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The impact of settleable atmospheric particulate on the energy metabolism, biochemical processes, and behavior of a sentinel mangrove crab

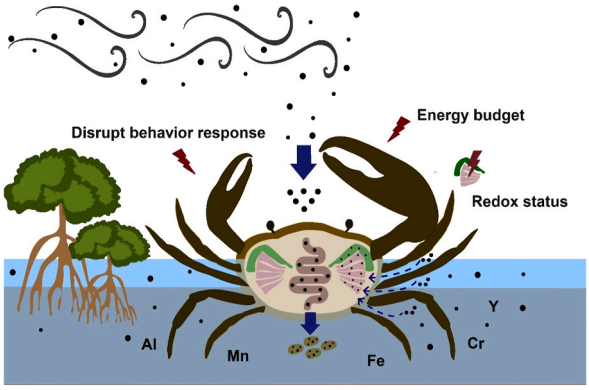
Anieli C. Maraschi [a], Cesar Rubio-Lopez [b], Solana M. Snitman [c], Iara C. Souza [a], Brian Pichardo-Casales [d], Guillermina Alcaraz [e], Magdalena V. Monferrán [f], Daniel A. Wunderlin [f], Claudia Caamal-Monsreal [g], Carlos Rosas [g], Marisa N. Fernandes [a], Mariana V. Capparelli [d,*]

[a] Department of Physiological Sciences, Federal University of São Carlos, Rod Washington Luis km 235, 13565–905 São Carlos, SP, Brazil
[b] Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Av. Ciudad Universitaria 3000, C.P. 04510 Coyoacán, Ciudad de México, Mexico
[c] IIMyC: Instituto de Investigaciones Marinas y Costeras, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-Universidad Nacional de Mar del Plata (UNMdP), Funes 3350, 7600 Mar del Plata, Argentina
[d] Estación El Carmen, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Carretera Carmen-Puerto Real km 9.5, 24157 Ciudad del Carmen, Mexico
[e] Laboratorio de Ecofisiología Animal, Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Av. Ciudad Universitaria 3000, C.P. 04510 Coyoacán, Ciudad de México, Mexico
[f] ICYTAC: Instituto de Ciencia y Tecnología de Alimentos Córdoba, CONICET, Departamento de Química Orgánica, Universidad Nacional de Córdoba, Bv. Medina Allende s/n, Ciudad Universitaria, 5000 Córdoba, Argentina
[g] Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, Sisal, Yucatán, Mexico

HIGHLIGHTS

- The metallic SePM affects physiology, biochemical and behavior of crab *M. rapax*.
- SePM exposure increased as a concentration-dependent Al, Fe, Mn, Cr and Y in crabs.
- The effects differ depend on submersion regime: access to dry surface or submerged.
- *MinUca rapax* accumulates more metals when completely submerged.
- Exposure to 1 $g.L^{-1}$ SePM reduces assimilation efficiency and available energy for activity.

GRAPHICAL ABSTRACT



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ABSTRACT

We use the sentinel mangrove crab, *Minuca rapax*, as a model to investigate the effects of metallic settleable particulate matter (SePM) on wetland. Multiple levels of energetic responses, including (i) metabolic rate and

* Corresponding author.
E-mail address: mcapparelli@cmarl.unam.mx (M.V. Capparelli).

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Metabolic rate
Righting time
MinUca rapax

energy budget, (ii) oxidative stress, and (iii) behavioral response by righting time, were assessed as well as the metal and metalloid content in crabs exposed to 0, 0.1 and 1 $g.L^{-1}$ of SePM, under emerged and submerged conditions over five days, simulating the rigors of the intertidal habitat. Al, Fe, Mn, Cr, and Y exhibited a concentration-dependent increase. Metal concentrations were higher in submerged crabs due to the continuous ingestion of SePM and direct exposure through gills. Exposure concentration up to 1 $g.L^{-1}$ decreased metabolic rate and enzymatic activities, reduced assimilation efficiency and energy for maintenance, and induces a slower response to righting time, probably by metal effects on nervous system and energy deficits. In conclusion, SePM exposure affects the redox status and physiology of *M. rapax* depending on he submersion regime and SePM concentration. The disruption to the energy budget and the lethargic behavior in *M. rapax* exposed to SePM implies potential ecological alterations in the mangrove ecosystem with unknown consequences for the local population.

1. Introduction

Air pollution from steel industries, biomass combustion, and increased vehicle traffic increased atmospheric particulate matter, which poses significant risks to aquatic ecosystems when settled [1]. The settleable atmospheric particulate matter (SePM) is characterized by a complex aggregation of particles containing various metals and metalloids, including rare earth elements identified as emerging contaminants. SePM exhibits a dissociation propensity in water, yielding nanoparticles measuring less than 200 nm in size [2]. This characteristic renders these metals susceptible to ingestion by aquatic organisms in the first levels of the food web and subsequent transfer to higher trophic levels [3,4], causing deleterious effects that range from the subcellular to the ecosystem level [5]. Souza et al., [4] suggest that the biomagnification of metals/metalloids in the plant-crab-fish trophic chain in estuaries is the most important metal transfer pathway and should be considered for management studies, especially when evaluating atmospheric contamination.

Mangrove wetland is considered an important repository and sink of pollutants, serving as a natural barrier to delivering land-based inorganic contaminants to the sea [6]. Also, they are highly vulnerable to climate change; some areas can experience extended periods of flooding caused by mangrove forest decline [7], creating physiologically limiting conditions for species with semi-terrestrial lifestyles [8]. Fiddler crabs interact closely with sediments, consume organic detritus, and play a vital role in bioturbation, thus contributing to ecosystem health [9]. Several studies indicate that fiddler crabs can be considered bioindicators of ecosystem health [10-13] due to their ability to bioaccumulate metals in their tissues at levels significantly higher than those in surrounding waters and sediments [14,12,15].

Although the toxic mechanisms of SePM, particularly regarding rare earth elements, in aquatic organisms remain insufficiently explored, it is established that trace metals can disrupt multi-level of energetic responses that can be evaluated by energy metabolism [16,17]. Metal exposure was demonstrated in crustaceans [18] as related to a drop in levels in the energy charge potential of tissues that might suggest a decrease in cellular energy production as a possible cause of toxicity. For example, metal contamination can impair the antioxidant system, increasing reactive oxygen species (ROS) [19]. Regarding the complex metal composition of SePM, the mixture may disrupt the balance between pro-oxidants and antioxidants, tipping it towards pro-oxidants [20-22].

Energetic endpoints can provide insights into how a pollutant modulates and affects the physiological mechanisms related to transforming food energy into biomass and activity or behavior [23]. Combining the evaluation of the scope for growth with the assessment of antioxidant defense mechanisms and oxidative damage, we can gain insights into whether SePM affects the metabolic status of individuals through the impairment of energy acquisition and allocation or by causing impairment in the antioxidant defense mechanisms, which at the end affects the cellular energy production process [24,25]. In this context, fine-scale behavioral and metabolic responses of mangrove crabs exposed to contaminants could contribute to understanding large-scale changes. Exposure to SePM can induce physiological and behavioral trade-offs. In general, semi-terrestrial crabs use a semi-terrestrial lifestyle to escape contaminants in the water [23]. However, when fully submerged, they may present behavioral changes such as lethargy or agitation in response to energy demands [26].

To bridge the knowledge gaps in the impact of SePM on mangrove bioindicators' health, and the higher biomagnification potential of mangrove crabs, we assess how SePM sublethal concentration affects the fiddler crab *Minuca rapax,* by examining in laboratory exposure the multi-level of the antioxidant defense mechanisms (SOD, CAT, and GST) and lipid damage (LPO), the energetic responses such as the energy budget (feeding rate - FR, assimilation efficiency - AE, energy intake - EI and scope for growth - SFG), alongside behavioral biomarkers, as righting time. Additionally, this study investigated the presence and accumulation of metals and metalloids in the tissues of crabs, both when exposed to a dry surface and when permanently submerged, to simulate the rigors of the intertidal habitat. Such alterations may subject mangrove inhabitants to prolonged periods of submersion or desiccation, highlighting the need to evaluate the tolerance thresholds of sentinel species in these altered environments [8,27]. Our assessment will underscore the considerable environmental risk posed by atmospheric contamination in sink mangrove ecosystems.

2. Materials and methods

2.1. Study model

Minuca rapax (Brachyura, Ocypodidae), a species of semiterrestrial generalist crab, inhabits sandy to muddy substrates within a geographical range extending from Florida to southeastern Brazil [28]. This species can be considered a sentinel species due to its high abundance and presence in both pristine and contaminated locations of tropical mangroves [29]. *Minuca rapax* accumulates significant quantities of metals and microplastics in its tissues from the surrounding environment [8,14]. This species can display exceptional hypo-osmoregulatory abilities [30], a trait crucial for their semi-terrestrial lifestyle [30], enabling it to maintain stable hemolymph osmolality even during periods of aerial exposure [11]. While these crabs are fully submerged only during high tides, alterations in tidal patterns due to anthropogenic impacts on mangrove ecosystems, as well as the effects of climate change and rising sea levels, can disrupt their natural rhythms [31].

2.2. SePM sampled

The metallic particulate matter released in the atmosphere, referred to as settleable particulate matter (SePM), was gathered in Ilha do Boi, located at coordinates 20°17′03.8″S and 40°14′24.9″W in Vitória city, state of Espírito Santo, Brazil. This region is renowned for its notable atmospheric pollution, primarily attributed to extensive industrial iron ore processing activities at the Tubarão Complex, subsequently utilized for steel production and export [2,32]. The SePM sampling took place in March 2021 in containers that were strategically positioned on the building rooftop, approximately 20 m above the ground. SePM is

composed of a complex mixture of particles of different sizes and when dissolved in water can disperse into metals and nanoparticles smaller than 200 nm. For a more detailed account of the collection methodology, see Souza et al. [2].

