

5-2024

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### Recommended Citation

Trinh, Bao-Son; Le, Lien Thi; Tran, Loc Minh; Rosen, Gunther; and Hale, Robert C., Comparative accumulation and effects of microplastics and microplastic-associated PCB-153 in the white hard clam (*Meretrix lyrata*) and giant river prawn (*Macrobrachium rosenbergii*) following chronic exposure (2024). *Environmental Technology & Innovation*, 34. <https://doi.org/10.1016/j.eti.2024.103581>

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# Comparative accumulation and effects of microplastics and microplastic-associated PCB-153 in the white hard clam (*Meretrix lyrata*) and giant river prawn (*Macrobrachium rosenbergii*) following chronic exposure

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## ARTICLE INFO

### Keywords:

Microplastics  
Polyethylene  
Polychlorinated biphenyl 153  
Accumulation  
*Macrobrachium rosenbergii*  
*Meretrix lyrata*

## ABSTRACT

Global environmental abundance of microplastics (MPs) is increasing. MPs may sorb hydrophobic organic contaminants (HOCs), accumulate in and cause deleterious effects on exposed organisms. This study investigated and compared the accumulation and effects of MPs and MP-associated PCB in the two indigenous aquatic organisms in Viet Nam, the white hard clams, *Meretrix lyrata*, and the giant river prawns, *Macrobrachium rosenbergii*. The test organisms were exposed to either polyethylene microbeads (PEMBs), waterborne polychlorinated biphenyl 153 (PCB-153), or PEMB-associated PCB-153 (PEMB-PCB) over 28 days. Organismal MP accumulation, survival, and weight gain were examined at various sampling intervals. In general, MP accumulation was significantly greater in the clams than in the prawns. A significant quantity of MPs was observed in the digestive systems of the organisms after 0.5-day and during 28-day exposure. Although the effect of MPs and MP-associated PCB-153 on mean survival rate was not statistically significant, this effect was significant towards the end of the 28-day exposure. In addition, while MP-associated PCB-153 did not significantly affect the weight gain of the prawns, it significantly reduced weight gain in clams. Given the global increases of MPs and HOCs (and in southeast Asia specifically), and the importance of the hard clams and giant river prawns in the Indo-Pacific region, this present study provides valuable data to enhance our understanding of the effects of MPs and HOCs on these species.

## 1. Introduction

Plastic production, use, and disposal are increasing. Consequently, resulting plastic pollution has become a global problem. It was estimated that floating plastic particles in the world oceans increased from 5 trillion to 170 trillion pieces in the last ten years (Eriksen et al., 2014, 2023). Plastic products in use and following disposal are fragmented into small particles, known as microplastics (MPs) and typically defined as 1 to either 1000 or 5000  $\mu\text{m}$  (Hale et al., 2020, Hoang, 2022), by UV irradiation, heat, chemical reactions, abrasion, and biodegradation (Zhang et al., 2021). MPs can be categorized as primary MPs, i.e., personal care products, coatings,

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<https://doi.org/10.1016/j.eti.2024.103581>

Received 16 January 2024; Received in revised form 22 February 2024; Accepted 23 February 2024

Available online 24 February 2024

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paints, drilling fluids, plastic pre-production pellets (nurdles), or secondary MPs (far more numerous), i.e., the fragmentations of larger plastic products such as aquaculture, fishing gear, textiles (Hale et al., 2020, Kurniawan et al., 2021). Wastewater effluents may carry substantial MPs to aquatic environment. According to Murphy et al. (2016), a MP abundance of 15,700 and 250 particles/m<sup>3</sup> were detected in the influent and final effluent of a Scottish wastewater treatment plant (WWTP), respectively. In Viet Nam, higher MP abundances were reported in both domestic and industrial WWTPs, i.e., up to 125,000 particles/m<sup>3</sup> in the influents and 140 – 813 particles/m<sup>3</sup> in the effluents of domestic WWTPs (Nguyen et al., 2023), and 183,000 – 443,000 particles/m<sup>3</sup> in the influents and 138,000 – 340,000 particles/m<sup>3</sup> in the effluents of the industrial WWTPs (Van Do et al., 2022). MP pollution in southeast Asia in general, and in Viet Nam in particular, is increasing at particularly high rates due to increased local use (in part due to a desire for a higher standard of living), inadequate waste handling infrastructure, waste imports from developed nations and a large coastline to land mass ratio (Jambeck et al., 2015, Lahens et al., 2018, Ng et al., 2023).

In aquatic environment, MPs can associate with hydrophobic organic contaminants (HOCs), be ingested by the exposed organisms, and can result in deleterious effects. These processes, known as the 'MP vector effect', have been well documented (Koelmans et al., 2016, Ziccardi et al., 2016, Mohamed Nor and Koelmans, 2019, Syberg et al., 2020). MP-associated HOCs in water and sediment pose risks to aquatic organisms via deleterious physical interactions or associated HOCs by ingestion, dermal or respiratory uptake pathways (Hale et al., 2020, Bank and Hansson, 2022). HOC uptake from MPs by exposed biota is mediated by their respective fugacities, and in turn their physico-chemical properties and ambient environment conditions (Koelmans et al., 2016, Rodrigues et al., 2019, Li et al., 2022). Hence further investigation of uptake of MPs and MP-associated HOCs by diverse aquatic species is merited.

Polychlorinated biphenyls (PCBs) are hydrophobic, lipophilic, persistent, bioaccumulative, and toxic organic contaminants (Safe, 1994, Norland et al., 2021). Although PCBs were banned from commercial use in the 1970 s, they continue to be released from poorly managed hazardous waste sites and illegal or improper dumping and burning of wastes in municipal and industrial incinerators (USEPA, 2023). It has been reported that substantial amounts of PCBs, dioxins, and other persistent organic pollutants (POPs) were released into the aquatic environments during the Vietnam War in 1955–1975 (Mai et al., 2007) and since (Anh et al., 2019). These war years coincide with peak global PCB manufacture and use (Melymuk et al., 2022). Because of their persistent properties, PCBs remain in Vietnamese ecosystems (Tham et al., 2016, Hanh et al., 2019). Among PCB congeners, PCB-153 (2,2',4,4',5,5'-hexachlorobiphenyl) is one of the most abundant and bioaccumulative congener (Safe, 1994) and thus has been used as a model for assessing the fate and toxicological effects of PCBs on aquatic organisms (Danis et al., 2005, Grilo et al., 2014, Norland et al., 2021, Phung et al., 2023).

In aquatic environments, both high-density and low-density polymers (following development and aggregation of biofilms on their surface) may eventually sink (Zettler et al., 2013, Hale et al., 2020). This argues for studies that consider both benthic and free-swimming species. The white hard clam (*Meretrix lyrata*) is a benthic and filter-feeding species. This species strains small food particles (e.g., phytoplankton, zooplankton, and other tiny organisms) from the water around them. It can filter large water volumes and therefore uptake great quantities of particles in a day (Zahra, 2022, Cole et al., 2023). In contrast, the giant river prawn (*Macrobrachium rosenbergii*) is a free-swimming, omnivorous, and opportunistic species. It uses its chelate legs aided, by third maxillipeds, to capture and convey small food particles such as algae, zooplankton, organic matter to its mouth (FAO, 2023). It is, therefore, expected to uptake fewer food particles than the clam per time. The white hard clams and giant river prawns are the ecologically and commercially important species in the Indo-Pacific region. They provide high economic and nutritional benefits, particularly for local people. Thus, these organisms are widely cultured in rivers, estuaries, and along coastal shores of Viet Nam (Hien et al., 1998, Chu and Kumar, 2008).

