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SPECIAL ISSUE-LETTER

Feeding behavior is the main driver for microparticle intake in mangrove crabs

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Scientific Significance Statement

Microplastics, which are plastic pieces smaller than 5 mm, are commonly found in the marine environment, where they are available for ingestion by marine fauna. Because of both their root structure and their geographic distribution, mangrove forests are known as sinks of microplastics, but very little is known about the factors controlling the microplastics ingestion by mangrove associated crabs, which are a dominant and ecologically relevant ecosystem component. We observed that both the level of plastic contamination in the mangrove and the feeding habit of the crab's species play critical roles in the abundance and type of microplastic ingested by mangrove crabs.

Abstract

As marine plastic debris is primarily sourced from terrestrial input, coastal environments are particularly affected by deposition. Because of their pneumatophores, mangroves have been recognized for their importance in confining plastic waste. Crabs are a dominant component of the mangrove ecosystem and play a critical role in maintaining healthy and resilient mangrove forests. Therefore, the presence of debris fragmented from waste, in their habitat is a potential threat. However, the potential ingestion of microplastic pieces by mangrove crabs has not yet been investigated. Here, we quantified microparticles found in the cardiac stomachs and gill chambers of four species of crabs. All specimens collected had anthropogenic microparticles present either via their digestive or respiratory systems. We observed significant variability in the abundance and types of anthropogenic microparticles across sites and species. Interspecific differences appear to be explained by their particular feeding habits, with less selective species ingesting more particles.

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Data Availability Statement: Data will be made available in the dryad data repository at the final submission of the manuscript.

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Marine debris, and in particular plastic debris, has been observed throughout large ranges of marine environments (Moore et al. 2001; Widmer and Hennemann 2010; Galgani et al. 2015). Rivers have been identified as the main sources of plastic litter, which results in considerable pollution in coastal regions. Hong Kong coastlines are no exception, as Hong Kong is located at the mouth of the Pearl River, the third largest riverine source of plastic into the ocean (Lebreton et al. 2017). Large plastic debris can be easily observed stranded on many coastal habitats of Hong Kong, but investigation of their abundance and composition is mostly limited to sandy beaches, with other coastal habitats remaining understudied (Cheung et al. 2018*b*; Ho and Not 2019). Macroplastics are fragilized by several environmental variables, including UV radiation, humidity, and wave action. As such, this debris is susceptible to fragmentation, eventually leading to the formation of microplastics (i.e., plastic pieces smaller than 5 mm; Cole et al. 2011). While microplastics have been found in all marine environments, coastal environments are particularly affected due to their typical proximities to river deltas and urbanized regions (Peters and Bratton 2016; Lebreton et al. 2017; Chan et al. 2019). In Hong Kong, microplastics have been identified in coastal surface waters (Cheung et al. 2018*a*; So et al. 2018), sandy beaches (Fok and Cheung 2015; Fok et al. 2017), marine sediments (Tsang et al. 2017), and also within marine organisms (Chan et al. 2019).

Because of their coastal habitats, mangroves are highly susceptible to debris exposure. Furthermore, the spatial complexity characteristic of mangrove ecosystems provides many opportunities for debris to become entangled, which enhances their role as sinks for plastic pollution (Garcés-Ordóñez et al. 2019; Martin et al. 2019). Macroplastics trapped within coastal forests are easily fragmented into microplastics, and recent studies have reported a high abundance of microplastic accumulation within mangrove roots systems (Garcés-Ordóñez et al. 2019; Li et al. 2019; Martin et al. 2019). With the exception of the abovementioned studies, there are still very few estimates of macro- and microplastic abundances within mangroves. These forests host diverse and highly specialized invertebrate fauna (Duke et al. 2007; Cannicci et al. 2008), which play critical roles in maintaining ecosystem functions and could be strongly impacted by plastic pollution.

