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Title

Determination of microplastics in the edible green-lipped mussel *Perna viridis* using an automated mapping technique of Raman microspectroscopy

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Keywords

Bivalves, mariculture, seafood, health risk, Hong Kong

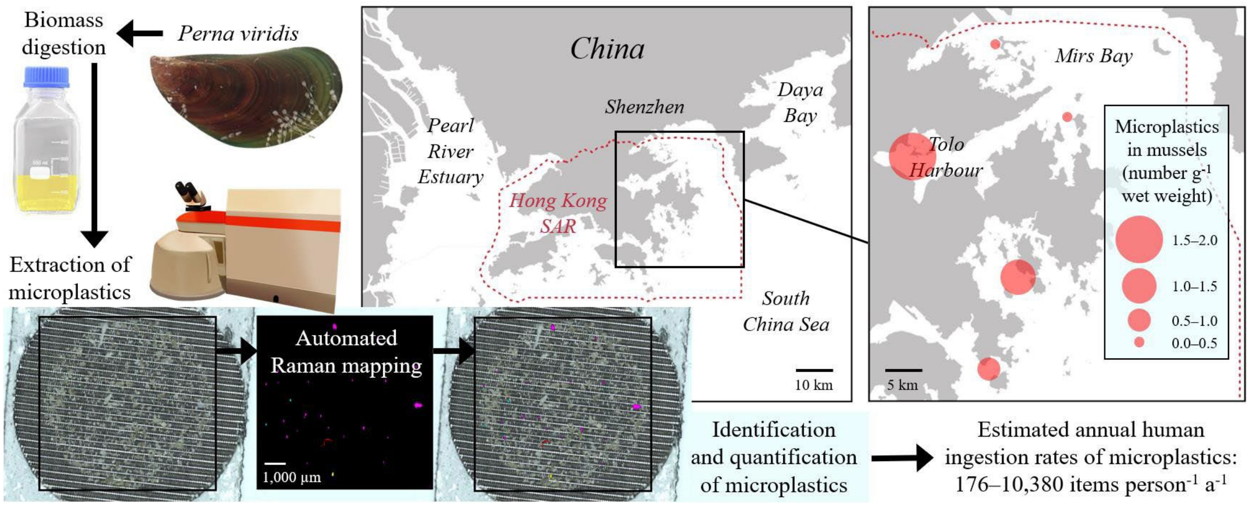
Highlights

- Automated Raman mapping approach to identify microplastics
- Abundance of microplastics in edible mussels
- High rates of estimated human ingestion of microplastics in Hong Kong

Abstract

Microplastics are prevalent in marine environments and seafood and thus can easily end up in human diets. This has raised serious concerns worldwide, particularly in Hong Kong where the seafood consumption per capita can be three times higher than the global average. This study focused on the green-lipped mussel *Perna viridis*, a popular seafood species which is subject to a high risk of contamination by microplastics due to its filter-feeding nature. *P. viridis* was collected from five mariculture sites in Hong Kong and assessed for its body load of microplastics using an automated Raman mapping approach. Microplastics were found in all sites, with an average of 1.60–14.7 particles per mussel per site, or 0.21–1.83 particles per g wet weight. Polypropylene, polyethylene, polystyrene and polyethylene terephthalate were detected among the microplastics, mainly as fragments or fibres in the size range of 40–1,000 µm. It was estimated that through consumption of *P. viridis*, the population in Hong Kong could ingest up to 10,380 pieces of microplastics per person per year. These estimated rates were high compared to the values reported worldwide, suggesting the potential human health risk of microplastics in Hong Kong and adjacent areas.

Graphical Abstract



Introduction

The global production of plastics has exceeded 359 million tonnes since 2018 and is expected to rise to meet the growing demand (PlasticsEurope, 2019). The increasing use of plastics is often accompanied by plastic pollution, particularly in marine environments. Diverse sorts of plastic waste are becoming pervasive, among which microplastics, i.e. plastic pieces < 5 mm, are posing some of the greatest threats to marine ecosystems (Nava et al., 2020; Wang et al., 2020; Zhang et al., 2021). These small-sized plastics are easily ingested and bioaccumulated, and thus could be transferred to higher-trophic animals along the food chain (Andray, 2011; Jambeck et al., 2015). A lot of these animals serve as seafood for humans, which allows microplastics to enter our diets (Nelms et al., 2018). This is worrying, particularly for Hong Kong, where the seafood consumption per capita can be three times higher than the global average (To and Cheung, 2016).

The sources of microplastics in Hong Kong waters include surface runoff and discharges from sewage treatment plants, along with the riverine input from the Pearl River Estuary (Fok and Cheung, 2015; Mak et al., 2020). It has been confirmed that microplastics are widely found in Hong Kong's coastal and marine environments, including the eastern side where most of the mariculture activities take place (Tsang et al., 2017; Li et al., 2020; Lo et al., 2018, 2020; Wu et al., 2020; Xu et al. 2020a, 2020b). Not surprisingly, microplastics have been found in a number of edible fish species collected from the same areas (Cheung et al. 2018; Chan et al. 2019). Other seafood species are also subject to contamination with microplastics. Among the different types of seafood, bivalve shellfish such as mussels represents a high-risk group of microplastic contamination due to its filter-feeding nature of capturing suspended particles from the water column. For instance, the green-lipped mussel *Perna viridis*, the test species in this study, can filter more than 10 L of seawater and the particles therein per hour per unit g dry weight, a filtration rate which is equivalent to more than 370 L per day per mussel (Tantanasarit et al. 2013). Through consumption of bivalve shellfish, the human ingestion rates of microplastics were estimated to be alarmingly high in some parts of Europe (Van Cauwenberghe and Janssen, 2014; Renzi et al., 2018). The situation may not be any better in Hong Kong, considering the heavy seafood consumption of its population.