The present study investigated MP accumulation and effects of MPs and MP-associated PCB-153 in the white hard clams (*M. lyrata*) and the giant river prawns (*M. rosenbergii*) following chronic exposure (28 days). We performed controlled lab exposures of the clams and prawns to polyethylene microbeads (PEMBs), water-borne PCB-153, and PEMB-associated PCB-153 (PEMB-PCB). A temporal comparison of the MP accumulation, survival, and weight gain of the exposed organisms is also discussed.

## 2. Materials and methods

### 2.1. Microplastics and chemicals

Green, fluorescent PEMBs (63–75  $\mu\text{m}$ ,  $5.81 \times 10^6$  beads/g,  $1.025 \text{ g/cm}^3$ ) were purchased from Cospheric Inc. (Santa Barbara, CA, USA). PCB-153 was obtained from Chem Service, Inc. (West Chester, PA, USA). Solvents, including acetone (HPLC grade, 99.8%), dichloromethane (DCM, HPLC grade, 99.6%), and methanol (HPLC grade, 99.8%), were purchased from Macron Fine Chemicals™ Inc. (Allentown, PA, USA). The surfactant Tween 20 (polyoxyethylenesorbitan monolaurate) was purchased from RPI Corp. (Mount Prospect, IL, USA). Chemicals used for making the moderately hard water (MHW, details in Supplementary Material), including  $\text{MgSO}_4$ ,  $\text{NaHCO}_3$ ,  $\text{KCl}$ , and  $\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$ , were purchased from Scharlab (Sentmenat, Barcelona, Spain).

PCB stocks: Two concentration stocks were prepared. Stock PCB 1000 (1000 mg PCB-153/L DCM) was prepared by dissolving 10 mg PCB-153 in 10 mL DCM. Stock PCB 10 (10 mg PCB-153/L DCM) was prepared by diluting 1 mL Stock PCB 1000 in 99 mL DCM.

*Polyethylene microbeads associated with PCB-153 (PEMB-PCB)*: The adsorption of PCB-153 on PEMBs was conducted based on the method of Phung et al. (2023). Briefly, 1.0490 g PEMBs were mixed with 100  $\mu\text{L}$  Stock PCB 1000 in 25 mL methanol (80%). The mixture was shaken by an orbital shaker (model GLF 3032) at 30 rpm for 28 days. PEMB-sorbed PCB samples ( $\times 3$  replicates) were collected at the intervals of 7, 14, 21, and 28 days. PCB extraction and analysis were conducted based on the methods presented in the Supporting Material. PCB concentrations after the 7-, 14-, 21- and 28-day exposures were  $8389 \pm 2930$ ,  $4714 \pm 1554$ ,  $10,225 \pm 4566$ , and  $6467 \pm 636$  ng PCB-153/g PEMB, respectively. These results suggested that PCB concentration was stable after 7-day exposure.

Therefore, PEMB-PCB with concentration of  $8389 \pm 2930$  ng PCB-153/g PEMB (7-day sorption) was used for the following MP exposure experiments with the aquatic organisms.

## 2.2. Test organisms and exposure media

### 2.2.1. Test organisms

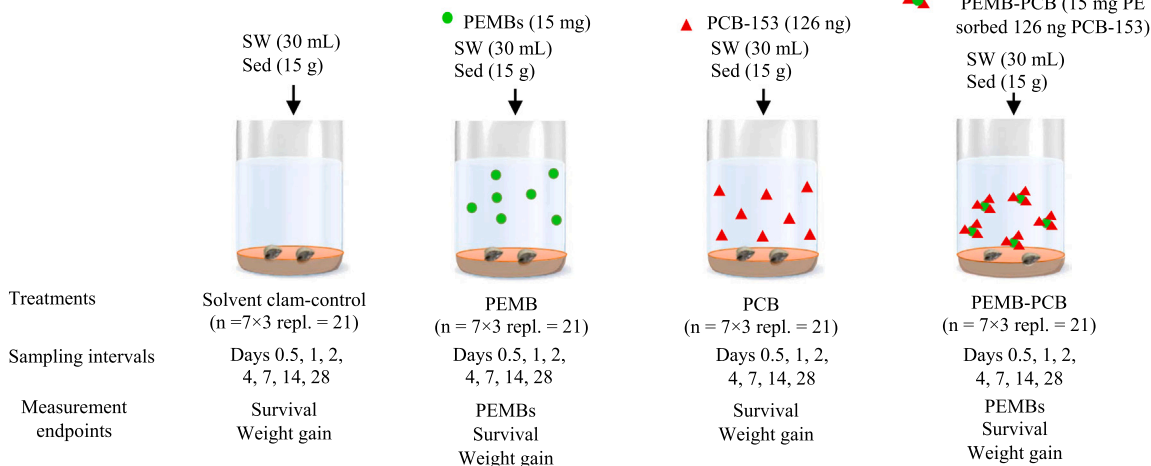
Juvenile stage, 6-month-old white hard clams (*M. lyrata*,  $429.5 \pm 72.4$  mg in wet weight and  $1.17 \pm 0.05$  cm in length, Table S1) were used in the experiments. Specimens were obtained from a clam farm located on Tan Thanh Beach ( $10^{\circ}17'06.9''$  N,  $106^{\circ}46'39.6''$  E), Tien Giang Province, Viet Nam, transported to the laboratory within 24 hours and held in a 20 L glass tank containing 5 L of seawater and 2 cm (depth) sediment (collected at the same position as the clams were obtained) for 7 days at  $26 \pm 1$  °C and 14:10 h light:dark photoperiod. The clams were fed with algae *Chlorella* sp. ( $2 \times 10^7$  cells/mL) twice daily. The tank was equipped with an aeration system that allowed oxygenation of the overlying water without disturbance of the sediment.

Post-larval, 11-day-old giant river prawns (*M. rosenbergii*,  $6.5 \pm 0.5$  mg in wet weight and  $1.01 \pm 0.05$  cm in length, Table S1) were used in the experiments. Specimens were obtained from the Research Institute for Aquaculture No. 2, Ho Chi Minh City, Viet Nam. These were held in a 20 L glass tank containing 5 L of MHW equipped with an aeration filter system for 4 days at  $26 \pm 1$  °C, 14:10 h light:dark photoperiod, and fed artificial shrimp food (Le Gouessant, Brittany, France) twice daily before the experiments.

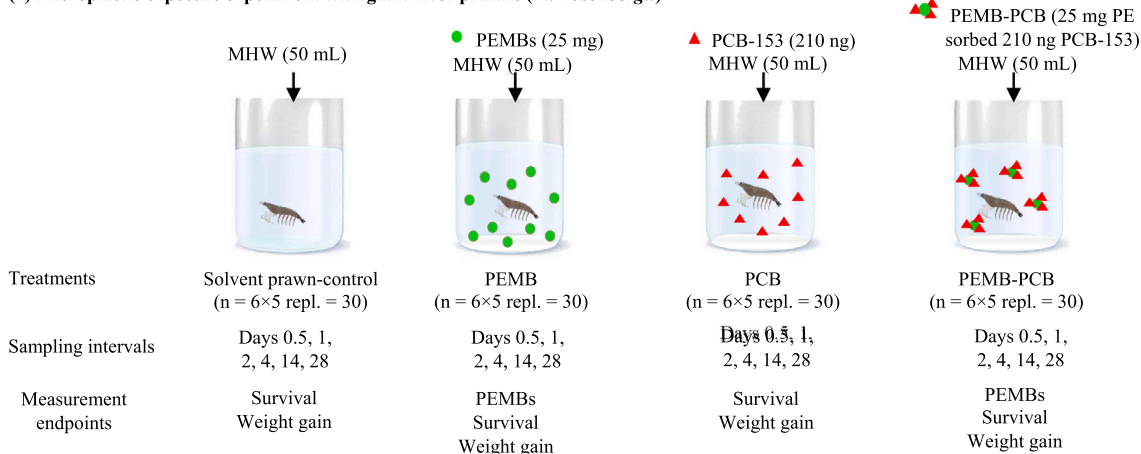
### 2.2.2. Exposure media

For the experiment with the clams, clean seawater and sediment were collected at Tan Thanh Beach ( $10^{\circ}17'06.9''$  N,  $106^{\circ}46'39.6''$  E) where the clams were obtained. Stock PCB 8-clam ( $8389$  ng PCB-153/L seawater) was made by mixing 0.800 mL Stock PCB

#### (1) Microplastic exposure experiment with white hard clams (*M. lyrata*)



#### (2) Microplastic exposure experiment with giant river prawns (*M. rosenbergii*)



**Fig. 1.** Experimental designs for chronic exposures to polyethylene microbeads (PEMBs), waterborne polychlorinated biphenyl 153 (PCB-153), and PEMB-associated PCB-153 (PEMB-PCB) of: (1) the white hard clams (*M. lyrata*) and (2) the giant river prawns (*M. rosenbergii*). Abbreviations: SW = seawater, Sed = sediment, MHW = moderately hard water.