The SePM utilized in this study was previously characterized by Monteiro et al., in 2023. Composing various metals and metalloids at varying concentrations, 18 out of the 26 analyzed metals were quantified in the SePM. Elevated levels of Fe (106,140 $\mu g.g^{-1}$) and Al (6997 $\mu g.g^{-1}$) were determined, with Ti (708 $\mu g.g^{-1}$), Mn (565 $\mu g.g^{-1}$), Zn (212 $\mu g.g^{-1}$), Ba (88.1 $\mu g.g^{-1}$), Sr (68.5 $\mu g.g^{-1}$), Cr (55.4 $\mu g.g^{-1}$), Cu (25.5 $\mu g.g^{-1}$), V (20.4 $\mu g.g^{-1}$), Ni (18.2 $\mu g.g^{-1}$), Ce (18.1 $\mu g.g^{-1}$), Pb (12.6 $\mu g.g^{-1}$), La (9.0 $\mu g.g^{-1}$), Y (5.3 $\mu g.g^{-1}$), Sn (4.9 $\mu g.g^{-1}$), Zr (3.8 $\mu g.g^{-1}$), and Rb (2.2 $\mu g.g^{-1}$) also present, albeit at decreasing concentrations. We selected the highest concentration of SePM used in our study (1 $g.L^{-1}$) based on its environmental relevance. Specifically, this concentration closely mirrors the values found in estuaries located near areas with significant steelmaking activity [33,32,34].

2.3. Crab collection

Adult crabs (weighing 2.6 ± 0.13 g) were manually collected during the summer of 2023 from their natural mangrove habitat in Laguna de T é rminos (18° 41′ N, 91° 40′ W), Isla del Carmen in Campeche, Mexico. Specimens (total n = 60) of the *M. rapax* were carefully transported in plastic containers, containing water from the collection site. In the laboratory, the crabs underwent a 5-day acclimatization period in tanks containing 1 L of water at a salinity of 10 ‰ and temperature-controlled at 25 °C. Crabs were fed daily with commercial food Lomas tropical slow sinking crumbles until the start of the experiment.

2.4. Experimental design

Following acclimatization, 60 crabs were exposed to experimental conditions for five days; based on previous studies by [8], the lethal time for 50 % of organisms was up to 120 h of submersion. The crabs were individually placed in plastic containers in submerged condition, with 200 mL of water and no access to air (under constant aeration), and in semi-submerged (emerged) condition with 50 mL and access to air. These conditions included three concentrations of SePM, specifically 0 (control condition), 0.1, and 1. $g.L^{-1}$ (10 crabs per concentration per condition). One crab died when exposed to 1 $g.L^{-1}$ of SePM under submerged conditions after 48 h of exposure.

After the 5-day experimental period, behavior responses were assessed in all crabs using the righting time (RT) (n = 10 crabs per concentration per condition). Then, crabs, including the control, 0.1, and 1 $g.L^{-1}$ of SePM under emerged and submerged conditions (n = 5 per concentration per condition) were transferred individually to 0.16 L plastic containers. Their metabolic rate was measured for 12 h using an intermittent respirometry system. After oxygen consumption measurements, crabs were returned to their experimental containers, and the energy budget parameters, as FR, AE, EI and SFG, were calculated. Subsequently, these animals used to obtain metabolic rate and scope for growth were sacrificed by freezing and used to measure metal and metalloid concentrations in whole crabs. The remaining specimens (n = 5 per concentration and condition), not used for the previously detailed analyses, were cryo-anesthetized. Fragments of gills and hepatopancreas were meticulously extracted and immediately frozen in liquid nitrogen and posteriorly stored in an ultra-freezer at −80 °C to assess the biochemical biomarkers: superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and lipid peroxidation (LPO).

2.5. Metal and metalloid concentration in whole crabs

Crabs were euthanized through cryofreezing, and the whole crab was subjected to a 10-day drying process in an oven at 40 °C for complete tissue dehydration. Following dehydration, samples (n = 5 per concentration per condition) underwent maceration and homogenization. Samples from homogenized dried crabs weighing 0.1 g dry weight were digested by combining 3 mL of nitric acid (69 %), 750 µL of hydrochloric acid, and 250 µL of hydrogen peroxide for 12 h at 100 °C. Subsequently, the samples were filtered through a 0.45 µm nitrocellulose filter (Millipore, Brazil), adhering to Agilent ICP-MP guidelines. A control was prepared using all the reagents employed in the digestion protocol. These samples were then stored at 4 °C for until further analysis. Twenty-eight elements (Ag, Al, As, Au, Ba, Bi, Cd, Ce, Cr, Cu, Fe^{56}, Fe^{57}, Hg^{202}, La, Mn, Mo, Nb, Ni, Pb, Rb, Se, Sn, Sr, Ti, V, W, Y, Zn, and Zr) were measured in triplicate using ICP-MS according to 200.8 (EPA, 1994). Quality control and assurance were ascertained by adding certified reference material (spike) to the crab sample (Certipur® Certified Reference Material multi-standard VI), with recoveries averaging 94.3 ± 9.4 %.

2.6. Biochemical markers

Gill and hepatopancreas were homogenized at a ratio of 100 mg to 1 mL in cold phosphate-buffered saline (PBS; 100 mM, pH 7.4) using a PRO250 homogenizer (Pro Scientific®) (n = 5 per concentration per condition). The homogenate underwent centrifugation at 12,000 g for 20 min at 4 °C and the resulting supernatant was transferred to a microtube to the determination of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities and to assess lipid peroxidation (LPO) levels. All spectrophotometric analyses were conducted using a multimode reader (Multiskan SkyHigh Microplate Spectrophotometer, Thermo Scientific).

The protein content was quantified utilizing a Bio-Rad protein assay (BioRad, Richmond, CA) following the protein-dye binding protocol established by Bradford [35], adapted to a microplate. Bovine γ-globulin served as the standard for calibration in this assay.

Superoxide dismutase activity was assessed using the method outlined by Misra and Fridovich [36], which relies on inhibiting the autoxidation of adrenaline to adrenochrome under alkaline pH conditions. Specifically, 30 µL of supernatant from the microplate was combined with 220 µL of the reaction solution (0.3 mM adrenaline in carbonate buffer, pH 10.2). A water blank was employed as a reference. After a 2-minute incubation period, the absorbance was measured at 480 nm. The SOD activity in the samples was quantified in units per milligram of protein (U.mg $protein^{-1}$).

Catalase activity was determined using the method outlined by Radi et al. [37], which involves the assessment of hydrogen peroxide (H_2O_2) dismutation at 240 nm after 0 and 60 s. In this assay, 200 µL of the supernatant was added to a microplate, followed by the addition of 100 µL of a reaction solution containing 20 mM H_2O_2 in buffer (0.3 M sucrose, 1 mM EDTA, 5 mM HEPES, and 5 mM KH_2PO_4). The CAT activity was expressed in units per milligram of protein (U.mg $protein^{-1}$).

Glutathione-S-Transferase was measured with a Sigma-Aldrich GST assay Kit (CS0410). This enzyme catalyzes the conjugation of the glutathione thiol group to compounds containing electrophilic centers. The kit utilizes a 1-chloro2,4-dinitrobenzene substrate (CDNB), which conjugates with the glutathione thiol group, forming a compound that can be read at 340 nm. The GST activity was expressed in units per milligram of protein (U.mg $protein^{-1}$).

Lipoperoxidation was quantified by assessing thiobarbituric acid reactive substances (TBARS), following the methodology described by Buege and Aust [38] with adaptations for microplate use. A 150 mM Tris-HCl pH 7.4 buffer solution was added to 250 µL of the sample without prior centrifugation. The mixture was incubated at 37 °C for 15 min, followed by the addition of a TCA-TBA solution (0.375 % thiobarbituric acid dissolved in 15 % trichloroacetic acid), and subjected to a thermal shock in boiling water for 45 min. After cooling, the solution was centrifuged at 3000 rpm for 10 min, and the absorbance at 535 nm was recorded. The results for LPO were expressed as nmol TBARS.mg $protein^{-1}$, utilizing a molar extinction coefficient of 1.56×10^5 $M.cm^{-1}$.

2.7. Energy budget

Oxygen consumption was monitored over 12 h using an intermittent respirometry system, ensuring that oxygen concentration in the chambers remained above 70 % saturation throughout the experiment. An empty chamber served to measure background oxygen consumption levels. Trials were conducted in a controlled environment, isolated and semi-dark, to mitigate potential stress on the animals. Room temperature was regulated using air conditioning. For precise measurements, dissolved oxygen levels in each chamber were recorded every 15 s using flow-through oxygen sensors from Loligo Systems, Denmark. These sensors were securely affixed to the chamber walls and connected via optical fiber to an OXY-10-Channel Oxygen Meter by PreSens, based in Regensburg, Germany. Calibration of the sensors was achieved using both saturated seawater (100 % DO) and a 5 % sodium sulfite solution (0 % DO) for accurate readings. Upon completion of the experiment, crabs were gently dried on a napkin to remove excess moisture and subsequently weighed. The metabolic rate (mg $O_2.g^{-1}.h^{-1}$) was calculated as:

$VO2 = [O2]i - \frac{[O2]f}{\Delta Tx} xVr/ww$, where $[O_2]$i and $[O_2]$f are the initial and final oxygen concentrations ($mg.L^{-1}$) respectively, ΔT represents the trial period in hours, Vr represents the water volume (L) in the respirometry chamber minus the animal volume (g), and the ww refers to the wet weight of the crabs in grams.