P. viridis is a popular seafood species in the Indo-Pacific region and is widely distributed in Hong Kong waters. This study aimed to quantify and characterise the microplastics in *P. viridis* collected from local mariculture areas, and to estimate the human ingestion rates of microplastics through consumption of *P. viridis*. These rates determined from the Hong Kong population are applicable to other areas in southern China given the similar eating habits and seafood availability in the region. The adoption of an automated mapping technique of Raman microspectroscopy was another highlight of this study. This approach allows the scanning and mapping of microplastics on the whole filter membranes (see Fig. 3), offering clear advantages over conventional processes that analyse particles one by one, which are time-consuming and prone to handling errors.

Materials and Methods

Collection of mussel samples

Mussel sampling was carried out in the mariculture areas of Hong Kong, namely five sites in the northern (N), northeastern (NE), eastern (E) and southeastern waters (SE) and in Tolo Harbour (TH; Fig. 1). The site NE is relatively remote from human activities and would serve as a reference site in this study. The other sites are closer to human settlements or waters contaminated with microplastics (Lo et al., 2018; Mak et al., 2020). In particular, the site TH is located in the vicinity of urbanised areas and is in a land-locked embayment with weak currents, and therefore hypothesised to be the most contaminated site with microplastics (Sin et al., 2003; Lee et al., 2006; Lei et al. 2018). Samples of *P. viridis* with shell lengths of about 80–84 mm (Table 1) were handpicked with cotton gloves and stainless-steel scissors at 0.1–2.0 m water depth from the five sites in August–September 2019 (n = 10 per site). Collected samples were transported in cooler bags and stored at –20 ºC in the laboratory until the extraction process of microplastics began.

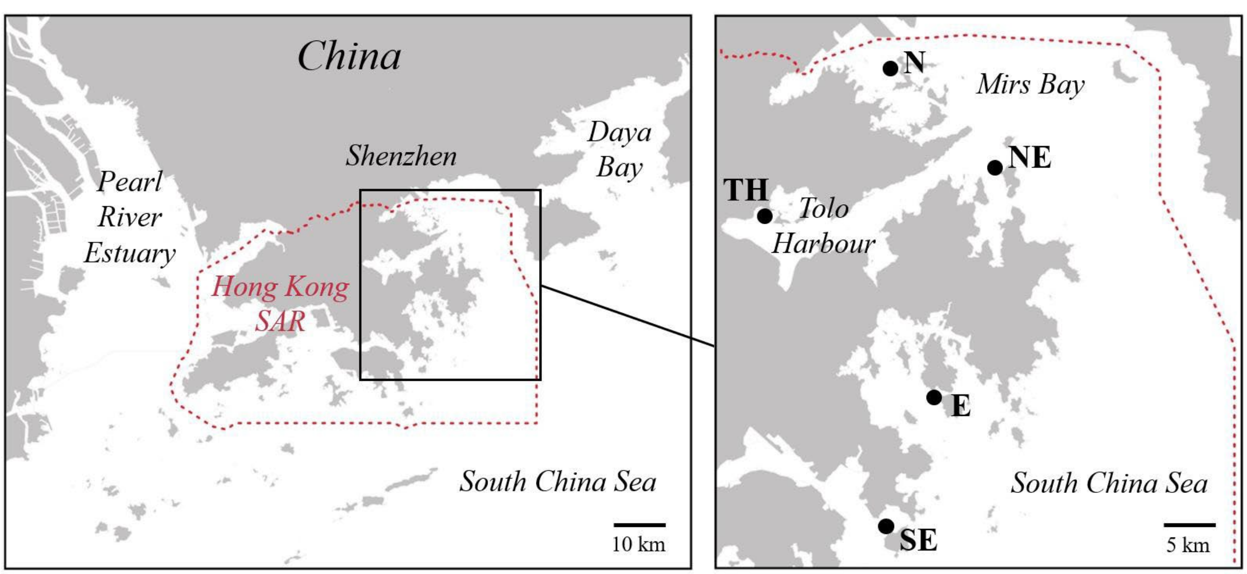


Fig. 1. Sampling sites of the green-lipped mussel *Perna viridis* in the northern (N), northeastern (NE), eastern (E) and southeastern waters (SE) and in Tolo Harbour (TH), Hong Kong Special Administrative Region (SAR), China. All sites are located within mariculture areas, while the site NE is the least affected by human activities.

Table 1. Biological parameters of *Perna viridis* collected from the five sites in Hong Kong and the number of microplastics in these mussels (mean ± standard deviation; n = 10). Refer to Fig. 1 for the site abbreviations and locations. The mussel condition index was estimated as 100 × the ratio of tissue wet weight (g) to shell length (mm). The number of microplastics was standardised per g wet weight or per individual (ind).

Sites	Sampling date in 2019	Shell length (mm)	Tissue wet weight (g)	Condition index	Microplastics (items g^{-1})	Microplastics (items ind^{-1})
N	27th August	84.4 ± 3.77	9.16 ± 2.23	10.8 ± 2.20	0.23 ± 0.26	2.50 ± 3.60
NE	27th August	84.8 ± 2.99	7.93 ± 1.38	9.34 ± 1.53	0.21 ± 0.16	1.60 ± 1.35
TH	27th August	83.9 ± 2.57	8.33 ± 1.33	9.91 ± 1.39	1.83 ± 2.52	14.7 ± 19.3
E	13th September	80.4 ± 3.94	8.06 ± 1.70	9.99 ± 1.80	0.94 ± 0.49	7.60 ± 4.45
SE	28th August	82.7 ± 3.52	10.7 ± 1.80	12.8 ± 1.80	0.54 ± 0.74	5.40 ± 7.17