10–0.770 mL acetone and 0.004 mL Tween 20 in 952 mL seawater. Stock solvent clam-control (without PCB-153) was prepared by mixing 1.600 mL DCM, 1.440 mL acetone, and 0.008 mL Tween 20 in 1904 mL seawater. For the experiment with the prawns, MHW was prepared by dissolving an equivalent amount of laboratory grade chemicals ( $\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$ ,  $\text{MgSO}_4$ ,  $\text{NaHCO}_3$ , and  $\text{KCl}$ ) in 16–18 M $\Omega$  Milli-Q water using the formula of the U.S. EPA Method for chronic toxicity testing (USEPA, 2002). Stock PCB 8-prawn (8389 ng PCB-153/L) was made by mixing 1.300 mL Stock PCB 10–2.000 mL acetone and 0.007 mL Tween 20 in 1546 mL MHW. Stock solvent prawn-control (without PCB-153) was prepared by mixing 1.730 mL DCM, 2.670 mL acetone, and 0.009 mL Tween 20 in 2000 mL MHW. The addition of Tween 20 (0.0002% by volume) was used to reduce the aggregation and enhance the dispersion of PEMBs in water. Tween 20 at this low concentration had been reported to have no effect on test organisms (Chae and An, 2020, Phung et al., 2023). Physico-chemical characteristics of the exposure media for the experiments with the clams and the prawns are presented in Table S2 of the Supplementary Material.

### 2.3. Experimental procedures

The experiments were performed at the Environmental Toxicology Laboratory of the Institute for Environment and Resources, Vietnam National University of Ho Chi Minh City. Fig. 1 presents two separate experimental designs for investigating the chronic exposure to PEMBs, waterborne PCB-153, and PEMB-associated PCB-153 of the clams and prawns.

#### 2.3.1. Microplastic exposure experiment with the white hard clams

The exposure design with clams followed APHA (2017) and Setälä et al. (2016). Three treatments of PEMB, PCB and PEMB-PCB and a clam-control were prepared in 80-mL wide mouth glass bottles (7 sampling intervals  $\times$  3 replicates = 21 glass bottles for each treatment). The PEMB treatment was prepared by loading 15 g sediment, 15 mL seawater mixed with 15 mg PEMBs, and 15 mL solvent clam-control in each glass bottle to generate a nominal PEMB concentration of 500 mg PEMB/L (or  $2.905 \times 10^6$  beads/L). The PCB treatment was prepared by loading 15 g sediment, 15 mL seawater, and 15 mL Stock PCB 8-clam in each glass bottle to generate a nominal PCB-153 concentration of 4195 ng/L. The PEMB-PCB treatment was prepared by loading 15 g sediment, 15 mL seawater mixed with 15 mg PEMB-PCB, and 15 mL solvent clam-control in each glass bottle to generate a nominal PEMB concentration of 500 mg PEMB/L and a nominal PCB-153 concentration in water to be 4195 ng/L. The addition of sediment aimed to mimic the realistic living microcosm of the clams and to enhance the survival of the clams over the chronic exposure (28 days). High concentrations of PEMBs in the PEMB and PEMB-PCB treatments might not be environmentally realistic but increased the mass for PCB-153 adsorption on PEMBs. The clam-control was prepared by mixing 15 g sediment, 15 mL seawater, and 15 mL solvent clam-control stock in each glass bottle.

The exposure was initiated by transferring two healthy clams (normal filtering) to each glass bottle. The bottles were gently aerated through a glass pipette (approx. 80 – 100 bubbles/min) to maintain appropriate dissolved oxygen (D.O.) concentrations for the organisms. Food was provided twice daily at 10:00 am and 4:00 pm with 50  $\mu\text{L}$  of green algae suspension *Chlorella sp.* ( $1 \times 10^8$  cells/mL). Renewals were conducted on days 7, 14, and 21 by carefully withdrawing 15 mL water from the top layer (avoiding plastic particles and sediment) and then adding 15 mL renewal solution (a mixture of 7.5 mL seawater and 7.5 mL solvent clam-control stock) for each glass bottle of the PEMB, PEMB-PCB, and the clam-control treatments. For the PCB treatment, a similar renewal procedure was conducted by gently withdrawing 15 mL water (without sediment) and adding 15 mL renewal solution (a mixture of 7.5 mL seawater and 7.5 mL Stock PCB-8 clam). The renewals did not change concentrations of PEMBs and PCB-153. Mortalities were observed daily. Dead organisms were removed immediately (1–2 hours) from the glass bottles and frozen ( $-20^\circ\text{C}$ ) until observing MP accumulation. This design allowed collection of six clams (3 replicates of 2 clams each) at seven sampling intervals of 0.5, 1, 2, 4, 7, 14 and 28 exposure days for analyzing MP accumulation and weight gain. Samples were frozen ( $-20^\circ\text{C}$ ) until analysis.

#### 2.3.2. Microplastic exposure experiment with the giant river prawns

The exposure design with prawns followed Grilo et al. (2014). Like the clam exposures, three treatments of PEMB, PCB, and PEMB-PCB and a prawn-control were prepared in 80-mL wide mouth glass bottles (6 sampling intervals  $\times$  5 replicates = 30 glass bottles for each treatment). The PEMB treatment was prepared by adding 25 mg PEMBs, 25 mL MHW, and 25 mL solvent prawn-control in each glass bottle to generate a PEMB concentration of 500 mg PEMB/L (or  $2.905 \times 10^6$  beads/L). The PCB treatment was prepared by adding 25 mL MHW and 25 mL Stock PCB 8-prawn in each glass bottle to generate a nominal PCB-153 concentration of approximately 4195 ng/L. The PEMB-PCB treatment was prepared by adding 25 mg PEMB-PCB, 25 mL MHW, and 25 mL solvent prawn-control in each glass bottle to generate a nominal PEMB concentration of 500 mg PEMB/L and a nominal PCB-153 concentration of approximately 4195 ng/L. The prawn-control was prepared by mixing 25 mL MHW and 25 mL solvent prawn-control stock in each glass bottle.