Hong Kong mangroves are characterized by a high density and diversity of crabs and molluscs (Lee 2000). These two taxa dominate the mangrove food web (Kristensen et al. 2017), and their activity has a significant engineering effect on the sediment and whole-forest functionality (Kristensen 2008). Although recent experimental evidence has emphasized the important role played by these bioengineers in maintaining healthy and functional mangrove forests, very few studies have investigated the impact of anthropogenic stressors (Cannicci et al. 2009), such as plastic pollution, on their physiology. However, based on experimental tests, crabs have been shown to uptake microplastic via dietary input and ventilation, leading to a reduction in their food consumption and, consequently, of their energy budget (Watts et al. 2015, 2016). In addition, transfer of microplastics from stomachs to hepatopancreas has been identified in the tropical fiddler crab, *Uca rapax* (Brennecke et al. 2015). Observations of microplastic ingestion by crabs in natural environments are, however, still limited (Wójcik-Fudalewska et al. 2016; Welden et al. 2018).

Over the last decades, mangroves have been under multiple threats and are disappearing worldwide at the alarming rate of 1–2% per year (Duke et al. 2007; Richards and Friess 2015; Friess et al. 2019), especially along the coast of the South China Sea. Hong Kong mangroves, however, have proved to be truly resilient, since about 60 mangrove stands, covering an area of about 500 ha, are still thriving along the coast (Tam et al. 1997). These remaining pockets of forest still provide paramount ecosystem services and functions (Duke et al. 2007). They support pelagic and benthic marine food webs, evidenced through their significant contributions of organic carbon (Meynecke et al. 2007), but also act as carbon sinks and thus mitigate climate change (Donato et al. 2011). Mangrove forests further reduce coastal erosion and act as filters for pollutants, which are trapped within their fine sediment and are not dispersed into open waters (MacFarlane et al. 2007). Due to these important ecological roles and the increasing threat of plastic litter, it is crucial to characterize the types and ecological implications of plastic litter within mangrove habitats. Here, we use a field approach to look at the ingestion of anthropogenic particles by four mangrove crab species occupying different levels of the food web. The transfer of anthropogenic debris as microplastic, from the environment into crab organs, has been primarily observed via feeding and gill ventilation. Therefore, we investigated the presence of anthropogenic debris in the cardiac stomach and gills of mangrove crabs, to better understand and characterize the main intake processes. Potential transfer from stomach to hepatopancreas has been suggested in experimental studies (Brennecke et al. 2015) but it is beyond the scope of this study, which focuses on intake processes rather than transfer among internal organs. Here, we concentrate on possible differences in intake among crab populations subject to different levels of plastic pollution and among crab species characterized by different feeding habits to test whether microdebris availability and feeding behavior affect the microplastic intake in these keystone species in mangrove habitats.

Materials and methods

Crabs were collected between October 21st, 2017 and November 3rd, 2017 from three mangrove forests in Hong Kong: (1) Ha Pak Nai (HPN, 14 individuals sampled), located in the north west of Hong Kong, (2) Pak Tam Chung (PTC, 19 individuals sampled), and (3) Yung Shue O (YSO, 16 individuals sampled), both located on the east coast (Fig. 1; Table 1).

The three sites are small patches (< 1 ha) of mangrove forests dominated by *Kandelia obovata* trees and located at the mouth of small rivers (Tam et al. 1997). However, HPN, situated within the estuary of the Pearl River, is characterized by higher salinity and nutrients compared to PTC and YSO (Duprey et al. 2016). Within each mangrove site, adult crabs, that is, morphologically and sexually mature individuals, belonging to four crab species were collected by hand during their low tide activity period by two expert researchers. The focal species were: a detritivorous sesarmid, *Parasesarma bidens* (n = 15, size range 13 mm < carapace width [CW], > 20 mm), known to ingest mainly plant material and organic sediment on the mangrove floor (Poon et al. 2010); the grapsid *Metopograpsus frontalis* (n = 14, size range 15 mm < CW > 20 mm), which is an opportunistic feeder able both to predate on small invertebrates and to feed on plant material (Fratini et al. 2000; Poon et al. 2010); the ocypodid *Paraleptuca splendida* (n = 13, size range 13 mm < CW > 18 mm), which is known as a floating feeder, since it places a small amount of substratum in its buccal cavity and sorts food particles by passing water through the buccal cavity (Crane 1975), and the predator portunid *Thalamita crenata* (n = 7, size range 25 mm < CW > 35 mm), which ambushes crabs and searches for bivalves on the mud flat (Cannicci et al. 1996). Although all of the above four species show some degree of terrestrialization and are active at low tide, physiological and morphological differences are evident. *Parasesarma bidens*, *M. frontalis*, and *T. crenata* strongly rely on gills to breath and have evolved various mechanisms to retain water into their gill chambers (Little 2009). On the other hand, *Paraleptuca splendida*, like most fiddler crabs, evolved a true lung within the gill chamber and mostly uses its gills for osmoregulation and excretion (Paoli et al. 2015). Due to the strong differences in abundances shown by the above species at the study sites, we could not obtain a balanced design, although we could standardize for sizes and sex. To minimize stress, the collected crabs were transported in buckets with leaves and sediments, rinsed with ultra-pure water (Milli-Q) to remove sediment from the surface of their carapaces. Finally, crabs were stored at −18°C until further processing.

