



## Research Article

# Microplastic impacts physiological mechanisms of marine, diadromous, and freshwater crustaceans

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## ABSTRACT

The effects of microplastic on species inhabiting different saline gradients remain unclear, particularly the impact of glitter particles. We investigated the effects of glitter on the physiological mechanisms of species from various saline environments using the following models: a marine/estuarine shrimp (*Penaeus vannamei*), a diadromous shrimp (*Macrobrachium amazonicum*), and an exclusively freshwater shrimp (*Macrobrachium potiuna*). The animals were exposed to varying glitter concentrations (0, 0.4, 4 and 40 mg.L<sup>-1</sup>) for 10 days, and to the salinities where they are found in nature. In addition, we evaluated the ability of one of the species (*P. vannamei*) to recover its physiology and morphology when transferred to glitter-free water after previous exposure to the pollutant for six days. We examined multiple mechanisms, including oxygen consumption, nitrogen excretion, hepatosomatic index, energy substrate oxidation, and osmoregulation. *P. vannamei* showed 13 % mortality at salinity 30. Physiological parameters exhibited specific variations in response to salinity and/or glitter concentration. Glitter exposure affected oxygen consumption in all three species, but with contrasting responses: *P. vannamei* exhibited increases up to ~200 % (depending on salinity), likely due to elevated energy demands, while *M. amazonicum* and *M. potiuna* showed reductions up to 70 %, potentially indicating gill damage. In *M. amazonicum*, glitter exposure enhanced the species' hyperosmotic regulatory capacity. *Penaeus vannamei* could not recover its hepatosomatic index and gill and intestinal morphologies after being transferred to glitter-free water. We conclude that glitter significantly alters physiological functions related to energy metabolism and osmoregulation. However, since these responses are salinity-dependent, the ability to migrate across different salinity gradients may provide an adaptive advantage for species exhibiting such behavior. The results obtained provide significant insights into the response of shrimps from different saline gradients to microplastics, which is still a major gap in our knowledge.

## 1. Introduction

Microplastics can bioaccumulate (Hossain et al., 2024; Lin et al., 2024), biomagnify (Pei et al., 2024), and impact several biological aspects of aquatic species, including structural and functional changes that lead to alterations in growth (Kudla et al., 2024; Uguen et al., 2024), reproduction (Castro et al., 2023), immunology (Guo et al., 2024), genotoxicity (Nugnes et al., 2022), and transgenerational effects (Song et al., 2022). It can be defined as synthetic polymer particles smaller

than 5 mm (Han et al., 2024). Generally, microplastics cause injuries primarily to the gills and digestive system (Xing et al., 2023), and the consequences include alterations in organ functions such as osmoregulation, respiration, nitrogen excretion, nutrient absorption, and energy acquisition (Capparelli et al., 2023; Rashid et al., 2024; Sun et al., 2024). Furthermore, microplastics can adsorb various contaminants, including persistent organic pollutants, antimicrobial agents, and hydrophobic chemical compounds (Singla et al., 2020; Uddin et al., 2020). Glitter, a microplastic particle widely used across various countries and cultures,

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is commonly found in cosmetics, make-up materials, body paints, nail polishes, and crafts (Tagg and Ivar do Sul, 2019; Yurtsever, 2019). Annually, tons of glitter, a primary, reflective microplastic ranging from 0.05 to 0.35 mm in size and resistant to degradation, are produced (Yu et al., 2018), with density of  $1.3 \text{ g}\cdot\text{cm}^{-3}$  at  $20 \text{ }^\circ\text{C}$  (de Oliveira et al., 2025). Glitter constitutes a distinct subclass of microplastics, generally engineered as a trilaminar structure comprising a core of biaxially oriented polyethylene terephthalate (BoPET), serving as a durable and flexible substrate, a vapor-deposited aluminum layer to enhance reflectivity, and an outer coating of thermoplastic polymer, to provide color and environmental resistance (Yu et al., 2018; Green et al., 2021). Compared to other microplastic fragments, the shape, composition, and density of the glitter allow the particles to remain suspended in aquatic systems. Their irregular laminar structure provides environmental relevance. This makes glitter suitable for controlled laboratory exposure experiments and representative of microplastics commonly found in the environment (de Oliveira et al., 2025). Although some studies have shown the toxicity of glitter, its effects on animal physiology and morphology are unknown (Tagg and Ivar do Sul, 2019; Abessa et al., 2023; Chen et al., 2022). Due to its complex chemical composition and structure, it can affect the morpho-physiology of animals and cause physical and chemical damage to tissues.

Research on the impacts of glitter on aquatic species' biology is relatively recent, with a key focus on understanding their effects across different saline gradients. It remains unclear whether coastal species are more severely affected by glitter pollution than freshwater organisms. However, it is known that glitter interacts with various environmental parameters, including salinity, temperature, soil type, precipitation, and wind (Malli et al., 2022). While salinity does not significantly alter glitter length and structure (Sait et al., 2021), it can influence their buoyancy, transport, and density (Joo et al., 2021).

Decapod crustaceans are important for comparative studies across species inhabiting different environments, as they are found in marine, brackish, and freshwater ecosystems. Additionally, some species are diadromous, migrating between freshwater and brackish waters as part of their life cycle. Decapods play crucial roles in coastal ecosystem functioning and are significant resources for fisheries and aquaculture (FAO - Food and Agriculture Organization of the United Nations, 2024). In this study, we investigated the effects of glitter in crustaceans from marine to freshwater habitats. We measured multiple physiological parameters (metabolism, ammonia excretion, hepatosomatic index, oxidized energy substrate type, and osmoregulation) in marine/estuarine (*Penaeus vannamei*), diadromous (*Macrobrachium amazonicum*), and exclusively freshwater (*Macrobrachium potiana*) shrimp exposed to varying glitter concentrations and to the salinities where they are found in nature. These species were selected to represent distinct ecological types along the marine-freshwater gradient, enabling comparative assessment of glitter effects across different salinity gradients. Additionally, we evaluated the ability of one of the species (*P. vannamei*) to recover its physiology and branchial and intestinal morphologies when transferred to glitter-free water after previous exposure to the pollutant. We hypothesize that exposure to glitter would cause morphological and physiological alterations in shrimps due to its chemical and structural composition. However, such effects may vary due to the biology of each species and the salinity in which they are found in their natural habitat.

