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Abundance of Microplastics in Two Venus Clams (*Meretrix lyrata* and *Paratapes undulatus*) from Estuaries in Central Vietnam

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Abstract: This study investigated the presence of microplastics in two common edible bivalves in Vietnam, the hard clam (*Meretrix lyrata*) and the undulate venus clam (*Paratapes undulatus*), from two estuaries in Da Nang city. Microplastics were detected in both species with relatively high concentrations—from 2.17 ± 0.43 to 2.38 ± 1.28 items g^{-1} in the undulate venus clams and from 4.71 ± 2.15 to 5.36 ± 2.69 items g^{-1} in the hard clams. Fibers were the most dominant form of microplastic in both clams, and a high proportion were fibers with sizes from 300 μm to 1500 μm. An estimation of microplastic intake in Vietnamese consumers' bodies from clam consumption was made, which showed an average ingestion of 2489 items $person^{-1}$ $year^{-1}$. Our study is also the first global record of microplastic distribution characteristics in the undulate venus clam *Paratapes undulatus*.

Keywords: microplastics; clam; estuary; *Paratapes undulatus*; *Meretrix lyrata*; Vietnam

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1. Introduction

Microplastics (plastic particles of size < 5 mm [1]) have emerged as one of the most worrisome pollutants in coastal and marine environments on a global scale [2,3]. It was reported that 4.8 to 12.7 million metric tons of plastic waste from 192 coastal countries reached marine environments by 2010 [4], making plastic debris predominant among marine debris (accounting for three-quarters of marine debris [2]). Worryingly, this figure might increase sharply in the near future because plastic products were predicted to increase to 33 billion tons by 2050 [2]. The abundance of plastic debris in the environment, together with its persistence, has led to the breaking down of larger plastics into microplastics (secondary microplastics), resulting in an overwhelming abundance of microplastics in all coastal and marine habitats, including estuaries, mangroves, lagoons, bays, and deep-sea areas [5–9]. Furthermore, primary microplastics—plastics produced for a particular application (e.g., plastic pellets for drugs and cosmetics products)—are also another important source of pollution in the environment through the discharge of products containing microplastics after use or accidents in plastic transportation [10]. The widespread distribution of microplastics in all coastal and marine environments has meant they have been introduced to every trophic-level organism in the food web. According to Gall et al. (2015) [11], 92% of all encounters between individual organisms and marine debris are with plastic, which affects these species by ingestion, entanglement, and causes habitat disturbance. The main effects of microplastics on aquatic ecosystems include physical injury, abnormal behavior patterns, interference in the nutrient cycle, cytotoxicity and genotoxicity, and an increase in mortality [10].

Filter feeders, such as bivalve mollusks, are among the species with the highest risk of being contaminated by microplastics due to their feeding habits, as they feed by straining suspended particles from the water column [12–14]. These microplastics could cause many negative direct impacts on bivalves, e.g., impairing their filtration activity and reproductive health, inducing genotoxic effects, and indirect effects related to changing their habitat structure and food resources [15]. It was reported that exposure to polyvinyl chloride (PVC) increased the mortality of Asian green mussels *Perna viridis*, while polystyrene (PS) exposure reduced the quality of oocytes, the motility of sperm, and the egg production of the Pacific oyster *Crassostrea gigas* [10]. Furthermore, bivalves are important prey for many species, including invertebrates, fish, birds, and mammals [16]. As such, they can translocate microplastics into higher trophic species in the food chain. More importantly, bivalves are also an important human nutrition source, with the production of more than 15 million tons per year, accounting for 14% of total global marine production [17]. Unlike the consumption manners of other seafood species, humans always eat the whole body of bivalves, making them possibly a major microplastic source for humans [15]. Microplastics have been detected in many commercially popular bivalves, including clams, scallops, oysters, and mussels from both wild habitats and aquaculture areas [18–23]. The abundance of microplastics in bivalves often ranges from 10^{-1} to 10^{1} items g^{-1} [24–26]. However, microplastic concentrations of up to 657.5 items g^{-1} were also reported in bivalve species in Canada [27]. An estimation by Van Cauwenberghe and Janssen (2014) showed that Europeans with average mollusk consumption of 11.8–72.1 g cap^{-1} day^{-1} might ingest up to 1800–11,000 microplastics per year, which can cause serious impacts on human health [23].

In Vietnam, bivalve mollusks are one of the favorite kinds of seafoods of both the local community and tourists. Bivalves, especially clams, are targeted by the government as one of Vietnam's key seafood exports to the world [28,29]. However, information on microplastic contamination levels in edible bivalves is still scarce, although microplastics have been reported to appear with rather high concentrations in both freshwater systems and coastal environments of the country [30–32]. As such, microplastics have a high probability of accumulating in living organisms and affecting consumers' health. This study investigated the occurrence and distribution characteristics of microplastics in two common edible bivalve mollusks in coastal Da Nang, Vietnam: the hard clam *Meretrix lyrata*, and the undulate venus clam *Paratapes undulatus*. This study may contribute to the knowledge about microplastic contamination in biota in Vietnam.

2. Materials and Methods

2.1. Samples Collection

Two bivalve species, *Meretrix lyrata* (Sowerby, 1851) (common name: Lyrate Asiatic hard clam or hard clam) and *Paratapes undulatus* (Born, 1778) (common name: undulate venus clam), were selected for an investigation of microplastics. They are among the favorite seafood in Vietnam and some other Asian countries (e.g., China, Philippines) and are also important export products with high commercial value to the world market (e.g., Europe, the US, Japan) [29,32,33]. In Vietnam, these two species are widely distributed in coastal areas, including the coastal area of Da Nang city—an important coastal city with strengths in tourism development and fishing [34].

Field sampling of *Meretrix lyrata* and *Paratapes undulatus* was conducted at the Han River Estuary and Cu De River Estuary in June 2021, the dry season in Da Nang (Figure 1). These rivers are the two main rivers in Da Nang, which flow across densely residential areas and pour their water into Da Nang Bay. Hard clams were collected by using metal rakes during the low tide at the intertidal zone. Meanwhile, for undulate venus samples, sampling was more difficult and required diving into deeper water of approximately 7–8 m depth. A total of 15 individuals of each species were collected at three sites as three replicates for each estuary. After collection, all bivalve samples were washed with filtered distilled water (water that was already filtered through GF/A filters), kept in silver zip bags, and then stored at −20 °C in the laboratory. In addition, the shell lengths of the bivalves were measured (Table 1).