The exposure was initiated by transferring one healthy prawn (normal swimming) to each glass bottle (1 prawn/bottle to prevent physical and behavioral effects due to interactions of individual prawns). The bottles were gently aerated through a glass pipette (approx. 80 – 100 bubbles/min) to maintain appropriate D.O. concentrations for the organisms. Artificial shrimp food was provided twice daily at 10:00 am and 4:00 pm. Renewals were conducted on days 7, 14, and 21 by carefully withdrawing 25 mL water (avoiding plastic particles) and then adding 25 mL renewal solution (a mixture of 12.5 mL MHW and 12.5 mL solvent prawn-control stock) for each glass bottle of the PEMB, PEMB-PCB, and the prawn-control treatments. For the PCB treatment, a similar renewal procedure was conducted by gently withdrawing 25 mL water and adding 25 mL renewal solution (a mixture of 12.5 mL MHW and 12.5 mL Stock PCB-8 prawn). The renewals did not change concentrations of PEMBs and PCB-153. Mortalities were recorded daily. Dead organisms were removed immediately (1–2 hours) from the glass bottles and frozen ( $-20^\circ\text{C}$ ) until observing MP accumulation. This design



allowed collection of five prawns (5 replicates of one prawn each) at six sampling intervals of 0.5, 1, 2, 4, 14 and 28 exposure days for analyzing MP accumulation and weight gain. Samples were frozen (-20 °C) until analysis.

## 2.4. Sample analysis

### 2.4.1. Survival and weight gain

Survival rates (%), mean  $\pm$  SD of the replicates) were calculated by subtracting the mortality rate (%) from 100. Mortality rates (%) were calculated by dividing the number of dead by the total initially alive organisms in each replicate. Dead organisms were recorded daily. A clam was considered dead when its shell was wide open, its siphons were not extended, and the clam did not react when gently touched by a thin glass stick. Similarly, a prawn was considered dead if it remained unresponsive when gently touched by a thin glass stick. Dead organisms were removed from the bottle immediately, weighed, and frozen (-20 °C) until MP accumulation analysis.

Weight gain (%), mean  $\pm$  SD of the replicates) was determined based on the different percentage of the wet weights between the initial (day 0) and the termination (day 28) of the experiment. The organismal samples were thoroughly rinsed with distilled water to remove MPs attached to the exterior. Cleaned samples were placed on dry filter paper for approximately 5 minutes and weighed by an analytical balance (Quintix224-1S, Sartorius, Germany; precision 0.0001 g). The organismal samples were then frozen (-20 °C) for MP accumulation analysis.

### 2.4.2. PEMB accumulation

Organismal accumulation of fluorescent PEMBs was determined by direct observation with a stereomicroscope equipped with an ultraviolet light (10–40 $\times$ , Olympus SD30, Japan) and a fluorescent microscope (40 $\times$ , Olympus BX51, Japan) followed the published methods (Setälä et al., 2016, Saborowski et al., 2019). For the clams, MPs accumulated in the mantle and the digestive system were identified as described in Figure S1a. Briefly, the shell was opened using a precleaned scalpel and tweezers. MPs accumulated in the mantle were quantified via microscopic enumeration. The clam's soft tissues were then separated from the shell, placed in a petri dish and dissected for counting MPs accumulated in the digestive system. Similarly for the prawns, MPs accumulated in the gills and digestive system (Figure S1b) were observed and quantified microscopically. MP accumulation in the organisms (beads/individual) was reported as mean  $\pm$  standard deviation (SD) of the replicates. Other units such as beads/g dry weight of the organisms, and  $\mu$ g plastic/g dw of the organisms were also reported.

### 2.4.3. PCB-153 analysis

The composition of PCB-153 was extracted from the water, sediment and MP samples following procedures described in the Supplementary Material. The extracts were then analyzed for PCB-153 concentration by a gas chromatography (GC) system equipped with an Agilent DB-XLB column (30 m  $\times$  320  $\mu$ m  $\times$  0.5  $\mu$ m) and an Agilent micro-electron capture detector (GC- $\mu$ ECD, Agilent 7890 A, Agilent Technologies, Inc.). The column was programmed at 6 °C/min to 320 °C. The Helium carrier gas flow rate was 2.62 mL/min, and injector and detector temperatures were 280 °C and 350 °C, respectively.

## 2.5. Statistical analysis

Values were reported as mean  $\pm$  SD of the replicates. Statistical analysis was performed using Minitab ® 20.2. Before evaluating significant differences, data groups were initially tested for normal distribution via the Ryan-Joiner method and homogeneity of variances using Null hypothesis and Levene's Method (Zar, 2010). If the data did not meet the assumption of normal distribution and homogenous variance, square-root or log transformations were applied. One-way ANOVA with Tukey's test was used to analyze for statistically significant differences among groups. A  $p$ -value  $\leq$  0.05 was considered significant.

## 3. Results and discussion

### 3.1. Chemistry of the exposure media

Nominal and measured concentrations of MPs and PCB-153 in the exposure media were determined before the exposure experiments were started. For the clam experiment (Table S3), the nominal MP concentrations in water of the PEMB and PEMB-PCB treatments were both 500 mg/L. The measured MP concentrations in water of the PEMB and PEMB-PCB treatments were  $447 \pm 17$  and  $427 \pm 50$  mg/L, respectively, i.e., between 85 – 89% of the nominal MP concentration. In the PCB treatment, the nominal waterborne PCB-153 concentration was 4195 ng/L (or 126 ng PCB-153 in 30 mL of each replicate). The measured waterborne PCB-153 concentration of this treatment was  $3840 \pm 166$  ng/L, i.e., 92% of the nominal waterborne PCB-153 concentration. In the PEMB-PCB treatment, PCB-153 was transferred to this treatment via PEMB-PCB beads with the initial concentration of 8389 ng PCB-153/g PEMB-PCB beads (Section 2.1). As a result, the mass of PCB-153 in 15 mg PEMB-PCB of each replicate was 126 ng. This value was equal to the PCB-153 mass in the PCB treatment. The measured PCB-153 concentration in water of the PEMB-PCB treatment initially was below the limit of detection.

Chemistry of the exposure media for the prawn experiment is presented in Table S4. The nominal MP concentrations in water of the PEMB and PEMB-PCB treatments were both 500 mg/L. The measured MP concentrations in water of the PEMB and PEMB-PCB treatments were  $472 \pm 10$  and  $469 \pm 17$  mg/L, respectively, i.e., 94% of the nominal PEMB concentration. In the PCB treatment, the nominal waterborne PCB-153 concentration was 4195 ng/L (or 210 ng PCB-153 in 50 mL of each replicate). The measured

waterborne PCB-153 concentration of this treatment was  $3842 \pm 166$  ng/L, i.e., 92% of the nominal waterborne PCB-153 concentration. Similar to the clam experiment, PCB-153 was transferred to the PEMB-PCB treatment via PEMB-PCB beads with an initial concentration of 8389 ng PCB-153/g PEMB-PCB beads (Section 2.1). As a result, the mass of PCB-153 in 25 mg PEMB-PCB of each replicate was 210 ng. This value was equal to the PCB-153 mass in the PCB treatment. The initial measured PCB-153 concentration in water of the PEMB-PCB treatment was below the limit of detection.

### 3.2. Microplastic accumulation

Exposure to MPs (the PEMB treatment) and MP-associated PCB-153 (the PEMB-PCB treatment) resulted in MP accumulation in the clams and prawns after 0.5-day (Fig. 2) and during the 28-day exposure (Tables 1 and 2). In general, MP accumulation in the clams was significantly greater than in the prawns. For instance, the average MP accumulations (mean  $\pm$  SD of the sampling intervals over the 28-day exposure) in the clams versus in the prawns were  $323 \pm 311$  beads/clams versus  $5 \pm 6$  beads/prawns in the PEMB treatments ( $p = 0.000$ , applying the log-transformation method for testing the equal variances and ANOVA one-way with Tukey method), and  $247 \pm 136$  beads/clams versus  $20 \pm 16$  beads/prawns in the PEMB-PCB treatments ( $p = 0.000$ , applying the log-transformation method for testing the equal variances and ANOVA one-way with Tukey method).