## 2. Material and methods

### 2.1. Glitter composition

Glitter samples (0.08 mm) were analyzed for polymer type using pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) according to methodologies adapted from Gimiliani et al., (Gimiliani et al., 2020), Tsuge et al. (Tsuge et al., 2011) and Zellner and Quarino (Zellner and Quarino, 2009). Before exposure, glitter particles were rinsed with distilled water, oven-dried at  $40 \text{ }^\circ\text{C}$  for 24 h, and stored in sterile glass

containers to avoid contamination. The choice of 0.08 mm glitter particles is based on their suitability for analytical procedures and environmental relevance. This particle size mimics the dimensions of microplastics commonly found in environmental samples, facilitating studies on dispersion, toxicity, and bioaccumulation. It also represents particles that can escape wastewater treatment systems, making them significant for environmental monitoring. Furthermore, it allows for more controlled laboratory analyses, especially in Py-GC/MS, where smaller particles undergo more efficient and uniform pyrolysis (Yurtsever, 2019). Py-GC/MS represents a robust approach for glitter characterization, requiring minimal sample preparation and allowing both chromatographic separation and mass detection of the pyrolysis products (Matsui et al., 2020). About 0.2 mg of sample was transferred to the sampler of the pyrolyzer with a stainless steel spatula to sample cup in the Py-GC/MS system. In the GC system, the column temperature was initially set at  $40 \text{ }^\circ\text{C}$  for 2 min, followed by an increase to  $295 \text{ }^\circ\text{C}$  at a rate of  $20 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$  over 13 min. In the MS system, the mass-to-charge ratio ranged from 25 to 200  $m/z$ , with a split ratio of 27 and electron impact ionization at 70 eV. The gases resulting from sample firing were transferred by helium carrier gas through (at a flow rate of  $1.5 \text{ mL}\cdot\text{min}^{-1}$ ) the Ultra ALLOY-5 (nonpolar) column, the dimensions of which were 0.25 mm in diameter, 30 m in length and 0.25  $\mu\text{m}$  in thickness. A Frontier Labs pyrolyzer (model EGA/PY-3030D) was used with a micro furnace in single shot mode, connected to a Shimadzu GC/MS system (model QP5000). The gases resulting from the firing were separated and quantified using the GC/MS system. In parallel to the analysis of the sample, an analysis of the standard polyvinyl chloride (PVC) was performed. Before each analysis, a blank sample was analyzed before each run under the same conditions to check for retention time interferences, and the chromatograms were compared to those reported by Tsuge et al., (Tsuge et al., 2011). The components identified in the glitter samples were: benzene, anthracene, hydrogen chloride, methyl acrylate, and toluene (increasing order of concentration) (Fig. 1).

### 2.2. Characterization of species

Since our objective was to compare the effects of glitter on species that live in different salinity gradients, we used as models a marine/estuarine shrimp (*P. vannamei*), a shrimp that migrates to fresh and brackish water (*M. amazonicum*) and an exclusively freshwater shrimp (*M. potiana*). *Penaeus vannamei* is native to the Eastern Pacific Ocean, and is the most cultivated marine shrimp in the world (FAO - Food and Agriculture Organization of the United Nations, 2024; Wang et al., 2019). In 2023, approximately 1.98 million tons of the species were

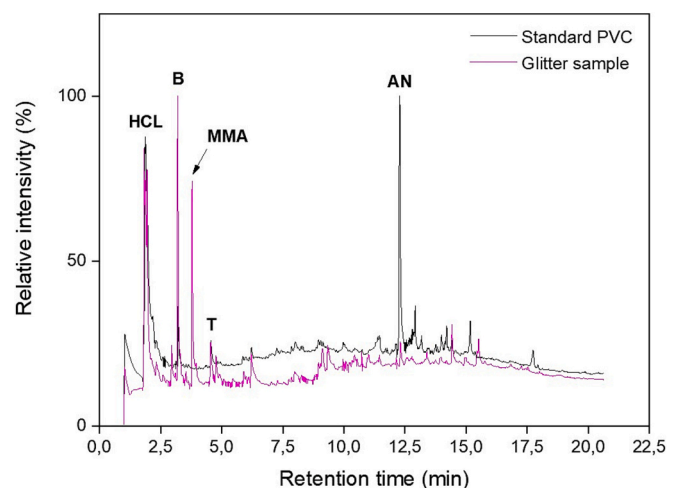


Fig. 1. Components in glitter identified by pyrolysis coupled with mass spectrometry: HCL (Hydrogen Chloride), B (Benzene), MMA (Methyl Acrylate), T (Toluene), AN (Anthracene).

farmed in several countries, including China, India, and Ecuador (FAO - Food and Agriculture Organization of the United Nations, 2024). It is an euryhaline species found in its natural environment at salinities ranging from 0.5 to 40S (Alves Neto et al., 2019). The genus *Macrobrachium* is a diverse group of freshwater and brackish water shrimps distributed throughout tropical and subtropical regions. *Macrobrachium amazonicum* is a shrimp endemic to South America (Pileggi et al., 2013), important for fishing and aquaculture, and is found in fresh and brackish water regions close to the sea (Maciel and Valenti, 2009; Moraes-Valenti and Valenti, 2007). *Macrobrachium amazonicum* is a diadromous species that depends on brackish water to complete its life cycle. In contrast, *M. potiuna* has a wide distribution from the States of Bahia to the Rio Grande do Sul, South America, but it is found only in freshwater and is independent of brackish water to complete its life cycle (Müller and Carpes, 1991).

### 2.3. Collection and acclimation of animals in the laboratory

The marine shrimp *P. vannamei* (about 4.5 g) was collected from farms in the state of Santa Catarina, Brazil (26°12'32.3"S 48°44'23.7"W). Shrimps *M. amazonicum* (about 1.5 g) and *M. potiuna* (about 0.5 g) were collected, respectively, in shrimp excavated tanks of Aquaculture Centre of UNESP/CAUNESP, Jaboticabal, SP, Brazil (21°14'19" S 48°17'36" W) and in the rivers of Itanhaém, SP, Brazil (24°10'09.4" S 46°56'35.7" W). All animals were adults in intermolt. The molt stage was verified based on the morphology of the uropod setae and confirmed by the absence of exuviae during acclimation, following Drach and Tchernigovtzeff (Drach and Tchernigovtzeff, 1967). The animals were transported in boxes containing water from the collection site with constant aeration to the Sustainable Aquaculture Laboratory/UNESP in São Vicente, Brazil (23°58'S 46°23'W).

Shrimps were acclimated to laboratory conditions for seven days in individual aquariums before the experiments began. The animals were kept in individual aquariums containing 4 L of water with constant aeration, photoperiod of 12 h light–12 h dark, at 25 °C, dissolved oxygen  $6.76 \pm 0.38$ , mg.L<sup>-1</sup> and ammonia  $0.14 \pm 0.02$  µg.L<sup>-1</sup>. During the acclimation phase to the laboratory, the animals were kept at salinities of the collection (*P. vannamei*: 30S, *M. amazonicum* and *M. potiuna*: ≤ 0.5S). The treatments were performed with *N* = 10, and each animal is considered a replicate (one animal per aquarium).

### 2.4. Exposure of shrimp to glitter in the laboratory for physiological evaluation

After acclimatization to laboratory conditions (see item 2.3), the animals were exposed to different concentrations of glitter [0 (control), 0.4, 4, and 40 mg.L<sup>-1</sup>] for 10 days (Ping et al., 2018). The white glitter was about of 0.08 mm in size, and the different concentrations of glitter represent real concentrations of microplastics found in the aquatic environment (Lenz et al., 2016; Freire et al., 2023). The animals were exposed to glitter for 10 days because crustaceans usually recover homeostasis after a chronic period of adjustment (Song et al., 2024; Augusto and Masui, 2014). The three species studied were kept in individual aquariums (one animal per aquarium) containing water with the salinities in which they are found in their natural habitat. Thus, *P. vannamei* was exposed to the salinities of 20, 30, and 35S, *M. amazonicum* to freshwater and brackish water of 10 and 20S, and *M. potiuna* was kept only in freshwater (Freire et al., 2023; Augusto et al., 2007; Liu et al., 2024). The brackish water was obtained by diluting seawater with freshwater. The water were filtered in a compressed air vacuum pump (Primatec, model 121). During the experiments, the animals were fed commercial shrimp feed (Guabi) containing 35 % Crude Protein and a controlled photoperiod and water quality which is in item 2.4. Water in the aquariums was changed every three days. All shrimp were in the inter-molt stage of the molting cycle.