Extraction of microplastics from mussels

Collected samples of *P. viridis* were thawed at room temperature. Shell length was measured with a digital calliper. Mussel soft tissue was collected with stainless-steel tools, blot-dried on paper towels for wet weight (WW) measurement, and then thoroughly rinsed with Milli-Q water (Merck, Darmstadt, Germany). Microplastics were extracted from the soft tissue of *P. viridis* using the digestion method of Teng et al. (2019), with modification of adding ethylenediaminetetraacetic acid (EDTA), which is commonly used for decalcification in histology and was adopted here to facilitate digestion of the mantle tissue containing calcium carbonate (Bancroft and Gamble, 2008). In brief, each rinsed tissue sample was digested in a 180 mL solution containing 10% potassium hydroxide (KOH) and 14% EDTA at 40 °C for 48 h, during which 10 mL of 30% hydrogen peroxide (H_2O_2) was added twice at 24 h and 42 h to boost the digestion performance. The digestion efficiency of this approach on *P. viridis* biomass was determined to be higher than 99%. Microplastics of of 250–5,000 µm in the solution after digestion were retained on a stainless-steel filter membrane with a pore size of 250 µm using vacuum filtration. The filtrate was furthermore filtered through 30 µm to retrieve microplastics of 30–250 µm.

Several quality assurance measures were implemented during the process of microplastic extraction to minimise contamination. First, all glassware, filtration kits and tools were thoroughly rinsed thrice with Milli-Q water prefiltered by a Merck Millipak 40 Gamma Gold Filter (pore size: 0.22 µm), before each sample analysis. Second, the solutions of 10% KOH, 14% EDTA and 30% H_2O_2 were made with Milli-Q water and filtered through Advantec GC-50 filter membranes (pore size: 0.50 µm; Tokyo, Japan) prior to the tissue digestion treatment. Third, cotton lab coats and nitrile gloves were worn at all times during sample processing. Fourth, the same digestion process was repeated without the mussel samples to serve as the procedural blank (n = 3). Microplastics in the mussel samples and procedural blank, if any, were characterised using Raman microspectroscopy.

Characterisation of microplastics

Microplastics (250–5,000 μm) that were retained on the 250 μm filter membrane were identified using the point acquisition mode of a Renishaw inVia confocal Raman microscope (Wotton-under Edge, UK) equipped with a Leica 10× objective (Wetzlar, Germany) and a 785 nm edge laser (300 mW output power). Raman spectra were acquired for 10 s using 0.1–1% laser power in the wavenumber range of 676–1767 cm^{-1}. Baseline correction, smoothing and cosmic ray removal of the acquired spectra were performed with the Renishaw WiRE 5.2 software. The polymer types of microplastics were identified from these Raman spectra using the Renishaw Polymeric Materials Database. Similarity of each sample spectrum to the reference spectrum was indicated by the matching index provided in WiRE 5.2, which ranged from 0 to 1. A higher value of the index indicated greater similarity, and when > 0.7, the identity of microplastics was accepted. For values of 0.4–0.7, the sample spectra were visually re-examined and were considered microplastics if they contained all characteristic peaks of the reference plastic polymers.

The smaller microplastics (30–250 μm) that were retained on the 30 μm filter membrane was assessed in an automated mapping mode of the Renishaw inVia system using a 785 nm streamline laser. The whole circle that was coated with microplastics (8 mm in diameter) on each filter membrane was scanned at 10% laser power and a spatial resolution of 28.4 μm. Raman spectra were acquired at 5 s per pixel. The mapping process generated more than 10,000 Raman spectra per sample, among which microplastics, if any, were identified using the Renishaw Polymeric Materials Database. Identified microplastics were colour-coded and illustrated in a two-dimensional panel. Other settings and criteria remained the same as in the point acquisition mode.

The shapes and sizes of the identified microplastics were visually verified under a stereomicroscope. Microplastics were categorised into five forms of shape including fragment, fibre, film, rod, and pellet. The size of microplastics in all shapes was expressed as the longest dimension across the area, except for fibre of which the size was measured in length along the central axis. The size measurements were performed on stereomicrographs of the microplastics using the software ImageJ (National Institutes of Health, MD).

Data analysis

The numbers of microplastics in *P. viridis* per unit WW and per individual were compared among the five sites. The data did not fulfil the assumptions of normality or homogeneity of variance, even after data transformation, and were analysed with the Kruskal-Wallis H test (Alam et al., 2019; Lin et al., 2020). If the site effect was significant, Dunn's pairwise comparisons were used to elucidate the spatial pattern of microplastic contamination in the mussels. The significance level was set at 0.05. The statistical procedures were performed with the statistical software SPSS, version 23 (Chicago, IL).

The human ingestion rates of microplastic through mussel consumption were estimated for the Hong Kong population. The calculation was based on the numbers of microplastics determined in *P. viridis* in the present study (on average 0.21–1.83 items g^{-1} WW; Table 1), and the consumption rates of bivalve shellfish determined from a local survey in which a total of 5,008 adults participated (see FEHD, 2010). The obtained data in the survey had been age- and genderweighted to represent a population of 5,394,000 aged 20–84. The mean daily consumption rate was derived to be 2.29 g person^{-1} d^{-1} for this population, among which 15% had reported to have consumed bivalve shellfish during the survey period, on average at 15.5 g person^{-1} d^{-1} (FEHD, 2010). The two values, 2.29 and 15.5 g person^{-1} d^{-1}, were equivalent to the annual rates of 836 and 5,672 g person^{-1} a^{-1} for the whole population and regular consumers, respectively. These annual rates were used to estimate the lowest end (0.21 × 836 items person^{-1} d^{-1}) and highest end (1.83 × 5,672 items person^{-1} d^{-1}) of the human ingestion rates of microplastics through mussel consumption, and were expressed as items person^{-1} a^{-1}. The rates of microplastic ingestion were also estimated as number per meal, assuming that a portion of 100–250 g WW of mussels was consumed in a meal (Van Cauwenberghe and Janssen, 2014; Renzi et al. 2018; Dowarah et al., 2020; Gündoğdu et al. 2020).