Different feeding strategies between the clams and prawns likely explain the difference in the observed MP accumulation (Setälä et al., 2016). The clams are filter-feeding species that consume great numbers of food particles, including MPs, via their incurrent siphon (Figure S1a). In contrast, the prawns consume fewer particles than the filter feeders because they capture and convey food particles using their chelate legs (Figure S1b). Our present finding is consistent with Setälä et al. (2016), who reported that bivalves (*Mytilus trossulus* and *Macoma balthica*) ingested significantly greater numbers of MPs than free-swimming crustaceans (*Gammarus* spp. and mysid shrimps), i.e., 300 – 600 beads/INDV versus 25 – 40 beads/INDV when exposed to polystyrene (PS) microbeads (10  $\mu$ m,  $250 \times 10^3$  beads/L) over a 1-day exposure, respectively. High MP accumulation in bivalves was also reported by others. For example, Trestrail et al. (2021) reported accumulation of PE and PS beads (20–75  $\mu$ m,  $50 \times 10^3$  beads/L, 7-day exposure) in marine Mediterranean mussels (*Mytilus galloprovincialis*, 6.09  $\pm$  0.35 cm) from 2000 to 10,000 beads/INDV/day. Cole et al. (2023) suggested that the propensity of blue mussels (*Mytilus edulis*) to filter microplastics (estimated at 40,146 microplastic particles/kg/h) might present a nature-based solution for the removal of microplastics from the water column. Others reported low MP accumulation in prawns or shrimps. For example, Hossain et al. (2022) reported MP accumulation in giant river prawn (*M. rosenbergii*) was  $9.33 \pm 0.82$  particles/prawn, in the Karnafully River in Bangladesh. Devriese et al. (2015) reported a mean of  $1.23 \pm 0.99$  MPs/shrimp was detected in the brown shrimp (*Crangon crangon*) obtained across the Channel area and Southern part of the North Sea between France, Belgium, the Netherlands and the UK.

Further investigation of MP accumulation inside the body of the exposed organisms, we observed a greater number of MPs accumulated in the digestive systems than in the mantle of the clams and in the gills of the prawns (Fig. 3). For instance, the average MP accumulations in the digestive system versus in the mantle of the clams in the PEMB treatment were  $287 \pm 294$  versus  $36 \pm 21$  beads/clam ( $p = 0.005$ ), and in the digestive system versus in the gills of the clams were  $4 \pm 5$  versus  $1 \pm 1$  beads/prawn ( $p = 0.876$ ). Even transient entry of MPs into either the digestive or respiratory systems could result in deleterious physical interactions. For example, Seeley et al. (2023) postulated that contact with MPs may have compromised the fill membrane integrity of exposed fish, resulting in greater to virus infection and resultant mortalities. In addition, higher trophic level organisms could also accumulate MPs

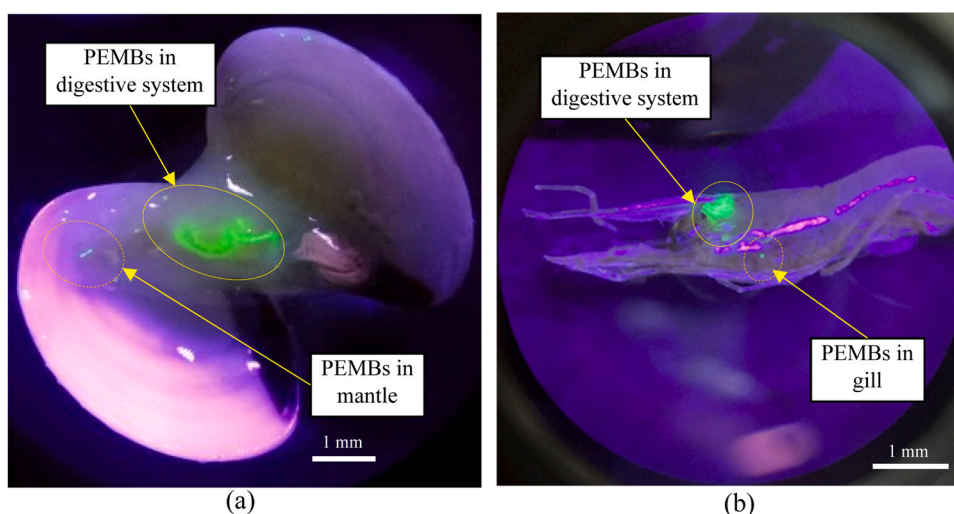


Fig. 2. Green, fluorescent polyethylene microbeads (PEMBs) were observed in: (a) white hard clams (*M. lyrata*) and (b) giant river prawns (*M. rosenbergii*) after 0.5-day exposure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**  
Accumulation and effects of microplastics (MPs), PCB-153, and MPs associated PCB-153 on white hard clam (*Meretrix lyrata*).

Treatments		Exposure time (day)									Average
		0	0.5	1	2	4	7	14	28		
		<b>Microplastic accumulation</b>									
Control		ND	N/A	N/A	N/A	N/A	N/A	N/A	ND	N/A	
PEMB	(beads/INDV)	ND	574 ± 572 <sup>ab</sup>	892 ± 382 <sup>a</sup>	89 ± 104 <sup>b</sup>	68 ± 50 <sup>b</sup>	355 ± 90 <sup>b</sup>	84 ± 80 <sup>b</sup>	202 ± 278 <sup>b</sup>	323 ± 311	
	(beads/g dw)	ND	2339 ± 2647 <sup>ab</sup>	2772 ± 1405 <sup>a</sup>	292 ± 382 <sup>b</sup>	220 ± 149 <sup>b</sup>	1040 ± 304 <sup>ab</sup>	242 ± 240 <sup>b</sup>	526 ± 725 <sup>b</sup>	1061 ± 1066	
	(ug PE/g dw)	ND	403 ± 456 <sup>ab</sup>	477 ± 242 <sup>a</sup>	50 ± 66 <sup>b</sup>	38 ± 26 <sup>b</sup>	179 ± 52 <sup>ab</sup>	42 ± 41 <sup>b</sup>	91 ± 125 <sup>b</sup>	183 ± 183	
PCB-153		ND	N/A	N/A	N/A	N/A	N/A	N/A	ND	N/A	
PEMB-PCB	(beads/INDV)	ND	334 ± 438 <sup>a</sup>	451 ± 518 <sup>a</sup>	222 ± 163 <sup>a</sup>	178 ± 143 <sup>a</sup>	352 ± 408 <sup>a</sup>	102 ± 144 <sup>a</sup>	92 ± 103 <sup>a</sup>	247 ± 136	
	(beads/g dw)	ND	1472 ± 2238 <sup>a</sup>	1363 ± 1548 <sup>a</sup>	803 ± 691 <sup>a</sup>	711 ± 495 <sup>a</sup>	1640 ± 1757 <sup>a</sup>	209 ± 275 <sup>a</sup>	165 ± 169 <sup>a</sup>	909 ± 599	
	(ug PE/g dw)	ND	234 ± 332 <sup>a</sup>	251 ± 298 <sup>a</sup>	145 ± 115 <sup>a</sup>	122 ± 85 <sup>a</sup>	221 ± 271 <sup>a</sup>	53 ± 70 <sup>a</sup>	42 ± 43 <sup>a</sup>	153 ± 86	
		<b>Survival rate (%)</b>									
Control		N/A	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	83.3 ± 28.9 <sup>a</sup>	83.3 ± 28.9 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	95.2 ± 8.1	
PEMB		N/A	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	83.3 ± 28.9 <sup>a</sup>	97.6 ± 6.3	
PCB-153		N/A	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0	
PEMB-PCB		N/A	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	83.3 ± 28.9 <sup>a</sup>	50.0 ± 0.0 <sup>b</sup>	90.5 ± 18.9	
		<b>Wet weight (mg)</b>									
Control		428.6 ± 47.8 <sup>cd</sup>	419.0 ± 48.8 <sup>d</sup>	422.8 ± 64.4 <sup>cd</sup>	436.4 ± 37.6 <sup>bcd</sup>	496.3 ± 14.3 <sup>abc</sup>	507.2 ± 30.5 <sup>ab</sup>	511.0 ± 32.0 <sup>ab</sup>	563.7 ± 34.1 <sup>a</sup>	32 ± 11	
PEMB		417.3 ± 65.7 <sup>ab</sup>	366.3 ± 56.6 <sup>b</sup>	497.5 ± 60.9 <sup>ab</sup>	486.2 ± 74.2 <sup>ab</sup>	427.6 ± 56.7 <sup>ab</sup>	511.2 ± 80.7 <sup>ab</sup>	537.9 ± 76.1 <sup>a</sup>	536.3 ± 70.9 <sup>a</sup>	30 ± 13	
PCB-153		448.5 ± 101.0 <sup>a</sup>	449.7 ± 96.4 <sup>a</sup>	498.0 ± 84.7 <sup>a</sup>	515.9 ± 53.1 <sup>a</sup>	445.5 ± 123.4 <sup>a</sup>	513.4 ± 127.4 <sup>a</sup>	512.9 ± 114.9 <sup>a</sup>	513.0 ± 125.0 <sup>a</sup>	14 ± 13	
PEMB-PCB		423.8 ± 81.4 <sup>a</sup>	389.3 ± 94.9 <sup>a</sup>	471.7 ± 42.9 <sup>a</sup>	448.7 ± 186.5 <sup>a</sup>	384.8 ± 151.1 <sup>a</sup>	458.0 ± 89.8 <sup>a</sup>	551.1 ± 122.2 <sup>a</sup>	493.7 ± 124.0 <sup>a</sup>	14 ± 5	