### 2.5. Exposure of *P. vannamei* to glitter followed by transfer to water free of glitter: Evaluation of the recovery capacity of homeostasis

After acclimatization to laboratory conditions (see item 2.3), *P. vannamei* were exposed to glitter (4 mg.L<sup>-1</sup>) or kept in filtered water without glitter (control) for 10 days. After 10 days, shrimps were transferred to filtered water free of glitter, and their physiology was evaluated after three, four, five, or six days. The morphology of the gills and intestine was assessed only on the sixth day. The concentration was chosen according to the results of pilot experiments in our laboratory, which showed that this concentration affects the metabolism, hepatosomatic index, and osmoregulation of *P. vannamei*. The animals were kept at 35S and in the conditions of water quality, food, photoperiod and temperature, which is in item 2.4.

### 2.6. Physiologic responses

#### 2.6.1. Survival

The survival of the animals in the aquariums was verified during the 30 days of experiments three times a day: at 8:00 am, 2:00 pm, and 8:00 pm. All animals that died were removed from the aquariums.

#### 2.6.2. Oxygen consumption, nitrogen excretion, and O: N ratios

The oxygen consumption was evaluated in closed individual respirometric chambers (Li et al., 2007; Ramaglia et al., 2018; Ramaglia et al., 2024). The chambers contained water with the same salinities, temperatures, and glitter concentrations as those in which the animals were kept in aquariums. The chambers were equipped with an oximeter (YSI, mod 52) and a probe with a precision of 0.01 mg.L<sup>-1</sup> (YSI, mod 5905). Every animal was subjected to 24-h starvation to reduce the calorogenic effect of food. After acclimation for 30 min under aeration, the first measurement of oxygen content within the chamber was made, and three hours later another measurement was made. Control chambers were also used, and the calculation of oxygen consumption was performed. Ammonia excretion (TAN = unionized plus ionized ammonia, as nitrogen) was measured from samples collected from the chambers at the end of the oxygen consumption procedure, including the control chamber (Ramaglia et al., 2024) and concentration was determined by colorimetry (Koroleff, 1976). By the end, animals were killed by a freezing meter, weighed (wet mass), oven-dried at 60 °C for 48 h, and weighed again (dry mass). Oxygen consumption and ammonia excretion were expressed dry mass rate (µg.mg DM<sup>-1</sup>.h<sup>-1</sup>). The major metabolic substrate for the production of energy used was estimated by atomic ratio O:N, dividing the gram atoms of oxygen consumed by the gram atoms of nitrogen excreted. Ratios between 3 and 16 indicate the use of proteins; values between 16 and 60 indicate the use of a mixture of proteins and lipids, and ratios over 60 indicate a predominant oxidation of lipids (Mayzaud and Conover, 1988).

#### 2.6.3. Hepatosomatic index

After euthanasia, the hepatopancreas was dissected and weighed (dry mass) (Metler toledo, 1 µg) to determine the Hepatosomatic Index based on the ratio below (da Costa et al., 2024):

Hepatosomatic Index (%) = (hepatopancreas mass x 100)/body mass.

#### 2.6.4. Hemolymph osmolality

After measuring oxygen consumption and euthanizing the animals (day 10th) hemolymph samples (30 µL) were taken from the region located at the cephalothorax to shrimps using an insulin syringe coupled to a #25–8 (Ramaglia et al., 2018). Hemolymph osmolality was measured using a vapor pressure osmometer (Wescor, Modelo 5500). The results are presented in mOsm.Kg<sup>-1</sup> water.

## 2.7. Morphology of *P. vannamei* transferred to water free of glitter: Histology of the gills and intestine

After the euthanasia of the animals in ice, the four posterior gill pairs and the intestine were removed for histological analysis. The methodology was adapted from Xing et al., (Xing et al., 2023). The tissues were fixed with formaldehyde (10 %), dehydrated in a gradient of ethyl alcohol (70 %, 80 %, 90 %, and absolute alcohol 99.5 %), and diaphanized with xylene. The samples were included in histological paraffin to facilitate the cutting and later, they were blocked in paraffin. The paraffin blocks were cut into 5  $\mu\text{m}$  sections in a rotating microtome (Lupetec, model MRP2015). The staining was done with hematoxylin and eosin (H&E staining). The slides were analyzed descriptively on a polarizing microscope with a camera (Leica, model DM 2500 P).

## 2.8. Statistical analysis

The effect of glitter and salinity on the physiology of *P. vannamei* and *M. amazonicum* was assessed by two-way ANOVA (salinity and microplastic). The effect of glitter on the physiology of *M. potiuna* was evaluated by one-way ANOVA (microplastic). Ability of the *P. vannamei* to recover its physiology when transferred to glitter-free water was evaluated by *t*-test. ANOVA and *t*-test were followed by the Student-Newman-Keuls multiple means test (SNK) to identify significant differences between groups. The analyses were performed using the program Sigma Stat 3.5 and a minimum significance level of  $P \leq 0.05$  was applied. The figures were presented through the data entered in the program Graphpad 5.01.

## 3. Results

### 3.1. Mortality

In *P. vannamei*, mortality was observed during exposure to 30 salinity: in the presence of glitter a single shrimp died at 4  $\text{mg.L}^{-1}$  and another at 40  $\text{mg.L}^{-1}$ . No mortalities occurred in *M. amazonicum* and *M. potiuna*.

