle in 2 mL of 80% ethanol and centrifuging the mixture at 1960×*g* for 15 min. Four milliliters of 0.2% anthrone reagent (in ice-cold H_2SO_4) were added to the supernatant in a test tube. The test tube was then placed in a boiling water bath for 10 min and cooled in a dark room. Using a UV–visible spectrophotometer, the color intensity was recorded at 620 nm against a blank solution and the glucose was used as a reference solution. The content of total lipid was extracted from 500 mg of crab tissue using 4 mL of (2:1 v/v) chloroform-methanol mixture (Folch et al., 1957). The extraction was added with 0.2 mL of 0.9% NaCl and the solution was kept overnight. The lipid layer was separated, dried, and dissolved in 0.5 mL of H_2SO_4. The mixture was placed in a water bath for 10 min, cooled at ambient room temperature, and of 5 mL sulpho-phospho-vanillin reagent was added. The mixture was well shaken and allowed to stand for 30 min. The color intensity was recorded using a UV–visible spectrophotometer against a blank solution at 520 nm and the olive oil was used as a reference to determine the lipid levels in samples.

2.6. Analysis of antioxidants and lipid peroxidation

After the end of 60 days, all experimental crabs were sedated with ice cubes and the muscle tissue was extracted by homogenizing within frozen tris buffer (pH 7.4, 10% w/v), separated by rotating a cooling centrifuge (2415×*g* for 20 min at 4 °C) and the supernatant was used as enzyme source to diagnose the level of superoxide dismutase (SOD) and catalase (CAT), and lipid peroxidation (LPO). The level of total soluble protein in each crab's tissue was estimated by the Lowry et al. (1951) method.

SOD level was done by the autoxidation of pyrogallol in the tris buffer described by Marklund and Marklund (1974). The reaction mixture consisted of 2 mL Tris–HCl (pH 8.2), 0.5 mL 2 mM pyrogallol, and 2.0 mL water. Primarily, the rate of autoxidation of pyrogallol was measured at an interval of 1 min for 3 min for complete autoxidation. The autoxidation reaction mixture contains 4.5 mL of distilled water, 2 mL of Tris-HCl buffer, 0.5 mL of 2 mM pyrogallol, and 2 mL of enzyme source. The rate of pyrogallol autoxidation was measured at 430 nm. In addition, 2.0 mL of Tris-HCl buffer and 2.5 mL of distilled water were combined to serve as a blank and the activity of SOD was expressed as unit per mg of protein.

The activity of CAT was done by the method described by Sinha (1972), and the substrate is considered as hydrogen peroxide (H_2O_2) in phosphate buffer. In short, the enzyme reaction was started by mixing 0.5 mL of crab muscle tissue homogenate with 1 mL of 0.01 M phosphate buffer (pH 7.1), 0.5 mL of 0.2 M hydrogen peroxide, and 0.4 mL of distilled water. After 60 seconds, the reaction stopped by adding 2 mL of dichromate-acetic acid, and the tubes were then boiled for 10 min before being cooled to room temperature. The chromophore absorption was measured at 620 nm. The CAT activity was quantified as μmol of H_2O_2 per min per mg of protein and a reaction without enzyme served as the control.

The activity of LPO level was done by the formation of thiobarbituric acid reactive substances prescribed by Ohkawa et al. (1979). Briefly, trichloroacetic acid (10%), 0.02 M Tris buffer (pH 7.5), and 1.5 mL of thiobarbituric acid (1.5% were added to the tubes containing 1 mL of tissue homogenate. The reaction was heated in a boiling water bath for 15 min before cooling to room temperature. The supernatant was separated once the content was centrifuged at 100×*g* for 20 min. The absorbance of the supernatant was measured at 535 nm in comparison to a reagent blank solution and the activity was expressed as nanomoles of malondialdehyde (MDA) per mg of protein.

2.7. Activity of metabolic and alkaline phosphatase enzyme analysis

Crab tissue (100 mg) was homogenized in a 0.25 M sucrose solution, followed by centrifuge at 2415×*g* for 25 min at 4 °C and the supernatant was used to determine the glutamic oxalate transaminase (GOT) and glutamic pyruvate (GPT) transaminase. These assays were performed by using the procedure of Reitman and Frankel (1957). Analysis of GOT was performed by the addition of 0.5 mL of substrate solution (L-aspartic acid: α-ketoglutarate) to a 100 μL tissue sample, incubated at 37 °C for 1 h, followed by adding 0.5 mL of 2, 4-dinitrophenyl hydrazine and allowed to stand for 20 min at ambient temperature. Three milliliters of freshly prepared 0.4 N NaOH was added to the reaction mixture and the color intensity was read at 505 nm against blank. Sodium pyruvate was used as a standard. Determination of GPT was done by adding 0.5 mL of L-alanine: α-ketoglutarate substrate) with 100 μL of tissue sample and incubated at 37 °C for 20 min. Precisely 0.5 mL of 2, 4-dinitrophenyl hydrazine was added to the above reaction mixture, allowed to stand at ambient temperature for 30 min and 3 mL of 4 N NaOH was added. The color development was measured at 505 nm against blank. Sodium pyruvate was used as a standard. The activity of both GOT and GPT was expressed as U/mL.

The alkaline phosphatase (ALP) level was estimated using Kind and King's (1954) procedure. In short, whole crab except carapace and eyestalk was ground in 1 mL of 50 mM cold distilled water, followed by centrifuging at 6350×*g* for 5 min at 4 °C. Separate test tubes were used as blank (B), standard (S), and control (C) and test (T). Approximately 1.05 mL double distilled water was poured in B and 1 mL in S, C, and T. Furthermore, 0.1 mL of disodium phenyl phosphate substrate reagent was added to all test tubes followed by shaking well and incubated at 35 °C for 5 min. The separated extract (0.05 mL) was poured into T, and 0.05 mL of phenol solution was poured into S as standard. Additionally, all test tubes were shaken well and allowed to stand for 15 min at 35 °C. At last, 1 mL of dye reagent (Phenol + 4-amino antipyrine) was added to all test tubes, and 0.05 mL of centrifuged supernatant was poured into test tube C. Finally, the intensity of all test tube reactions was measured at 510 nm in a UV spectrophotometer.

2.8. Enumeration of total haemocytes

Accurately, 100 μL haemolymph was withdrawn from the joint of the merus of crab using a 1 mL insulin syringe pre-filled with 900 μL of anticoagulant (1:1:1 ratio of 10 mM of sodium citrate with pH 7.5, 10 mM Tris-HCl, and 250 M of sucrose respectively). From the above stock, 50 μL of anti-coagulated haemolymph was added to 10% of 50 μL formalin and incubated for 30 min. After incubation, 50 μL of formalin was mixed with anti-coagulated blood and diluted using a 50 μL phosphate buffer solution. From this, 50 μL diluted haemolymph was mixed with 20 μL rose Bengal stain (0.01 g rose Bengal stain diluted in 50% ethanol) and incubated for 10 min. Finally, the haemocytes was counted by using a haemocytometer (Neubauer improved, Germany) under a light microscope at RP10*X* (Labomed, OPTIcx), and calculated by using the formula.

$$\mathrm{THC}\left(\times 10^{6}\ \mathrm{cells\ mL^{-1}}\right) = \frac{Counted\ cells \times depth\ of\ chamber \times dilution\ factor}{Number\ of\ 1mm\ square}$$

2.9. Statistical analysis

The data were all symbolized as mean ± SD. The significant difference between OA experiments (different pH treatments) and OA + Cd

experiments (different pH treatments with Cd) was determined using one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). The noticeable difference between each parameter of each OA and OA + Cd was analyzed using a two-tailed paired sample 't'-test. Moreover, two-way ANOVA was subjected to transformed data to know the interactions of pH and Cd. However, the parameters like salinity, temperature, DO, SGR, FI, FCR, carbohydrate, lipid, SOD, CAT, LPO, GOT, GPT, and ALP retained only one-way ANOVA and "t" test due to their significant difference during normal distribution analysis. All the analyses were achieved using IBM SPSS (20.0) software.

3. Results

3.1. Physicochemical parameters of seawater

In the present investigation, pH, carbonate (CO_3^{2-}), calcite (ΩCa), and aragonite (ΩAr) of the seawater were considerably decreased from pH 7.8 to 7.0 in both OA alone and OA + Cd treatments when equated to the respective normal seawater at pH 8.2 (control) and seawater with Cd at pH 8.2. The experimental setup's salinity and temperature exhibited an insignificant variation in all pH treatments (pH 7.8 to 7.0) when matched to control seawater without and with Cd in pH 8.2 of OA alone and OA + Cd experiments respectively. The level of dissolved oxygen level in OA treatment was insignificantly altered in all acidified seawater environments, whereas, total alkalinity was decreased considerably in pH 7.2 and 7.0 compared to seawater at pH 8.2. In this context, dissolved oxygen and total alkalinity showed a significant decline in pH 7.4 to 7.0 when matched to the Cd alone treatment in OA + Cd experiments. The level of total carbon dioxide (TCO_2), partial pressure of carbon dioxide (pCO_2), and bicarbonates (HCO_3^-) showed significant increases in pH 7.8 to 7.0 in both OA alone and OA + Cd experiments when matched to the control seawater and seawater with Cd at pH 8.2 (Supplementary Table S1). In the seawater chemistry such as alkalinity, TCO_2, pCO_2, CO_3^{2-}, ΩCa, and ΩAr, considerable changes were observed in OA + Cd treatments when compared to the OA alone treatments as per paired sample *t*-test (Supplementary Table S1). In addition, the two-way ANOVA indicated that the OA process influenced the pH of seawater, however, pH and its interaction with Cd influenced the alkalinity, TCO_2, pCO_2, CO_3, ΩCa and ΩAr of experimental seawater (Supplementary Table S2).

3.2. Accumulation of Cd in seawater and crab tissue

The Cd level was insignificantly varied among different pH exposures of OA and OA + Cd seawater experiments. In this circumstance, a significant accumulation of the Cd in crabs under all OA and OA + Cd was noticed compared to crabs under control seawater and Cd alone experiments (pH 8.2) respectively. The paired sample *t*-test indicated significant elevations in Cd bioaccumulation of crabs in all OA + Cd experiments compared to respective OA experiments (Fig. 1).