Components of the energy budget were measured (feeding rate, assimilation efficiency, energy intake, scope for growth) from individual crabs (n = 5 per concentration per condition). The crabs were starved for 48 h and fed once with pre-weighted and pre-dried pellets in the laboratory (1 mm, New Life Spectrum ® Thera + A) of 10 % of their body mass. Containers of each treatment but without crabs and with pellet samples were used as controls for leaching. The crabs were kept in their containers for four hours, after which the food leftovers were filtered by vacuum filtration with pre-weighted and pre-dried 10 μm pore filter papers. The samples were rinsed with distilled water to reduce salt residues. The samples were oven-dried at 60 °C for 24 h and their dry mass was measured. The feeding rate (I) was determined as the amount of food uneaten by the crabs (grams of food per day per gram of wet mass of crab: g d^{-1} g^{-1} wm).

The crabs were returned to their containers and their feces were siphoned every 12 h for 48 h after the feeding experiments. Feces (F) were filtered the same way as the food leftovers and their dry weight was measured (grams of feces per day per gram of wet mass of crab [g d^{-1} g^{-1} wm]). Feces samples were used to calculate the assimilation efficiency (AE), which is a measure of how much of the energy in the food has been absorbed across the gut wall [39]. AE (%) was calculated with the equation: AE = [(I - F) / I] * 100 [40,41] where I is the feeding rate and F is the rate of feces excreted.

The energy assimilated (A) rate was calculated by multiplying the feeding rate by the AE of each treatment. Energy assimilated was converted into energy units using the factor of 4463 cal g^{-1} of food. The energy content of the food was measured by burning a known quantity in a micro bomb calorimeter (Semi-micro PARR 1425). The calorimeter was calibrated with a standard of 0.2 g of benzoic acid. Energy intake was converted to Joules using the caloric conversion factor of 4.186 J. cal^{-1} [42] and expressed as $J.day^{-1}.g^{-1}$ wm. The equation determines the scope for growth (SFG) = A - (R + E), where A is the rate of energy assimilated (i.e. the energy assimilated from food), R is the energy used for maintenance and E is the energy excreted as nitrogen [25].

Metabolic rates were converted to Joules by using the oxycaloric conversion factor of 450 kJ mol O_2^{-1} or 28.125 J.mg O_2^{-1} [42] and expressed as $J.day^{-1}.g^{-1}$ wt. The energy wasted as ammonium excretion was not measured as it represents less than 5 % of the energy used for maintenance [43]. SG was then calculated with the simplified equation: SFG = A - R [25] and expressed as $J.day^{-1}.g^{-1}$.

2.8. Righting time

Righting time (RT) was determined as the duration, in seconds, required for the crab to return to an upright position after being flipped onto its dorsal decubitus (180°) [44]. Using a stopwatch, timing commenced upon full inversion of the crab and finished when it regained its upright stance. This experimental procedure lasted a short time, not exceeding 1 min in duration per crab.

2.9. Statistical analysis

All statistical analyses were carried out in R (version 4.3.3, R Development Core Team) [45]. After ascertaining the normality of the data distributions (Shapiro-Wilk test) and the homogeneity of their variances (Levene's test), the effects of SePM concentration and submerged or emerged conditions were evaluated on: (i) physiological parameters; (ii) biochemical biomarkers; (iii) righting time; and (iv) assimilation efficiency and energy intake. The data sets were evaluated using two-way analyses of variance followed by the Tukey test post-hoc multiple means procedure to detect significant differences. A minimum significance level of $p = 0.05$ was used, and data are given as the mean ± standard error of the mean. The graphs in the results section represent variables that exhibited a significant effect. The graphical representations were generated utilizing the ggplot2 package.

The effect of SePM concentration and experimental condition on the non-parametric data, as the metals and metalloid concentration in whole-body crabs was determined by permutational analysis of variance (PERMANOVA) with the packages adonis and pairwiseAdonis. To assess the variation in univariate metals and metalloids concentration in whole-body crabs, the dataset was compared against background bioconcentration levels in control (0 $g.L^{-1}$ SePM) in both emerged and submerged conditions, using a statistical approach known as 'data analysis with bootstrap-coupled estimation', implemented through the R package dabestr [46]. Principal Component Analysis (PCA) was conducted with the FactoMineR and factoextra packages.

3. Results

3.1. Metal and metalloid concentration in whole crabs

The analysis of 27 metals and metalloids was conducted on whole-body crabs, with 13 metals below the limit of detection (LOD) or limit of quantitation (LOQ) of the device (Table 1). Metals such as Fe, Al, Cr, Mn, Cu, Zn, Se, Rb, Sr, Y, Zr, Ag, Ba, and As were identified in at least three readings within a group. The PERMANOVA revealed significant differences in metals and metalloid levels in whole crabs depending on the SePM concentration ($F = 20.82$, $p = 0.001$), exposure condition ($F = 26.66$, $p = 0.001$), and their interaction ($F = 18.44$, $p = 0.001$). The highest values for Al, Fe, Mn, Cr, and Y, particularly in submerged conditions, were found (Fig. 1). Aluminum and Fe levels in crabs exposed to 1 $g.L^{-1}$ SePM were higher compared to the control in both emerged ($p < 0.002$) and submerged ($p < 0.002$) conditions. Manganese and Y levels were higher in crabs exposed to 1 $g.L^{-1}$ only when permanently submerged ($p < 0.001$). Chrome levels in crabs exposed to 0.1 and 1 $g.L^{-1}$ SePM were higher than the control in both emerged conditions ($p < 0.009$) and submerged ($p < 0.021$) (Fig. 1).

Strontium and Rb levels were higher in emerged crabs to 1 $g.L^{-1}$ ($p = 0.0036$ and $p < 0.0001$, respectively) while Ba and Sr levels were higher in submerged crabs to 0.1 $g.L^{-1}$ of SePM ($p < 0.001$). Zirconium, Zn, and As levels were high in emerged crabs to 0.1 $g.L^{-1}$ SePM (Zr, $p = 0.0001$), and in submerged crabs at 1 $g.L^{-1}$ (Zn, $p = 0.003$; As, $p < 0.0001$). Copper level was reduced in crabs exposed to 0.1 and 1 g. L^{-1} in emerged ($p = 0.007$) and submerged ($p < 0.001$) conditions. Selenium level was not different between groups ($p > 0.234$) (Table 1).

Table 1
Metal and metalloid concentration (μg.g^{-1} dry mass) in whole crabs exposed to SePM (0, 0.1, and 1 g.L^{-1}) under emerged and submerged conditions for 5 days. The data are presented as median (minimum-maximum value). LOD: limit of detection; LOQ: limit of quantification of each metal analyzed in the ICP-MS.

Whole crabs metal content (μg/g dm)	Emerged			Submerged				
	SePM (g.L^{-1})						ICP-MS	
	0	0.1	1	0	0.1	1	LOD	LOQ
Al	<LOQ	3.2 (0.3 - 9.4)	15.4 (5.9 - 16.5)	<LOQ	1.6 (1.4 - 1.7)	27.2 (18.7 - 34.4)	0.116	0.313
Ti	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.063	0.170
V	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.009	0.025
Cr	0.29 (0.03 - 2.44)	2.44 (1.71 - 13.56)	7.58 (0.30 - 20.15)	<LOQ	0.08 (0.03 −7.64)	8.76 (3.56 - 20.57)	0.012	0.030
Mn	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7.3 (7.0 - 8.4)	1.126	3.019
Fe	11.1 (9.2 - 13.2)	17.3 (9.7 - 84.4)	116.6 (67.0 - 218.0)	7.1 (3.9 - 10.1)	47.8 (23.5 - 76.6)	279.2 (111.4 - 338.0)	0.381	1.023
Ni	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.113	0.273
Cu	72.5 (63.7 - 78.2)	63.88 (62.0 - 67.9)	62.5 (55.5 - 63.4)	50.9 (49.0 - 52.2)	44.5 (30.0 - 47.1)	55.7 (49.5 - 56.6)	0.013	0.035
Zn	39.5 (28.8 - 40.6)	39.1 (33.5 - 41.9)	39.7 (36.1 - 42.7)	34.7 (30.5 - 37.2)	35.7 (22.3 - 36.9)	39.32 (34.5 - 41.5)	0.224	0.609
As	7.5 (7.2 - 8.1)	7.3 (6.8 - 7.6)	7.3 (6.9 - 7.6)	5.9 (5.6 - 6.1)	5.0 (4.4 - 5.3)	7.1 (6.5 - 7.2)	0.119	0.280
Se	<LOQ	0.97 (0.52 - 1.18)	0.91 (0.52 - 1.28)	<LOQ	<LOQ	<LOQ	0.261	0.520
Rb	1.5 (1.4 - 1.6)	1.4 (1.4 - 1.5)	1.7 (1.6 - 1.7)	1.5 (1.4 - 1.6)	1.6 (1.4 - 1.7)	1.3 (1.2 - 1.4)	0.010	0.026
Sr	2005 (1906 - 2079)	2005 (1974 - 2044)	2072 (2044 - 2185)	2351 (2298 - 2373)	2556 (2501 - 2634)	2212 (2185 - 2235)	0.003	0.008
Y	<LOQ	0.007 (0.001 - 0.009)	0.006 (0.003 - 0.010)	<LOQ	0.003 (0.000 - 0.009)	0.016 (0.009 - 0.021)	0.002	0.005
Zr	<LOQ	0.50 (0.21 - 0.82)	0.08 (0.03 - 1.74)	0.14 (0.07 - 0.18)	<LOQ	<LOQ	0.036	0.078
Nb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.019	0.046
Mo	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.159	0.406
Ag	0.13 (0.11 - 0.15)	0.13 (0.11 - 0.16)	0.12 (0.11 - 0.14)	0.10 (0.09 - 0.14)	0.12 (0.06 - 0.13)	0.15 (0.14 - 0.18)	0.026	0.062
Cd	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.085	0.185
Sn	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.036	0.093
Ba	21.6 (20.3 - 21.9)	20.5 (19.5 - 21,0)	21.8 (21.1 - 23.0)	28.7 (28.4 - 29.2)	41.0 (40.4 - 41.4)	25.0 (24.7 - 25.6)	0.115	0.305
La	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.002	0.003
Ce	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.001	0.002
W	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.007	0.017
Hg	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.105	0.286
Pb	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.242	3.156
Bi	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.507	1.292