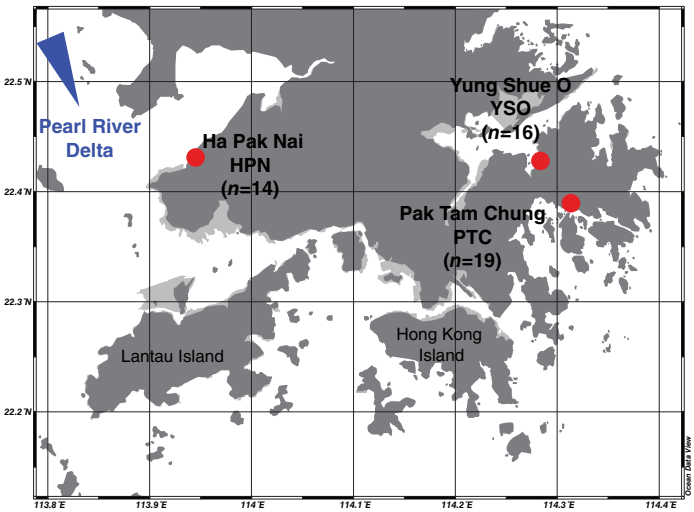

Fig. 1. Sampled mangrove sites within Hong Kong. n represents the number of crabs sampled.

Stomachs and gills were dissected and digested by a 10-min sonication treatment followed by a hydrogen peroxide (35%) bath heated at 80°C for 2 h. The digested solution was filtered through a 0.3 μm pore size glass filter and dried. Finally, 0.5 mL of 1% Rose Bengal was dropped on the filter to facilitate the identification of remaining organic material. Each filter was examined under a stereomicroscope (Carl Zeiss Stemi 305) and particles ≥ 10 μm were identified, categorized, and counted as fragment, fiber, pellet, and bead. Fragments were categorized as angular-shaped pieces, fibers as elongate cylindric pieces, pellets as oval-shaped pieces, and beads as spherical particles (Supporting Information Fig. S1). Because categorical distinctions of particles smaller than 10 μm were difficult, especially between bead and pellet, and because such particles are unidentifiable via fourier transform infrared spectroscopy (FTIR) analysis, we limited our investigation to particles bigger than 10 μm. Finally, selected particles, based on their sizes and shapes, were analyzed with a Bruker Lumos

Table 1. Number of microparticles found in gills and stomachs by crab species and by location and number of crab individuals analyzed for each species and location. Notes: Nb microparticles refers to the total number of particles found across all crabs investigated for a particular species and location. Nb ind. refers the number of individual crabs analyzed.

	YSO		PTC		HPN		Total	
Species/sites	Nb microparticles	Nb ind.	Nb microparticles	Nb ind.	Nb microparticles	Nb ind.	Nb microparticles	Nb ind.
Parasesarma bidens	365	3	393	6	615	6	1373	15
Paraleptuca splendida	86	6	53	4	194	3	333	13
M. frontalis	534	5	197	6	238	3	969	14
T. crenata	49	2	99	3	143	2	291	7
Total	1034	16	742	19	1190	14	2966	49

FTIR microscope using the attenuated reflectance transmission attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) method to identify their compositions. Spectra were identified using the Bruker spectrum library. To reduce potential contamination, all equipment were rinsed with MilliQ and methanol, all experiments were run under a fume hood, unnecessary opening of the filtration setup and petri dish were avoided, and cotton laboratory coats were used. Additionally, a procedural blank was used during each set of samples, with a total of nine replicates.