3.3. Survival, development, molting, and food indices

In the present findings, in both acidification treatments (OA and OA + Cd), the crab's survival rate notably dropped in pH 7.2 and 7.0 when matched to the respective control seawater (pH 8.2) and seawater with Cd (pH 8.2) treatments (Fig. 2). Meanwhile, the survival rate showed an insignificant variation between the crabs exposed to all OA and OA + Cd treatments as per paired sample 't'-test. In OA treatments, the crab's length and length gain notably decreased in pH 7.6 to 7.0 when matched to the control seawater pH 8.2 (Fig. 2). In OA + Cd treatments, the length and length gain of the crab was notably decreased in pH 7.8 to 7.0 and pH 7.6 to 7.0, respectively when compared to the Cd only treatment (Fig. 2). Paired sample 't'-test indicates that the length and length gain of crabs were observed to be notably dropped in Cd treatments at pH 8.2

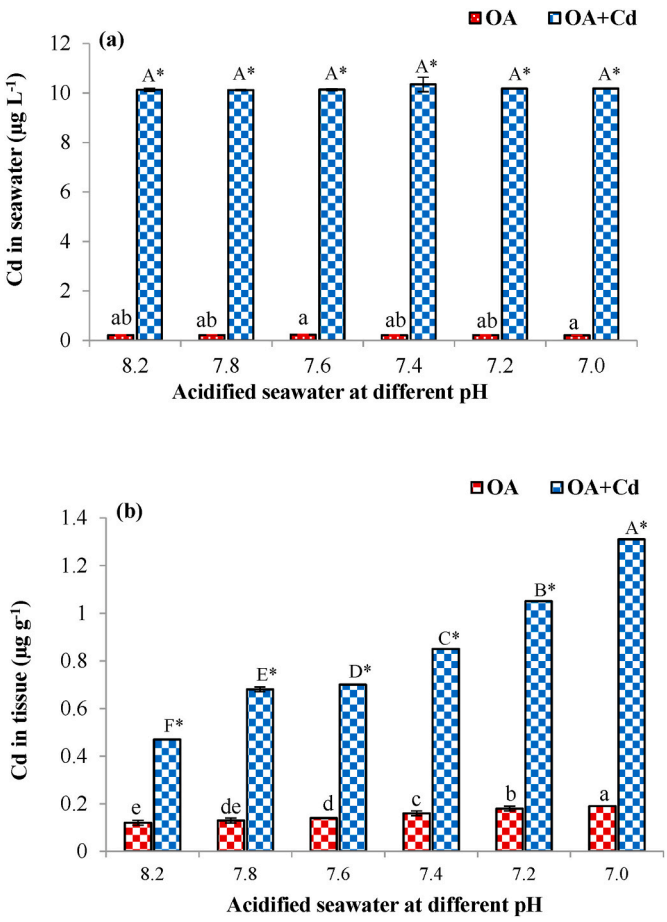


Fig. 1. (a) Level of Cd in seawater and (b) Accumulation of Cd in tissue, of *S. serrata* exposed to CO_2 driven acidified seawater. n = 9, mean ± SD; bars sharing different letters are considered as significant at $p < 0.05$ among OA alone (lowercase letters) and OA + Cd (uppercase letters) treatments; * indicates the significant difference between the respective each pH of OA and OA + Cd as per paired sample 't' test.

and 7.8 compared to that of same seawater pH without Cd groups. In both OA and OA + Cd treatments, the weight and weight gain notably dropped from pH 7.8 to 7.0 when equated to respective control seawater without and with Cd at pH 8.2 (Fig. 2). Moreover, the weight and weight gain showed a significant reduction in pH 7.8 to 7.0 in crabs under OA + Cd when compared to the OA alone group crabs as per paired sample 't'-test. The molting rate of crabs in OA treatments shows a notable drop in pH 7.6 to 7.0 compared to the ambient seawater pH of 8.2. In OA + Cd treatments, the molting rate showed significant variations in pH 7.0 only when related to the other pHs (pH 8.2 to 7.2). Meanwhile, an insignificant difference was found between the OA and OA + Cd groups as per the 't'-test. The specific growth rate (SGR) and feed intake (FI) of OA treatments showed a considerable reduction in pH 7.8 to 7.0 and pH 7.4 to 7.0, respectively, compared to seawater at pH 8.2 (control) (Fig. 2). In the case of the OA + Cd experiment, the SGR and FI were notably dropped in pH 7.8 to 7.0, and pH 7.0, respectively, when compared to Cd alone treatments at pH 8.2. Moreover, a considerable reduction in SGR (pH 7.4 to 7.0) and FI (pH 7.8 to 7.0) of crabs was noted in OA + Cd treatments compared to that in OA alone treatments as per the 't'-test. . Besides, a notable upsurge was found in the feed conversion ratio (FCR) of crab treated at pH 7.2 and 7.0 when matched to that in the control seawater and seawater with Cd (pH 8.2) respectively. Also, no significant changes were observed in the FCR of the crabs exposed to the OA and OA + Cd treatments as per the paired sample 't'-test. (Fig. 2). Further, the two-way ANOVA indicated that the OA process influenced

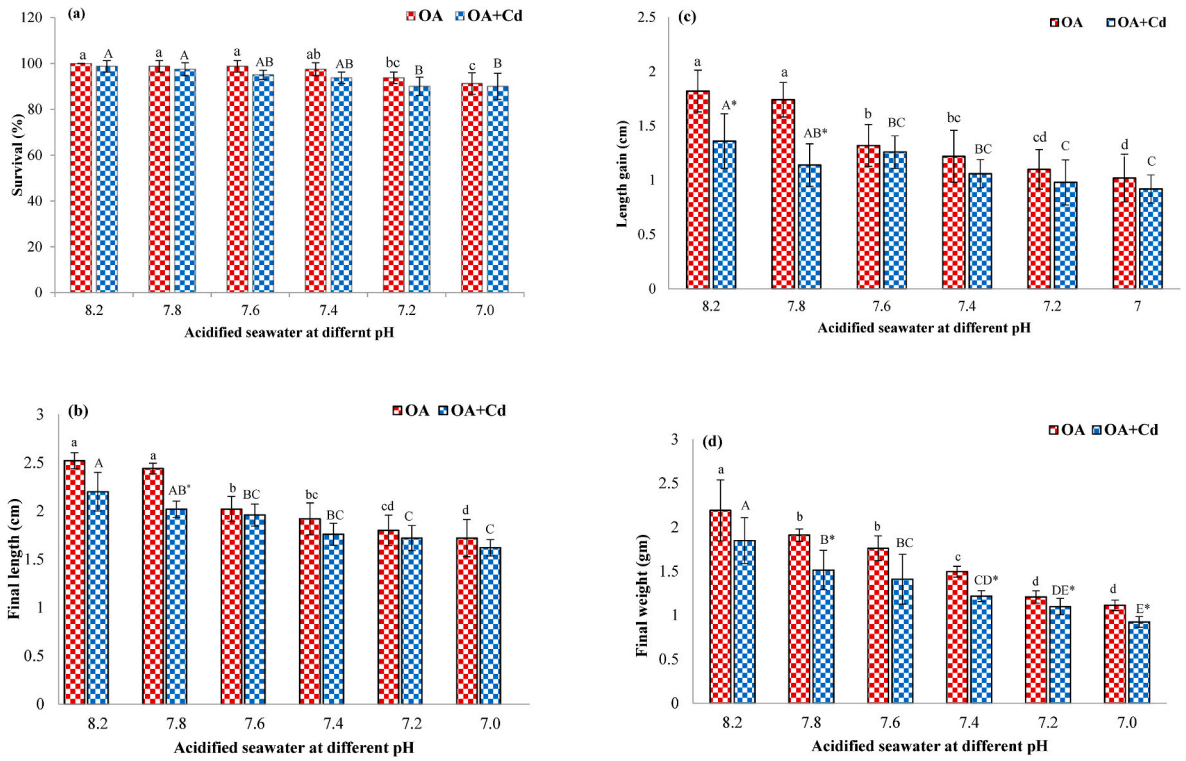


Fig. 2. (a) Survival, (b) final length, (c) LG, (d) final weight, (e) WG, (f) Molting rate, (g) SGR, (h) FI and (i) FCR of *S. serrata* exposed to CO_2 driven acidified seawater with and without Cd. n = 60 for survival, molting, FI, and FCR; n = 15 for LG, WG, and SGR; mean ± SD; bars sharing different letters are considered as significant at $p < 0.05$ among OA alone (lowercase letters) and OA + Cd (uppercase letters) treatments; * indicates the significant difference between the respective each pH of OA and OA + Cd as per paired sample 't' test. LG, Length gain; WG, weight gain; SGR, specific growth rate; FI, feed intake; FCR, feed conversion ratio.

the crab *S. serrata,* while Cd and OA + Cd did not influence the survival. Whereas, OA, Cd, and OA + Cd influenced length and weight gain in crabs. Moreover, OA and Cd notably influenced crabs' molting rate (Supplementary Table S3).

3.4. Biochemical elements

In both OA and OA + Cd treatments, the protein, carbohydrate, amino acid, and lipid levels notably declined in crabs exposed to pH 7.8 to 7.0 compared to the respective ambient seawater and seawater with Cd alone exposures at pH 8.2. The carbohydrate level of the crabs subjected to pH 7.8 and 7.6 showed an insignificant difference in both OA and OA + Cd experiments, respectively. Moreover, lipid content was insignificantly altered in crabs reared in OA + Cd treatments at pH 7.8 to 7.4 and 7.2 and 7.0. Meanwhile, the level of protein and amino acids was found to be decreased in crabs reared in all OA + Cd treatments compared to OA treatments alone , however, a considerable decrease was noticed in carbohydrate and lipid levels of crabs exposed to OA + Cd at pH 7.4 and 7.2 to 7.0, respectively compared to respective OA exposures as per the paired sample 't'-test (Fig. 3). In this context, individual exposure to OA, Cd, and Cd interact with OA were influenced the protein and amino acid levels of the *S. serrata* as per two-way ANOVA (Supplementary Table S3).