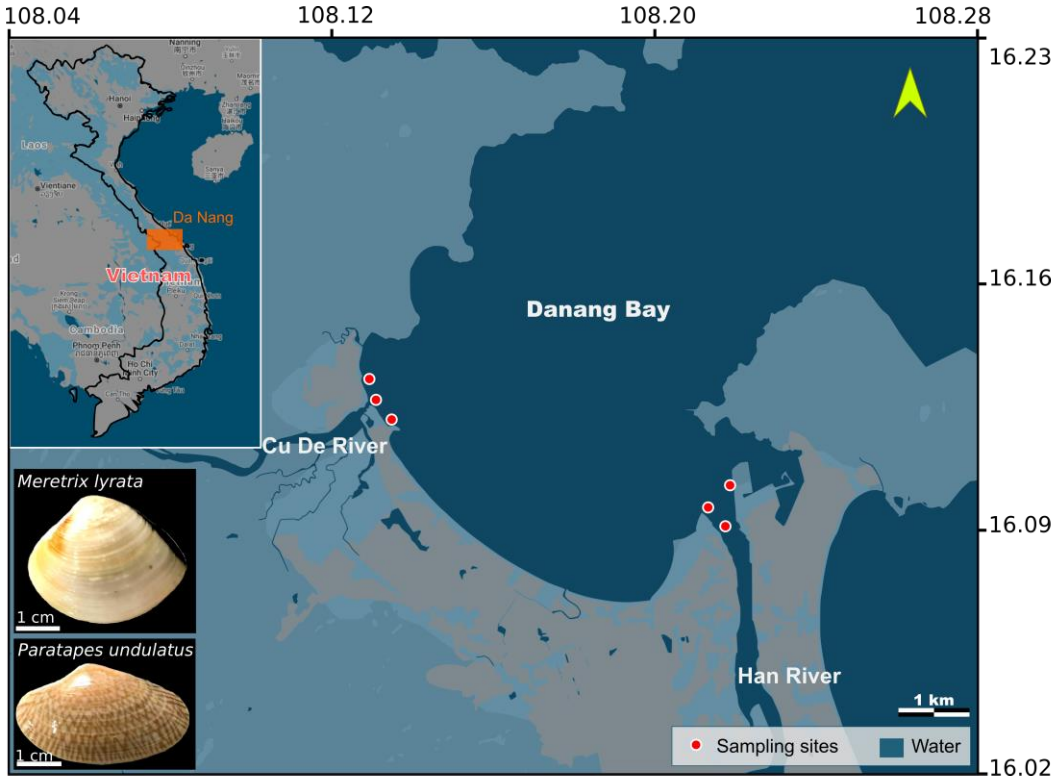

Figure 1. Sampling sites and photos of the two bivalve species.

Table 1. Information of two bivalve species collected from coastal Da Nang.

Species	Sampling Site	Number	Shell Length (cm)	Wet Tissue Weight (g)
Meretrix lyrata	Han River Estuary	15	3.6 ± 0.4	2.80 ± 1.05
	Cu De River Estuary	15	3.8 ± 0.4	2.40 ± 0.73
Paratapes undulatus	Han River Estuary	15	3.8 ± 0.5	1.56 ± 0.25
	Cu De River Estuary	15	3.5 ± 0.7	1.58 ± 0.49

Along with the bivalve samples, water samples from the Cu De and Han River Estuaries were also collected to determine the contents of microplastics in the water environment. Approximately 100 L of a bulk water sample was taken from each estuary, with 3 replicates, using a stainless-steel bucket. Then, the bulk water samples were filtered through a plankton net with a mesh size of 80 µm to obtain a sample volume of 300 mL for each site, and then preserved in glass bottles at 4 °C.

2.2. Microplastics Extraction and Analysis

First, the shells of the bivalves were removed to obtain the inner soft tissue, which was weighted (Table 1) and rinsed with filtered distilled water to remove all fine particles clinging to the outside. The soft tissue samples were then digested in a 10% potassium hydroxide (KOH) solution (Thermo Fisher Scientific, Cranbury, NJ, USA) and kept at 60 °C for 48 h [25,35]. Subsequently, the samples were sieved through a two-stage stainless steel sieve with mesh sizes of 5 mm and 50 µm to obtain the target microplastic size for the next steps. The samples retained on the surface of the second sieve stage were transferred to a 500 mL glass beaker using a filtered NaCl solution (density: 1.18 g mL^{-1}). After that, the NaCl solution was further poured into the beaker to create overflows that contained microplastics. Finally, the overflowed solution was vacuum filtered through glass fiber filters (Whatman GF/A, 1.6 µm pore size), and the filters were kept in closed Petri dishes for further analysis.

For the water samples, the extraction procedure followed the protocol developed by Strady et al. (2021) [30]. Briefly, the water samples were treated with 1 g of sodium dodecyl sulfate (50 °C, 24 h), 1 mL of bioenzyme F, 1 mL of bioenzyme SE (40 °C, 48 h), and 15 mL of H_2O_2 30% (40 °C, 48 h). Then, the samples were filtered through a 250 µm mesh-sized sieve to obtain a sample fraction greater than 250 µm, which continued to be density-separated and filtered in the same way as for the bivalve samples to obtain the final filters with microplastics attached.

The abundance and physical characteristics (shape, color, and size) of the microplastics were identified visually using a stereoscopic microscope equipped with a digital camera (Leica S9i), and their chemical properties were verified by a µ-Raman microscope (Confocal Raman Microscope XploRA™ PLUS of HORIBA Scientific). Specifically, the microplastics were recorded if they had properties as described by GESAMP (2019) [36] and Strady et al. (2021) [30] and classified based on their morphology (fibers, fragments, films, foams, and pellet) and color. The sizes of the microplastics were measured using LASX software® based on the captured images, with observation sizes limited to between 200 µm and 5000 µm for fibers, and from 25,000 $µm^2$ (300 µm × 150 µm) to 25,000,000 $µm^2$ (5000 µm × 5000 µm) for fragments.

To prevent microplastic contamination of the samples, a series of measures was carried out throughout the sample treatment and analysis process. The main measures included: carefully cleansing the working areas and all equipment before use, avoiding the use of material made of plastics, filtering all solutions (distilled water, KOH, NaCl) through glass fiber filters, and covering the samples right after each step of treatment. Additionally, one blank sample was run through the whole process, from removing the shells to the final filtration, and the results showed that there were no microplastics present on the filter.