Results

Spatial comparison of microplastics in mussels

The procedural blank of our method was found to contain 1.33 ± 0.58 items of microplastics (mean ± standard deviation; n = 3). This level of contamination was regarded insignificant and the data obtained from mussels were not corrected. A total of 321 pieces of microplastics were identified in 47 out of 50 mussels collected from the five sites. In these 47 mussels, the abundance of microplastics ranged from 0.08 to 8.60 items g^{-1}, or 1.00 to 66.0 items per individual. The lowest and highest numbers of microplastics in the mussels were detected at the reference site NE and the inner-harbour site TH, respectively. Kruskal-Wallis H test and Dunn's multiple comparisons were used to compare the spatial levels of microplastics in the mussels, which were significantly higher at the sites TH and E than the sites N and NE (Fig. 2).

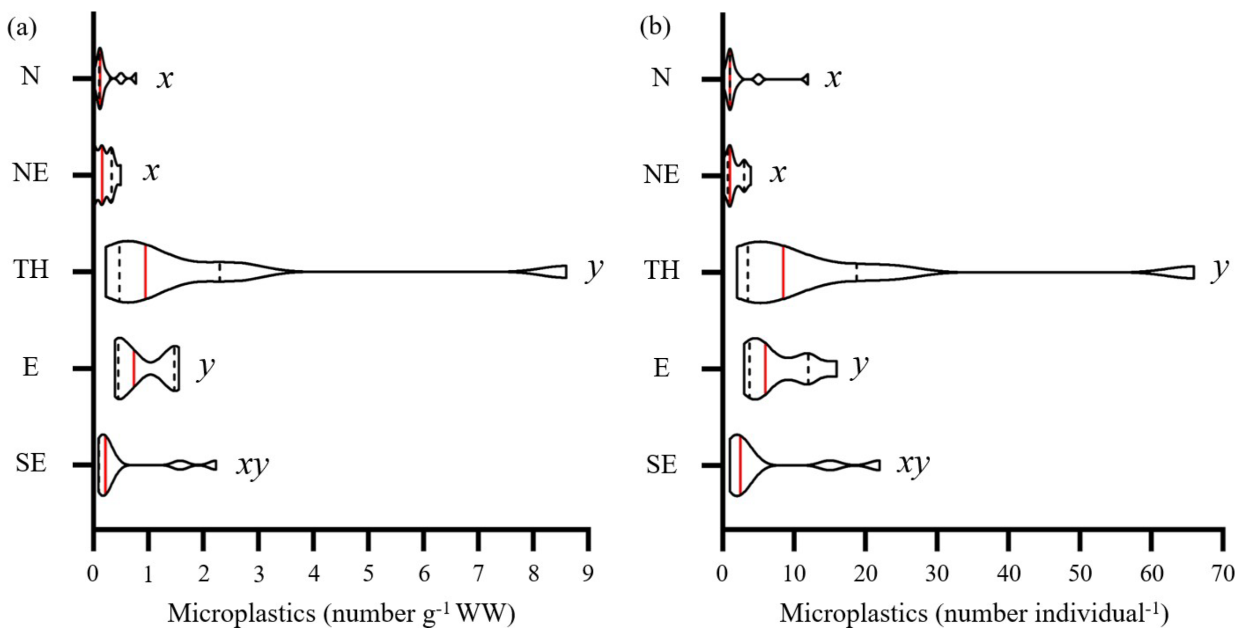


Fig. 2. Violin plots of the number of microplastics, (a) per g wet weight (WW) and (b) per individual, in *Perna viridis* sampled from the five sites reported in Fig. 1 (n = 10). A thicker part of the violin implies a higher frequency of that section of data. The median value is indicated by the red line, while the lower quartile and upper quartile are represented by the dashed black lines. The values of mean and standard deviation among the five sites are reported in Table 1. The

values at sites indicated with different italic letters (*x*, *y*) were significantly different from each other, as revealed in the Kruskal-Wallis H test for the data (a) per unit WW ($\chi^2(4) = 22.3$, $p < 0.05$) and (b) per individual ($\chi^2(4) = 21.8$, $p < 0.05$), followed by Dunn's multiple comparisons ($p < 0.05$).

Characterisation of microplastics

Microplastics extracted from the mussel samples were assessed using an automated Raman mapping approach (Fig. 3). The particle size range of the identified microplastics was 41.7–4,679 µm, but the majority, i.e. 292 pieces out of 321, was smaller than 1,000 µm, while the peak abundance occurred at 90–110 µm (Fig. 4). Polypropylene (PP; 56%) was identified to be the most common type of microplastics in total, followed by polyethylene (PE; 25%), polyethylene terephthalate (PET; 10%), and polystyrene (PS; 9.0%). The microplastics mostly existed as fragments (89%) and fibres (9.7%), along with small amounts of films (1.0%) and rods (0.3%). These proportions varied spatially among the five sites (Fig. 5a, 5b). When the shapes were sorted according to the polymer types, fragments accounted for 93–97% of the numbers of PP, PE and PS microplastics (Fig. 6). However, 77% of the PET microplastics were in the form of fibres (Fig. 5c).