3.2. Biochemical responses

Catalase activity in gill was affected by the SePM concentration ($F = 3.42$, $p = 0.05$), the exposure condition ($F = 10.23$, $p = 0.004$), and the interaction between these factors ($F = 3.57$, $p = 0.045$). The CAT activity was higher in crabs submerged in 1 g.L^{-1} SePM (↑ 144 %) ($p = 0.016$) compared to the control (Fig. 2 A). GST activity of crabs was not altered under any condition ($F = 0.62$, $p = 0.439$) or concentration ($F = 0.32$, $p = 0.730$) (Fig. 2 B). There was no lipid peroxidation in gills exposed to SePM concentration ($F = 0.06$, $p = 0.946$), nor different conditions ($F = 0.29$, $p = 0.597$) (Fig. 2 C).

In hepatopancreas, the SePM concentration ($F = 2.68$, $p = 0.091$), the exposure condition ($F = 0.134$, $p = 0.718$), nor the interaction between these factors ($F = 0.18$, $p = 0.839$) affected the CAT activity (Fig. 3 A). The condition ($F = 6.8$, $p = 0.016$), and the interaction between SePM concentration and condition ($F = 17.84$, $p < 0.001$) affected the SOD activity. There was an increase in the SOD activity in emerged crabs to 1 g.L^{-1} SePM ($p = 0.021$). Conversely, a reduction concentration-dependent was observed in the SOD activity in crabs submerged to 0.1 (↓ 58.9 %) ($p = 0.041$) and 1 g.L^{-1} SePM (↓ 82.5 %) ($p < 0.001$) compared to control and between them (↓ 57.5 %) ($p = 0.046$) (Fig. 3 B). GST activity of crabs was not altered under any condition ($F = 1.71$, $p = 0.204$) or concentration ($F = 1.38$, $p = 0.274$) (Fig. 3 C). Lipid peroxidation in hepatopancreas was affected by concentration ($F = 3.79$, $p = 0.039$), evidenced by the reduction (↓ 5 %) in submerged crabs exposed to 0.1 compared to 1 g.L^{-1} SePM (Fig. 3 D).

3.3. Energy budget

The metabolic rate (MR) was significantly influenced by the interaction between SePM concentration and exposure condition ($F = 6.82$, $p = 0.005$). MR in emerged conditions was higher (↑ 50.8 %) in crabs exposed to 0.1 g.L^{-1} SePM compared to crabs exposed to 1 g.L^{-1} SePM ($p = 0.019$). Conversely, MR was lower (↓ 59.7 %) in crabs exposed to 0.1 g.L^{-1} SePM under submerged conditions compared to 1 g.L^{-1} SePM ($p = 0.029$). Furthermore, emerged crabs showed higher MR compared to those submerged to 0.1 g.L^{-1} of SePM ($p = 0.002$) (Fig. 4).

The SePM concentration and exposure condition affected the energy balance of crabs. There was an increase in feeding rate (FR) in emerged crabs exposed to 0.1 g.L^{-1} SePM compared to the control (↑ 51.9 %) ($p = 0.009$). Unlike, a decrease in submerged crabs to 1 g.L^{-1} SePM compared to control (↓ 44.2 %) ($p = 0.006$) and 0.1 g.L^{-1} SePM (↓ 51.9 %) ($p < 0.001$). There was a difference in FR in submerged and emerged crabs to 0.1 ($p = 0.019$) and 1 g.L^{-1} SePM ($p < 0.001$) (Fig. 5 A). The assimilation efficiency (AE) decreased only in submerged crabs exposed to 1 g.L^{-1} SePM compared to control (↓ 18.4 %) ($p < 0.001$) and 0.1 g.L^{-1} SePM (↓ 15.9 %) ($p = 0.001$). There was a decrease in AE submerged regarding emerged crabs to 1 g.L^{-1} SePM ($p < 0.001$) (Fig. 5 B).

The EI and the SFG were affected by SePM concentration, exposure condition, and the interaction between these two factors ($p < 0.001$). The EI was higher in emerged crabs in 0.1 g.L^{-1} SePM compared to control (↑ 82.4 %) and 1 g.L^{-1} SePM (↑ 57.7 %) (both $p < 0.001$). Conversely, the EI of submerged crabs decreased in 1 g.L^{-1} SePM

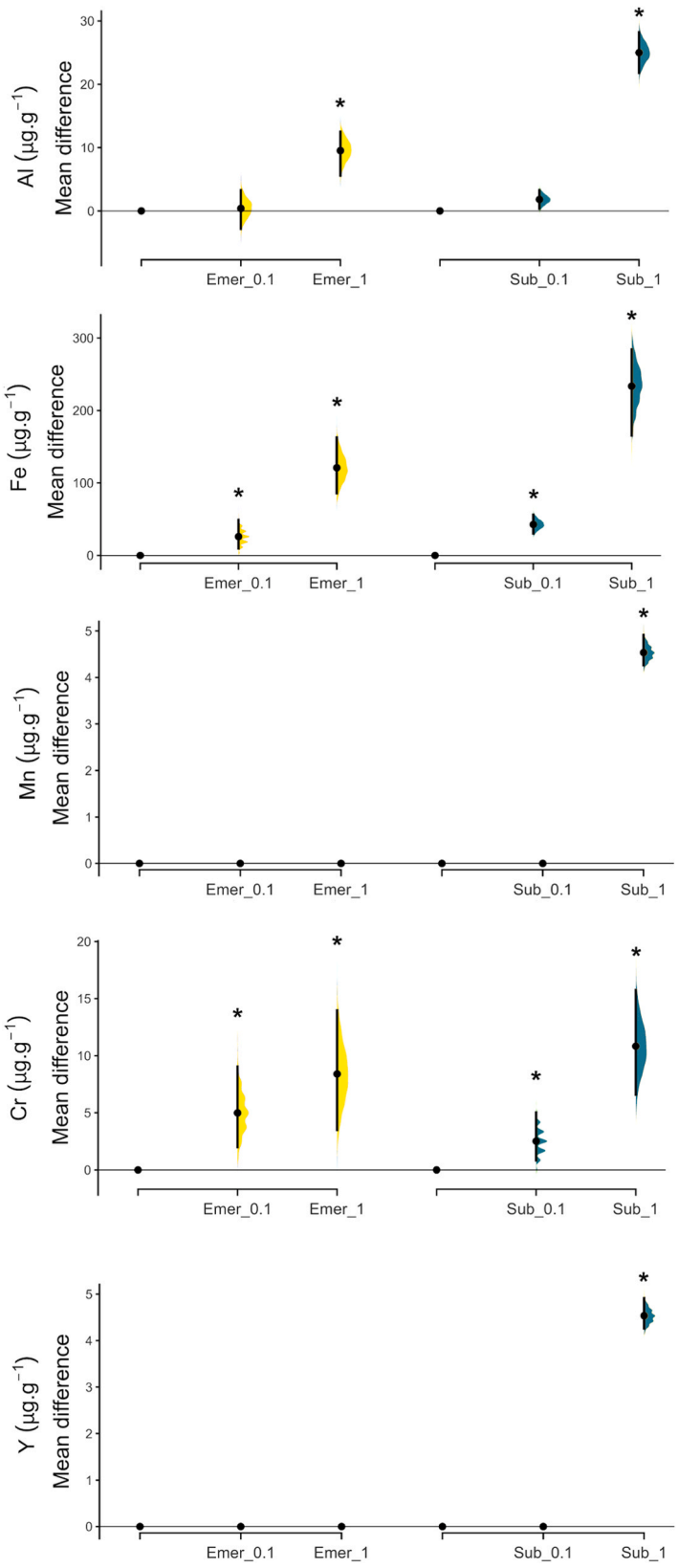

Fig. 1. The mean difference of metals and metalloid accumulation in whole-body crabs exposed to varying concentrations of SePM (0, 0.1, and 1 g.L^{-1}) under both emerged (yellow) and submerged (blue) conditions. The data are presented using cumming estimation plots, wherein dots represent mean differences, and the vertical error bars signify the 95 % confidence interval for each comparison. The bootstrap sampling distributions show the metals and metalloid concentration across different experimental conditions and control groups. *Indicates the difference between concentrations of SePM compared to the control condition.