2.3. Data Analysis

All data preprocessing, analysis, and visualization were performed on Google Colab (colab.research.google.com, accessed on 15 December 2022) using Python 3.6.9 programming language (Python Software Foundation; http://www.python.org, accessed on 15 December 2022). The concentration of microplastics in the bivalves is expressed as the number of microplastic items per individual (items $individual^{-1}$) and per gram of soft tissue (items g^{-1}) and presented as the mean ± standard deviation (SD). Meanwhile, the concentration of microplastics in the water is expressed in items L^{-1}. One-way ANOVA was used to examine the differences in the microplastic concentration between species and between sampling sites, for which a statistically significant difference was acceptable at $p < 0.05$ for all cases.

3. Results

3.1. Distribution Characteristics of Microplastics in the Hard Clam Meretrix lyrata

Microplastics were detected in the hard clam *Meretrix lyrata* with concentrations of 4.71 ± 2.15 items g^{-1} (12.73 ± 4.49 items $individual^{-1}$) in the Han River Estuary and 5.36 ± 2.69 items g^{-1} (13.20 ± 7.66 items $individual^{-1}$) in the Cu De River Estuary (Figure 2a). The statistical results show that there was no significant difference in the microplastic concentrations in this species between the two estuaries ($p > 0.05$). Fibers and fragments were the most common shapes in the clams in both estuaries, which, respectively, accounted for 52.9% and 46.6% of the total microplastics in the Han River Estuary and for 60.6% and 38.4% in the Cu De River Estuary (Figures 2b and 3). Films only appeared at a very small ratio (less than 1%), while foams and pellets were not found.

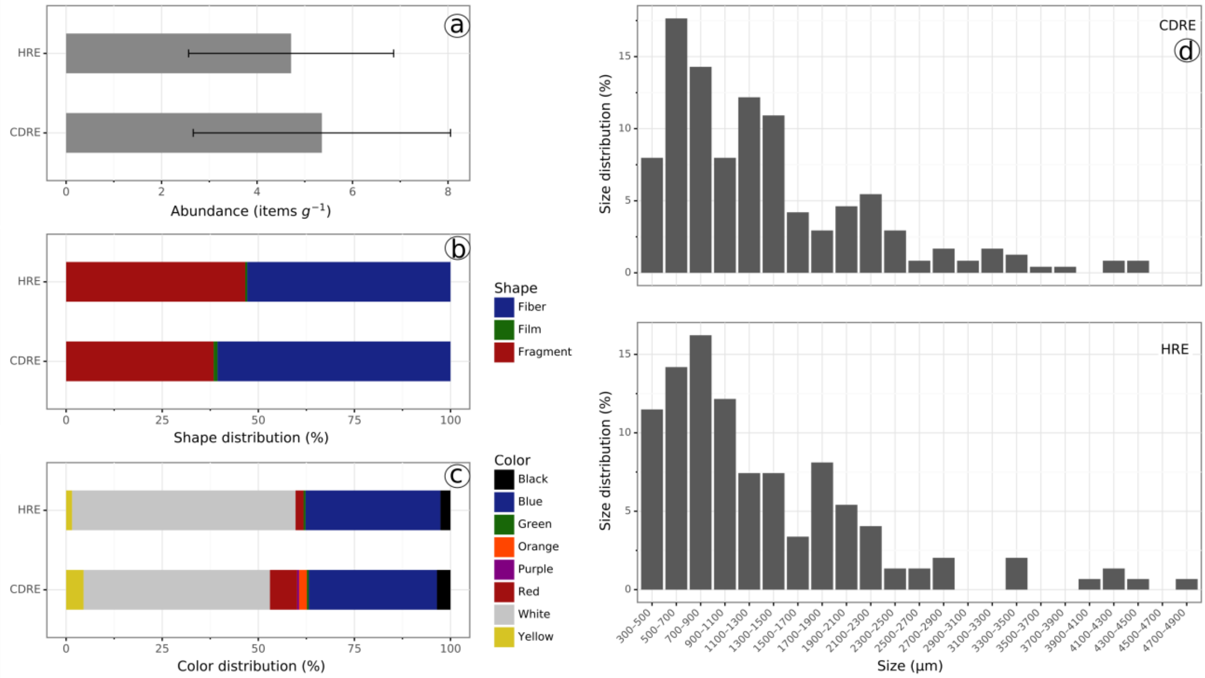

Figure 2. Microplastic characteristics in the hard clam *Meretrix lyrata* from Han River Estuary (HRE) and Cu De River Estuary (CDRE): (**a**) abundance; (**b**) shape distribution; (**c**) color distribution; and (**d**) size distribution.