The total amount of recorded particles and the different amount of particle types found were analyzed with univariate and multivariate permutational analysis of variance (PERMANOVA, Anderson et al. 2008) designs, respectively. Both analyses were applied to test for differences in microplastic presence across locations (three levels, fixed and orthogonal), species (four levels, fixed and orthogonal), and between gills and stomach (fixed and orthogonal). In particular, the total amount of particles found, and the overall composition of particle types present were analyzed with univariate and multivariate PERMANOVA designs, respectively. To standardize for possible relationships between the specimen size and the amount of bodily microdebris found, the number of particles belonging to each of the selected categories found in each specimen was divided by their CW, a standard measure of size for crabs. Due to their heteroscedasticity assessed via PERMDISP, a multivariate version of the Leven test (Anderson et al. 2008), the data were log-transformed and the Euclidean distance was used to calculate the dissimilarity matrix. When appropriate, post hoc pairwise tests were performed to examine significant differences among levels of factors. A principal component analysis (PCA) on previously normalized data was performed as an unconstrained ordination to visualize patterns of particle composition in stomach and gills. To further analyze the differences in the number of particles of the various categories found in the stomach and gills of the different species analyzed, we performed a canonical analysis of principal coordinates (CAP). All analyses were based on 9999 permutations and were carried out using the software PRIMER 7 and its add-on package PERMANOVA+ (Anderson et al. 2008).

Results and discussion

All 49 crab specimens had particles present either in their stomach or in/on their gills, with only one individual containing particles exclusively in its stomach, reflecting the high contamination of mangrove crabs by particles. From the sampled specimens, we retrieved a total 2966 microparticle pieces, with almost 90% of the pieces present in the stomach and 10% in the gills, indicating that ingestion is the main driver of microparticle intake (df = 1; $F = 75.1$; $p < 0.0001$, three-way PERMANOVA, Table 1, Supporting Information Table S1). Based on the method used for microparticles recovery from gills (dissection and then digestion), it is not possible to distinguish particles that were trapped at the surface vs. the ones inside the gills; however, only 10% of the microparticles retrieved where found in the gills, suggesting that the ventilation system is not a main path of microparticles intake, at least for microparticles bigger than 10 μm. No significant correlation was observed between the content of microparticles recorded in stomach, gills, or both and the size of the individual crab, both measured as carapace width and length. An average of two microparticles was observed in procedural blanks and was thus used to correct the number of microparticles in crabs. We tested 46 pieces with ATR-FTIR but could only confirm the composition of nine of them. All of the other pieces were either too small for signal-to-background differentiation, or the presence of rose Bengal or remaining organic matter altered the readability of the spectrum. Of these nine particles, one fiber was identified as polyethylene (PE), one fragment as polyethylene terephthalate (PET), four particles as rayon, two particles as paint, and one as cobalt. Based on these results, we ascertained that most of the microparticles identified were derived from human-made material (89%, considering that cobalt may come from a natural source). If a majority of these microparticles can be considered as originating from human material, only 22% are identified as plastic polymer (PE and PET). However, this is based on a limited number of analyses and we recognize the need for additional investigations to further verify the abundance of microplastics in mangrove crab organs. Therefore, we use the term "microparticles" here to describe the combination of plastic and nonplastic micropieces from anthropogenic sources.