### 3.2. Oxygen consumption

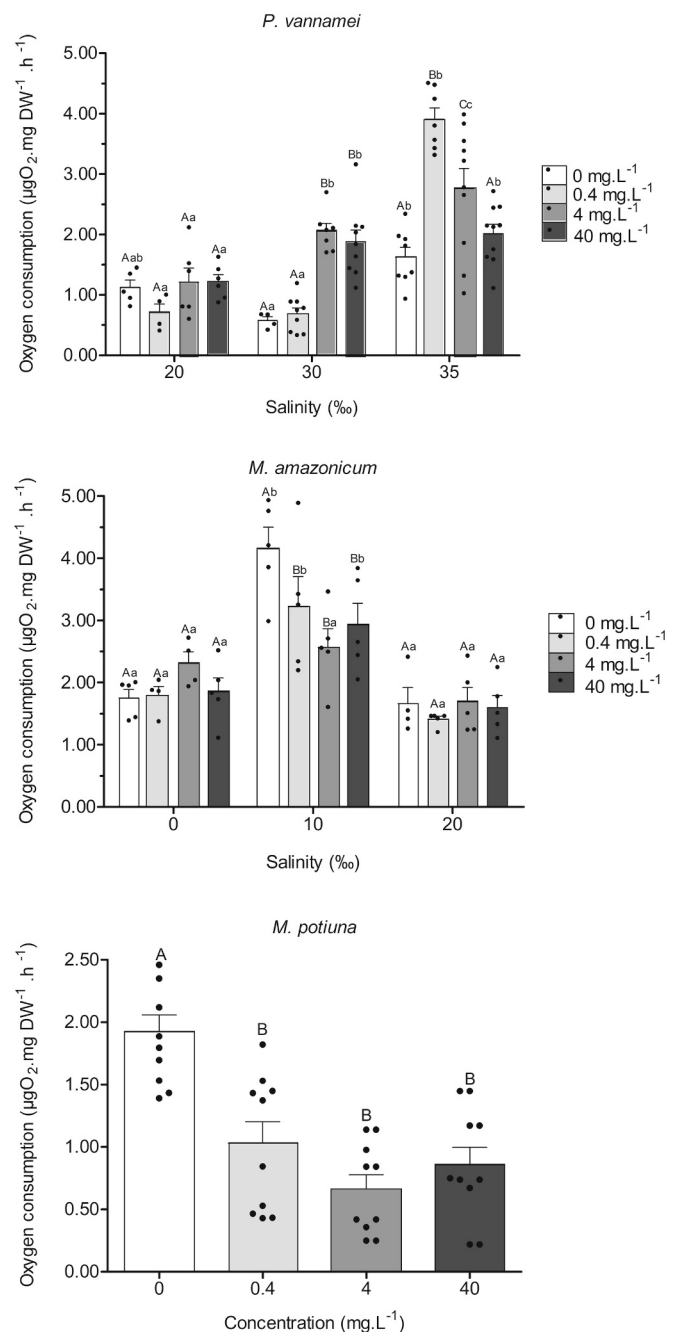
In *P. vannamei*, exposure to glitter caused increases in oxygen consumption at 30S (of up to +225 %) and 35S (of up to +140 %) (Fig. 2). In contrast, the exposure to glitter caused a decrease in oxygen consumption in *M. amazonicum* (10S: approximately -70 %) and *M. potiuna* (freshwater: -20 %).

### 3.3. Ammonia excretion

The ammonia excretion showed specific increases in the three species, during exposure to 0.4  $\text{mg.L}^{-1}$  of glitter. In *P. vannamei*, ammonia excretion increased only at 35S, the salinity where excretion is higher than the others (up to +5500 %). In *M. amazonicum* the ammonia excretion suffered alterations in brackish water 10S (of up to +155 %) and 20S (of up to +100 %) in some concentrations of glitter. In *M. potiuna* the ammonia excretion increased only at the concentration of 0.4  $\text{mg.L}^{-1}$  (~240 %). (Fig. 3).

### 3.4. Type of oxidized energy substrate and hepatosomatic index

*Penaeus vannamei* oxidizes proteins in the absence of glitter, but it can also begin to use a mixture of proteins and lipids, depending on the concentration of the pollutant in the water (Table 1). *M. amazonicum* is a species that alternates between the oxidation of lipids or a mixture of both energy substrates, depending on salinity and the presence of glitter. In contrast, *M. potiuna* oxidizes proteins in all treatments. Overall, the hepatosomatic index of *P. vannamei* changed when exposed to 40  $\text{mg.L}^{-1}$

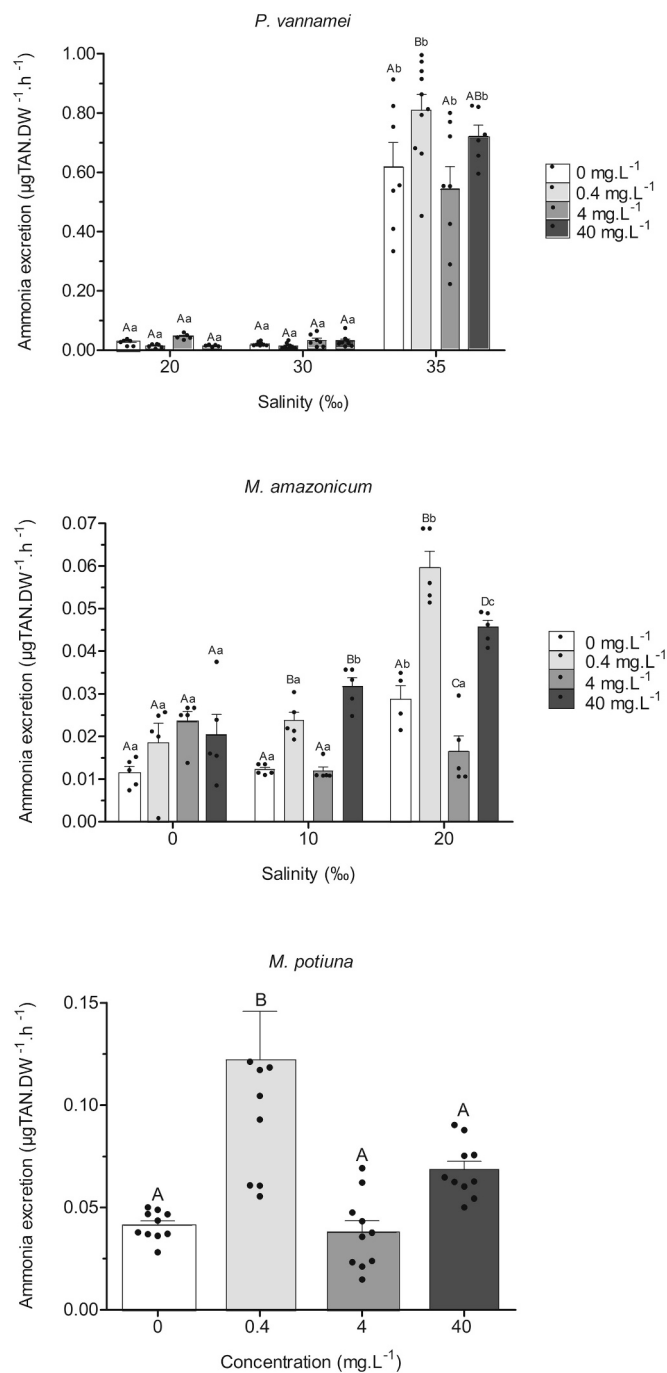


**Fig. 2.** Oxygen consumption ( $\mu\text{g.mg}^{-1}$  dry mass. $\text{h}^{-1}$ ) of *P. vannamei*, *M. amazonicum*, and *M. potiuna* exposed to different salinity and concentrations of glitter. Capital letters indicate statistical differences between animals exposed to different concentrations of glitter but kept in the same salinity. Lowercase letters indicate statistical differences in animals kept at different salinity levels but exposed to the same glitter concentration ( $M \pm \text{SEM}$ ,  $5 \leq N \leq 10$ ,  $F_{P. vannamei} = 14.75$ ,  $F_{M. amazonicum} = 2.94$ ,  $F_{M. potiuna} = 6.07$ ,  $P \leq 0.05$ ).

of glitter (Table 2).