3.5. Antioxidants and lipid peroxidation

The level of SOD was remarkably elevated in *S. serrata* exposed to the high pCO_2 seawater (pH 7.8 to 7.0) of the OA experiment compared to the control seawater at pH 8.2. While CAT and LPO showed a significant elevation in crabs at pH 7.6 and 7.0 related to crabs in normal seawater at pH 8.2 in OA experiments. However, an insignificant change was observed in the CAT and LPO of crabs exposed between pH 7.8 and 8.2 (control seawater) of the OA experiment. In OA + Cd treatment, the SOD, CAT, and LPO of *S. serrata* showed significant elevation in pH 7.8 to 7.0 when related to the crabs in Cd alone treatment (pH 8.2). While in LPO, an insignificant variation was observed in pH 7.8 to 7.2 of OA + Cd exposures. Moreover, paired sample 't'-test revealed that the level of SOD and CAT of the crabs' group was noted to be significantly elevated in OA + Cd exposure when compared to the OA alone groups. Besides the level of LPO showed a notable increase in pH 7.8 and 7.0 of OA + Cd when compared to the respective OA alone groups (Fig. 4).

3.6. Metabolic enzymes and alkaline phosphate activity

In the OA trial, a notable elevation was recorded in crabs treated to pH 7.8 to 7.0 compared to seawater at pH 8.2. While an insignificant difference was noted in GOT and GPT at pH 7.8 and 7.6, pH 7.6 to 7.0, respectively. In OA + Cd treatments, the GOT and GPT showed a notable elevation in pH 7.8 to 7.0 when matched to Cd only treatments at pH 8.2. Nonetheless, an insignificant change was observed in GOT at pH 7.8 to 7.0 in the OA + Cd experiment. Moreover, in GOT and GPT activity, an insignificant variation was noted in all OA + Cd treatments. In this context, the GOT and GPT activities were noted to be a considerable elevation in pH 7.8 to 7.4, and 8.2 to 7.0, respectively, in OA + Cd treatments compared to respective OA only treatments as per the 't'-test (Fig. 5). The alkaline phosphatase activity of crabs exposed in both OA and OA + Cd treatment, showed a noteworthy decrease in pH 7.8 to 7.0 when compared to the respective control seawater and seawater with Cd at pH 8.2. In both OA and OA + Cd treatment, an insignificant difference was observed in pH 7.8 to 7.0 and 7.8 to 7.4, respectively. Furthermore, no significant variations were observed between the OA and OA + Cd experiments as per the paired sample 't'- test (Fig. 5).

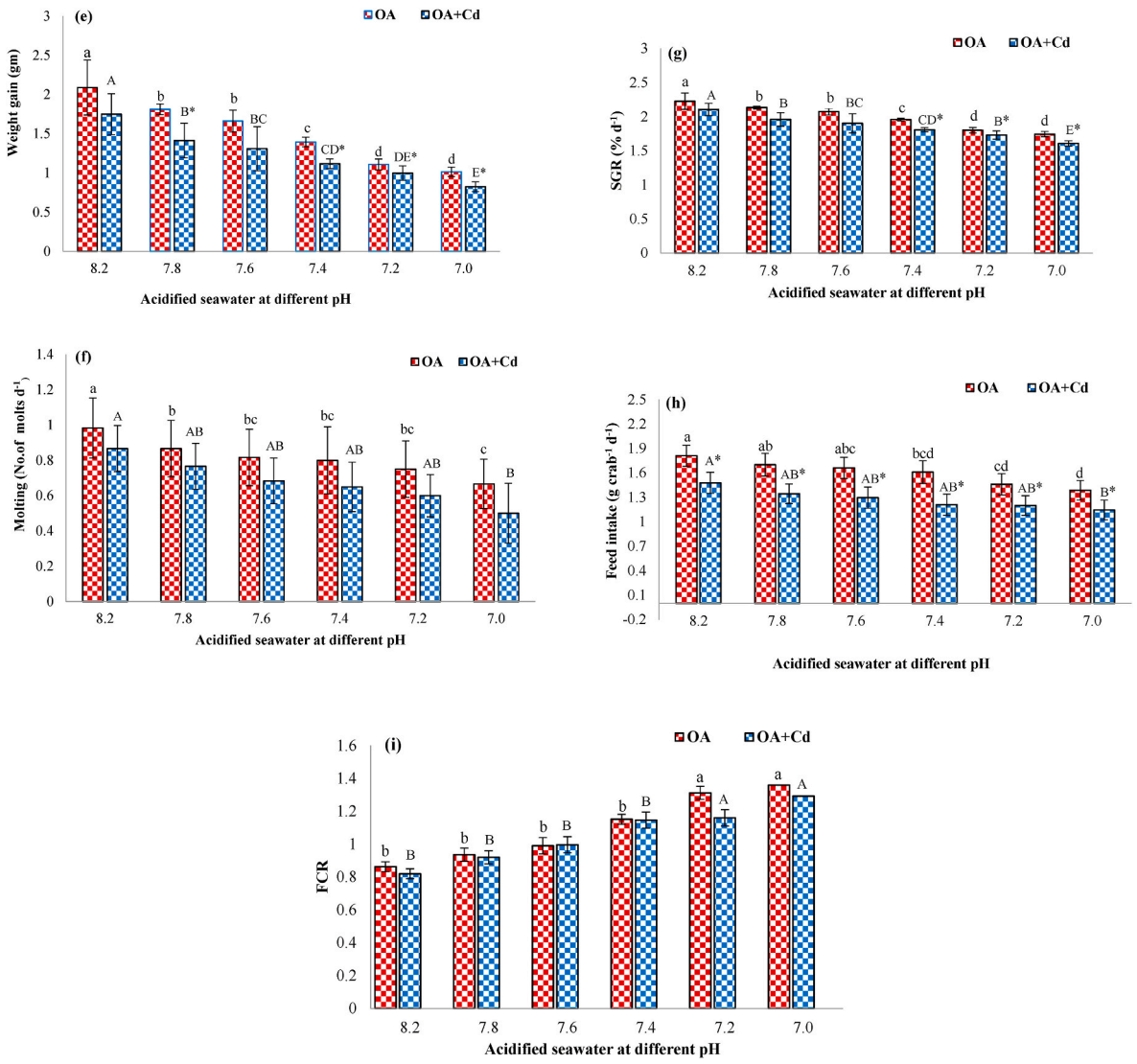


Fig. 2. (*continued*).

3.7. Haemocyte counts

The total count of the haemocytes population of *S. serrata* was remarkably decreased in all OA and OA + Cd experiments when compared to respective control seawater and seawater with Cd alone treatments at pH 8.2. Moreover, the population of haemocytes was significantly decreased in all OA + Cd exposed groups when compared to the respective OA groups as per the paired sample 't'-test. In addition, two-way ANOVA calculation shows that pH significantly influenced the haemocyte counts of *S. serrata*. Whereas, Cd and its interaction with pH failed to influence the haemocyte counts (Fig. 5). As per the two-way ANOVA, the OA, Cd, and OA + Cd influenced the haemocyte population of the crab *S. serrata* (Supplementary Table S3).

4. Discussion

Seawater quality is crucial in maintaining their ambient physiological activities in marine life. The excess amount of CO_2 released into the atmosphere is one of the main factors to creates OA, which disturbs the carbon content of the seawater that influences the water quality parameter, including dissolved inorganic carbon , pCO_2, aragonite, and calcite (Long et al., 2017; Thangal et al., 2022). Heavy metal pollution is another severe problem, which increases heavy metal availability in seawater and bioaccumulation in organisms. The increases in threats, including OA and metal pollution, disturb the normal physiological activities of the crustaceans, which create ecological implications in the food web (Whiteley, 2011; Adeleke et al., 2020), followed by significant threats to higher tropic animals. In the present study, a significant decline in pH, pCO_2, total CO_2, carbonate, calcite, and aragonite of the all acidified seawater in OA experiments and OA + Cd experiments compared to the respective control seawater (pH 8.2) and seawater with Cd alone (pH 8.2 + Cd) suggests that the CO_2 driven ocean acidification can alter the physicochemical properties of seawater thereby lacking essential elements for marine calcareous species. An insignificant variance in salinity and temperature of acidified seawater in OA and OA + Cd experiments compared to the control seawater and seawater with Cd only experiments that CO_2 driven acidification did not produce any significant changes in these properties of seawater under a laboratory environment. Similarly, alterations in the pCO_2, HCO_3^-, ΩCa, and ΩAr have been observed in the CO_2 driven acidified seawater (Long et al., 2017; Anand et al., 2021). Seawater acidification experiments have recorded significant variations in salinity and temperature (Muralisankar et al., 2021; Shi et al., 2016; Cao et al., 2018a, 2018b; Thangal et al., 2021, 2022).

Seawater acidification may alter heavy metals' toxicity and increase toxic metal accumulations (Campbell et al., 2016). In the present study,

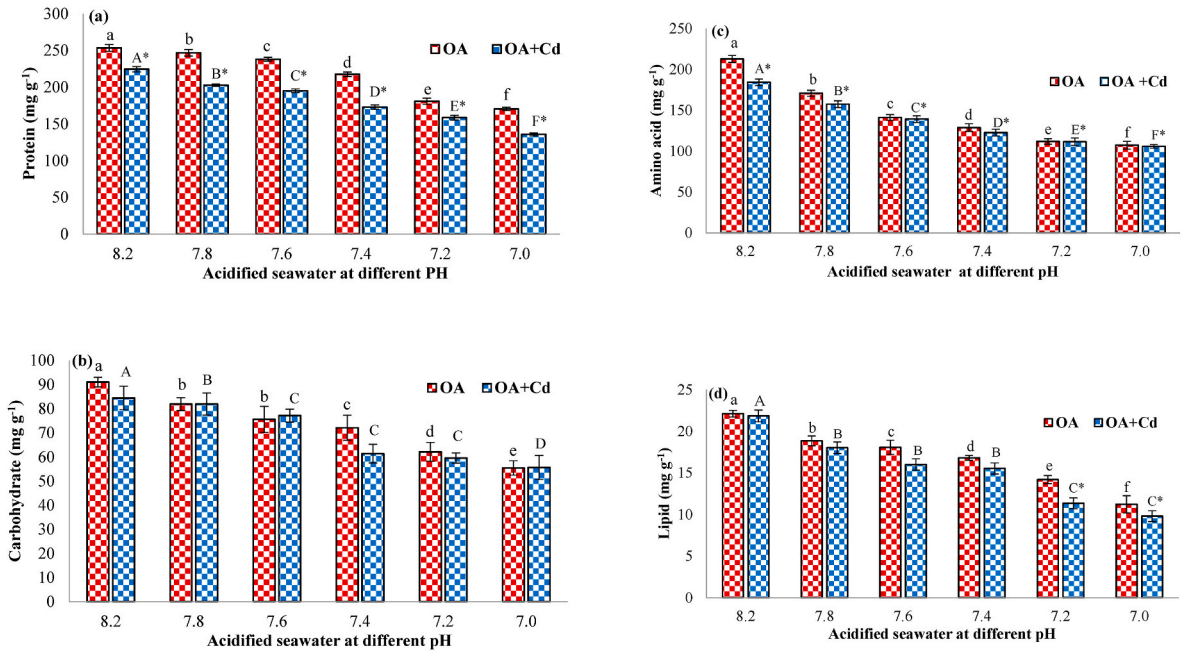


Fig. 3. (a) Protein, (b) Carbohydrate (c) Amino acid and (d) Lipid of *S. serrata* exposed to CO_2 driven acidified seawater with and without Cd. n = 9, mean ± SD; bars sharing different letters are considered as significant at $p < 0.05$ among OA alone (lowercase letters) and OA + Cd (uppercase letters) treatments; * indicates the significant difference between the respective each pH of OA and OA + Cd as per paired sample 't' test.