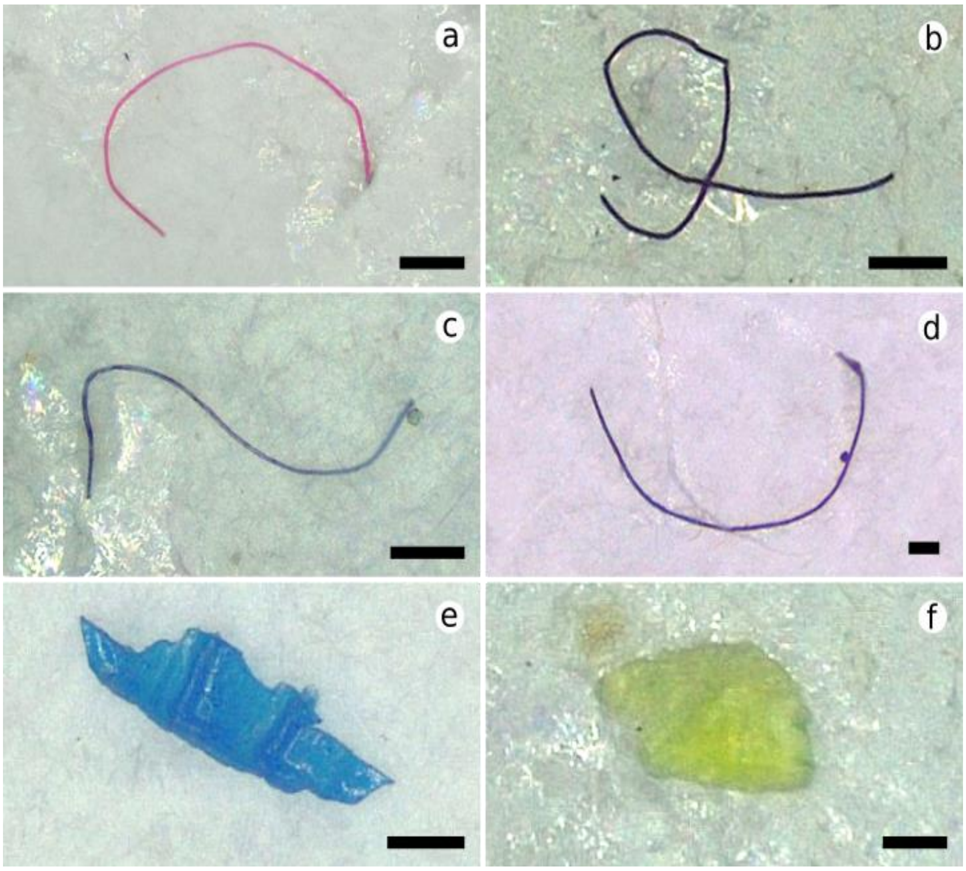

Figure 3. Examples of microplastics found in clams from the two estuaries in Da Nang: (**a**–**d**) fiber; and (**e**,**f**) fragment. All scale bars represent 100 μm.

Fibers in the hard clams were found with a wide range of sizes, of which 70% had a length of less than 1500 μm (Figure 2d). The average length was 1331.1 ± 1009.1 μm (median: 999.3 μm) in the Han River Estuary and 1183.5 ± 847.8 μm (median: 859.1 μm) in the Cu De River Estuary. For the fragments, the average areas were, respectively, 133,202.5 ± 129,415.8 μm^2 (median: 76,813.3 μm^2) in the Han River Estuary and 76,113.6 ± 88,689.6 μm^2 (median: 43,001.1 μm^2) in the Cu De River Estuary. Seventy percent of the fragments in the Han River Estuary clams had a size of less than 146,000 μm^2, while those in the Cu De River Estuary clams had a size of less than 64,000 μm^2.

Multiple colors (white, blue, black, green, orange, yellow, red, and purple) were observed for microplastics in the hard clams, and of them, white was found to be dominant, at 58.1% and 48.5% in the Han River Estuary and the Cu De River Estuary, respectively, followed by blue (35.1% in the Han River Estuary and 33.3% in the Cu De River Estuary) (Figure 2c). The other colors accounted for small ratios (0.5%–7.1%). Polyester, polyethylene (PE), and polypropylene (PP) were the main polymer types in the hard clams. Polyester accounted for 22.2–30% of the total identified microplastics, while both PE and PP accounted for 20–22.2% each. Additionally, low-density polyethylene (LDPE) was also detected in the clams from the Cu De River Estuary, at 20%. Polyvinyl chloride (PVC), polyethylene terephthalate (PET), and high-density polyethylene (HDPE) were also found.

3.2. Distribution Characteristics of Microplastics in the Undulate Venus Clam Paratapes undulatus

The microplastic abundance in the undulate venus clam *Paratapes undulatus* was 2.17 ± 0.43 items g^{-1} (3.43 ± 0.98 items $individual^{-1}$) in the Han River Estuary. In the Cu De River Estuary, this species accumulated a similar level of microplastics, with 2.38 ± 1.28 items g^{-1} (3.30 ± 0.94 items $individual^{-1}$) ($p > 0.05$) (Figure 4a). The microplastic abundances in the undulate venus clam *Paratapes undulatus* were significantly lower than those in the hard clam *Meretrix lyrata* in both estuaries ($p < 0.05$). Fibrous microplastics dominated the other forms, accounting for more than 90% of the total microplastics in both estuaries (94.2% in the Han River Estuary, 99.2% in the Cu De River Estuary). Fragments appeared at a very small ratio in this species in both estuaries (5.8% in the Han River Estuary, 0.8% in the Cu De River Estuary), while films, foams, and pellets were not found (Figure 4b).

The average lengths of the fibrous microplastics in the Han River Estuary and Cu De River Estuary clams were, respectively, 1429.7 ± 1215.1 μm (median: 983.6 μm) and 1286.4 ± 1159.6 μm (median: 889.6 μm). Similar to the hard clams, most fibers in the undulate venus clams (67–72%) fell in the size range of 300–1500 μm (Figure 4d). For fragments, the average sizes were, respectively, 133,202.5 ± 129,451.8 μm^2 (median: 76,813.3 μm^2) and 76,113.6 ± 88,689.6 μm^2 (median: 43,001.0 μm^2) in the Han River Estuary and Cu De River Estuary clams.

The microplastics in the estuarine undulate venus clams were mainly blue (Han River Estuary: 65.7%, Cu De River Estuary: 61.2%) and black (Han River Estuary: 12.4%, Cu De River Estuary: 18.9%). The ratios of the remaining colors ranged from 0.6% to 7.3% (Figure 4c). The most common polymer type in the undulate venus clams was PP (25–33.3%), followed by PE (11.1–25.0%), PET (11.1–25.0%), and polyester (12.5–22.2%). Additionally, polyamide (PA) (22.2%) and HDPE (12.5%) were also detected in this species.

3.3. Distribution Characteristics of Microplastics in Estuarine Waters

The concentration of microplastics in the water was 0.50 ± 0.36 items L^{-1} in the Cu De River Estuary, which was significantly higher than that in the Han River Estuary (0.21 ± 0.08 items L^{-1}) ($p < 0.05$) (Figure 5a). Fibers predominated the microplastics in both estuaries (accounting for 75.0% and 78.8% in the Han River Estuary and the Cu De River Estuary, respectively), followed by fragments (Han River Estuary: 23.4%, Cu De River Estuary: 17.2%), film (Cu De River Estuary: 4.0%), and foam (Han River Estuary: 1.6%) (Figure 5b). Blue and white dominated the other colors, with blue accounting for 50.0%

and 39.1% and white accounting for 43.7% and 43.1% in the Han River Estuary and the Cu De River Estuary, respectively (Figure 5c).