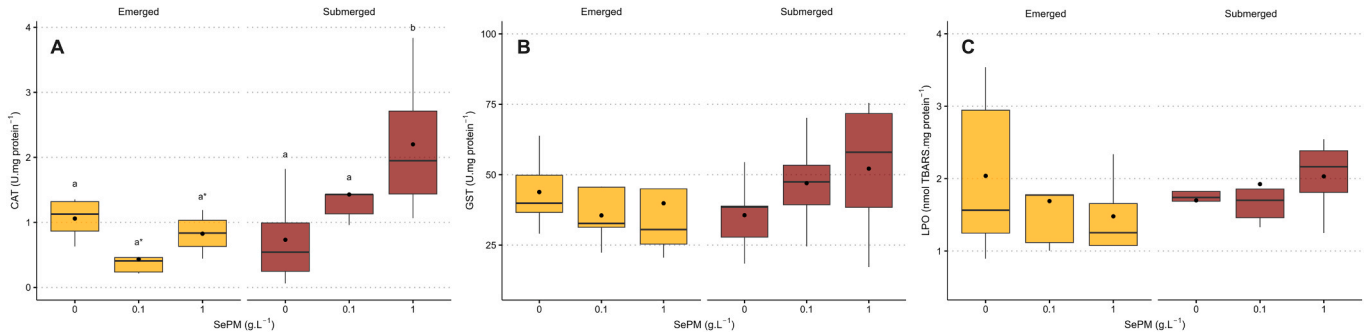

Fig. 2. Catalase activity (U.mg protein^{-1}) (A), GST activity (U.mg protein^{-1}) (B), and LPO levels (nmol TBARS.mg protein^{-1}) (C) in the gill of crabs exposed to 0, 0.1 and 1 g.L^{-1} SePM under both emerged and submerged conditions. Boxplots represent the distribution of data for each experimental group, where the median line indicates the median of the data, the bottom of the box represents the first quartile (Q1), and the top of the box represents the third quartile (Q3). The black dot indicates the mean value for each group. Different letters indicate differences between concentrations within the same condition. The absence of letters represents the absence of differences. *Indicates the difference between conditions within a concentration.

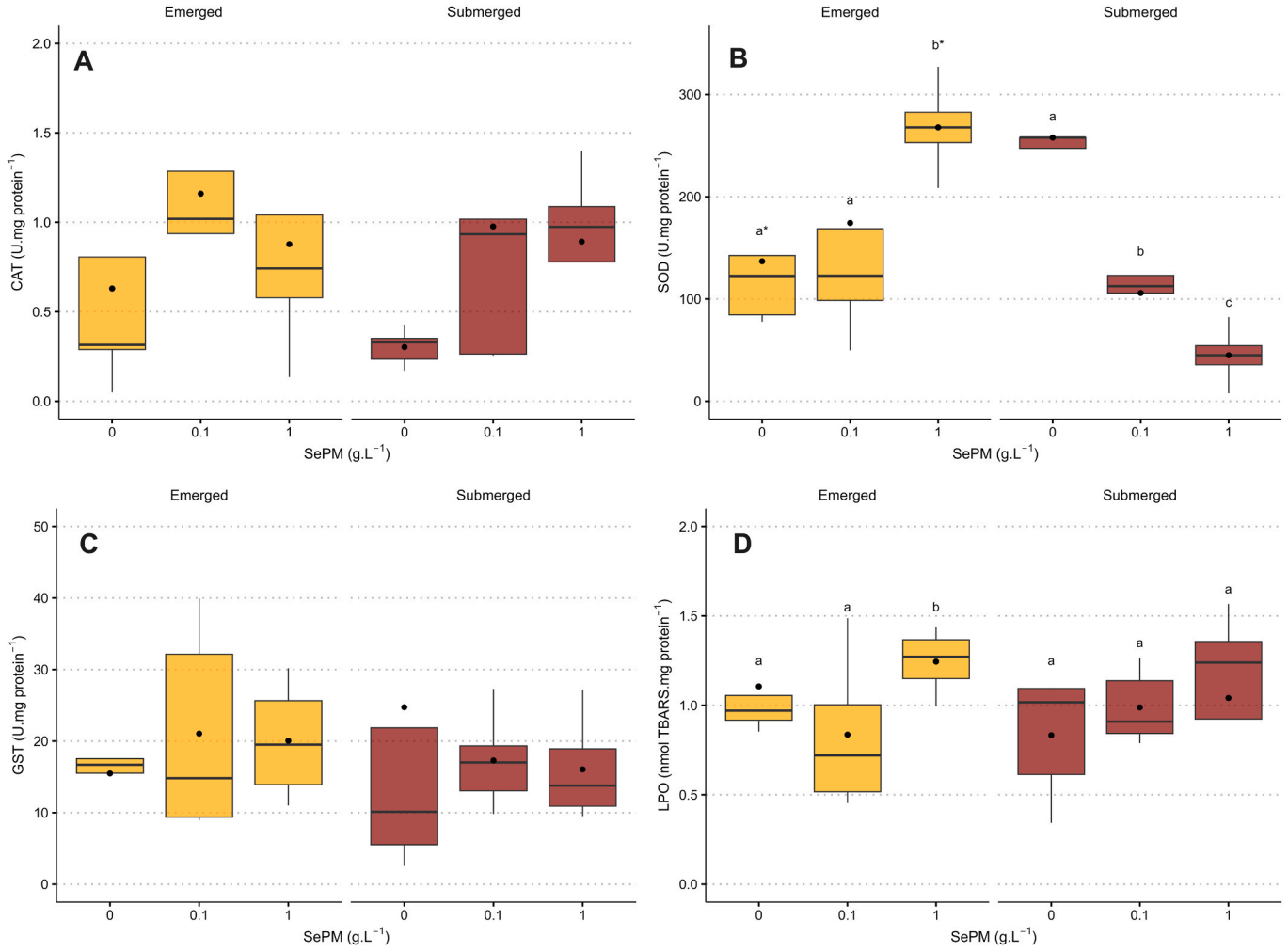

Fig. 3. Catalase (U.mg protein^{-1}) (A), SOD (U.mg protein^{-1}) (B), GST (U.mg protein^{-1}) activities (U.mg protein^{-1}) (C), and LPO levels (nmol TBARS.mg protein^{-1}) (D) in the hepatopancreas of crabs exposed to 0, 0.1, and 1 g.L-1 SePM under both emerged and submerged conditions. Boxplots represent the distribution of data for each experimental group, where the median line indicates the median of the data, the bottom of the box represents the first quartile (Q1), and the top of the box represents the third quartile (Q3). The black dot indicates the mean value for each group. Different letters indicate differences between concentrations within the same condition. The absence of letters represents the absence of differences. *Indicates the difference between conditions within a concentration.

compared to control (↑ 54.1 %) and to 0.1 g.L^{-1} SePM (↑ 59.6 %) (both $p < 0.001$) (Fig. 5 C). In emerged crabs to 0.1 g.L^{-1} SePM the SFG was higher than control (↑ 85.2 %) and 1 g.L^{-1} SePM (↑ 62 %) ($p < 0.001$). In submerged crabs to 1 g.L^{-1} SePM, the SFG was lower than control (↓ 64.5 %) and 0.1 g.L^{-1} SePM (↓ 68.9 %) ($p < 0.001$) (Fig. 5 D). There was a difference between the EI and SFG in emerged crabs compared to submerged crabs in 1 g.L^{-1} SePM ($p < 0.001$).

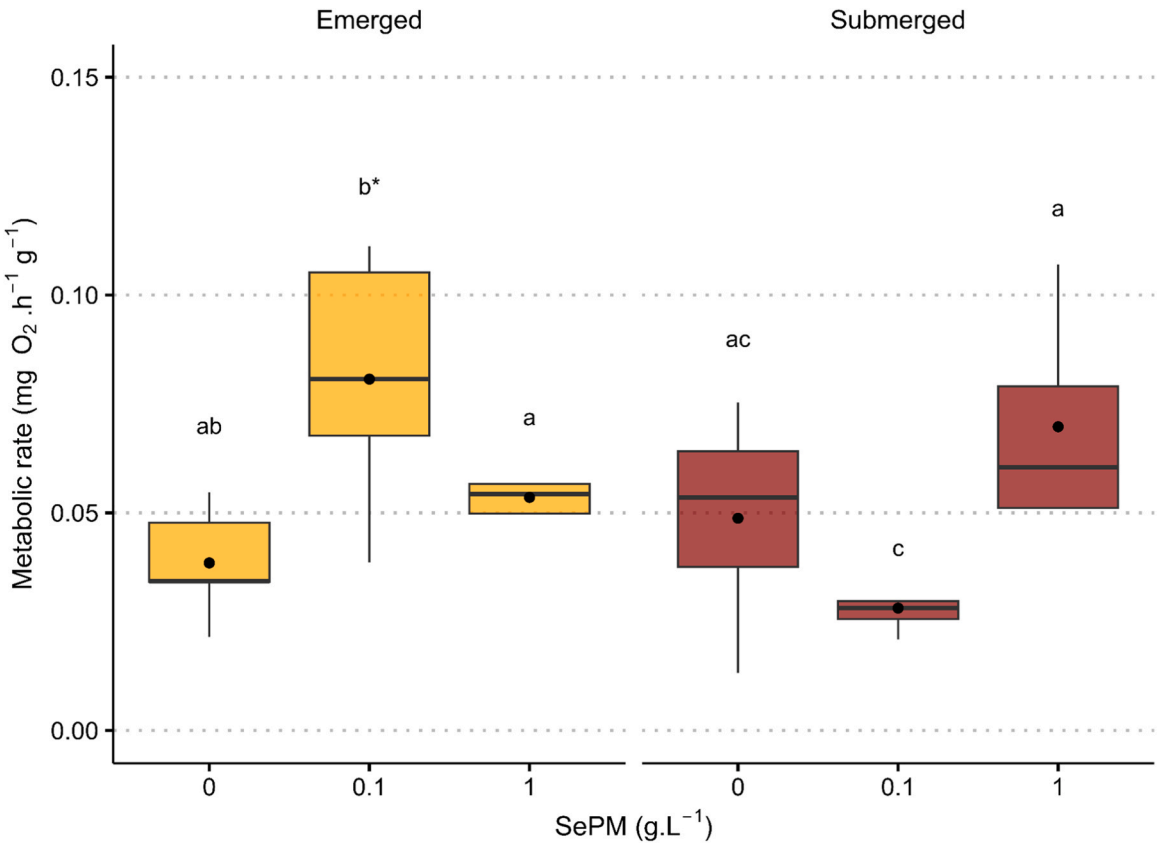

Fig. 4. Metabolic rate (mg. $O_2.h^{-1}.g^{-1}$) in crabs exposed to SePM under emerged and submerged conditions. Boxplots represent the distribution of data for each experimental group, where the median line indicates the median of the data, the bottom of the box represents the first quartile (Q1), and the top of the box represents the third quartile (Q3). The black dot indicates the mean value for each group. Different letters indicate differences between the concentrations within the same condition. *Indicates the difference between conditions within a concentration.