### 3.5. Hemolymph osmolality

In general, the glitter did not alter the osmoregulation of *P. vannamei*, a strong hyperosmoregulator up to the isosmotic point (22S:  $682 \pm 0.01$   $\text{mOSm.kg}^{-1}$  water) (Fig. 4). In this species, the only change in osmolality occurred in animals exposed to 35S and 4  $\text{mg.L}^{-1}$  of glitter (+8.9 %). In *M. amazonicum* the glitter reduced the osmolality of hemolymph of animals kept in freshwater when exposed to 40  $\text{mg.L}^{-1}$  of glitter (-70 %)



**Fig. 3.** Excretion of ammonia ( $\mu\text{g}.\text{mg}^{-1}$  dry mass. $\text{h}^{-1}$ ) of *P. vannamei*, *M. amazonicum*, and *M. potiuna* exposed to different salinity and glitter concentrations. Capital letters indicate statistical differences between animals exposed to different concentrations of glitter but kept in the same salinity. Lowercase letters indicate statistical differences in animals kept at different salinity levels but exposed to the same glitter concentration ( $M \pm \text{SEM}$ ,  $5 \leq N \leq 10$ ,  $F_{P. vannamei} = 3.30$ ,  $F_{M. amazonicum} = 7.17$ ,  $F_{M. potiuna} = 18.38$ ,  $P \leq 0.05$ ).

and increased by 10S (all concentrations of glitter). In *M. potiuna* the osmolality of hemolymph in freshwater was  $392.2 \pm 19.2$  mOsm. $\text{kg}^{-1}$  of water and the glitter did not affect the osmoregulation of this species ( $P = 0.331$ ).

### 3.6. Ability of *P. vannamei* to recover homeostasis after transfer to water free of glitter

#### 3.6.1. Oxygen consumption, hepatosomatic index, and osmolality

The animals recovered oxygen consumption ( $2.2 \pm 0.4$   $\mu\text{g}.\text{mg}^{-1}$  dry mass $^{-1}.\text{h}^{-1}$ ) on the sixth day after transfer to water free of glitter (Fig. 5). However, the six-day period was not sufficient for the animals to recover the hepatosomatic index ( $4.7 \pm 0.5$ ) and the hemolymph osmolality ( $850.7 \pm 11.4$  mOsm. $\text{kg}^{-1}$  of water), which remained higher than the control animals, respectively, 151 % and 109 %.

#### 3.6.2. Morphology of the gills and intestine

The lamellae of the gills of the control group of *P. vannamei* had a complete and ordered tissue structure (Fig. 6A). Exposure to glitter for 10 days caused damage to the branchial tissue, including branchial lamellae bifurcation with lamellar epithelium detachment and interstitial edema (Fig. 6B). In the experimental group where animals were transferred to glitter-free water after previous exposure to the glitter, edema disappeared and bifurcation decreased, but a narrowing of most lamellas can still be observed after six days (Fig. 6C).

In the intestine of *P. vannamei*, the villi of the control group were organized, presented in sequence, and grouped (Fig. 7A). After exposure to the glitter for 10 days, we observed tissue damage, including rupture of the intestinal mucosa in which the villi and the absorbent cells are located, as well as muscle tissue disruption and an increase in the submucosa (Fig. 7B). The transfer of *P. vannamei* to glitter-free water for six days allowed the villi to resemble the control group, but it was still possible to observe a greater thickening of the submucosa and muscle tissue (Fig. 7C).

## 4. Discussion

All studied species exhibited physiological effects due to glitter, with salinity significantly influencing these effects. The physiological impact on these organisms could result from physical tissue damage, and exposure to glitter components such as toluene and benzene identified in our samples. Moreover, we observed that the marine shrimp *P. vannamei* can partially restore its homeostasis when transferred to a glitter-free environment.

Although physiological aspects were affected by glitter exposure in all three studied species, mortality was only observed in *P. vannamei* in 30S (about 13 %), underscoring the role of salinity in pollutant effects. Mortality occurred one day after ecdysis, and during this period crustaceans may be more susceptible to the toxic effects of pollutants (da Costa et al., 2024). However, *P. vannamei*'s ability to migrate across saline gradients may help mitigate such impacts in natural environments. Other authors also found that microplastics have been shown to impact survival in crustaceans such as *Artemia franciscana* (40 % mortality) and *Macrobrachium nipponense* (40 % mortality) (Eom et al., 2020; Li et al., 2021). Extended exposure periods may not affect survival in the species, as pollutant-induced mortality in crustaceans typically occurs within the first 72 h of exposure (Houssou et al., 2024).

### 4.1. Oxygen consumption

Metabolism can be defined as the sum of all chemical reactions that occur in an organism and can be quantified by measuring the animal's oxygen consumption (da Costa et al., 2024). Overall, the three species exhibited divergent metabolic responses to glitter exposure (increases and decreases), with salinity playing an important role. In the case of the penaeid shrimp *P. vannamei*, the observed increases in oxygen consumption at 30 and 35S may reflect either an energy acquisition strategy or heightened locomotor activity induced by glitter in the aquarium. In contrast, maintaining oxygen consumption at 20S may be linked to the species' isosmotic point (Fig. 4), a salinity where energy expenditure for ion absorption/secretion is lower. Among the studied palaemonid

**Table 1**

Type of oxidized energy substrate (O:N) by *P. vannamei*, *M. amazonicum*, and *M. potiuna* exposed to different salinities and concentrations of glitter. M  $\pm$  SEM; 5  $\leq$  N  $\leq$  10,  $F_{P. vannamei} = 17.35$ ;  $F_{M. amazonicum} = 10.91$ ;  $F_{M. potiuna} = 9.48$ .  $P \leq 0.05$ .

O:N Glitter (mg.L <sup>-1</sup> )	<i>P. vannamei</i>			<i>M. amazonicum</i>			<i>M. potiuna</i>
	20S	30S	35S	0S	10S	20S	0S
	0.0	15.20 $\pm$ 2.80 P	14.40 $\pm$ 3.20 P	2.80 $\pm$ 2.40 P	70.60 $\pm$ 10.30 L	186.30 $\pm$ 11.40 L	21.30 $\pm$ 8.80 P/L
0.4	3.70 $\pm$ 0.70 P	25.90 $\pm$ 2.90 P/L	2.80 $\pm$ 2.40 P	41.90 $\pm$ 8.90 P/L	71.10 $\pm$ 8.80 L	13.20 $\pm$ 9.90 P	6.10 $\pm$ 1.80 P
4.0	14.70 $\pm$ 2.30 P	31.40 $\pm$ 2.90 P/L	2.50 $\pm$ 2.40 P	62.10 $\pm$ 10.30 L	81.40 $\pm$ 11.40 L	44.30 $\pm$ 11.40 P/L	5.70 $\pm$ 2.70 P
40.0	45.10 $\pm$ 2.80 P/L	2.10 $\pm$ 3.20 P	1.60 $\pm$ 2.90 P	44.20 $\pm$ 8.90 P/L	65.10 $\pm$ 8.80 L	18.80 $\pm$ 8.80 P/L	3.50 $\pm$ 1.40 P

P: protein and L: lipids. Ratios between 3 and 16: proteins; values between 16 and 60: mixture of proteins and lipids; ratios over 60 lipids (Mayzaud & Conover, 1988).

**Table 2**

Hepatosomatic index (HSI) of *P. vannamei*, *M. amazonicum*, and *M. potiuna* exposed to different salinities and concentrations of glitter. M  $\pm$  SEM; 5  $\leq$  N  $\leq$  10,  $F_{P. vannamei} = 6.55$ ;  $F_{M. amazonicum} = 3.08$ ;  $F_{M. potiuna} = 0.94$ .  $P \leq 0.05$ .