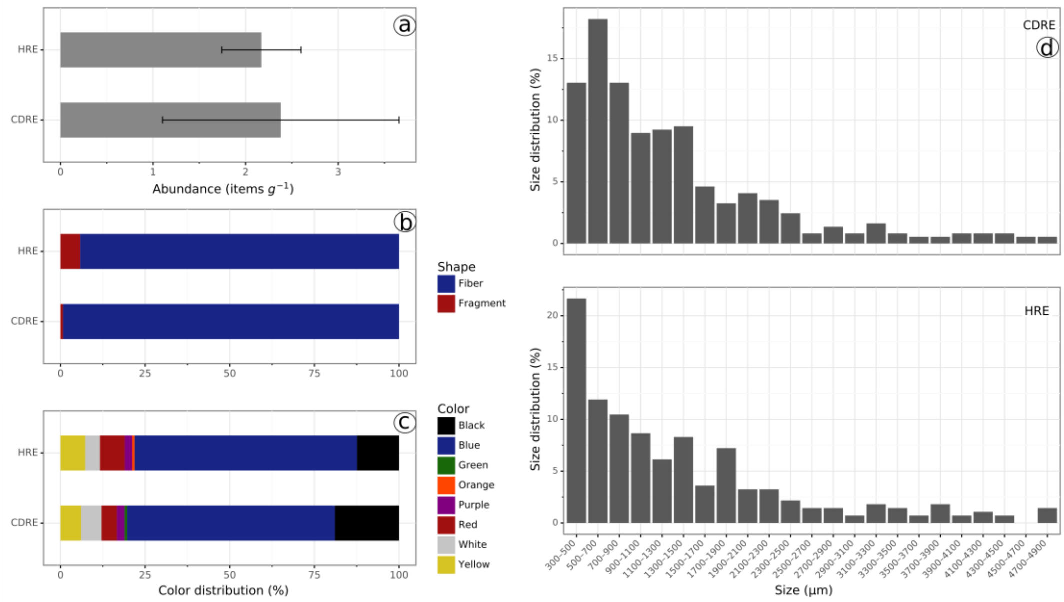

Figure 4. Microplastic characteristics in the undulate venus clam *Paratapes undulatus* from Han River Estuary (HRE) and Cu De River Estuary (CDRE): (**a**) abundance; (**b**) shape distribution; (**c**) color distribution; and (**d**) size distribution.

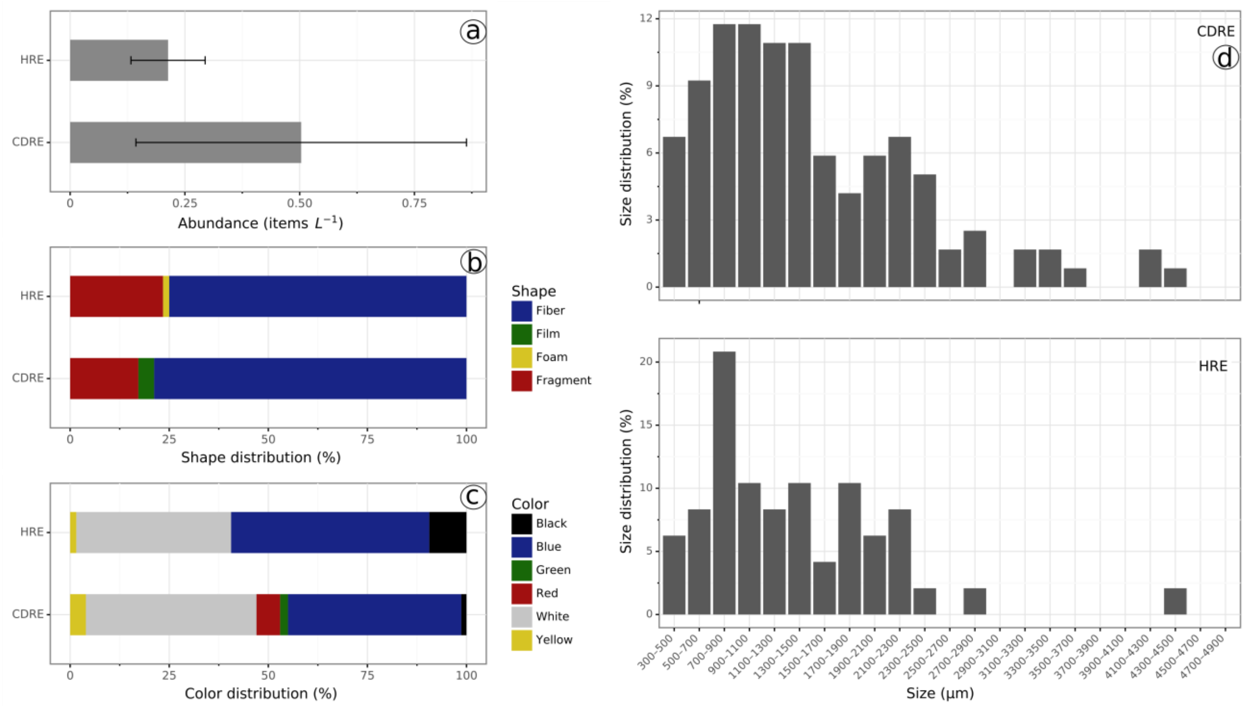

Figure 5. Microplastic characteristics in the water of Han River Estuary (HRE) and Cu De River Estuary (CDRE): (**a**) abundance; (**b**) shape distribution; (**c**) color distribution; and (**d**) size distribution.

In the Han River Estuary, the fibers had an average length of 1350.4 ± 747.7 µm (median: 1214.6 µm), and the fragments had an average area of 109,511.1 ± 117,116.9 µm^2 (median: 66,157 µm^2). In the Cu De River Estuary, the average fiber length was

1507.5 ± 874.1 µm (median: 1277 µm), and the average fragment area was 116,593.8 ± 66,279.5 µm^2 (median: 97,753 µm^2). In the two estuaries, approximately seventy percent of the fibers had a length of less than 1900 µm, and ninety percent of fibers had a length of less than 2500 µm (Figure 5d).

Various polymer types were found in the Han River Estuary water, including PP, polyester, PET, PE, PA, polyacrylonitrile (PAN), and poly-ethylene vinyl alcohol copolymers (PVOH), which accounted for 10–20% each. The composition of the microplastics in the Cu De River Estuary water was quite similar to those in the Han River Estuary, with PE, PET, PA, polyester, PVC, LDPE, and polydimethylsiloxane (PDMS) and no dominant type (10–20% each).