Each value is mean ± standard deviation (n = 6). Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at  $p < 0.05$  (one-way ANOVA with Tukey procedure). Abbreviations: PEMBs = polyethylene microbeads, ND = not detected, BLD = below the limit of detection, N/A = not applicable, and INDV = individual.



**Table 2**  
Accumulation and effects of microplastics (MPs), PCB-153, and MPs associated PCB-153 on giant river prawn (*Macrobrachium rosenbergii*).

Treatments		Exposure time (day)							Average
		0	0.5	1	2	4	14	28	
		Microplastic accumulation (beads/INDV)							
Control		ND	N/A	N/A	N/A	N/A	N/A	ND	N/A
PEMB	(beads/INDV)	ND	15 ± 16 <sup>a</sup>	1 ± 1 <sup>a</sup>	0 ± 1 <sup>a</sup>	1 ± 1 <sup>a</sup>	4 ± 8 <sup>a</sup>	9 ± 13 <sup>a</sup>	5 ± 6
	(beads/g dw)		10,612 ± 10,660 <sup>a</sup>	358 ± 329 <sup>b</sup>	161 ± 359 <sup>b</sup>	748 ± 788 <sup>b</sup>	2251 ± 3906 <sup>ab</sup>	2158 ± 2948 <sup>ab</sup>	2715 ± 3971
	(ug PE/g dw)		1826 ± 1835 <sup>ab</sup>	7747 ± 7222 <sup>a</sup>	28 ± 62 <sup>b</sup>	129 ± 136 <sup>b</sup>	388 ± 672 <sup>b</sup>	371 ± 507 <sup>b</sup>	1748 ± 3011
PCB-153		ND	N/A	N/A	N/A	N/A	N/A	ND	N/A
PEMB-PCB	(beads/INDV)		1 ± 1 <sup>a</sup>	2 ± 3 <sup>a</sup>	24 ± 48 <sup>a</sup>	17 ± 24 <sup>a</sup>	34 ± 68 <sup>a</sup>	39 ± 42 <sup>a</sup>	20 ± 16
	(beads/g dw)		1013 ± 480 <sup>a</sup>	994 ± 1703 <sup>a</sup>	15,091 ± 30,274 <sup>a</sup>	10,117 ± 15,165 <sup>a</sup>	25,591 ± 52,905 <sup>a</sup>	11,281 ± 11,948 <sup>a</sup>	10,681 ± 9271
	(ug PE/g dw)	ND	174 ± 83 <sup>a</sup>	171 ± 293 <sup>a</sup>	2597 ± 5211 <sup>a</sup>	1741 ± 2610 <sup>a</sup>	4405 ± 9106 <sup>a</sup>	1942 ± 2057 <sup>a</sup>	1838 ± 1596
		Survival rate (%)							Average
Control		N/A	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
PEMB		N/A	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	80.0 ± 44.7 <sup>a</sup>	96.0 ± 8.9
PCB-153		N/A	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
PEMB-PCB		N/A	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	80.0 ± 44.7 <sup>a</sup>	96.0 ± 8.9
		Wet weight (mg)							Weight gain (%)
Control		6.1 ± 0.8 <sup>c</sup>	5.9 ± 0.3 <sup>c</sup>	6.2 ± 0.4 <sup>c</sup>	6.1 ± 0.3 <sup>c</sup>	6.7 ± 0.5 <sup>c</sup>	9.9 ± 0.9 <sup>b</sup>	16.6 ± 3.4 <sup>a</sup>	171 ± 19
PEMB		6.7 ± 0.2 <sup>b</sup>	6.3 ± 0.6 <sup>b</sup>	7.4 ± 1.0 <sup>b</sup>	8.6 ± 1.9 <sup>b</sup>	7.6 ± 0.4 <sup>b</sup>	9.1 ± 1.3 <sup>b</sup>	18.3 ± 3.2 <sup>a</sup>	179 ± 38
PCB-153		6.6 ± 0.2 <sup>c</sup>	7.1 ± 1.0 <sup>c</sup>	6.0 ± 1.5 <sup>c</sup>	7.6 ± 1.6 <sup>c</sup>	8.8 ± 2.6 <sup>bc</sup>	11.8 ± 1.5 <sup>ab</sup>	13.3 ± 1.8 <sup>a</sup>	101 ± 27
PEMB-PCB		6.7 ± 0.4 <sup>b</sup>	6.8 ± 0.8 <sup>b</sup>	7.4 ± 1.6 <sup>b</sup>	7.5 ± 1.1 <sup>b</sup>	7.6 ± 1.3 <sup>b</sup>	8.4 ± 1.5 <sup>b</sup>	16.3 ± 1.7 <sup>a</sup>	152 ± 20

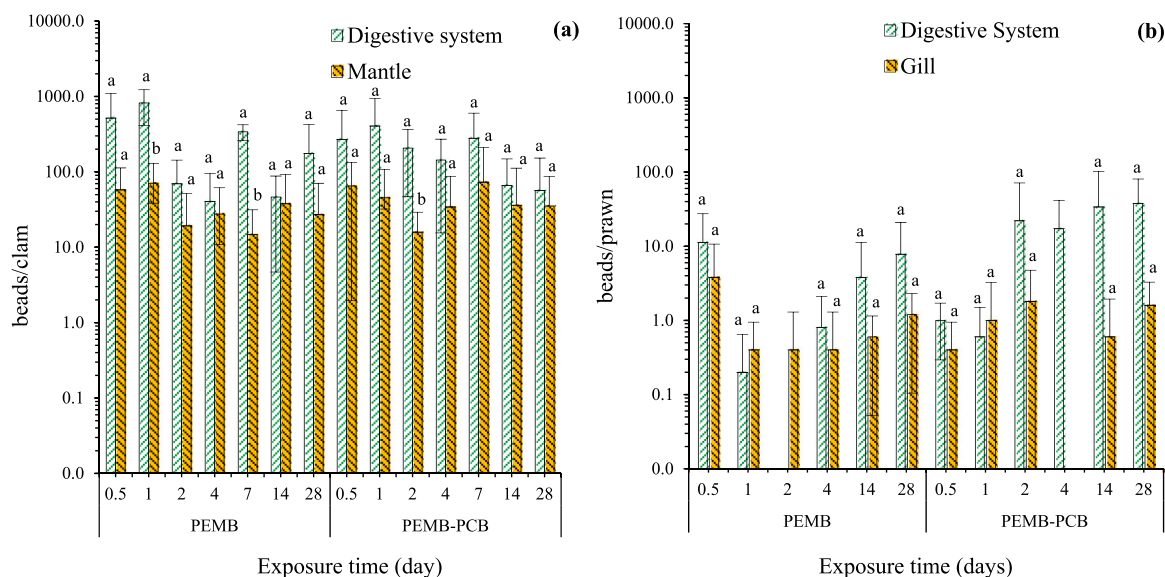
Each value is mean ± standard deviation (n = 5). Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at  $p < 0.05$  (one-way ANOVA with Tukey procedure). Abbreviations: PEMBs = polyethylene microbeads, ND = not detected, BLD = below the limit of detection, N/A = not applicable, and INDV = individual.