3.4. Righting time

The SePM concentration ($F = 2.96$, $p = 0.061$), the exposure condition ($F = 3.97$, $p = 0.052$), nor the interaction between these factors ($F = 2.69$, $p = 0.078$) affected the righting time response (RT). Nevertheless, the RT unpaired mean difference comparing crabs submerged in 1 $g.L^{-1}$ SePM and the control was 0.271 [95 %CI 0.121, 0.53] ($p = 0.012$). The mean difference between emerged crabs in 0.1 $g.L^{-1}$ SePM and control was 0.164 [95 %CI 0.061, 0.278] ($p = 0.034$) (Fig. 6).

3.5. Principal component analysis (PCA)

The principal component analysis (PCA) incorporating metals and metalloid concentrations in whole crabs and physiological, biochemical, and behavioral responses effectively revealed 42.4 % of the total variation within the two principal components for emerged crabs (Fig. 7 A). The PC1 dimension explained 22.2 % of the total variation, while the PC2 dimension explained 20.2 %. There was an association among Al, Fe, Mn, Sr, Rb, and Zn levels in whole crabs and SOD in the hepatopancreas of crabs exposed to 1 $g.L^{-1}$ SePM. Conversely, Zr, Se, GST, and CAT in the hepatopancreas, metabolic rate, energy budget components, and righting time were associated with each other.

For submerged crabs, the total variation within the two principal components totaled 64.3 % (Fig. 7 B). Within the PC1 dimension (48.4 %), there was a significant correlation among Al, Fe, Mn, Cr, righting time, and lipid peroxidation (LPO) in the gills of crabs exposed to 1 $g.L^{-1}$ SePM. Additionally, on the same PC1 dimension, the concentration of Rb and the energy budget parameters, such as FR, EI, and SFG, were oppositely associated with the grouping of 1 $g.L^{-1}$ SePM. The juxtaposition of these contrasting associations emphasizes the discernible impact of SePM exposure. It underscores that the high concentrations of Al, Fe, Mn, Cr, and Y correlate with a reduction in both SOD activity and energy budget parameters. The correlation between Zr, SOD, and GST in the hepatopancreas, opposite to CAT and GST in the gills, as well as CAT and LPO in the hepatopancreas, contributed to the variation in PC2 (15.9 %).

3.6. Canonical correspondence analysis (CCA)

To assess the effect of metal and metalloid concentrations on biological responses in crabs, the CCA was conducted utilizing paired data matrices to submerged and emerged groups. The CCA results demonstrated a correlation between canonical components representing metal and metalloid concentrations in crabs and the biochemical biomarkers in gill and hepatopancreas (emerged: $r^2 = 0.97$, $p < 0.001$; submerged: $r^2 = 0.99$, $p < 0.001$), as well as the energy budget components, metabolic rate, and behavior response (emerged: $r^2 = 0.97$, $p < 0.001$; submerged: $r^2 = 1$, $p < 0.001$).

4. Discussion

Using both bivariate and multivariate approaches, we successfully unveiled a discernible pattern of bioconcentration of the constituent metals within SePM and their potential effects on multiple energetics treats of a bioindicator crab. We sought to ascertain the impact of two concentrations of SePM in water on the fiddler crab *M. rapax* when challenged by submergence regimes. Furthermore, we demonstrated that the increase in Al, Fe, Mn, Cr, and Y in crabs depended on SePM concentration, with their highest concentrations observed under prolonged submerged conditions. The increase in these metal

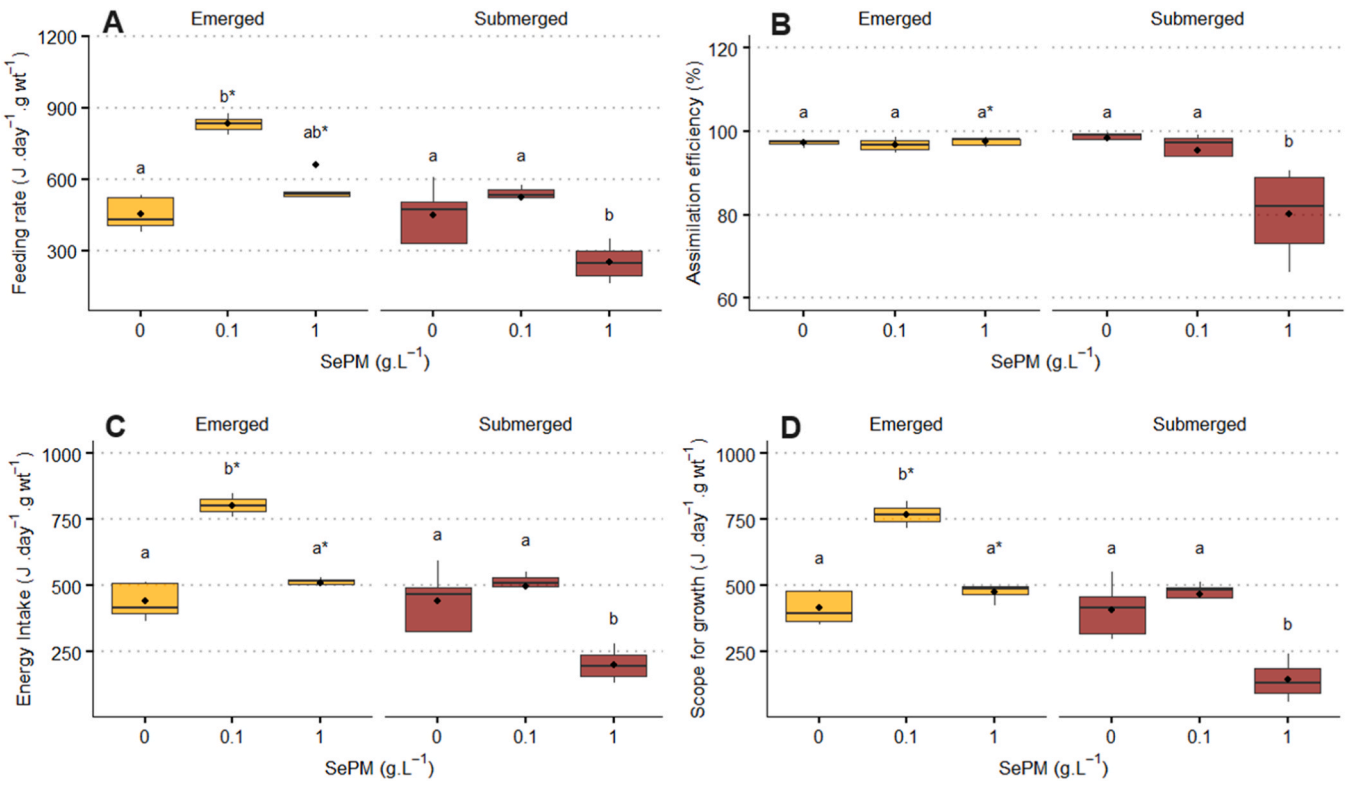

Fig. 5. Feeding rate (FR) (J.day^{-1}.g wt^{-1}) (A), assimilation efficiency (AE) (%) (B), Ingested energy (I) (J.day^{-1}.g wt^{-1}) (C) and scope for growth (PPg + Pr) (J. day^{-1}.g wt^{-1}) (D) in crabs exposed to increasing concentrations of SePM under both emerged and submerged conditions are showed. Boxplots represent the distribution of data for each experimental group, where the median line indicates the median of the data, the bottom of the box represents the first quartile (Q1), and the top of the box represents the third quartile (Q3). The black dot indicates the mean value for each group. Different letters indicate differences between concentrations within the same condition. *Indicates the difference between conditions within a concentration.

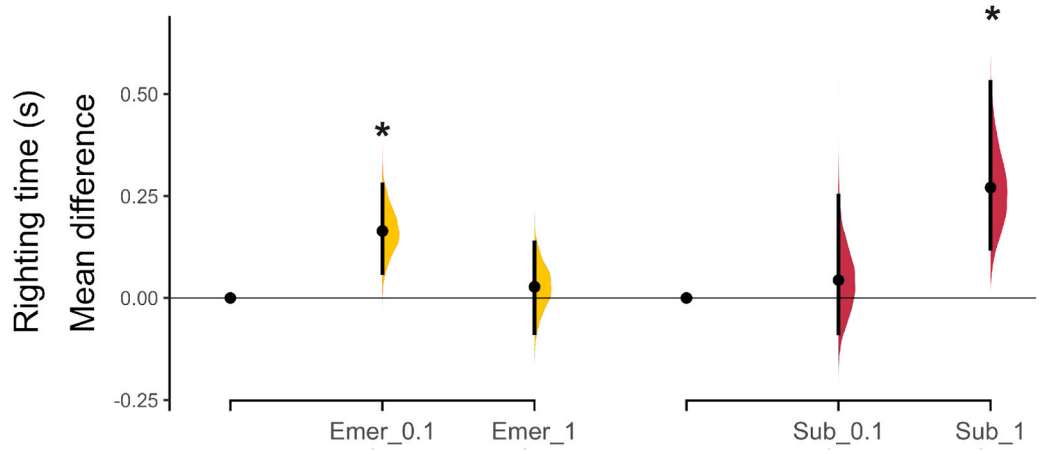

Fig. 6. The mean difference in righting time (RT) (s) is measured in crabs exposed to varying concentrations of SePM under both emerged (yellow) and submerged (red) conditions. The data is presented using Cumming estimation plots, wherein dots represent mean differences, and the vertical error bars signify the 95 % confidence interval for each comparison. The bootstrap sampling distributions provide a view of the behavior across different experimental conditions. *Indicates the difference between concentrations of SePM compared to the control condition.

concentrations may be related to the suppression of the activity of antioxidant system enzymes, such as SOD in the hepatopancreas of *M. rapax,* as well as the reduction in the feeding rate, assimilation efficiency, energy intake, and scope for growth and consequently less energy to maintain homeostasis or behavior responses reflected by the increase in time for straightening the position of crabs. Also, the total submergence experience shows a higher stress level compared to non-submerged regimes by crabs [12,47]. Fiddler crabs exhibit escape behavior, with a clear preference to avoid water when it is contaminated by metals [23]. Thus, the simulation of different flooding regimes, common in mangrove wetlands and exacerbated in degraded mangrove areas, was instrumental in understanding the effects of SePM.