the gradual increase of Cd accumulation in carbs under all OA and OA + Cd environments indicates that the OA can trigger the bioaccumulation of Cd in *S. serrata*. Exclusion of Cd is a more energy-consuming process in aquatic animals. OA can hinder Cd exclusion by organisms due to high energy demand for physiological maintenance under acidic stress that can cause more bioaccumulation (Roberts et al., 2013). A similar result was observed earlier in crab *Dotilla fenestrate* exposed to the OA + Cd at pH 7.2, 7.4, and 7.6 for 96 h (Adeleke et al., 2020). Accumulation of Cd in the gills and digestive glands of three bivalve species, *Mytilus edulis, Tegillarca granola,* and *Meretrix meretrix* was observed previously at pH 7.8 and 7.6 for 30 days (Shi et al., 2016). Also, the accumulation of Cd in the gills and digestive glands of *Crassostrea gigas* exposed to OA and Cd exposure for 31 days was observed earlier (Cao et al., 2018a).

Crustaceans are connecting links between benthic and pelagic species performing an important role in the marine ecosystem food chain. Many economically important commercial fishes and invertebrates depend on larval forms of crustaceans for their dietary needs. Particularly, decapods are commercially vital for their delicious taste and easy assimilability. Crustaceans are affected by biotic and abiotic factors, which can influence their regular physiological activities. Therefore, crustaceans are considered bioindicators to find the variation in aquatic ecosystems (Flint et al., 2021). Due to numerous anthropogenic activities, marine ecosystems are being polluted, which may lead to critical issues, including acidification and heavy metal contamination. A substantial decline in the survival, length, length gain, weight, weight gain, SGR, and feed intake of the crabs in OA and OA + Cd of this current study indicated that the OA and OA + Cd could produce adverse effects in studied experimental crabs. In this context, the significant decreases in growth and feed intake of crab *S. serrata* under OA + Cd exposure revealed that the effect of OA with heavy metal Cd can produce more adverse effects on crabs than OA alone exposures. In this study, the increased feed conversion ratio of crabs in both OA treatments and OA + Cd treatments matched to ambient seawater and seawater with Cd at pH 8.2 indicates the low nutrient utilization of crabs under these stress environments. However, insignificant differences recorded between OA and OA + Cd experiments indicated that both experiments had similar adverse effects on crabs. The digestive glands of aquatic animals are adversely affected by pH stress, which may cause consequences in appetite and feed intake (Liang et al., 2022). Also, under stressful environments animals suffer from sense feeds that cause poor feeding, assimilation, high metabolic demand, and reduced growth (Heydarnejad et al., 2013; Durant et al., 2023). Likewise, the reduced hatching, survivorship, and growth in Dungeness crab *Cancer magister* and golden king crab *Lithodes aequispinus* when reared at pH 7.1 for 45 days and pH 7.5 for 127 days have been recorded respectively (MillerO et al., 2009; Long et al., 2021). Also, a commendable reduction in the survival , growth and calcification of the Tanner crab *Chionoecetes bairdi* and juvenile red crab *Paralithodes camtschaticus* lived at pH 7.5 has been stated earlier by Long et al. (2013). Moreover, a study observed that acidified seawater and heavy metal toxicity decrease marine organisms' growth and survival in bivalve *Scrobicularia plana* in estuaries (Ivanina and Sokolova, 2015). Increased mortality in bivalves *M. edulis* exposed to pH 6.2 with different heavy metals (Cd, Pb, and Cu) was observed previously, which indicates that the heavy metals under acidified conditions can alleviate metallothionein in soft tissues that can cause high toxicity of heavy metals (Han et al., 2014).

Molting is a vital process in arthropods, which contributes to animal development, expansion, breeding, and regeneration. Both biotic factors (inhibit the molting hormone and development of gonads) (Gong et al., 2015) and abiotic factors (salinity, temperature, light, and nutrients) (Zhao et al., 2015) affect molting in animals. In the present investigation, the significant decrease in crabs' molting suggests that the decreasing seawater pH can reduce the molt formation, followed by poor growth. Acidified seawater can reduce carbonates, decreasing $CaCO_3$, which is essential for forming calcareous shells. In the present study, reductions in the molting rate of crabs exposed to OA + Cd compared to OA alone experiments indicate a more negative effect on studied crabs. OA can suppress chitinolytic enzymes (chitinase and *N*-acetyl β-D-glucosidase), which adversely affects crustaceans' molt regulation (Luo et al., 2015; Chen et al., 2019). Besides, Cd toxicity can stimulate the release of molt inhibition hormone (MIH) in animals, including crustaceans which can affect the molting process (Ortega et al., 2022). Earlier studies also recorded the reduction in molting in shrimp *L. vannamei* (pH 7.8 to 7.0 for 60 days) and hermit crab *Pagurus criniticornis* (pH 7.7 for 120 days) under pCO_2 rich seawater (Turra et al., 2020; Muralisankar et al., 2021). Hence, the present study **is** an evidence for the inhibition of

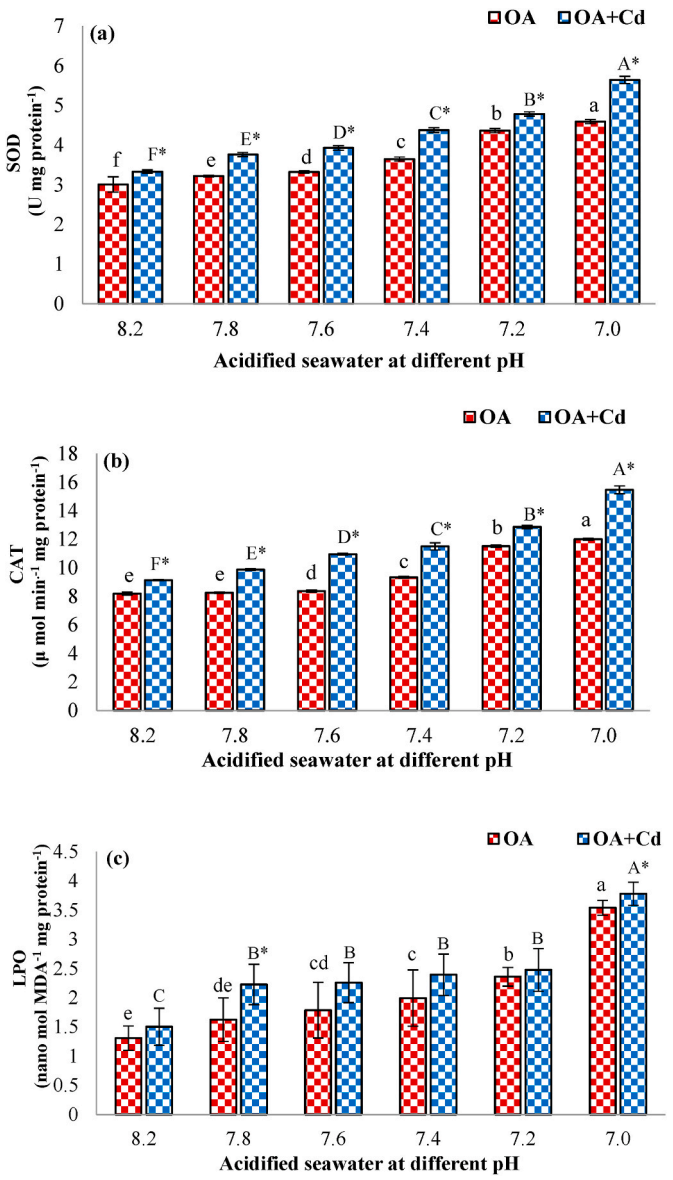


Fig. 4. (a) SOD, (b) CAT, and (c) LPO of *S. serrata* exposed to CO_2 driven acidified seawater. n = 9, mean ± SD; bars sharing different letters are considered as significant at $p < 0.05$ among OA alone (lowercase letters) and OA + Cd (uppercase letters) treatments; * indicates the significant difference between the respective each pH of OA alone and OA + Cd as per paired sample 't' test. SOD, superoxide dismutase; CAT, catalase; LPO, lipid peroxidation.

molting in the crab *S. serrata* under the synergetic toxicity of both OA and Cd.

Biochemical properties have a vital role in the nutritional index of an animal, which influences the physiology and biology of the animal. In the present investigation, the notable drop in biochemical constituents like protein, amino acid, carbohydrate, and lipid of crabs exposed to OA and OA + Cd revealed that the production of biochemical elements might be negatively affected in crabs at OA and OA + Cd environments compared to normal (control) seawater environment. Moreover, the considerable decreases in biochemical elements in crabs exposed to OA + Cd treatments compared to OA treatments alone showed that combining OA with Cd can produce more adverse effects on those biochemical productions. Proteins, carbohydrates, and lipids are the main constituents of animals, which play a significant role in energy metabolism and growth. Animals need high-energy demands under stressful environments that lead to more utilization of biochemical elements for maintaining regular physiological activities. Poor feeding and digestion under both acidic and heavy metal stress (Heydarnejad et al., 2013) leads to decreased protein, carbohydrate, and lipid in experimental crabs. A previous study by Turra et al. (2020) reported a noticeable decline in the lipid content of the hermit crab *P. criniticornis* after being reared at pH 7.70 for 120 days. Similar reductions in total protein, amino acid, lipid, and carbohydrate have been observed in the shrimp *L. vannamei* and the brine shrimp *A. franciscana* when exposed to varying levels of acidified environments (pH 7.8 to 6.8) as reported by Muralisankar et al. (2021) and Thangal et al. (2021). Furthermore, Hsieh et al. (2021) reported a decrease in amino acid content in tiger shrimp *P. monodon* after exposure to pH 7.5 for four weeks. In this context, the present study reveals that the combined adverse effect of OA and heavy metal Cd on tissue biochemical elements of marine carbs. The observation indicates the low nutrient profile of marine edible species under the OA and Cd toxicity.