by consuming lower trophic levels such as prawns or clams prior to subsequent egestion.

MP accumulation in the digestive system of the clams observed in our present study was higher than that described for some field-collected organisms. For example, Ding et al. (2018) reported that MP in the digestive systems of the field-collected *Chlamys farreri* and *M. galloprovincialis*, varied from 5 to 19 items/INDV and from 2 to 10 items/INDV, respectively. Lam et al. (2022) reported a MP abundance of 35 particles/INDV in the stomach and intestine of marine-cultured fishes (*Epinephelus fuscoguttatus* and *Epinephelus lanceolatus*) in the Pearl River Estuary of China. These were likely due to the difference in MP abundance in exposure waters, i.e., the water MP concentration used in our study exceeded reported by Ding et al. (2018) and Lam et al. (2022). That said, reporting of MP concentrations in field samples are prone to substantial underestimations due to analytical bias derived from a focus on select polymer classes and a subset of MP sizes (Hale et al., 2020).

Studying the temporal accumulation of MPs over the 28-day exposure in each treatment indicated that the clams and the prawns accumulated MPs differently. The clams rapidly ingested a great number of MPs during the initial exposure periods and decreased MP accumulation afterwards. For instance, MP accumulations in the clams decreased from  $892 \pm 382$ – $202 \pm 278$  beads/clam in the PEMB treatment and from  $451 \pm 518$ – $92 \pm 103$  beads/clam in the PEMB-PCB treatment after 1- and 28-day exposure, respectively (Table 1). In contrast, the prawns ingested a small number of MPs initially and then steadily increased. For instance, MP accumulations in the prawns increased from  $1 \pm 1$ – $9 \pm 13$  beads/prawn in the PEMB treatment and from  $2 \pm 3$ – $39 \pm 42$  beads/prawn in the PEMB-PCB treatment, after 1- and 28-day exposure, respectively (Table 2). Weber et al. (2021) reported rapid initial (after 1 h) PEMB ingestion by the freshwater bivalve, *D. polymorpha*, but their later egestion (within 12 h).

Our present results also indicated that the association with PCB-153 of MPs may have affected MP accumulation differently in the exposed organisms. While the association with PCB-153 of MPs likely reduced MP accumulation in the clams, i.e.,  $323 \pm 311$  beads/clam in the PEMB treatment versus  $247 \pm 136$  beads/clam in the PEMB-PCB treatment ( $p = 0.261$ ), the association with PCB-153 of MPs likely enhanced MP accumulation in the prawns, i.e.,  $5 \pm 6$  beads/prawn in the PEMB treatment versus  $20 \pm 16$  beads/prawn in the PEMB-PCB treatment ( $p = 0.210$ ). Although the variability MP accumulations were high, this suggests that there could be a difference in palatability associated with PCB-153. Savoca et al. (2016) reported that weathered plastics, i.e., those that had sorbed dimethylsulfide, were more palatable to seabirds.



**Fig. 3.** Microplastic accumulation in: (a) digestive system (DS) and mantle of white hard clams (*M. lyrata*) and (b) DS and gills of giant river prawns (*M. rosenbergii*) over 28-day exposure to polyethylene microbeads (PEMBs) and PEMB-associated PCB-153 (PEMB-PCB). Error bars are  $\pm$  standard deviations. Mean values of each sampling intervals sharing different alphabetical letter superscripts are statistically significant at  $p < 0.05$  (one-way ANOVA with Games-Howell procedure).

### 3.3. Effects on the survival

Clam and prawn survival varied following exposure to MPs and MP-associated PCB-153. In the clam experiment (Table 1), the average survival rate of the clam control was  $95.2 \pm 8.1$  (mean  $\pm$  SD of the sampling intervals over the 28-day exposure). The survival rates of the PEMB and PEMB-PCB treatments were not significantly different from the survival rate of the control, i.e.,  $97.6 \pm 6.3\%$  and  $90.5 \pm 18.9\%$  versus  $95.2 \pm 8.1\%$  ( $p = 0.560$  and  $p = 0.390$ ), respectively. This finding suggested that exposure to MPs and MP-associated PCB-153 over 28 days did not significantly affect clam survival. Nevertheless, examining the temporal clam survival in each separate treatment of PEMB or PEMB-PCB over the 28-day exposure (from days 0–28) indicated that the survival rates significantly decreased at termination of the experiments. For example, in the PEMB treatment, the rate decreased from 100% (during days 0–14) to  $83.3 \pm 28.9\%$  (days 15–28); or in the PEMB-PCB treatment, the rate decreased from 100% (days 0–7) to  $83.3 \pm 28.9\%$  (days 8–14), and finally to  $50.0 \pm 0.0\%$  (days 15–28). These findings indicated that MP exposure, especially to MP-associated PCB-153, reduced clam survival during the later periods.

In the prawn experiment (Table 2), the average survival rate of the prawn control was  $100.0 \pm 0.0$ . Similar to the clam survival, the average survival rates of the treatments with MPs (the PEMB and PEMB-PCB treatments) were not statistically significant different from the average survival rate of the control, i.e.,  $96.0 \pm 8.9\%$  versus  $0.0 \pm 0.0\%$  ( $p = 0.321$ ). On the other hand, investigating the temporal prawn survival in each treatment of PEMB or PEMB-PCB indicated that the survival rates decreased at termination of the experiments. For example, in both of the PEMB and PEMB-PCB treatments, the rates decreased from 100% (during days 0–14) to  $80.0 \pm 44.7\%$  (days 15–28). These findings also indicated that MP exposure likely decreased prawn survival during the later periods.