4. Discussions

4.1. Microplastic Concentration in Bivalves

To the best of our knowledge, this is the first time that microplastic abundance has been reported for the clam *Paratapes undulatus*. The concentrations of microplastics in *Paratapes undulatus* from two Da Nang estuaries (2.17 ± 0.43 items g^{-1}–2.38 ± 1.28 items g^{-1}) were comparable to those in other bivalves, including *Venerupis philippinarum* from an aquaculture farm in British Columbia (1.7 ± 1.2 items g^{-1}; [21]) and *Mytilus galloprovincialis* from the natural area in Qingdao, China (2.0 items g^{-1}; [25]). In the case of the hard clam *Meretrix lyrata*, the microplastic level in our study site (4.71 ± 2.15 items g^{-1}–5.36 ± 2.69 items g^{-1}) was higher than that in clams grown in the Can Gio mangrove in Ho Chi Minh City, Vietnam (2.7 ± 2.4 items g^{-1}; [37]), and Bandon Bay, Thailand (0.28 ± 0.06–0.03 ± 0.01 items g^{-1}; [38]) (Table 2).

The microplastic level in the two clams from the Da Nang estuaries was approximately one order of magnitude higher than that in many other bivalve species from different worldwide habitats, e.g., *Perna viridis* in Thanh Hoa, Vietnam (0.29 ± 0.14 items g^{-1}; [39]), *Tapes philippinarum* in Korea (0.34 ± 0.31 items g^{-1}; [24]), *Venerupis philippinarum* (wild) in British Columbia (0.9 ± 0.9 items g^{-1}; [21]), and *Mytilus edulis* in Korea (0.12 ± 0.11 items g^{-1}; [24]), Belgium (0.26–0.51 items g^{-1}; [40]), France (0.23 ± 0.20 items g^{-1}; [39]; 0.15 ± 0.06–0.25 ± 0.16 items g^{-1}; [41]), and Germany (0.36 ± 0.07 items g^{-1}; [23]) (Table 2).

The abundance of microplastics in bivalves is related to many factors, and thus, it is quite complicated to understand. In the study by Phuong et al. (2018) [39] on two bivalves from the French Atlantic coasts, the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*), three factors, including the sampling location, the season, and the mode of life of organisms (wild and cultivated organisms), did not significantly affect the microplastic concentration, but the species characteristics, specifically the filtration rate, caused a difference in the microplastic abundance between the two species. Related to species characteristics, Setälä et al. (2016) [12] also reported the role of the behavior and feeding modes of bivalves in affecting the amount of microplastic being ingested. Meanwhile, according to Hermabessiere et al. (2019) [41], the location was believed to be a more important factor in influencing microplastic pollution levels in bivalves compared to the species. This finding is also in accordance with the studies of Jin-feng et al. (2018) [25], Li et al. (2019) [14], Qu et al. (2018) [18], and Cho et al. (2019) [24], who have reported that the pollution degree of microplastics in the environment among different areas causes great differences in the microplastic concentrations in bivalves. Additionally, the differences in bivalves' habitats and the utilized analytical methods were found to influence the variation in the microplastic concentrations in bivalves [24]. The role of the habitat was also mentioned in the study of Baechler et al. (2020) [42], in which oysters grown in an estuary accumulated microplastics higher than razor clams that lived along the open coast.

In this study, no significant differences in the microplastic concentrations in clams were found between the Han River Estuary and the Cu De River Estuary for either species, although the microplastic concentration in the water of the Cu De River Estuary was approximately two times higher than that of the Han River Estuary. However, in the same estuary, the microplastic concentration in the hard clam *Meretrix lyrata* was detected to be significantly higher than that in the undulate venus clam *Paratapes undulatus*. This demonstrates that species characteristics are possibly important factors that influence the microplastic abundance in bivalves from Da Nang. Therefore, it is suggested that the impact of the species morphology and the mechanisms of particle uptake and accumulation of the two species should be further investigated in future studies. Moreover, aside from species characteristics, the habitat where bivalves live is possibly an important factor that affects the microplastic concentrations in these two species. Although living in the same river estuary, these two clams have different habitats, specifically, the hard clams *Meretrix lyrata* settle in the intertidal flats, whereas the undulate venus clams *Paratapes undulatus* were found in deeper water—the riverbed at a depth of approximately 7 m. In comparison to the deeper water layers, the intertidal zone and the surface water were found to have higher microplastic concentrations because of the impact of tidal currents that cause the suspension of particles, including microplastics, in the water column [24,43]. Moreover, the tidal flat is also the zone that is directly impacted by many waste streams from the inland area, thus accumulating a significant amount of microplastics [44]. This is consistent with the case in our study sites, where many activities take place along the two rivers that discharge solid wastes and wastewater containing microplastics.

Table 2. Microplastic concentrations in some popular edible bivalves from different areas.

Group	Species	Area	Sampling Site	Digestion Solution	MPs Concentration		Ref.
					(Items g^{-1})	(Items Individual^{-1})	
Clam	*Meretrix lyrata*	Danang, Vietnam	Wild, Han River Estuary	KOH 10%	4.71 ± 2.15	12.73 ± 4.49	This study
Clam	*Meretrix lyrata*	Danang, Vietnam	Wild, Cu De River Estuary	KOH 10%	5.36 ± 2.69	13.20 ± 7.66	This study
Clam	*Meretrix lyrata*	Ho Chi Minh city, Vietnam	Farm, Can Gio beach sand	KOH 10%	2.7 ± 2.4	3.6 ± 2.1	[37]
Clam	*Meretrix lyrata*	Surat Thani, Thailand	Wild, Bandon Bay	H_2O_2 30%	0.28 ± 0.06–0.03 ± 0.01	0.67 ± 0.15–0.23 ± 0.09	[38]
Clam	*Paratapes undulatus*	Danang, Vietnam	Wild, Han River Estuary	KOH 10%	2.17 ± 0.43	3.43 ± 0.98	This study
Clam	*Paratapes undulatus*	Danang, Vietnam	Wild, Cu De River Estuary	KOH 10%	2.38 ± 1.28	3.30 ± 0.94	This study
Clam	*Tapes philippinarum*	South Korea	Market	KOH 10%	0.34 ± 0.31	1.15 ± 0.74	[24]
Clam	*Venerupis philippinarum*	British Columbia	Wild	HNO_3 69–71%	0.9 ± 0.9	n.a.	[21]
Clam	*Venerupis philippinarum*	British Columbia	Farm	HNO_3 69–71%	1.7 ± 1.2	n.a.	[21]
Mussel	*Perna viridis*	Thanh Hoa, Vietnam	Wild	KOH 10%	0.29 ± 0.14	2.60 ± 1.14	[39]
Mussel	*Mytilus edulis*	South Korea	Market	KOH 10%	0.12 ± 0.11	0.68 ± 0.64	[24]
Mussel	*Mytilus edulis*	Belgium	Wild	HNO_3 65%: HClO4 68% (4:1 v:v)	0.26–0.51	n.a.	[40]