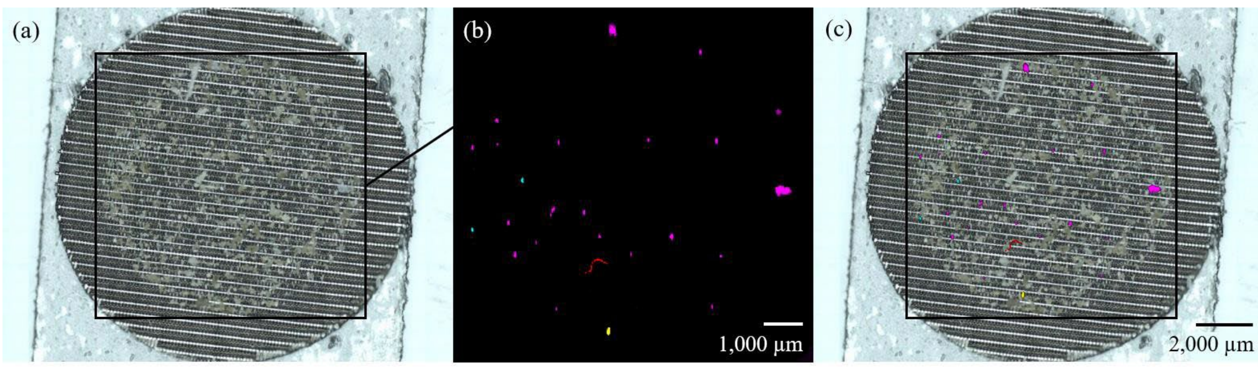


Fig. 3. (a) Microplastics extracted from *Perna viridis* on a stainless-steel filter membrane (pore size: 30 µm), (b) their colour-coded identification using an automated Raman mapping technique, and (c) the superimposed image of (a) and (b). All particles including microplastics within the black square were scanned and mapped at a spatial resolution of 28.4 µm, from which polypropylene (magenta), polyethylene (cyan), polystyrene (yellow) and polyethylene terephthalate (red) were identified in this sample.

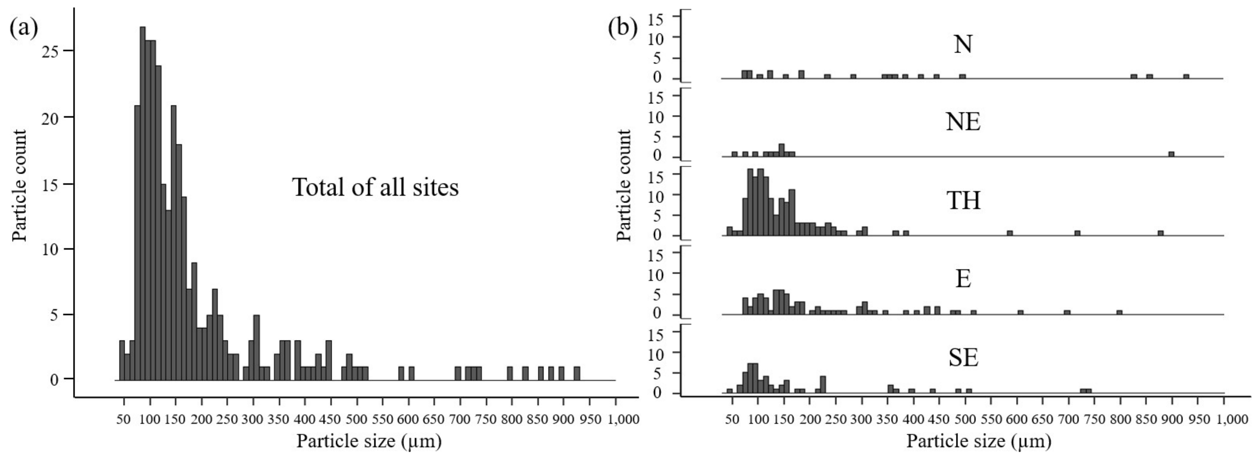


Fig. 4. Particle size distribution of microplastics extracted from *Perna viridis* (a) across all sampling sites (n = 50) and (b) at each of the five sites (n = 10). The site abbreviations and locations are provided in Fig. 1. Microplastics in the size range of 40–1,000 µm accounted for 94% of all microplastics.