Antioxidants have a special role in protecting cells. Remarkably, some marine animals can live in a polluted environment due to the shielding response against the scavenger free radicals delivered by the antioxidant cellular defense system (Rani et al., 2013). The antioxidant enzyme SOD converts into free radicle superoxide ion (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2) to protect cells (Jazayeri, 2012). Furthermore, CAT effectively transforms hydrogen peroxide into water and oxygen due to its capacity to eradicate cytotoxic hydrogen peroxide and improve the organism's life span (Arockiaraj et al., 2012). Lipid peroxidation (LPO) is a process that occurs when oxygen free radicals rob electrons from polyunsaturated fatty acids from cell membranes, which initiate a self-replicating chain reaction that is harmful to the viability of cells. It is a sign of free radicals in cells and tissues or oxidative stress (Salaenoi et al., 2015). Antioxidant defense systems of animals could alter by pH and Cd stress and stimulate the production of reactive oxygen species, which cause oxidative stress by reacting with cellular macromolecules like lipids, proteins, and DNA that lead to apoptosis and membrane lipid peroxidation (Liu et al., 2011; Wang et al., 2011). In the present investigation, significant elevation of CAT and SOD of *S. serrata* under acidified seawater suggested that the more amount of antioxidants produced to reduce the toxic reactive oxygen species that produced by acidic and Cd stress. Moreover, the substantial elevation of LPO in crabs under OA and OA + Cd, when matched to control seawater and seawater with Cd at pH 8.2 showed cell membrane damage. H^+ ions are essential for the production of ATP from ADP and inorganic phosphate (Pi) by the enzyme ATP synthase. Under an acidic environment, more availability of H^+ ions enters body fluids through gills. These excess H^+ ions may inhibit the active ion transport of cells that disrupts Na^+/H^+ and Cl^-/HCO_3^- exchanges, thus disturbing the acid-base balance followed by the development of blood acidosis in aquatic animals (Ultsch et al., 1981; Heisler, 1982) and altering the regular cellular metabolism. This altering cellular metabolism might produce more free radicals, elevating crabs' antioxidants. Earlier studies reported that the elevation of SOD, CAT, and LPO in shrimp *L. vannamei* (Muralisankar et al., 2021) and brine shrimp *A. franciscana* (Thangal et al., 2021) exposed in the acidified seawater. The considerable elevation in antioxidants SOD and CAT of crabs exposed to OA + Cd indicates that the seawater acidification with the heavy metal Cd had produced more adverse effects on crabs than the crabs exposed to OA treatments due to these combined stress environments. Similarly, a significant elevation in SOD and CAT has been observed in the Chinese mitten crab, *E. sinensis,* exposed in the acidified seawater (pH 7.8, 7.3, and 6.5) along with Cd (1 mg L^{-1}) for 7, 14, and 21 days (Zhao et al., 2021). Cao et al. (2018a) noted the increment of LPO level in the oyster *C. gigas* exposed to pH 7.8 and 7.6 along with 10 µg L^{-1} of Cd.

The usage and metabolism of nutrients, such as protein and carbohydrates, depend greatly on metabolic enzymes such as GOT and GPT. Massive amounts of transaminase are released into the haemolymph and stressors by tissue cells under liver injury during toxic stress. The increases in the concentration of heavy metals like Cu and Cd, and

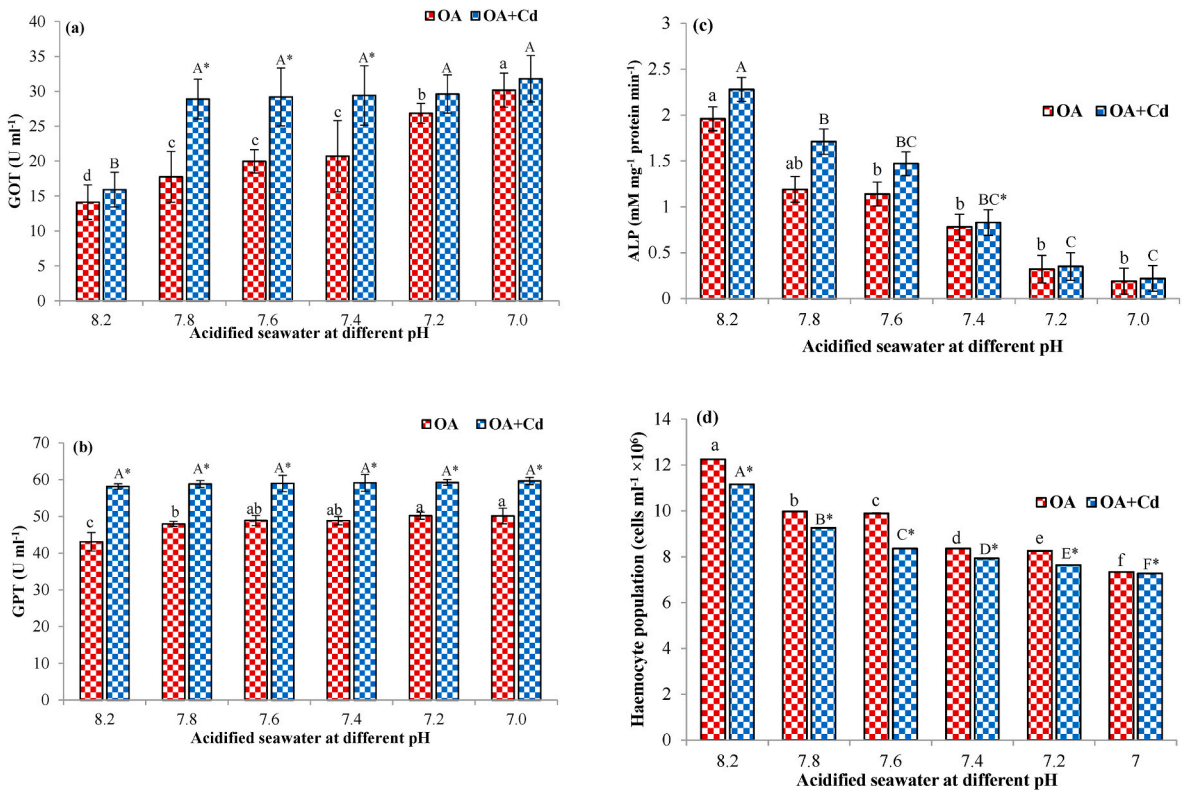


Fig. 5. (a) GOT, (b) GPT, (c) ALP, and (d) haemocyte population of *S. serrata* exposed to CO_2 driven acidified seawater. n = 9, mean ± SD; bars sharing different letters are considered as significant at $p < 0.05$ among OA alone (lowercase letters) and OA + Cd (uppercase letters) treatments; * indicates the significant difference between the respective each pH of OA and OA + Cd as per paired sample 't' test. GOT, Glutamic oxalic transaminase; GPT, Glutamic pyruvic transaminase catalase; ALP, Alkaline phosphate.

decreased water pH can cause energy transformations in animals, including crustaceans, to manage toxic stress, thereby changing GPT and GOT activity (Kim et al., 2021; Thangal et al., 2022). In the OA trial, commendable elevation in crabs subjected to pH 7.8 to 7.0 in both OA alone and OA + Cd treatments reveals the negative impact of these stressors on the hepatopancreas of crabs. Further, significant improvements in GOT and GPT of crabs treated with OA + Cd compared to OA alone treatment show these two stressors' synergistic effect on the experimental crab due to high energy requirement under this stressful environment. The previous studies publicized the increment of GOT and GPT activity in the brine shrimp *A. franciscana* and shrimp *L. vannamei* exposed to the acidified seawater (Muralisankar et al., 2021; Thangal et al., 2021). Therefore, the present study indicates the adverse combined effect of OA and Cd heavy metal on the metabolic enzyme activity of *S. serrata.*

Alkaline phosphatases are present in several living organisms. They are essential for several vital processes, including DNA synthesis, protein synthesis, carbohydrate metabolism, bone calcification, and the reduction of inflammatory responses by the innate immune system. They also hydrolyze various physiological substances, including phosphate. Alkaline phosphatase (ALP) is strongly active in the cells of mineralized tissue and is essential for developing hard tissue (Vimalraj, 2020). In the current study, a significant decrease in ALP at OA and OA + Cd treatments when matched to the crabs reared in normal seawater pointed out the unfavorable impact of OA and Cd on crabs that lead to poor physiological activities like decreased acid-base balance, biomineralization, metabolism, innate immune system, and growth. A similar result was observed in crab *S. serrata*, *Cardiosoma armatum*, and *Tachypleus tridentatus* exposed to the acidified seawater (Lawal-Are et al., 2021; Liu et al., 2022; Thangal et al., 2022). ALP is a nonspecific enzyme that hydrolyzes various phosphate-containing compounds and considerable changes in ALP in animals indicate abnormal hepatic activity and liver damage under the stress environment (Zhang et al., 2018).

Hematological indicators like total blood cells can be used to determine an organism's physiological and immune state. Three types of blood cells are present in green crabs' haemolymph: hyaline cells and semi and large-granule haemocytes. Hyaline cells are naturally small and have a high nucleocytoplasmic ratio and thin cytoplasmic granule content. Semi and large granule haemocytes are distinguished by the dominance of small and large electron-thick granules in their cytoplasm (Clare and Lumb, 1994). The haemocytes of crustaceans play a critical function in host immune responses, including identifying pathogens, melanization, phagocytosis, cytotoxicity, and cell communication (Johansson et al., 2000). In the present investigation, the considerable declining level of haemocyte populations of studied crabs in OA + Cd reveals the detrimental effect compared to crabs reared in OA alone experiment. It has been assumed that under a low pH environment, acidosis may occur in the crabs' haemolymph, which seems to alter the physiology of haemocytes and cell death by apoptotic processes (Meseck et al., 2016). This present outcome study is consistent with previous studies in *L. vannamei*, which showed that the count of blood cell populations was reduced once shrimp were reared in CO_2 driven acidic seawater (pH 7.8, 7.6, 7.4, 7.2, and 7.0) for 60 days (Muralisankar et al., 2021). An earlier study denotes that OA, acidification, and Cd exposure in Pacific oyster *C. gigas* have decreased the number of haemocytes in lower pH (7.8 and 7.6 for 31 days) compared to the control (Cao et al., 2018b).