Table 2. *Cont.*

Group	Species	Area	Sampling Site	Digestion Solution	MPs Concentration		Ref.
					(Items g^{-1})	(Items Individual^{-1})	
Mussel	*Mytilus edulis*	Belgium	Market	HNO3 65%: HClO4 68% (4:1 v:v)	0.35	n.a.	[40]
Mussel	*Mytilus edulis*	France	Wild and farm	KOH 10%	0.23 ± 0.20	0.60 ± 0.56	[20]
Mussel	*Mytilus edulis*	France	Wild	KOH 10%	0.15 ± 0.06–0.25 ± 0.16	0.76 ± 0.40–0.78 ± 0.30	[41]
Mussel	*Mytilus edulis*	Germany	Farm	HNO3 69%	0.36 ± 0.07	n.a.	[23]
Mussel	*Mytilus galloprovincialis*	Qingdao, China	Wild	KOH 10%	2.0	0.53	[25]
Mussel	*Mytilus galloprovincialis*	Qingdao, China	Market/farm	KOH 10%	3.17	1.9	[25]
Mussel	*Mytilus galloprovincialis*	Italy	Market	H_2O_2 30%	4.4–11.4	3.6–12.4	[26]
Mussel	*Mytilus galloprovincialis*	Italy	Wild	H_2O_2 30%	7.2	3.0	[26]
Oyster	*Crassostrea gigas*	France	Wild and farm	KOH 10%	0.18 ± 0.16	2.10 ± 1.71	[20]
Oyster	*Crassostrea gigas*	South Korea	Market	KOH 10%	0.07 ± 0.06	0.77 ± 0.74	[24]
Scallop	*Patinopecten yessoensis*	South Korea	Market	KOH 10%	0.08 ± 0.08	1.21 ± 0.71	[24]
Scallop	*Argopecten purpuratus*	Lima, Peru	Market	KOH 10%	0.13 ± 0.03	2.25 ± 0.54	[45]

4.2. Characteristics of Microplastics in Bivalve Species

Fibers and fragments were the dominant forms in the hard clam *Meretrix lyrata* in both estuaries in Da Nang. In the undulate venus clam *Paratapes undulatus*, fibers had outstanding dominance, accounting for more than 90% of the total microplastics. The predominance of fibers, followed by fragments, has been reported in many other bivalve species collected in wild habitats, farming areas, and markets, for instance, eight commercial bivalves from China (fibers: 52–82%; fragments: 10–40%; [46]), the oyster *Saccostrea cucullata* from the Pearl River Estuary, China (fibers: 69.4%; fragments: 20%; [47]), and the two clams *Amiantis umbonella*, *Amiantis purpuratus*, and the Pearl oyster *Pinctada radiata* from the Persian Gulf, Iran (fibers: 58%, fragments: 26%; [19]). Moreover, similar to the clam *Paratapes undulatus* in our study site, some bivalves even contain fiber proportions of up to more than 90%, e.g., the Manila clam *Venerupis philippinarum* from Baynes Sound, British Columbia (90%; [21]), the Pacific oyster *Crassostrea gigas* and razor clam *Siliqua patula* from Oregon, U.S.A. (over 99%; [42]), the *Chlamys farreri* (98.08%) and *Mytilus Galloprovincialis* (97.06%) from Qingdao, China [25], and the clam *Meretrix lyrata* (95%) and the mussel *Perna viridis* (100%) from Bandon Bay, Thailand [38].

The popularity of fibers in many bivalve species could be explained by the fact that fibers are also a common microplastic form found in both the natural estuarine environment [38,48,49] and the farming areas where plastic-based ropes and nets often been used for growing bivalves [21,50] and, thus, have a high possibility of being eaten by bivalves. In our study sites, fibers were also the prevalent form found in the waters of the Han and Cu De River Estuaries (75.0% and 78.8%, respectively), and in the beach sediments of the

Da Nang Bay (99.2%) [32]. These fibers potentially mainly originate from municipal solid wastes and wastewater discharging into the rivers and from fishery activities taking place at the two estuaries. Moreover, another reason for the predominance of fibers in bivalves is that they are much more difficult to eliminate than other forms once entering the bivalve bodies [46].

Microplastics detected in bivalves are reported to be dominated by small-sized particles of less than 500 µm in length in many areas (e.g., China: [22,25,46,47]; South Korea: [24]; Middle East: [19]) because they are thought to more easily enter and accumulate in bivalves' bodies. In this study, small fibers with sizes from 300 to 500 µm also accounted for a considerable proportion, especially in the undulate venus clam *Paratapes undulatus* (*Meretrix lyrata*: 8–11.5%, *Paratapes undulatus*: 13–21.7%). However, our findings show that larger-size fibers can still be ingested by bivalves, demonstrated by the high proportion of fibers from 500 µm to 1500 µm in both species (*Meretrix lyrata*: 57.4–63%, *Paratapes undulatus*: 45.5–59%); fibers longer 1500 µm were still detected, although at a small ratio. Similarly, Chinfak et al. (2021) [38] also reported that 86% of the fibers in the large-size clam *Meretrix lyrata* from Bandon Bay, Thailand were greater than 1000 µm (average: 1748 ± 168 µm). Fibers even longer than 5000 µm and up to 11,000 µm were also detected in *Meretrix lyrata* grown in the Can Gio mangrove, Vietnam (average: 1085.0 ± 783.7 µm; median: 627 µm; [37]). Additionally, fibers in other species, such as the blue mussel *Mytilus edulis* from Belgium [40] and the Pacific oyster *Crassostrea gigas* and razor clam *Siliqua patula* from the Oregon coast, U.S.A [42], have also been reported to commonly have a size of 1000 to 1500 µm. Although the mechanism of microplastic accumulation in bodies of each specific bivalve species has not been well studied, a study by Brillant and MacDonald (2000) on scallops showed that larger particles were retained longer in their bodies than smaller particles, which might explain the high ratio of large-size fibers detected in the clams in our study sites and other areas [51].