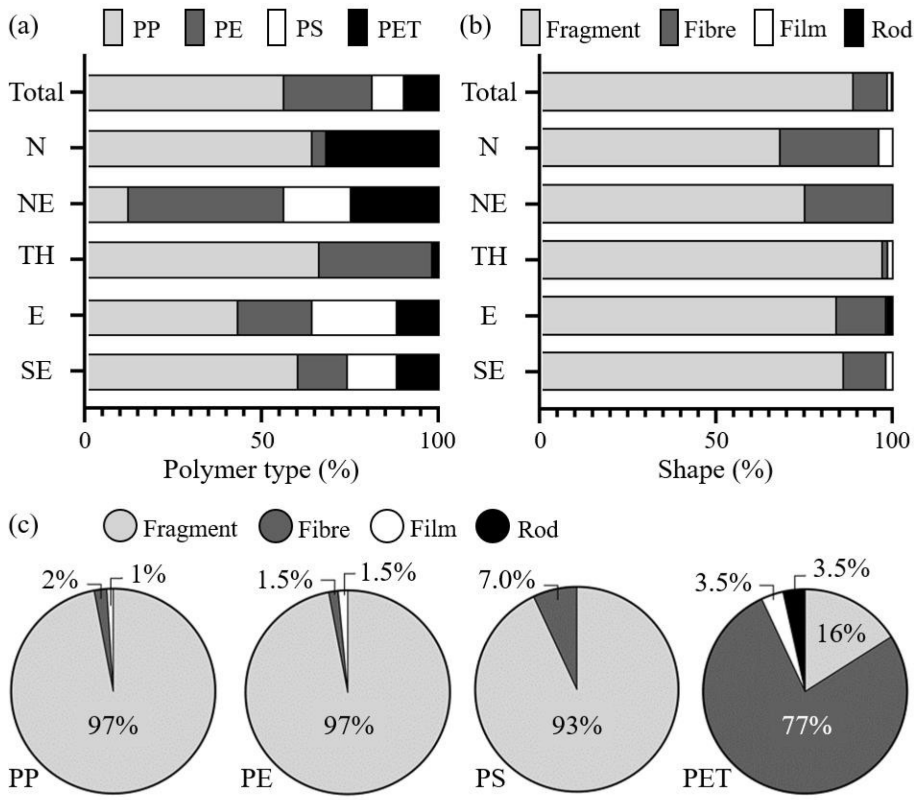


Fig. 5. The proportions of (a) polymer types, including polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyethylene terephthalate (PET), and (b) shapes of microplastics, including fragment, fibre, film and rod, determined in *Perna viridis* collected from the five sites, and (c) the relative amounts of the four shapes in each polymer type. The pellet shape was not found among the microplastics. Refer to Fig. 1 for the abbreviations and locations of the mussel sampling sites.

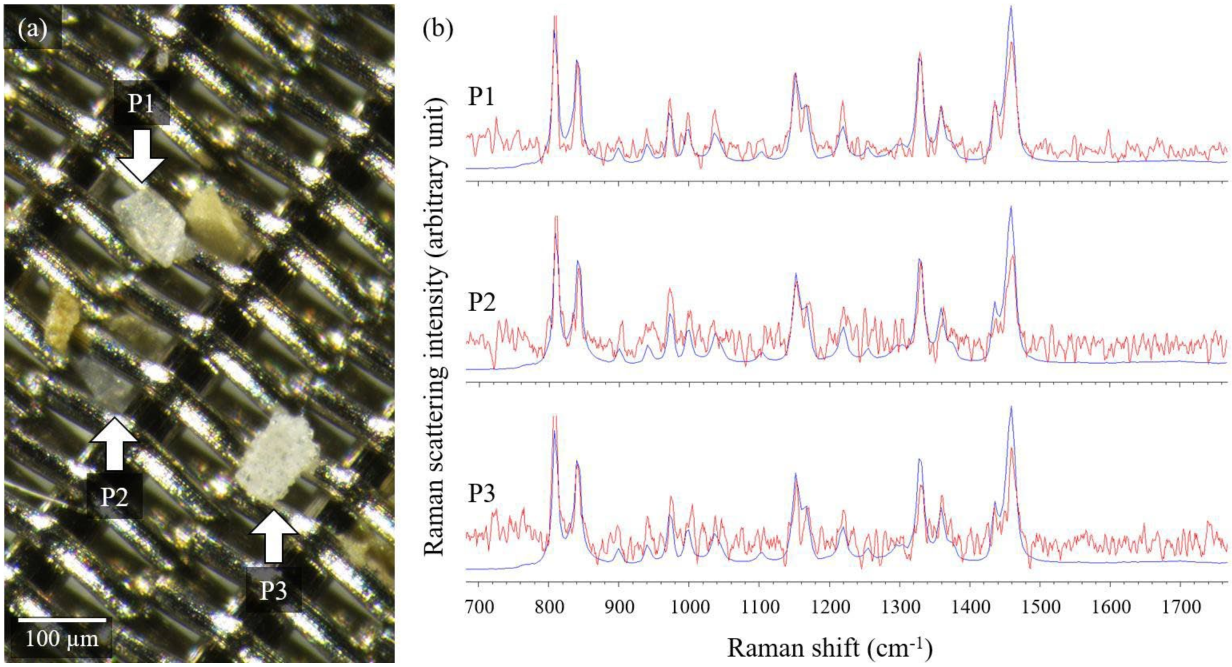


Fig. 6. (a) Three microplastic fragments extracted from *Perna viridis*, namely P1, P2 and P3, retained on a stainless-steel filter membrane with a plain Dutch weave pattern. (b) The three fragments are identified to be polypropylene by comparing their Raman spectra (red) to the reference spectrum of polypropylene (blue). The two brownish objects are undigested biological materials.

Human ingestion of microplastics through mussel consumption

The lowest and highest mean values of microplastics in *P. viridis* were found to be 0.21 and 1.83 items g^{-1} WW at the sites NE and TH, respectively (Table 1). Given the mean consumption rates of bivalve shellfish as 836–5,672 g $person^{-1}$ a^{-1} (FEHD, 2010), the annual human ingestion rates of microplastics through mussel consumption were estimated to be 176–10,380 items $person^{-1}$ a^{-1} in Hong Kong. Moreover, assuming a portion of 100–250 g WW of mussels was consumed in a meal, the human ingestion rates of microplastics were calculated to be 21–458 items $meal^{-1}$ (Table 2).

Table 2. Estimated annual human ingestion rates of microplastics through consumption of bivalve shellfish worldwide. The edible tissue of bivalves was expressed as g wet weight (WW). Different size ranges of microplastics were sampled among studies. Data obtained from the present study are bold.