5. Conclusion

The present study revealed the detrimental effect of OA (pH 7.8 to 7.0) with Cd (0.01 mg L^{-1}) on growth, tissue biochemical elements, and haemocytes population with signs of considerable improvements in stress biomarkers (antioxidants and metabolic enzymes) and Cd

accumulation in *S. serrata*. Moreover, the interaction effects of OA and Cd significantly affect the growth, protein, amino acid, and haemocytes population of *S. serrata,* which indicates the synergistic activities of these two stressors on the studied crab species. Hence, the present study suggests the toxicity of Cd can be higher in the mud crab *S. serrata* instars under decreased ocean pH. Nonetheless, the combined effect of OA and Cd showed minimal parameters, therefore, it indicates the selected Cd dose was minimal for this crab species, and future studies are required to study the effect of OA on the environmental level of Cd on marine crabs. Besides, the biochemical and molecular pathway of the interactive effect of OA and Cd in *S. serrata* also needs to the studied further.

CRediT authorship contribution statement

Said Hamid Thangal: Investigation, methodology, writing – review & editing. **Ramamoorthy Nandhinipriya:** Investigation, sample preparation and data curation. **Chandrasekaran Vasuki:** Investigation, sample preparation and data curation. **Velusamy Gayathri:** Sample preparation. **Krishnan Anandhan:** Formal analysis and graphical illustration. **Arumugam Yogeshwaran:** Sample preparation. **Thirunavukkarasu Muralisankar:** Funding acquisition, supervision, draft editing. **Mathan Ramesh:** Draft editing. **Rajendran Rajaram:** Sample preparation, formal analysis. **Perumal Santhanam:** Draft editing. **Balu Alagar Venmathi Maran:** Draft 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

The authors are thankful to the Department of Science and Technology (DST), Government of India, New Delhi for the infrastructure support to the Department of Zoology, Bharathiar University in the form of the DST-FIST programme (SR/FST/LS-II/2017/111 (c); dt.: January 25, 2019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.140447.

References

Adeleke, B., Andersson, R.D., Moodley, G., 2020. The effects of near-future coastal acidification on the concentrations of Cd and Pb in the crab *Dotilla fenestrata*. Heliyon 6 (9), e04744. https://doi.org/10.1016/j.heliyon.2020.e04744.

Alava, V.R., Sumile, J.D., Parado-Estepa, F.D., 2017. Nursery culture of mud crab Scylla serrata using different feeding rates. In: Quinitio, E.T., Parado-Estepa, F.D., Coloso, R.M. (Eds.), Conference Paper. Aquaculture department, Southeast Asian Fisheries Development Center. http://hdl.handle.net/10862/3199.

Anand, M., Rangesh, K., Maruthupandy, M., Jayanthi, G., Rajeswari, B., Priya, R.J., 2021. Effect of CO_2 driven ocean acidification on calcification, physiology and ovarian cells of tropical sea urchin *Salmacis virgulata*–A microcosm approach. Heliyon 7 (1), e05970. https://doi.org/10.1016/j.heliyon.2021.e05970.

APHA (American Public Health Association), 2005. Standard Methods for the Examination of Water and Wastewater, twenty-first ed.

Arockiaraj, J., Easwvaran, S., Vanaraja, P., Singh, A., Othman, R.Y., Bhassu, S., 2012. Molecular cloning, characterization and gene expression of an antioxidant enzyme catalase (MrCat) from *Macrobrachium rosenbergii*. Fish Shellfish Immunol. (5), 670–682. https://doi.org/10.1016/j.fsi.2012.01.013.

Arumugam, G., Rajendran, R., Shanmugam, V., Sethu, R., Krishnamurthi, M., 2018. Flow of toxic metals in food-web components of tropical mangrove ecosystem, Southern India. Hum Ecol Risk Assess. Int. J. 24 (5), 1367–1387. https://doi.org/10.1080/10807039.2017.1412819.

Barker, S., Ridgwell, A., 2012. Ocean acidification. Nature Education Knowledge 3 (10), 21. https://www.nature.com/scitable/knowledge/library/ocean-acidification-25822734.

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total' lipids. J. Exp. Mar. Biol. Ecol. 12 (1), 103–118. https://doi.org/10.1016/0022-0981(73)90040-3.

Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A., Dar, S.A., 2020. Concerns and threats of contamination on aquatic ecosystems. Bioremediat. Biotechnol. 1–26. https://doi.org/10.1007/978-3-030-35691-0_1.

Caldecott, J., 2008. Water: Life in Every Drop. Random House.

Caldeira, K., Wickett, M.E., 2003. Anthropogenic carbon and ocean pH. Nature 425 (6956), 365. https://doi.org/10.1038/425365a.

Campbell, A.L., Levitan, D.R., Hosken, D.J., Lewis, C., 2016. Ocean acidification changes the male fitness landscape. Sci. Rep. 6 (1), 1–8. https://doi.org/10.1038/srep31250.

Cao, R., Liu, Y., Wang, Q., Dong, Z., Yang, D., Liu, H., Zhao, J., 2018a. Seawater acidification aggravated cadmium toxicity in the oyster *Crassostrea gigas*: metal bioaccumulation, subcellular distribution and multiple physiological responses. Sci. Total Environ. 642, 809–823. https://doi.org/10.1016/j.scitotenv.2018.06.126.

Cao, R., Liu, Y., Wang, Q., Zhang, Q., Yang, D., Liu, H., Qu, Y., Zhao, J., 2018b. The impact of ocean acidification and cadmium on the immune responses of Pacific oyster, *Crassostrea gigas*. Fish Shellfish Immunol. 81, 456–462. https://doi.org/10.1016/j.fsi.2018.07.055.

Chen, H., Gu, X., Zeng, Q., Mao, Z., Liang, X., Martyniuk, C.J., 2019. Carbamazepine disrupts molting hormone signaling and inhibits molting and growth of *Eriocheir sinensis* at environmentally relevant concentrations. Aquat. Toxicol. 208, 138–145. https://doi.org/10.1016/j.aquatox.2019.01.010.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Thornton, P., 2014. Carbon and other biogeochemical cycles. In: Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, pp. 465–570.

Clare, A.S., Lumb, G., 1994. Identification of haemocytes and their role in clotting in the blue crab, *Callinectes sapidus*. Mar. Biol. 118 (4), 601–610. https://doi.org/10.1007/BF00347507.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.L., Fichefet, T., Friedlingstein, P., Booth, B.B., 2013. Long-term climate change: projections, commitments and irreversibility. In: Climate Change 2013-The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, pp. 1029–1136.

Dodd, L.F., Grabowski, J.H., Piehler, M.F., Westfield, I., Ries, J.B., 2015. Ocean acidification impairs crab foraging behaviour. Proc. Royal Soc. B 282 (1810), 333. https://doi.org/10.1098/rspb.2015.0333.

Doney, S.C., Busch, D.S., Cooley, S.R., Kroeker, J., J, K., 2020. The impacts of ocean acidification on marine ecosystems and reliant human communities. Annu. Rev. Environ. Resour. 45, 83–112. https://doi.org/10.1146/annurev-environ-012320-083019.

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO_2 problem. Ann. Rev. Mar. Sci 1, 169–192. https://doi.org/10.1146/annurev.marine.010908.163834.

Durant, A., Khodikian, E., Porteus, C.S., 2023. Ocean acidification alters foraging behaviour in Dungeness crab through impairment of the olfactory pathway. Global Change Biol. 29 (14), 4126–4139. https://doi.org/10.1111/gcb.16738.

Dutta, H., Dutta, A., 2016. The microbial aspect of climate change. Energy Ecol. Environ. 1 (4), 209–232. https://doi.org/10.1007/s40974-016-0034-7.

El-Kahawy, R., El-Shafeiy, M., Helal, S., Aboul-Ela, N., AbdEl-Wahab, M., 2021. Correction to: benthic ostracods (crustacean) as a nearshore pollution bio-monitor: examples from the red sea coast of Egypt. Environ. Sci. Pollut. Res. 31994–31995. https://doi.org/10.1007/s11356-021-13374-y.

FAO, 2022. The State of World Fisheries and Aquaculture. https://www.fao.org/3/ca9229en/ca9229en.pdf.

Fitzer, S.C., Cusack, M., Phoenix, V.R., Kamenos, N.A., 2014. Ocean acidification reduces the crystallographic control in juvenile mussel shells. J. Struct. Biol. 188 (1), 39–45. https://doi.org/10.1016/j.jsb.2014.08.007.

Flint, N., Anastasi, A., De Valck, J., Chua, E.M., Rose, A.K., Jackson, E.L., 2021. Using mud crabs *Scylla serrata* as environmental indicators in a harbour health report card. Australas. J. Environ. Manag. 28 (2), 188–212. https://doi.org/10.1080/14486563.2021.1923579.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. boil. Chem 226 (1), 497–509.

Ganesh, K., Raj, Y.C.T.S., Perumal, S., Srinivasan, P., Sethuramalingam, A., 2015. Breeding, larval rearing and farming of mangrove crab, *Scylla serrata* (Forskal, 1775). In: Perumal, S., T, A.R., Pachiappan, P. (Eds.), Advances in Marine and Brackishwater Aquaculture. https://doi.org/10.1007/978-81-322-2271-2_14.

Gong, J., Ye, H., Xie, Y., Yang, Y., Huang, H., Li, S., Zeng, C., 2015. Ecdysone receptor in the mud crab *Scylla paramamosain*: a possible role in promoting ovarian development. J. Endocrinol. 224, 273–287. https://doi.org/10.1530/JOE-14-0526.

Götze, S., Matoo, O.B., Beniash, E., Saborowski, R., Sokolova, I.M., 2014. Interactive effects of CO_2 and trace metals on the proteasome activity and cellular stress response of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Aquat. Toxicol. 149, 65–82. https://doi.org/10.1016/j.aquatox.2014.01.027.

Han, Z.X., Wu, D.D., Wu, J., Lv, C.X., Liu, Y.R., 2014. Effects of ocean acidification on toxicity of heavy metals in the bivalve *Mytilus edulis L*. Syn. React. Inorg. Metaorg. Nanometal. Chem. 44 (1), 133–139. https://doi.org/10.1080/15533174.2013.770753.