4.1. *The metals and metalloid concentration in Minuca rapax*

The complex mixture of metallic particles in SePM can disperse upon settling in water, releasing and dissociating into smaller particles, including metals and metallic nanoparticles, available as dissolved in

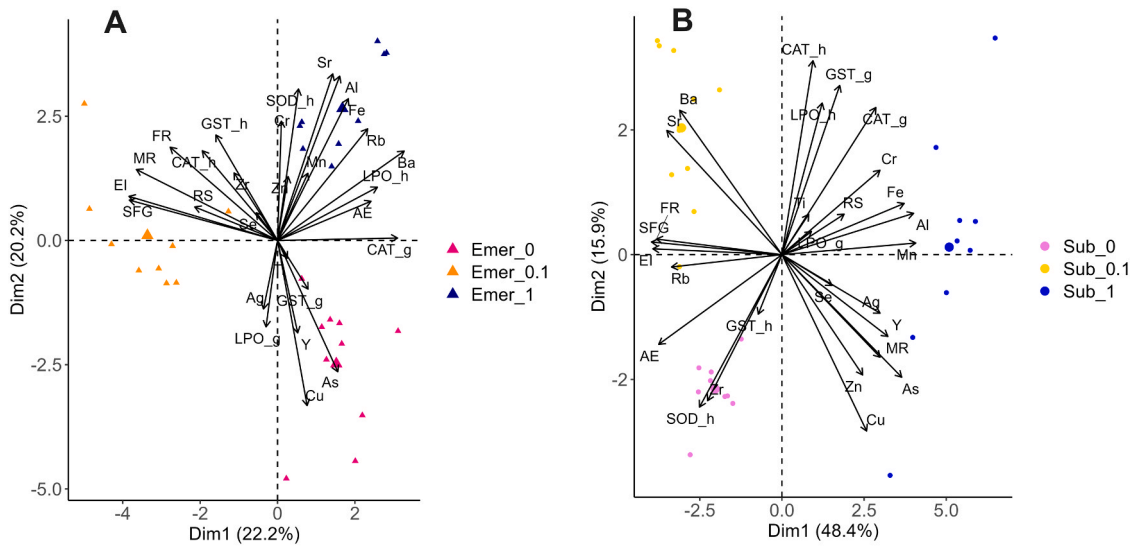

Fig. 7. Principal component analysis (PCA) between metal and metalloid concentration (Al, Ag, Ba, Cr, Cu, Fe, Mn, Rb, Se, Sr, Y, Zn, Zr, and As) in whole crab, metabolic rate (MR), righting time (RT), feeding rate (FR), assimilation efficiency (AE), energy intake (EI), scope for growth (SFG), oxidative stress enzymes in gill (catalase [CAT_g] and glutathione S-transferase [GST_g]) and hepatopancreas (catalase [CAT_h], glutathione S-transferase [GST_h] and superoxide dismutase [SOD_h]), as well as lipid peroxidation in gill (LPO_g) and hepatopancreas (LPO_h) in *M. rapax* exposed to emerged (A) and submerged (B) conditions. The components PC1 (horizontal axis) and PC2 (vertical axis) represent the total data variation (eigenvalues) (42.4 % in emerged crabs and 64.3 % in submerged crabs). Legend contains the combination of factors condition exposure (sub = submerged, and emer = emerged) and SePM concentration.

water or deposited in the sediment [2]. Metallic components can lead to diverse interactions with the biota, depending on their affinity with different particles in the water and their physicochemical parameters, such as salinity, temperature, and pH (reviewed in [48]). Since low salinities increase metal uptake in estuarine organisms [49], we added the SePM mixture to water with a salinity of 10 ‰ in our study. In this salinity, crabs are ion hyper-regulating, meaning their ionic regulation mechanisms favor the uptake of ions. Bioavailable metals have a positive monovalent or divalent charge, similar to many dissolved ions, such as Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+}. These ions are primarily taken up from the environment through the gills and reabsorption by the antennal gland [50]. Once uptake, metals can interfere with various physiological pathways, disrupting homeostasis and necessitating allostatic mechanisms to restore balance.

The SePM exposure (1 $g.L^{-1}$) mainly resulted in high Al, Fe, Mn, Cr, and Y levels in tissue crabs, particularly in submerged conditions. The bioavailability of these metals resulting from ore extraction activities has been characterized in numerous studies involving other crustaceans associated with sediment, such as penaeid shrimps [51], *M. rapax* [52] and *Ucides cordatus* [53], and different trophic levels, from lower levels such as plankton [54], to higher levels such as fish [55]. Our findings, consistent with Monteiro et al. [22], elucidated that the notable concentrations of metals found in crabs exposed to SePM, irrespective of their emersion/submersion condition, are primary constituents of SePM.

Several studies have explored the effects of SePM in 1 $g.L^{-1}$ concentration on metal and metalloid concentration in aquatic organisms for 96 h. In fish, Cr, Zn, and Ni were bioaccumulated in the heart and erythrocytes of the freshwater *Oreochromis niloticus* [20] and high levels of Zn and Fe were found in all organs of the estuarine *Centropomus parallelus* [22], both exposed to SePM for 96 h. The tadpoles *Lithobates catesbeianus* also presented elevated levels of Fe and Al in whole blood [21]. The long-term exposure (30 days) to SePM highlighted the persistent bioaccumulation of Fe, Al, and Mn as the three most abundant metals in the viscera of *O. niloticus* [56], Fe, Ni, Cu, Zn, Rb, Sr, Cd, and Ba in the brown mussels (*Perna perna*) [5], and Al, Fe, Cu, Zr, Nb in soft tissues of the mangrove crab *Ucides cordatus* [53].

The semi-terrestrial crab *M. rapax* can endure prolonged periods without aquatic exposure, potentially circumventing contamination risks [11]. In perspective, crabs used in the present study are from an area greatly affected by climate change in the northwestern Yucatan Peninsula [57]. This area is susceptible to extreme hydroclimatological phenomena like floods and drought. Mangroves suffer from prolonged periods of drought or flooding [58], resulting in sedimentary conditions that are physiologically intolerable for organisms inhabiting these mangroves [57]. The respective physiological limitations and flexibilities provide insights into how fiddler crabs might respond to environmental change in synergy with contaminants.

Burrowing crabs are inherently linked with sediment and are detritivores; as observed in this study, the animals indiscriminately foraged on food remains or particles associated with SePM settled at the container bottom. The same response pattern was observed with *M. rapax* exposed to particles of microplastics [59]. Crabs can considerably influence rates and pathways of organic matter mineralization and remove contaminants, such as metals, making them more available [60], and species closely associated with sediment face heightened exposure to metal concentrations tending to accumulate in their tissues at greater levels. Heightened genotoxicity tends to correlate with species interacting with multiple environmental compartments [4], including sediment, as observed in the crab species *Goniopsis cruentrata*, in contrast to arboreal and specialist herbivore crab *Aratus pisoni*, and the digger generalist herbivore crab *Ucides cordatus* [61]. Elevated concentrations of metals stemming from mining activities, such as Fe, Al, and Mn, persisting within animal tissues, align with predominant findings in benthic species inhabiting regions impacted by these activities [51,62]. High concentrations of essential metals (Al, Fe, and Mn) can trigger toxicity in crustaceans [19]. Accumulation of Y (Rare-earth elements) in *M. rapax*, highlights the use of fiddler crabs as a sentinel species, as observed in other studies [63,64]. To summarize, metal bioaccumulation by SePM exposure seems to be a time-dose-response, and a complex mixture of metals requires further study.

4.2. *Effects depend on the exposure regime*

The effects of SePM were largely dependent on the exposure regime. The metal content in submerged crabs was higher compared to emerged crabs, especially at higher concentrations (1 $g.L^{-1}$). This response may be attributed to the prolonged contact with the contaminant dissolved in the water when fully submerged, leading to more pronounced adverse

effects on the energy budget components in submerged crabs exposed to SePM.

The submersion regime can interfere with the metabolic rate, osmoregulation, and acid-base balance in semi-terrestrial crabs [65]. The role of the gills in all these processes is specific to the mechanisms that enable bimodal respiration in crabs, along with osmoregulation and acid-base balance [66]. In response to stressful conditions, crabs may attempt to minimize contact with the external environment by reducing the entry and exit of circulating water in the gill chamber [47]. However, this behavior is limited by the need for gaseous and ionic exchange with the external environment, resulting in different metabolic rates and redox balance responses between submerged and emerged crabs.