Species	Sampling area	Mean human consumption rates of bivalves	Mean human ingestion rates of microplastics	Target or detected size range of microplastics	Reference
(a) Rates per year		(g $person^{-1}$ a^{-1})	(items $person^{-1}$ a^{-1})	(µm)	
Magallana gigas	Local market, South Korea	307	21.5	> 43	Cho et al. (2019)
Magallana gigas	Bizerte Lagoon, Tunisia	27.2–3,060	40.3–4,537	> 50	Abidli et al. (2019)
Magallana gigas	Local markets,	10,350–14,000	910–1,231	> 20	Chen et al.

and *Meretrix lusoria* (and a squid)	Taiwan				(2020)
Magallana gigas and *Mytilus edulis*	E coast, France and NW coast, Germany	4,307–26,317	1,800–11,000	> 5.0	Van Cauwenberghe and Janssen (2014)
Mytilus edulis	Local market, South Korea	245	29.4	> 43	Cho et al. (2019)
Mytilus galloprovincialis	W, NW, N and NE coast, Turkey	8,322	1,918	> 70	Gedik and Eryaşar (2020)
Mytilus galloprovincialis	Bizerte Lagoon, Tunisia	31.2–3,510	24.5–2,757	> 50	Abidli et al. (2019)
Mytilus galloprovincialis	Bizerte Lagoon, Tunisia	2.01	4.20	Not reported	Wakkaf et al. (2020)
Mytilus spp. and *Modiolus modiolus*	NW, N and E coast, Scotland	82.0–3,080	123–4,620	> 200	Catarino et al. (2018)
Patinopecten yessoensis	Local market, South Korea	91.3	7.30	> 43	Cho et al. (2019)
Perna viridis	**E coast, Hong Kong**	**836–5,672**	**176–10,380**	**> 30**	**Present study**
Ruditapes decussatus	Bizerte Lagoon, Tunisia	30.4–3,420	43.7–4,920	> 50	Abidli et al. (2019)
Tapes philippinarum	Local market, South Korea	456	155	> 43	Cho et al. (2019)
(b) Rates per meal		(g meal^{-1})	(items meal^{-1})		
Mytilus galloprovincialis	Local markets, Italy	225	1,395–1,620	> 750	Renzi et al. (2018)
Mytilus galloprovincialis (as stuffed mussels)	Local markets, Turkey	100–250	5.80–14.4	Not reported	Gündoğdu et al. (2020)
Perna viridis	**E coast, Hong Kong**	**100–250**	**21–458**	**> 30**	**Present study**
Perna viridis and *Meretrix meretrix*	SE coast, India	121	152	Not reported	Dowarah et al. (2020)

Discussion

This study investigated the contamination levels of microplastics in *P. viridis* collected from the mariculture areas of Hong Kong. Microplastics extracted from the mussels were counted and characterised using an automated mapping technique of Raman microspectroscopy. Our work revealed higher numbers of microplastics in *P. viridis* at sites closer to human settlement, and that the local human population could ingest up to 10,380 items of microplastics per person per year. These findings highlight the severity of microplastic contamination in seafood and its potential ecological and human health impacts in Hong Kong and adjacent areas.

Spatial comparison of microplastics in mussels

The mean numbers of microplastics in Hong Kong waters were determined to be 51–27,909 items per 100 m^3 (Tsang et al. 2017, 2020; Cheung et al. 2018b). Along the coastline of Hong

Kong, microplastics were also found in a diversity of fauna including 42 species of bivalves, barnacles, gastropods and crabs, at up to 9.68 items per g WW or 18.4 items per individual on average (Xu et al. 2020a, 2020b). Likewise, microplastics were present in *P. viridis* investigated in this study. The mussels collected from the sites TH and E contained significantly higher numbers of microplastics among the five sites in Hong Kong, both per unit WW and per individual (Fig. 1, 2; Table 1). The greater proximity of the sites TH and E to human settlement and activities that can be associated with plastic pollution possibly led to the more abundant microplastics determined in *P. viridis* (Hantoro et al., 2019). Bioaccumulation of xenobiotics in mussels including *P. viridis* has been widely used for pollution monitoring purposes (e.g. Leung et al., 2011; Pinto et al., 2015; Yeung et al., 2017). As for microplastics, recent studies identified a close alignment between the number of microplastics found in mussels and that in the surrounding water, suggesting the applicability of using mussels in the environmental assessment of microplastics (Qu et al., 2018; Li et al., 2019; Wakkaf et al., 2020). In this connection, our findings from *P. viridis* indicated the abundance of microplastics in the mariculture areas of Hong Kong. Other local mariculture species are also subject to microplastic contamination. For instance, microplastics were detected in the flathead grey mullet *Mugil cephalus* collected near the site TH (Cheung et al. 2018a). In their study, PP and PE were the two most common polymer types identified in the fish, a result that was in line with our findings from *P. viridis* (Fig. 5a).

Characterisation of microplastics

The present study applied a Raman mapping technique to count and characterise microplastics, a method that showed clear advantages over the conventional visual sorting step which is prone to observer bias and often associated with high variance in the estimation of particle numbers. Meanwhile, given the increasing difficulty to handpick smaller-sized particles, the common analytical procedure that identified microplastics one by one could be labour intensive and subject to handling errors (e.g. Kershaw and Rochman, 2015; Lavers et al., 2016). These concerns were herein resolved by an automated mapping approach, in which Raman spectra were acquired from the whole area coated with particles and, from these spectra (> 10,000 spectra on an 8 mm circle), microplastics were identified and colour-coded (Fig. 3). This approach allowed the size distribution pattern of microplastics to be elucidated at a higher resolution (Fig. 4). The size range of microplastics determined in *P. viridis* was aligned with those from other mussel species, the majority of which were smaller than 200 µm (Digka et al., 2018; Li et al., 2018b; Hermabessiere et al.,2019; Ding et al., 2020; Gedik and Eryaşar, 2020). The uptake and bioaccumulation of microplastics in mussels may be size-dependent. For instance, the Mediterranean mussel *Mytilus galloprovincialis* showed the longest gut retention time for PS particles of 90 µm, but particles of 1–10 µm were rapidly excreted (Kinjo et al., 2019). Another study reported a higher uptake rate on phytoplankton of < 100 µm, compared with the 200 µm by the New Zealand green-lipped mussel *P. canaliculus* (Webb et al., 2019). Likewise, a peak abundance of microplastics at 90–110 µm was determined in *P. viridis* in the present study (Fig. 4).