Heisler, N., 1982. Transepithelial ion transfer processes as mechanisms for fish acid–base regulation in hypercapnia and lactacidosis. Can. J. Zool. 60 (5), 1108–1122. https://doi.org/10.1139/z82-155.

Heydarnejad, M.S., Hemamai, M.K., Nematollahi, A., 2013. Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout *Oncorhynchus mykiss*. Ir. Vet. J. 20 (1), 11. https://doi.org/10.1186/2046-0481-66-11.

Hoegh-Guldberg, O., 2014. Coral reef sustainability through adaptation: glimmer of hope or persistent mirage? Curr. Opin. Environ. Sustain. 7, 127–133. https://doi.org/10.1016/j.cosust.2014.01.005.

Hoegh-Guldberg, O., Poloczanska, E.S., Skirving, W., Dove, S., 2017. Coral reef ecosystems under climate change and ocean acidification. Front. Mar. Sci. 4, 158. https://doi.org/10.3389/fmars.2017.00158.

Hsieh, H.H., Weerathunga, V., Weerakkody, W.S., Huang, W.J., Muller, F.L.L., Benfield, M.C., Hung, C.C., 2021. The effects of low pH on the taste and amino acid composition of tiger shrimp. Sci. Rep. 11 (1), 1–9. https://doi.org/10.1038/S41598-021-00612-Z.

IPCC, 2014. Climate Change 2014 Synthesis Report Summary for Policymaker. https://www.ipcc.ch/site/assets/uploads/2018/02/AR5_SYR_FINAL_SPM.pdf.

Ivanina, A.V., Sokolova, I.M., 2015. Interactive effects of metal pollution and ocean acidification on physiology of marine organisms. Curr. Zool. 61, 653–668. https://doi.org/10.1093/czoolo/61.4.653.

Jazayeri, A., 2012. The importance of antioxidants with the marine origin in inhibit free radicals. Life Sci. 9 (2), 1128–1132 w.lifesciencesite.com.

Johansson, M.W., Keyser, P., Sritunyalucksana, K., Söderhäll, K., 2000. Crustacean haemocytes and haematopoiesis. Aqua (Oxf.) 191 (1–3), 45–52. https://doi.org/10.1016/S0044-8486(00)00418-X.

Keenan, C., Davie, P.J., Mann, D.L., 1998. A revision of the genus *Scylla* de Haan, 1833 (Crustacea: Decapoda: Brachyura: portunidae). Raffles Bull. Zool. 46 (1), 217–245.

Khan, Z., Elahi, A., Bukhari, D.A., Rehman, A., 2022. Cadmium sources, toxicity, resistance and removal by microorganisms-A potential strategy for cadmium eradication. J. Saudi Chem. Soc. 26, 101569 https://doi.org/10.1016/j.jscs.2022.101569.

Kim, J.H., Kim, S.R., Kim, S.K., Kang, H.W., 2021. Effects of pH changes on blood physiology, antioxidant responses and Ig M of juvenile olive flounder, Paralichthys olivaceus. Aquac. Rep. 21, 100790 https://doi.org/10.1016/j.aqrep.2021.100790.

Kim, J.M., Farmer, D.F., Bertram, T.H., 2014. A controlling role for the air−sea interface in the chemical processing of reactive nitrogen in the coastal marine boundary layer. Proc. Natl. Acad. Sci. USA 111, 3943–3948. www.pnas.org/cgi/doi/10.1073/pnas.1318694111.

Kind, P.R., King, E.J., 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. J. Clin. Pathol. 7 (4), 322–326.

Lacoue-Labarthe, T., Martin, S., Oberhänsli, F., Teyssié, J.-L., Markich, S., Jeffree, R., Bustamante, P., 2009. Effects of increased pCO_2 and temperature on trace element (Ag, Cd and Zn) bioaccumulation in the eggs of the common cuttlefish. Sepia officinalis, Biogeoscinces 6, 2561–2573. https://doi.org/10.5194/bg-6-2561-2009.

Landrigan, P.L., Stegeman, J.J., Fleming, L.E., Allemand, D., Anderson, D.M., Backer, L. C., Brucker-Davis, F., Chevalier, N., Corra, L., Czerucka, D., Bottein, M.D., Demeneix, B., Depledge, M., Deheyn, D.D., Dorman, C.J., Fénichel, P., Fisher, S., Gaill, F., Galgani, F., Gaze, W.H., Giuliano, L., Grandjean, P., Hahn, M.E., Hamdoun, A., Hess, P., Judson, B., Laborde, A., McGlade, J., Mu, J., Mustapha, A., Neira, M., Noble, R.T., Pedrotti, M.L., Reddy, C., Rocklöv, J., Scharler, U.M., Shanmugam, H., Taghian, G., J Water, J.A.J., V D, M., Vezzulli, L., Weihe, P., Zeka, A., Raps, H., Rampal, P., 2020. Human health and ocean pollution. Ann. Glob. Health 86 (1), 1–64. https://doi.org/10.5334/aogh.2831.

Lawal-Are, A.O., Moruf, R.O., Olaniyi, I.O., Okafor, D.S., 2021. Effects of water acidification on immune responses of the gercacinid, *Cardiosoma armatum* (Herklots, 1851). Nig. J. Pure. Appl. Sci. 34 (2), 4006–4013. https://doi.org/10.48198/NJPAS/21.B01.

Lemasson, A.J., Fletcher, S., Hall-Spencer, J.M., Knights, A.M., 2017. Linking the biological impacts of ocean acidification on oysters to changes in ecosystem services: a review. J. Exp. Mar. Biol. Ecol. 492, 49–62. https://doi.org/10.1016/j.jembe.2017.01.019.

Liu, D., Yan, B., Yang, J., Lei, W., Wang, L., 2011. Mitochondrial pathway of apoptosis in the hepatopancreas of the freshwater crab *Sinopotamon yangtsekiense* exposed to cadmium. Aquat. Toxicol. 105, 394–402. https://doi.org/10.1016/j.aquatox.2011.07.013.

Liu, X., Liu, J., Xiong, K., Zhang, C., Fang, J.K.H., Song, J., Tai, Z., Zhu, Q., Hu, M., Wang, Y., 2022. Effects of ocean acidification on molting, oxidative stress, and gut microbiota in juvenile Horseshoe crab *Tachypleus tridentatus*. Front. Physiol. 12, 813582 https://doi.org/10.3389/fphys.2021.813582.

Long, W.C., Swiney, K.M., Foy, R.J., 2021. Effects of ocean acidification on young of the year golden king crab *Lithodes aequispinus* survival and growth. Mar. Biol. 168 (8), 1–11. https://doi.org/10.1007/s00227-021-03930-y.

Long, W.C., Swiney, K.M., Harris, C., Page, H.N., Foy, R.J., 2013. Effects of ocean acidification on juvenile red king crab *Paralithodes camtschaticus* and Tanner crab *Chionoecetes bairdi* growth, condition, calcification, and survival. PLoS One 8 (4), e60959. https://doi.org/10.1371/journal.pone.0060959.

Long, W.C., Van Sant, S.B., Swiney, K.M., Foy, R.J., 2017. Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time. ICES J. Mar. Sci. 74 (4), 1033–1041. https://doi.org/10.1093/icesjms/fsw197.

Lowry, O., Rosebrough, N., Farr, A.L., Randall, R., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.

Luo, J., Pei, S., Jing, W., Zou, E., Wang, L., 2015. Cadmium inhibits molting of the freshwater crab Sinopotamon henanense by reducing the hemolymph ecdysteroid content and the activities of Chitinase and N-acetylbeta- glucosaminidase in the epidermis. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 169, 1–6. https://doi.org/10.1016/j.cbpc.2014.10.003.

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47 (3), 469–474.

Meng, P.Y., Guo, Z., Yao, H., Yeung, K.W.K., Thiyagarajan, V., 2019. Calcium carbonate unit realignment under acidification: a potential compensatory mechanism in an edible estuarine oyster. Mar. Pollut. Bull. 139, 141–149. https://doi.org/10.1016/j.marpolbul.2018.12.030.

Meseck, S.L., Alix, J.H., Swiney, K.M., Long, W.C., Wikfors, G.H., Foy, R.J., 2016. Ocean acidification affects hemocyte physiology in the tanner crab *Chionoecetes bairdi*. PLoS One 11 (2), e0148477. https://doi.org/10.1371/journal.pone.0148477.

MillerO, F.J., Woosley, R., DiTrolio, B., Waters, J., 2009. Effect of ocean acidification on the speciation of metals in seawater. Oceanography 22 (4), 72–85. http://www.jstor.org/stable/24861025.

Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T.B., Nainu, F., Khusro, A., Idris, A.M., Khandaker, M.U., Osman, H., Alhumaydhi, F.A., Simal-Gandara, J., 2022. Impact of heavy metals on the environment and human health: novel therapeutic insights to counter the toxicity. J. King Saud Univ. Sci. 34, 101865 https://doi.org/10.1016/j.jksus.2022.101865.

Moore, S., Stein, W.H., 1948. Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem. 176 (1), 367–388.

Muralisankar, T., Kalaivani, P., Thangal, S.H., Santhanam, P., 2021. Growth, biochemical, antioxidants, metabolic enzymes and hemocytes population of the shrimp *Litopenaeus vannamei* exposed to acidified seawater. Comp. Biochem. Physiol., C: Toxicology 239, 108843. https://doi.org/10.1016/j.cbpc.2020.108843.

NOAA, 2020. Was earth's 2nd-hottest year, just behind 2016.". NOAA 2021. https://www.noaa.gov/news/2020-was-earth-s-2nd-hottest-year-just-behind-2016.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 (2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Ortega, P., Vitorino, H.A., Green, S., Zanotto, F.P., Chung, J.S., Moreira, R.G., 2022. Experimental effects of cadmium on physiological response of Callinectes danae (Crustacea, Portunidae) from environments with different levels of Cd contamination. Comp. Biochem. Physiol., C 251, 109210. https://doi.org/10.1016/j.cbpc.2021.109210.

Paterson, B.D., Mann, D.L., 2011. Mud crab aquaculture. In: Fotedar, R.K., Philips, B.F. (Eds.), In Recent Advance and New Species in Aquaculture. Wiley-Blackwell, Oxford, pp. 115–135.