4.3. Enzymes of the antioxidant system and lipid peroxidation

The uptake of potentially toxic metals in crabs can occur across the gills or other body surfaces by absorption, or via the gut epithelia after food ingestion. In turn, metals may be excreted across the same epithelia [67], eliminated via carapace after ecdysis [68], or detoxified via the hepatopancreas. In animals permanently submerged, the gill epithelium is in direct contact with dissolved metals in the surrounding water, which is the main route of metal uptake from the dissolved phase. Consequently, the gill epithelium can be considered the first target interface of metal toxicity in submerged crabs according to the induction of CAT activity in submerged gill crabs by SePM exposure. The scenario of prolonged submergence is challenging for the physiology of *M. rapax*, as observed by the difference in CAT activity in the gills and SOD in the hepatopancreas between the emerged and submerged crabs when in control conditions. Adding the feed behavior exhibited, common to a bioturbator crab, a detritivore that exploits particulate organic matter, resulting in higher levels of metals in tissues and biochemical responses, such as the induction or inhibition of SOD activity depending on emerged or submerged conditions as well as the LPO high levels, in hepatopancreas.

The antioxidant defense strategies exhibited by decapod crustaceans when in response to the presence of metals vary according to the concentration and speciation of metals and their combination in a mixture [69]. Iron, Cr, and Cu are redox-active metals due to their ion capacity to change their valence state, which participates in the Fenton reaction [70]. The manganese dynamic (commonly associated with the Fe cycle) is also controlled mainly by redox processes. Not by chance, Fe, Cr, and Mn added to Al stood out among the metals that make up SePM, exhibiting high levels in the tissues of exposed crabs.

These elements are often neglected regarding their potentially toxic effect on organisms because they are considered micronutrients [71]. High Mn concentrations may cause severe adverse health effects, such as physiological disorders in fish and crabs [72,73]. Exposure to Cr for 96 h caused a decrease in AChE activity in hemolymph and GST in the hepatopancreas of *C. maenas* [74]. Although Al is the most abundant element on the planet, it is a non-essential metal, and its accumulation can cause severe damage to biota [19]. Crustaceans exposed to Al showed oxidative stress, histopathological damage, disruption of biochemical homeostasis, and immune system suppression [75-77]. These results suggest that the exposure regime in semi-terrestrial species also causes different antioxidant defense responses, as observed by [29], with other fiddler crab species.

While the increase in Fe, Mn, and Cr levels in crabs was concentration-dependent, there was a reduction in Cu concentration in animals exposed to SePM. The lower concentration of SePM (0.1 $g.L^{-1}$) resulted in a smaller amount of material settling at the container bottom, reducing ingestion by the crabs. Also, the lowest SePM concentration in water changes the interaction between the metallic components, facilitating or hindering entry and the effect of some metals on the gills. Different studies have demonstrated the intrinsic association between changes in Cu concentration in water and the direct impact on respiration and osmoregulation in crustaceans [11,78,79]. Another study using *M. rapax* not completely submerged revealed that this species appears to employ a behavioral strategy to avoid containing media in free access experiments from contaminated water [11], which would explain the lower toxicity found in the present study and reported with other species of fiddler crabs [23]. Copper is present in crustaceans in the hemocyanin pigment and is responsible for oxygen transport in the respiratory system; this explains the high concentrations, which do not have detoxification mechanisms, of this element in whole crabs irrespective of the SePM concentration.

The rare earth element Y was only found in submerged crabs exposed to 1 $g.L^{-1}$ SePM, alongside an abundance of Al, Fe, Mn, and Cr. Variations in the accumulation of Y and its proportions directed towards detoxification processes were described as species-dependent [80]. For instance, substantial Y accumulation can predominantly be sequestered within detoxifying subcellular fractions or highly sensitive subcellular fractions, such as mitochondria [80]. Despite fiddler crabs being a good indicator of rare earth elements in mangroves worldwide [63], understanding how organisms manage rare earth elements in natural aquatic ecosystems remains limited.

4.4. Energy budget

All components of the energy budget of *M. rapax* were affected by exposure to SePM. Submerged crabs exposed to SePM exhibit a remarkable reduction in energy budget components. Exposure to SePM has been shown to elevate the respiratory frequency in *O. niloticus* under normoxia and restrict respiratory adjustments during hypoxia [81]. It is well reported that metal contamination in crabs can cause damage to the gills and consequently has adverse effects on oxygen consumption, acid-base balance, and osmoregulation [14], leading to oxidative stress [60]. However, the impact on the metabolic scope and the subsequent ecological consequence for the species remains poorly understood.

The passive influx of metals can be enhanced by the route ingested from the sediment [12]. The lack of energy value in *M. rapax* may be attributed to SePM ingestion, which reduces the scope for growth as the available energy for metabolic processes, maintenance, and growth of organisms. Furthermore, when contaminants enter the organism through the intestine, it triggers heightened responses. The hepatopancreas acts directly on detoxification mechanisms to control the influx of these substances [82] and can also be negatively impacted by metals obtained from the diet. In mud crab *Scylla paramamosain*, the toxicity of Cd in the intestine was in a concentration-dependent manner, inducing oxidative damage, decreasing the immune system, disrupting metabolic function, and altering intestinal microbial composition [83]. Cadmium and Pb also caused intestinal mucosa damage and epithelial cell exfoliation, disturbance in intestinal antioxidant capacity, and changed the diversity of intestinal microbial of the white shrimp *Litopenaeus vannamei* [84]. Other metals such as Cu also affected the intestinal microbiota and caused structural damage in the hepatopancreas of the white shrimp *Litopenaeus vannamei* [85].

Considering that detritivore crabs like *M. rapax* deliberately feed on organic particles [8,12], the metal intake by gill and dietary contamination is particularly relevant in these semi-terrestrial crabs where waterborne exposure is periodic and often occurs only during high tide. Dietary metal exposure affects mainly energy metabolism [86] and reproduction [87]. The dietary contamination route should be considered in existing regulations regarding environmental contamination in risk assessments [88], especially in areas with metallurgical industries near mangrove ecosystems. The main data findings suggest that *M. rapax* is more vulnerable to the effects of SePM when completely submerged. Mangroves can experience long periods of flooding, creating physiologically limiting conditions for their inhabitants [58]. Prolonged flooding events in mangroves can enhance the negative effect of complex mixtures as the presence of metals and metalloids in the environment. These results are noteworthy contributors to understanding the dynamics of SePM exposure in mangrove habitats, highlighting their

consistent presence and potential ecological implications.

4.5. Behavior response: righting time

Exposure to SePM led to an increase in RT, subsequently affecting the behavior response of *M. rapax*. As highlighted, the metals Al, Cr, and Cu alter the activity of acetylcholinesterase, which is functionally associated with neuronal synapses and communication in the motor plate [19], and indirectly can alter behavioral responses based on their effect under synaptic communication. Furthermore, the decompensation in the energetic scope reduces the energy available for essential metabolic functions and can indirectly harm the crabs' behavioral responses [89]. Additional research has suggested that exposure to contaminated environments not only diminishes coordination [90] but also has the potential to disrupt critical life history behaviors such as mating and predator evasion [91], as well as foraging [92]. Crabs showed decreased righting time response and feeding, decreasing the capacity to capture and consume active prey species [89]. Therefore, our data reveal that the effect of exposure to SePM has potential ecological damage in addition to the specific biochemical and physiological effects already well characterized in aquatic species.

5. Conclusion

Minuca rapax exhibits a propensity to accumulate metals irrespective of the submersion regime. Our research indicates that at lower concentrations of SePM, crabs can display physiological compensation mechanisms to deal with the presence of SePM, however in higher concentrations (up to 1 $g.L^{-1}$), they seem to surpass a critical threshold, detrimentally affecting *M. rapax* by a multi-level of energetic biomarkers. Consequently, SePM exposure suppresses antioxidant enzyme activity, disrupting the physiological equilibrium of *M. rapax*. Furthermore, alongside biochemical and physiological impairments, the diminished righting response in crabs underscores the ecological ramifications of SePM contamination. In our assessment of the effects of SePM exposure on sentinel crabs, a warning is raised about the presence of mangrove sediments- SePM contamination in sentinel crabs is worthy of attention for health and the health of marine and estuarine ecosystems.

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CRediT authorship contribution statement

Mariana V. Capparelli: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Anieli C Maraschi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cesar Rubio-Lopez:** Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis. **Solana M Snitman:** Methodology, Formal analysis, Data curation, Conceptualization. **Guillermina Alcaraz:** Writing – review & editing, Visualization, Supervision, Resources. **Magdalena V Monferrán:** Writing – review & editing, Resources, Methodology, Formal analysis. **Iara C Souza:** Writing – review & editing, Resources, Project administration, Methodology, Investigation. **Brian Pichardo-Casales:** Writing – review & editing, Methodology, Data curation. **Carlos Rosas:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis. **Marisa N Fernandes:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Daniel A Wunderlin:** Writing – review & editing, Software, Resources, Methodology. **Claudia Caamal-Monsreal:** Supervision, Resources, Methodology.

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.

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Environmental implication

This is the first on-laboratory study that determined the effect of metallic settleable atmospheric material (SePM) on multi-level energetic responses on the sentinel fiddler crab *Minuca rapax*. SePM exposure increases, in a concentration-dependence way, Al, Fe, Mn, Cr, and Y, decreases metabolic rate, assimilation efficiency, energy for maintenance, enzymatic activities and increases the right-time response in this crab species. SePM in mangrove sediment raises an alert as burrower crabs contribute to top-down translocation of contaminants, have high bioaccumulation capacity and significant trophic chain biomagnification potential which highlight high impact at the organismal-level with unknown consequences to the local population.

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