HSI Glitter (mg.L <sup>-1</sup> )	<i>P. vannamei</i>			<i>M. amazonicum</i>			<i>M. potiuna</i>
	20S	30S	35S	0S	10S	20S	0S
	0.0	5.10 $\pm$ 0.40 <sup>Aa</sup>	4.30 $\pm$ 0.30 <sup>ABa</sup>	3.10 $\pm$ 0.30 <sup>Ab</sup>	6.90 $\pm$ 0.70 <sup>Aa</sup>	4.80 $\pm$ 0.70 <sup>Aa</sup>	5.70 $\pm$ 0.70 <sup>Aa</sup>
0.4	3.90 $\pm$ 0.40 <sup>Ba</sup>	3.60 $\pm$ 0.30 <sup>Aa</sup>	3.70 $\pm$ 0.30 <sup>Aa</sup>	6.90 $\pm$ 0.70 <sup>Aa</sup>	4.80 $\pm$ 0.70 <sup>Ab</sup>	8.00 $\pm$ 0.70 <sup>Ba</sup>	7.60 $\pm$ 0.70 <sup>A</sup>
4.0	4.80 $\pm$ 0.40 <sup>Aa</sup>	4.60 $\pm$ 0.30 <sup>ABa</sup>	5.90 $\pm$ 0.30 <sup>Bb</sup>	7.70 $\pm$ 0.70 <sup>Aa</sup>	6.40 $\pm$ 0.70 <sup>Aa</sup>	4.70 $\pm$ 0.70 <sup>Ab</sup>	7.60 $\pm$ 0.90 <sup>A</sup>
40.0	3.70 $\pm$ 0.40 <sup>Ba</sup>	5.10 $\pm$ 0.30 <sup>Bb</sup>	5.10 $\pm$ 0.30 <sup>Bb</sup>	8.40 $\pm$ 0.70 <sup>Aa</sup>	5.30 $\pm$ 0.70 <sup>Ab</sup>	5.40 $\pm$ 0.70 <sup>Ab</sup>	7.20 $\pm$ 0.50 <sup>A</sup>

Values with different uppercase letters indicate statistical differences in animals kept at the same salinity; values with different lowercase letters indicate differences in animals kept at different salinities.

shrimps, we highlight the absence of glitter effects on the metabolism of *M. amazonicum* maintained in freshwater, in contrast to the 20 % reduction observed in *M. potiuna* under the same salinity conditions. Several hypotheses may explain this difference between the two species of palaemonids shrimp, including species-specific variations, activity patterns in response to stressors (Li et al., 2018; Kangarloe et al., 2021; Duan et al., 2022) and the diadromous nature of *M. amazonicum*. Additionally, possible differences in branchiostegite and scaphognathite structures, which protect the gills and promote water circulation across this organ, could influence the animals' exposure to glitter (Li et al., 2022; Xie et al., 2024).

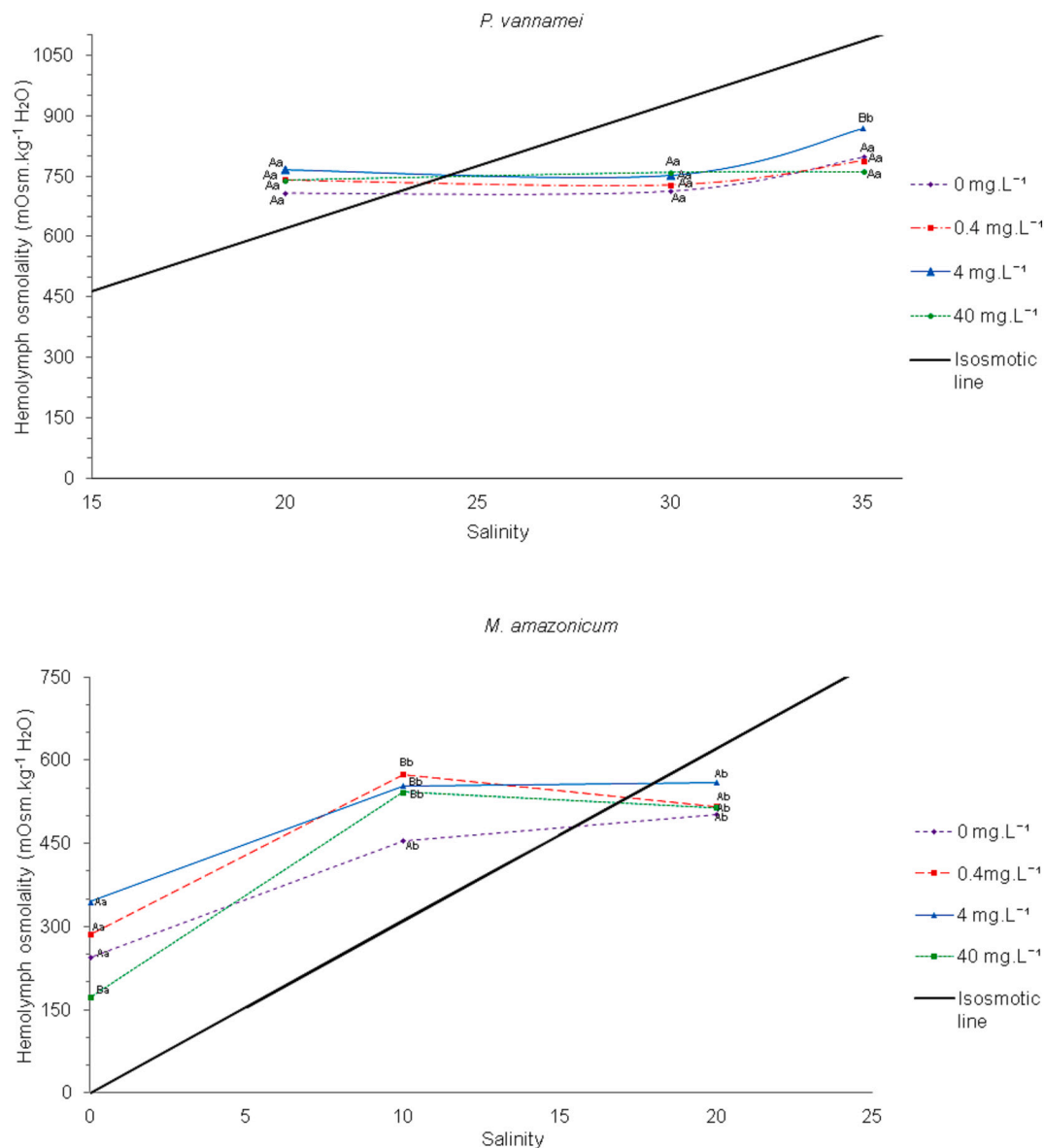
Considering the results presented here and by other authors (Duan et al., 2022; Li et al., 2022; Xie et al., 2024), a question that remains to be fully understood concerns the differential effects of microplastic exposure on aquatic species' metabolism. While some species show increased metabolic rates (Wu et al., 2022), others exhibited decreased metabolic activity (Abessa et al., 2023; Mayzaud and Conover, 1988). Initially, metabolic increases may respond to increased energy demands or swimming activity (Duan et al., 2022; De Felice et al., 2019) and reductions are commonly associated with gill and intestinal injuries, impairing nutrient absorption and decreasing energy expenditure on these physiological processes (Li et al., 2022; Xie et al., 2024; Liu et al., 2019). In *Artemia parthenogenetica*, histological analysis revealed damage to epithelial cells exposed to microplastics. This suggests that the destruction of the intestinal epithelium may impair digestive function, reducing energy metabolism and nutrient absorption (Suman et al., 2020). In the long term, changes in oxygen consumption may lead to reduced food intake, which could result in alterations in the growth of economically important species, as already observed in mussels *Mytilus coruscus* (Zhong et al., 2024; Gu et al., 2020). Following this hypothesis, diadromous (*M. amazonicum*) and freshwater (*M. potiuna*) shrimps appear more susceptible to the adverse effects of glitter exposure because of decreasing oxygen consumption, whereas *P. vannamei* responds to glitter presence in water by adjusting oxygen consumption.