Pinzón-Bedoya, C.H., Pinzón-Bedoya, M.L., Pinedo-Hernández, J., Urango-Cardenas, I., Marrugo-Negrete, J., 2020. Assessment of potential health risks associated with the intake of heavy metals in fish harvested from the largest estuary in Colombia. Int. J. Environ. Res. Publ. Health 17 (8), 2921. https://doi.org/10.3390/ijerph17082921.

Quéré, L.C., Andrew, R.M., Friedlingstein, P., Sitch, S., Hauck, J., Pongratz, J., Le Quéré, C., Andrew, R.M., Friedlingstein, P., Sitch, S., Hauck, J., Pongratz, J., Pickers, P.A., Korsbakken, J.I., Peters, G.P., Canadell, J.G., Arneth, A., Arora, V.K., Barbero, L., Bastos, A., Bopp, L., Chevallier, F., Chini, L.P., Ciais, P., Doney, S.C., Gkritzalis, T., Goll, D.S., Harris, I., Haverd, V., Hoffman, F.M., Hoppema, M., Houghton, R.A., Hurtt, G., Ilyina, T., Jain, A.K., Johannessen, T., Jones, C.D., Kato, E., Keeling, R.F., Goldewijk, K.K., Landschützer, P., Lefèvre, N., Lienert, S., Liu, Z., Lombardozzi, D., Metzl, N., Munro, D.R., Nabel, J.E.M.S., Nakaoka, S., Neill, C., Olsen, A., Ono, T., Patra, P., Peregon, A., Peters, W., Peylin, P., Pfeil, B., Pierrot, D., Poulter, B., Rehder, G., Resplandy, L., Robertson, E., Rocher, M., Rödenbeck, C., Schuster, U., Schwinger, J., Séférian, R., Skjelvan, I., Steinhoff, T., Sutton, A., Tans, P.P., Tian, H., Tilbrook, B., Tubiello, F.N., Laan-Luijkx, I.T.V.D., Werf, G.R.V.D., Viovy, N., Walker, A.P., Wiltshire, A.J., Wright, R., Zaehle, S., Zheng, B., 2018. Global carbon budget 2018. Earth Syst. Sci. Data 10 (4), 2141–2194. https://doi.org/10.5194/essd-10-2141-2018.

Rajan, K.C., Li, Y., Dang, X., Lim, Y.K., Suzuki, M., Lee, S.W., Vengatesen, T., 2023. Directional Fabrication and Dissolution of Larval and Juvenile Oyster Shells under Ocean Acidification, vol. 290, p. 20221216. https://doi.org/10.1098/rspb.2022.1216.

Rani, S.D., Kavitha, R., Padmaja, M., 2013. Histological and biochemical changes in reproductive organs of mud crab *Scylla olivacea* (Herbst, 1796) exposed to cadmium nanoparticle. J. Acad. Ind. Res. 2 (7), 391–396, 2278-5213.

Rehman, A., Ma, H., Ozturk, I., Murshed, M., Dagar, V., 2021. The dynamic impacts of CO_2 emissions from different sources on Pakistan's economic progress: a roadmap to sustainable development. Environ. Dev. Sustain. 23 (12), 17857–17880. https://doi.org/10.1007/s10668-021-01418-9.

Reitman, M.D.S., Frankel, A.S., 1957. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56–63. https://doi.org/10.1093/ajcp/28.1.56.

Revathi, P., Vasanthi, L.A., Munuswamy, N., 2011. Effect of cadmium on the ovarian development in the freshwater prawn *Macrobrachium rosenbergii* (De Man). Ecotoxicol. Environ. Saf. 74 (4), 623–629. https://doi.org/10.1016/j.ecoenv.2010.08.027.

Riebesell, U., Fabry, V.J., Lina, H., Jean-Pierre, G., 2010. Guide to Best Practices for Ocean Acidification Research and Data Reporting. Publications office of the European Union, Luxembourg, p. 258.

Robbins, L.L., Hansen, M.E., Kleypas, J.A., Meylan, S.C., 2010. CO_2calc: a user friendly carbon calculator for Windows, Mac OS X and iOS (iPhone). USA Geol. Surv. 1280, 1210–1280.

Roberts, D.A., Birchenough, S.N.R., Lewis, C., Sanders, M.B., Bolam, T., Sheahan, D., 2013. Ocean acidification increases the toxicity of contaminated sediments. Global Change Biol. 19 (2013), 340–351. https://doi.org/10.1111/gcb.12048.

Roe, J.H., 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. 212 (1), 335–343.
Salaenoi, J., Setthamongkol, P., Srimeetian, P., Satapornvanit, K., 2015. Lipid peroxidation and antioxidant activity in molting stages of mud crab *Scylla serrata*. Agric. Nat. Resour. 49 (3), 422–432. https://li01.tci-thaijo.org/index.php/anres/article/view/243585.
Shi, W., Zhao, X., Han, Y., Che, Z., Chai, X., Liu, G., 2016. Ocean acidification increases cadmium accumulation in marine bivalves: a potential threat to seafood safety. Sci. Rep. 6 (1), 1–8. https://doi.org/10.1038/srep20197.
Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem. 47 (2), 389–394. https://doi.org/10.1016/0003-2697(72)90132-7.
Su, Y.Q., Zhao, Y.J., Zhang, W.J., Chen, G.C., Qin, H., Qiao, D.R., Chen, Y.E., Cao, Y., 2020. Removal of mercury (II), lead (II) and cadmium (II) from aqueous solutions using *Rhodobacter sphaeroides* SC01. Chemosphere 243, 125166. https://doi.org/10.1016/j.chemosphere.2019.125166.
Sun, M., Ting Li, Y., Liu, Y., Chin Lee, S., Wang, L., 2016. Transcriptome assembly and expression profiling of molecular responses to cadmium toxicity in hepatopancreas of the freshwater crab *Sinopotamon henanense*. Sci. Rep. 6 (1), 1–14. https://doi.org/10.1038/srep19405.
Tang, D., Guo, H., Shi, X., Wang, Z., 2019. Comparative transcriptome analysis of the gills from the Chinese mitten crab *Eriocheir japonica sinensis* exposed to the heavy metal Cadmium. Turk. J. Fish. Aquat. Sci. 20 (6), 467–479. https://doi.org/10.4194/1303-2712-v20_6_05.
Tasoff, A.J., Johnson, D.W., 2019. Can larvae of a marine fish adapt to ocean acidification? Evaluating the evolutionary potential of California grunion *Leuresthes tenuis*. Evol. Appl. 12 (3), 560–571. https://doi.org/10.1111/eva.12739.
Thangal, S.H., Muralisankar, T., Anandhan, K., Gayathri, V., Yogeshwaran, A., 2022. Effect of CO2 driven ocean acidification on the mud crab *Scylla serrata* instars. Environ. Pollut. 312, 119995 https://doi.org/10.1016/j.envpol.2022.119995.
Thangal, S.H., Nivetha, M., Muttharasi, C., Anandhan, K., Muralisankar, T., 2021. Effects of acidified seawater on biological and physiological responses of *Artemia franciscana*. Mar. Pollut. Bull. 169, 112476 https://doi.org/10.1016/j.marpolbul.2021.112476.
Thévenod, F., 2009. Cadmium and cellular signaling cascades: to be or not to be? Toxicol. Appl. Pharmacol. 238 (3), 221–239. https://doi.org/10.1016/j.taap.2009.01.013.
Triollet, R., Francisco, A.M.A., 2020. Luxembourg, 2021. In: James, K. (Ed.), Publications Office of the European Union. https://doi.org/10.2760/503158. JRC123071.
Turra, A., Ragagnin, M.N., McCarthy, I.D., Fernandez, W.S., 2020. The effect of ocean acidification on the intertidal hermit crab *Pagurus criniticornis* is not modulated by cheliped amputation and sex. Mar. Environ. Res. 153, 104794 https://doi.org/10.1016/j.marenvres.2019.104794.
Ultsch, G.R., Ott, M.E., Heisler, N., 1981. Acid-base and electrolyte status in carp Cyprinus carpio exposed to low environmental pH. J. Exp. Biol. 93 (1), 65–80. https://doi.org/10.1242/jeb.93.1.65.
Vimalraj, S., 2020. Alkaline phosphatase: structure, expression and its function in bone mineralization. Gene 754, 144855. https://doi.org/10.1016/j.gene.2020.144855.
Vinothkannan, A., Charles, P.E., Rajaram, R., 2022. Ecological risk assessment and seasonal variation of heavy metals in water and sediment collected from industrially polluted Cuddalore coast, Southeastern India. Reg. Stud. Mar. Sci. 49, 102134 https://doi.org/10.1016/j.rsma.2021.102134.
Wang, L., Xu, T., Lei, W.W., Liu, D.M., Li, Y.J., Xuan, R.J., Ma, J.J., 2011. Cadmium-induced oxidative stress and apoptotic changes in the testis of freshwater crab, *Sinopotamon henanense*. PLoS One 6 (11), e27853. https://doi.org/10.1371/journal.pone.0027853.
Whiteley, N.M., 2011. Physiological and ecological responses of crustaceans to ocean acidification. Mar. Ecol. Prog. Ser. 430, 257–271.
Wurl, O., Ekau, W., Landing, W.M., Zappa, C.J., 2017. Sea surface microlayer in a changing ocean – a perspective. Elem. Sci. Anth. 5, 31. https://doi.org/10.1525/elementa.228.
Zhang, E., Dong, S., Wang, F., Tian, X., Gao, Q., 2018. Effects of l-tryptophan on the growth, intestinal enzyme activities and non-specific immune response of sea cucumber (*Apostichopus japonicus* Selenka) exposed to crowding stress. Fish Shellfish Immunol. 75, 158–163. https://doi.org/10.1016/j.fsi.2018.01.009.
Zhao, J., Wen, X., Li, S., Zhu, D., Li, Y., 2015. Effects of dietary lipid levels on growth, feed utilization, body composition and antioxidants of juvenile mud crab *Scylla paramamosain* (Estampador). Aqua (Oxf.) 435, 200–206. https://doi.org/10.1016/j.aquaculture.2014.09.018.
Zhao, X., Yang, Z., Cheng, Y., 2021. Effects of cadmium alone and in combination with pH on bioaccumulation, tissue structure, and enzyme activity of the Chinese mitten crab, *Eriocheir sinensis*. Comp. Biochem. Physiol., C: Toxicology 245, 109025. https://doi.org/10.1016/j.cbpc.2021.109025.