Four polymer types, PP (56%), PE (25%), PS (9.0%) and PET (10%), were identified among the microplastics in the forms of fragment (89%), fibre (9.7%), film (1.0%) and rod (0.3%; Fig. 5a, b). PP, PE and PS are commonly used in food packages, single-use cutlery and carrier bags among others, which are of disposable nature, often end up in waterways and seas, and can be fragmented into microplastics through mechanical and photochemical degradation processes

(Andrady, 2011). These PP, PE and PS microplastics are in general positively or neutrally buoyant in nature, suspended in the water column where they can easily encounter with intertidal animals including *P. viridis*. The fragment form was the dominated one among the PP, PE and PS particles (93–97%; Fig. 5c). However, only 16% of the PET microplastics occurred as fragments while the majority was confirmed to be fibres (77%; Fig. 5c). Apart from disposable bottle waste, a more significant source of PET microplastics may be the domestic laundry process in which a single garment could shed up to 1,900 fibres per wash (Browne et al., 2011). A lot of these fibres are made of PET that can be released into marine environments via municipal wastewater discharge (Browne et al., 2011; Napper and Thompson, 2016; Jönsson et al., 2018). PET is generally negatively buoyant, but the sinking rates can be influenced by the particle sizes and shapes, among which fibre-shaped microplastics appeared to sink more slowly than other shapes with the same density (Kowalski et al., 2016). The likely longer retention time of PET fibres in surface water provided an explanation for their presence in *P. viridis* sampled in < 2.0 m depth.

Human ingestion of microplastics through mussel consumption

Seafood consumption is known as a significant pathway for microplastics to enter human diets, particularly for the coastal populations that rely on seafood as a primary source of protein. In the case of Hong Kong, our findings indicated that an adult could ingest 21–458 items of microplastics per meal, or 176–10,380 items per year, through consumption of bivalve shellfish as estimated from *P. viridis*. These numbers were considered high among the values reported elsewhere (Table 2). However, compared with mussel consumption, it has been suggested that the household dust fallout during a meal could lead to an even higher rate of microplastic ingestion (Catarino et al. 2018). Nevertheless, to us the two estimates are not mutually exclusive but additive, as the former determines the amount of microplastics in food ingredients while the latter concerns about the contamination of microplastics in food preparation or in the dining environment.

Another point to be noted is that the predicted values summarised in Table 2 represented only rough estimates that relied on assumptions in the calculation, e.g. using an assumed annual consumption rate of bivalves, and that the target size ranges of microplastics varied among studies. Nevertheless, these estimates are important in the primary screening to compare microplastic contamination in human diets worldwide and, from these estimates, Hong Kong can be identified as a hotspot of microplastics. There is growing evidence that ingestion of microplastics can cause particle toxicity by inducing immune responses, or chemical toxicity due to leaching of plastic additives or other chemicals (reviewed by Wright and Kelly, 2017). The chronic human health effects, which are of greater concern in the dietary exposure to microplastics, however remain largely unclear. Overall, our results from *P. viridis* suggest an abundance of microplastics in the mariculture environment. These findings warrant further investigation on microplastic contamination in other seafood species and, on a longer term, the risk that microplastics pose to human health through seafood consumption.

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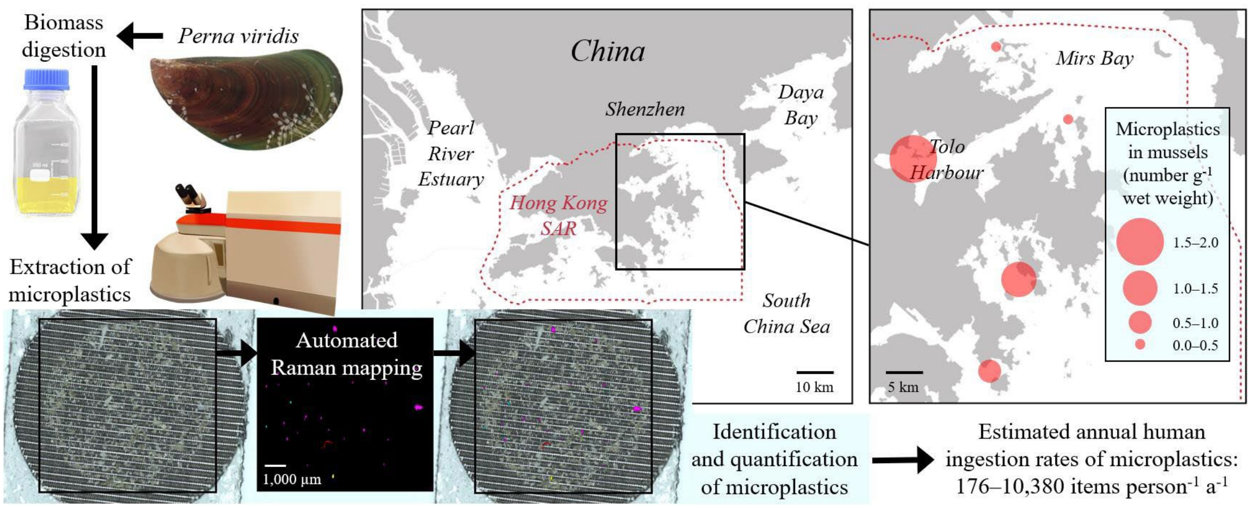
Graphical abstract



Highlights

- Automated Raman mapping approach to identify microplastics
- Abundance of microplastics in edible mussels
- High rates of estimated human ingestion of microplastics in Hong Kong