The microplastics found in the two clams in the Da Nang estuaries were mainly polyester, PP, PE, PET, LDPE, and PA. These polymer types have also been the major polymer types found in four bivalve species (*Crassostrea gigas*, *Mytilus edulis*, *Tapes philippinarum*, and *Patinopecten yessoensis*) from South Korea [24], the blue mussel *Mytilus edulis* and cockles from the Channel coastlines of France [41], the oysters *Saccostrea cucullata* from the Pearl River Estuary of China [47], three bivalves species (*Amiantis umbonella*, *Amiantis purpuratus*, and *Pinctada radiata*) from the Persian Gulf of Iran [19], a mussel (*M. edulis*) and oyster (*C. gigas*) from French Atlantic coasts [20], and two mussels (*Perna viridis*, *Mytilus edulis*) from coastal China [18]. This is not surprising, since these polymers are widely used and have been found to be rather common in the coastal environment worldwide, including Da Nang [49,52,53].

4.3. Health Risk Posed to Bivalve Consumers

Bivalve species, especially clams, are among the important aquatic products that are increasingly being consumed. The consumption of bivalves containing microplastics poses a major risk to human health since all the microplastics in bivalves' bodies will be transferred to and accumulated in human bodies. The amount of microplastic introduced to the bodies of Vietnamese consumers was estimated based on the average microplastic content found in clams in this study (3.66 items g^{-1}) and the per capita domestic consumption of clams by wet weight (680 g $person^{-1}$ $year^{-1}$). The per capita domestic consumption of clams by wet weight was calculated based on the domestic consumption of clams, the ratio of the whole-body weight and soft tissue weight (3.7:1, from our investigation), and the national population in the year 2021 (98.51 million people; [54]). Because there are no published data on the domestic consumption of clams in Vietnam, the production of bivalve mollusks for domestic consumption, which is mainly clams and oysters (250,000 tons in 2020; [55]) was used for the calculation. Our estimation shows that Vietnamese people might ingest up to 2489 items $person^{-1}$ $year^{-1}$ of microplastic via clam consumption.

The dietary exposure of Vietnamese consumers to microplastics is higher than that in South Korea, the UK, France, and Ireland, but lower than that in China, Iran, Canada, Belgium, and Italy (Table 3). However, this comparison only provides a glimpse into the level of risk for seafood consumers because of the inconsistency among studies, from data on the weight of bivalves consumed per capita to the protocol of sampling and analyzing microplastics in bivalves. For instance, in our study and in the study of Cho et al. (2019) [24], the annual amount of bivalve consumption used for the calculation was the wet weight of bivalve soft tissue, while these data in other studies have included the whole body weight of bivalves (including the shell and soft tissue), which means the estimated levels of microplastics entering consumers in these areas might be higher.

Table 3. Estimation of microplastic intake in consumers' bodies from bivalve consumption in different areas.

Area	Species	Microplastic Concentration in Bivalves (Items g^{-1})	Bivalve Consumption (g $Person^{-1}$ $Year^{-1}$)	Microplastic Concentration in Consumer (Items $Person^{-1}$ $Year^{-1}$)	Ref.
Vietnam	Clams (*Meretrix lyrata* and *Paratapes undulatus*)	3.66	680 [a]	2489	This study
South Korea	Bivalves (oyster *Crassostrea gigas*, mussel *Mytilus edulis*, Manila clam *Tapes philippinarum*, scallop *Patinopecten yessoensis*)	0.15	3475 [a]	521	[24]
China	Mussel	2.4	2765 [a]	6636	[24]
Iran	Mollusc (*A. umbonella*, *A. purpuratus*, *P. radiata*)	2.0	2400 [b]	4800	[19]
Belgium	Mollusc (*M. edulis* and *C. gigas*)	0.42	26,316 [b]	11,053	[23]
France and Ireland	Mollusc (*M. edulis* and *C. gigas*)	0.42	4307 [b]	1809	[23]
Canada	Mussel	7.42	1133 [a]	8407	[24]
Italy	Mussel	8.33	1437 [a]	11,970	[24]
UK	Mussel	0.9	379 [a]	341	[24]

Notes: [a] The wet weight of bivalve soft tissue. [b] The whole-body weight of bivalves, including the shell and soft tissue.

Bivalves were identified as a major source of microplastics to humans because they are among the favorite seafoods in many areas, and people consume the whole body [14]. Although the human body can excrete microplastics via feces [56], they still can impact human health during the time that they stay in the human digestive system, with effects mainly from the toxicity of microplastic additives or chemicals adhered to them from their surrounding environments [57]. For example, polyurethane (PU), PAN, and PVC are listed as carcinogenic, mutagenic, and toxic for reproduction [58]. It is necessary to have solutions to minimize the accumulation of microplastics in bivalve species and seafood to protect the health of consumers. These solutions should include controlling the farming environment (e.g., do not use plastic rope or net in aquaculture) and handling microplastic waste sources in marine environments (e.g., removing plastic and microplastics from domestic and industrial wastewater before being discharged into water bodies). Additionally, methods to reduce microplastic concentration in bivalves before consumption should also be noted, for instance, the depuration of bivalves in freshwater for some period of time is an effective method, as recommended by Baechler et al. (2020) [42]. Similarly, Van Cauwenberghe and Janssen (2014) reported that microplastic concentrations decreased by 30% in mussels and oysters after three days of depuration [23].

5. Conclusions

The appearance of microplastics in bivalves from the Da Nang estuaries further confirms the widespread distribution of microplastics in the biota in Vietnam and worldwide. There was no significant difference in the microplastic concentrations in the clams between the two estuaries, but differences between the two species in each estuary were observed, in which the hard clam (*Meretrix lyrata*) accumulated microplastics much more than the undulate venus clam (*Paratapes undulatus*). The microplastics detected in the two clams were dominated by fibers, with a high percentage of sizes from 300 μm to 1500 μm, which are rather similar to the microplastic characteristics in the estuarine waters. From our estimation, approximately 2489 items person^{-1} year^{-1} of microplastic might enter Vietnamese consumers' bodies via clam consumption, which has raised concerns for food safety and human health. It is suggested that further studies should be conducted to better clarify major factors affecting the microplastic abundance in these two clam species, thus providing appropriate measures for reducing the accumulation of microplastics within them.

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Abbreviations

HRE	Han River Estuary
CDRE	Cu De River Estuary
PE	polyethylene
PP	polypropylene
LDPE	low-density polyethylene
HDPE	high-density polyethylene
PVC	polyvinyl chloride
PET	polyethylene terephthalate
PA	polyamide
PAN	polyacrylonitrile
PVOH	poly-ethylene vinyl alcohol copolymers
PDMS	polydimethylsiloxane
PU	polyurethane

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