Additionally, while salinity-influenced metabolic changes in all three shrimp species exposed to glitter, *M. potiuna* appears to be the most vulnerable. As an exclusively freshwater species, it exhibits adverse effects from glitter exposure even at the lowest tested concentration (0.4

mg.L<sup>-1</sup> glitter). In contrast, *P. vannamei* and *M. amazonicum* could potentially avoid glitter's effects by migrating to more favorable salinity. For example, *P. vannamei* maintained a stable metabolism at 20S, while *M. amazonicum* exhibited no metabolic alterations in freshwater or 20S conditions. These salinity levels could be more advantageous for these species when considering metabolic maintenance requirements alone.

#### 4.2. Ammonia excretion, type of oxidized energy substrate and hepatosomatic index

Ammonia is the result of the catabolism of free amino acids and is toxic in high concentrations, mainly due to its damaging effect on enzymatic activity (da Costa et al., 2024). Although ammonia excretion increased in all three species studied, the atomic ratio of oxygen consumed to ammonia excreted indicated that they oxidize different energy substrates. Generally, crustaceans can change the energy substrates they oxidize when faced with stressful conditions as part of a strategy to maximize energy production (Ramaglia et al., 2018; Wang et al., 2021a; Han et al., 2022). For example, the oxidation of lipids provides more energy than proteins. Our findings demonstrate that while *P. vannamei* shifts to oxidizing a mixture of proteins and lipids to maximize energy production when exposed to glitter, *M. potiuna* does not use this strategy, maintaining proteins as the sole energy substrate across all treatments. Furthermore, in *P. vannamei*, the hepatosomatic index, which indicates energy storage, digestive enzyme synthesis, and absorption, varied according to glitter concentration (see Table 2). The decreases observed at 20S may be linked to stored reserves or reduced feeding rate, while the increases at 30 and 35S could result from pollutant accumulation in the organ (Wang et al., 2021b). Since energy substrate type and the hepatosomatic index are closely tied to species energetics (Watts et al., 2016), we infer that depending on salinity, glitter can induce alterations, particularly in *P. vannamei*. These effects may arise from tissue damage or adaptive responses by the species to pollutant-induced damage.



**Fig. 4.** Hemolymph osmolality ( $\mu\text{g}\cdot\text{mg}^{-1}$  dry mass. $\text{h}^{-1}$ ) of *P. vannamei* and *M. amazonicum*, exposed to different salinity and glitter concentrations. Capital letters indicate statistical differences between animals exposed to different concentrations of glitter but kept in the same salinity. Lowercase letters indicate statistical differences in animals kept at different salinity levels but exposed to the same glitter concentration ( $M \pm \text{SEM}$ ,  $5 \leq N \leq 10$ ,  $F_{P. vannamei} = 0.77$ ,  $F_{M. amazonicum} = 0.24$ ,  $F_{M. potiuna} = 1.28$ ,  $P \leq 0.05$ ).

#### 4.3. Hemolymph osmolality

While some studies have shown that microplastics have little effect on crustacean osmoregulation (Capparelli et al., 2023; Watts et al., 2016; Capparelli et al., 2024), the present study revealed specific glitter-induced osmoregulatory alterations in *P. vannamei* and more pronounced effects in *M. amazonicum*. Glitter exposure increased hemolymph osmolality, a phenomenon that several hypotheses may explain. One possible explanation is that the physical structure of the glitter facilitates its role as a carrier of substances, such as salts, thereby promoting the transport of ions into the animal's bodies (Gu et al., 2020). Alternatively, the increase could result from an upregulation in the number of salt transporters within the epithelia (Gu et al., 2020). These osmoregulation-related hypotheses warrant further investigation in future studies, as the effects of glitter on species' osmoregulation remain largely unexplored.

#### 4.4. Glitter characterization

The size of glitter particles (0.08 mm) is a factor that may also have contributed to the different responses observed in the three species of shrimps. While smaller animals such as *M. amazonicum* (~1.5 g) and *M. potiuna* (~0.5 g) may have experienced more significant tissue damage compared to *P. vannamei* (~4.5 g), the latter species may have ingested a larger quantity of the pollutant. Studies have demonstrated that ingested glitter can accumulate in the digestive system, leading to satiety, injuries, and intestinal obstruction (Xie et al., 2024; Provenza et al., 2022; Maraschi et al., 2021; Hirt and Body-Malapel, 2020; Korez et al., 2020; Almeda et al., 2021; Yin et al., 2022). Additionally, microplastics can transport adsorbed pollutants into the body, impacting multiple levels of biological organization (Karakolis et al., 2018; Sun et al., 2020).

While we observed that glitter influences various physiological parameters in shrimp under different salinity conditions, it is important to acknowledge that the chemical compounds present in glitter may have

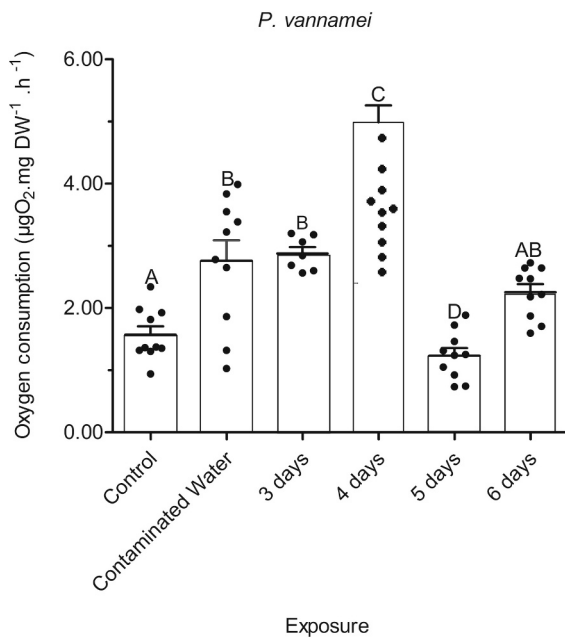


Fig. 5. Oxygen consumption ( $\mu\text{g mg}^{-1}$  dry mass  $\text{h}^{-1}$ ) in *P. vannamei* maintained in water without glitter (control), exposed to contaminated water containing  $4 \text{ mg.L}^{-1}$  of glitter for 10 days, and subsequently transferred to glitter-free water for up to six days. Different letters indicate a statistical difference between treatments.  $M \pm \text{SEM}$ ;  $N = 7$ ,  $F_{P. vannamei} = 6,13$ ,  $P \leq 0.05$ ).

also contributed to the alterations discussed earlier. For example, benzene exposure has been linked to immunosuppression (Guo et al., 2021) morphological alterations (Nascimento et al., 2020), and hepatopancreatic cell loss (Shukla et al., 2021). Anthracene has been shown to reduce filtration rates and increases lipid peroxidation in the mussel *Mytilus galloprovincialis* (Badreddine et al., 2017). Furthermore, Abessa et al., (Green et al., 2021) identified methyl acrylate in white glitter formulations, a toxic compound lethal to fish and invertebrates such as *Daphnia magna*, *Mysidopsis bahia*, and *Oncorhynchus mykiss* (Green et al., 2021; Staples et al., 2000).