Galloway et al. (2017) noted that MP ingestion alone (at levels commonly encountered in the environment) might not cause obvious mortalities, but could impact feeding, resulting in reduced energy stores, and “knock-on” effects for fecundity and growth. Likewise, Weber et al. (2021) reported exposure of freshwater mussel *Dreissena polymorpha* to PS fragments ( $\leq 63 \mu\text{m}$ ,  $6.4 - 100,000$  particles/L) caused no mortality in acute lab exposures (1, 3, and 7 days) and that mortality remained low (within 10% in the treatments with 160 and 4000 particles/mL) during chronic exposure (42 days). In contrast, Jaikumar et al. (2021) reported that exposure of juvenile giant river prawn *M. rosenbergii* to polystyrene microspheres (PSM,  $0.5 - 1.0 \mu\text{m}$ ) and polyethylene microparticles (PEM,  $30.0 - 15.0 \mu\text{m}$ ) caused significant dose-dependent declines in survival, varying from  $61.11 \pm 1.92 - 82.22 \pm 1.92\%$ , in those fed PSM and PEM incorporated foods. Similarly, Hsieh et al., 2023 reported that exposure of the shrimp *L. vannamei* ( $5.0 \pm 0.3$  g) to polystyrene nanoplastics (PS-NPs,  $0.05 \mu\text{m}$ ) also resulted in significant declines in survival, i.e., the shrimp that had received injections of PS-NPs at 0.5 and  $1.0 \mu\text{g/g}$  shrimp had percent survival rates of 81.10% and 70.00% versus 96.67% of the control after 7-day exposure, respectively. Other studies reported significant mortalities following MP exposure, e.g., 100% mortality of Asian green mussels (*Perna viridis*) after exposure to 2160 mg/L polyvinylchloride (PVC,  $1 - 50 \mu\text{m}$ ) for 44-days (Rist et al., 2016); 100% mortality of common goby (*Pomatoschistus microps*) when exposed to a mixture of polyethylene ( $184 \mu\text{g/L}$ ,  $1 - 5 \mu\text{m}$ ) and pyrene ( $200 \mu\text{g/L}$ ) for 60-h exposure (Oliveira et al., 2013); and 80% mortality of *Daphnia magna* when exposed to PEMBs ( $500 \text{ mg/L}$  or  $2.909 \times 10^6$  beads/L) over a 21-days (Phung et al., 2023).

### 3.4. Effects on the weight gain

In our experiments, exposure to MPs and MP-associated PCB-153 resulted in different effects on the weight gain of the test organisms. In the clam experiment (Table 1), the weight gain in the PEMB treatment was not significantly lower than that in the control, i.e.,  $30 \pm 13$  versus  $32 \pm 11\%$ , respectively ( $p = 0.772$ ). In contrast, the weight gain in the PEMB-PCB treatment was significantly lower than that in the control, i.e.,  $14 \pm 5$  versus  $32 \pm 11\%$ , respectively ( $p = 0.035$ ). These findings indicated that while MPs (the PEMB treatment) did not affect the weight gain, MP-associated PCB (the PEMB-PCB treatment) significantly lowered the weight gain of the clams. Notably, the weight gain in the PEMB-PCB treatment was not significantly different from the weight gain in the PCB treatment, i.e.,  $14 \pm 5$  versus  $14 \pm 13\%$  ( $p = 0.964$ ), respectively. It is noted that these two treatments contained the same quantity of PCB-153 (126 ng/glass bottle – see Section 3.1). However, while PCB-153 in the PCB treatment was in waterborne state (injected via Stock PCB 8-clam), PCB-153 in the PEMB-PCB treatment was adsorbed on solid microplastic particles (injected via the PEMB-PCB beads) and could be desorbed during the 28-day exposure. This finding suggested that PCB-153 bioavailable concentration could be similar in both treatments of PCB and PEMB-PCB, and MPs could transfer the associated PCB-153 to the clams.

In the prawn experiment (Table 2), exposure to MPs and MP-associated PCB-153 over 28 days did not statistically affect weight gain. For instance, the weight gains were not significantly greater in the control than in the PEMB treatment and in the PEMB-PCB treatment, i.e.,  $171 \pm 19\%$  versus  $179 \pm 38\%$  ( $p = 0.697$ ) and  $152 \pm 20\%$  ( $p = 0.174$ ), respectively. Particularly, when exposed to the same PCB-153 quantity (210 ng/glass bottle – see Section 3.1) via Stock PCB 8-prawn (in the PCB treatment) and via PEMB-PCB beads (in the PEMB-PCB treatment), the prawn weight gains were statistically significantly greater in the PEMB-PCB treatment than in the PCB treatment, i.e.,  $152 \pm 20$  versus  $101 \pm 27\%$  ( $p = 0.016$ ). These findings indicated that while exposure to MPs only (the PEMB treatment) and MP-associated PCB-153 (the PEMB-PCB treatment) did not significantly affect weight gain of the prawns, MPs reduced the negative effects of PCB-153 on the weight gain of the prawns by strongly associated with PCB-153.

These findings, therefore, did not support that consumption of MPs reduces growth rates (Galloway et al., 2017, Murphy and Quinn, 2018, Jaikumar et al., 2021). Jaikumar et al. (2021) reported that exposure to PSM and PEM significantly reduced the weight gains in juvenile *M. rosenbergii*, varying from 14% to 34% and from 5% to 23% less than the control, respectively. Galloway et al. (2017) reported that chronic exposure to MP is rarely lethal, but can adversely affect individual animals, reducing feeding and depleting energy stores, with knock-on effects for growth. Notwithstanding, a few other publication is in agreement with our present findings. Gerdes et al. (2019) reported that, given low concentration of MPs in the environment, the effects of MPs on growth in zooplankton were most likely negligible. When studying the interference of PCBs on the effects of MPs, the exposure of PCB contaminated daphnids to MP facilitated elimination of the high-molecular-weight PCB by 4-fold compared to the animals receiving only algal food, which provided proof-of-principle that MPs can act as a sink for HOCs (Gerdes et al., 2019). Phung et al. (2023) also suggested that the presence of PEMBs diminished the bioaccumulation of PCB-153 in *Daphnia magna*. The mechanism is not clear. The affiliation between HOC and MP might be stronger than the affiliation between HOC and the tissue of organisms. This assumption has been proposed by scientists (Gouin et al., 2011, Granby et al., 2018). However, more research on the affiliation between MP-HOC versus organism's tissue-HOC should be conducted to better understand the interference of HOCs on MPs that would result in less additive effects on the growth or weight gain of living organisms.

## 4. Conclusions

Increasing MP pollution has been reported globally. However, studies of interactions between MPs, HOCs, and indigenous species are limited. In laboratory studies, when either was exposed to MPs only or MP-associated PCB-153, we found that MP accumulation was significantly greater in the clams than in the prawns. MP accumulation was observed mostly in the digestive system of the test organisms. Although effects of MPs or MP-associated PCB-153 exposure on the mean survival rates of these organisms (28-day exposure) were not statistically significant, effects did significantly increase during the final stages of the experiments. In addition, while MP-associated PCB-153 did not significantly affect the weight gain of the prawns, it significantly reduced weight gain in clams. Given the increases of MPs and HOCs in the globe and in southeast Asia specifically, and the ecological and commercial importance of the hard clams and giant river prawns in the Indo-Pacific region, this present study provides valuable data to enhance our understanding of the effects of MPs and HOCs on these species.

### Ethical statement

The white hard clam (*M. lyrata*) and giant river prawn (*M. rosenbergii*) are the low-trophic level animals. They are popular indigenous animals in coastlines and rivers in Viet Nam. They are not listed in group IB (endangered and critically endangered species) or IIB (threatened and rare species) of the National Regulations for the Use of Animals in Research in Viet Nam. The experiments were complied with the Animal Research: Reporting of In Vivo Experiments Guidelines (ARRIVE Guidelines, 2024) and were carried out in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and, as applicable, the Animal Welfare Act (National Institutes of Health, 2015).

### CRedit authorship contribution statement

**Gunther Rosen:** Writing – review & editing, Conceptualization. **Robert C. Hale:** Writing – review & editing. **Lien Thi Le:** Methodology, Investigation, Data curation. **Tran Minh Loc:** Investigation, Data curation. **Bao-Son Trinh:** Writing – review & editing,

Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization.

## 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

I have shared the link to my data/code at the attach file step

## Acknowledgements

We thank Dr. Tham Hoang, Dr. Barnett Rattner, Dr. Doan Nhu Hai, Dr. Ning Wang, and Dr. Nguyen Phuoc Dan for professional discussions and comments on early versions of this manuscript. This research was funded by Viet Nam National University Ho Chi Minh City (VNU-HCM) under grant number B2021-24-03.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2024.103581](https://doi.org/10.1016/j.eti.2024.103581).

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