#### 4.5. Recovery capacity of *P. vannamei*

Few studies have investigated the functional and structural recovery of crustaceans after their transfer to unpolluted environments following exposure to contaminants (Zhang et al., 2021; Su et al., 2024). However, such evaluations are crucial for understanding the benefits of species migration to less impacted areas and restoring degraded habitats. Our experiments with *P. vannamei* showed that gill and intestinal structures remained altered six days after transfer to glitter-free water. In the present study, tissue regeneration was not observed because the six-day period was short. Furthermore, although crustaceans can regenerate tissue after ecdysis, our animals were in the intermoult period (Zhang et al., 2021; Su et al., 2024). This finding is particularly significant, as the gills are multifunctional organs involved in respiration, osmoregulation, excretion, and pH regulation, and the intestine is the site of nutrient absorption (Bacci et al., 2023; Lucu and Turner, 2024). The previously discussed impacts on osmoregulation and hepatosomatic index in *P. vannamei* may be attributed to gill and intestinal injuries, respectively, as these parameters did not fully recover six days after transfer to glitter-free water. However, the metabolic recovery of

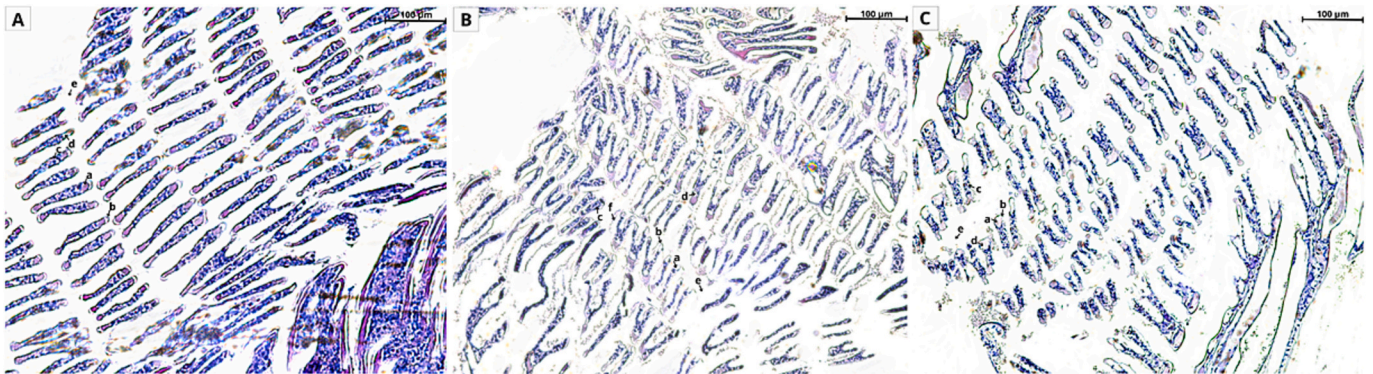


Fig. 6. Histological changes in *P. vannamei* gills after exposure to glitter. (A) control group; (B) exposure to glitter for 10 days; (C) exposure to glitter-free water after six days of contaminant exposure. (a) Epithelial cell; (b) Angular cortex; (c) Blood cell; (d) Outlet of the gill vessels; (e) Space between the lamellas; (f) Edema. The magnification is  $400\times$ .

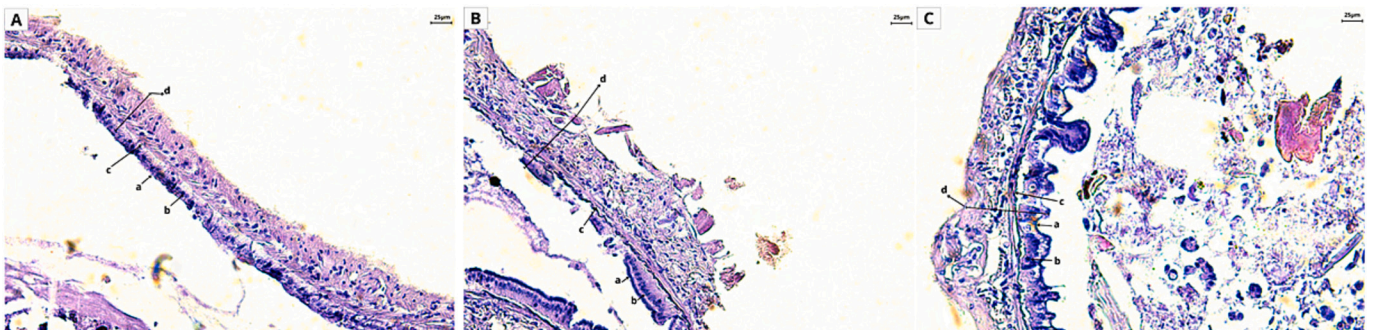


Fig. 7. Histological changes in the intestine of *P. vannamei* after exposure to glitter. (A) control group; (B) exposure to glitter for 10 days; (C) exposure to water free of glitter after six days of exposure to the contaminant. (a) Villi; (b) Cells; (c) Submucosa; (d) Muscle tissue. The magnification is  $1000\times$ .

*P. vannamei* is not dependent on the morphological restoration of gill tissue (see Fig. 5). Truchet et al., (Lucu and Turner, 2024) identified gills as the primary sites of glitter accumulation in decapod crustaceans, where histopathological damage such as hemocyte infiltration, edema, and necrosis can occur (Truchet et al., 2023). Future research should focus on assessing the long-term recovery potential of *P. vannamei* after being transferred to glitter-free environments. Additionally, it should investigate the recovery of the other studied species, with emphasis on the pre- and post-molt periods.

## 5. Conclusion

Physiologists have long recognized salinity as a key factor shaping species distribution in natural ecosystems (Teng et al., 2021; Cadotte et al., 2019). Our findings underscore that salinity is a critical modulator of glitter impacts on crustacean physiology. This implies that species capable of migrating across varying saline gradients may exhibit greater resilience to avoid pollutant effects. However, it remains uncertain which of the three species studied is most susceptible to waterborne glitter. Among them, *M. potiuna*, an exclusively freshwater species with limited capacity to escape polluted environments, may be particularly vulnerable to these effects.

## CRedit authorship contribution statement

**Héllen Siqueira Leite:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **Juliana Rodrigues da Costa:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Barbara Bernardes Calbo:** Writing – review & editing. **Mariana Capparelli:** Writing – review & editing. **Claudia Neves:** Methodology. **Giovanna Teixeira Gimiliani:** Methodology. **Alessandra Augusto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Hellen Siqueira Leite reports financial support was provided by State of Sao Paulo Research Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpa.2025.111946>.

## Data availability

Data will be made available on request.

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