Both the abundance and composition of microparticle types found in crabs significantly vary between species (df = 3; $F = 5.4$; $p < 0.01$ and df = 3; $F = 9.0$; $p < 0.0001$ for abundance and composition, respectively, three-way PERMANOVA) and mangrove sites from which they were collected (df = 2; $F = 3.4$; $p < 0.05$ and df = 2; $F = 4.6$; $p < 0.01$ for abundance and composition, respectively, three-way PERMANOVA; Fig. 2). Crabs from the HPN mangrove located on the west coast of Hong Kong were shown to have significantly higher abundances of microparticles compared to crabs from the two other mangroves (HPN > PTC = YSO, PERMANOVA post hoc tests). A distinction in microplastic content in Hong Kong waters between the east and west side of the region has been previously shown to be linked to the influence of the Pearl River (Fok and Cheung 2015). Since our sampling sites do not differ in terms of structural characteristics, tree dominance, or area, the east–west difference observed in the abundance of microparticles in crab organs is likely linked to the influence of the Pearl River.

We also observed a significant difference in the abundance of microparticles ingested by different species, with *Parasesarma bidens* and *M. frontalis* characterized by similar amounts of microdebris ($t = 1.16$, $p = 0.25$, PERMANOVA post hoc test), and with both species higher than the ocypodid

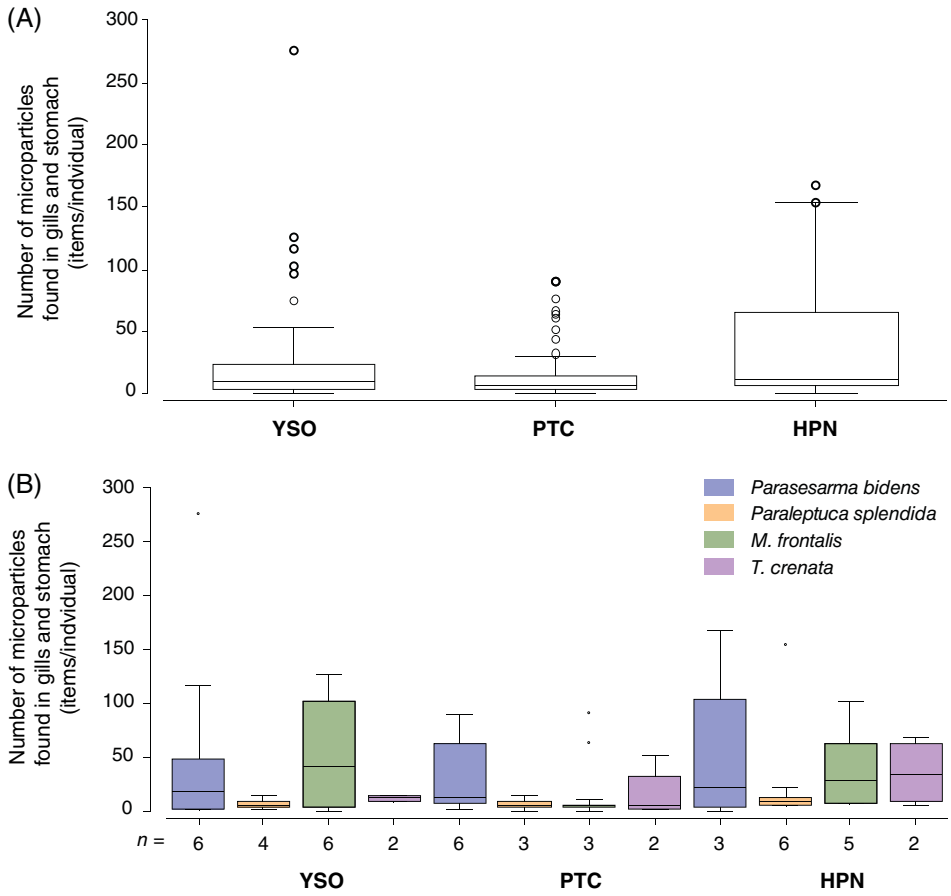

Fig. 2. Box plot showing the abundance microparticles found in gills and stomach of individual mangrove crabs at different locations (**A**) and in different species across locations (**B**). Sites are YSO, PTC, both located in the east side of Hong Kong, and HPN located in the north west of Hong Kong. The number of collected specimens per location and per species is shown below the bar. The box plots represent the nonparametric parameters, such as medians, quartiles, and 95% ranges.

Paraleptuca splendida ($t = 5.43$, $p < 0.001$ and $t = 3.92$, $p < 0.01$, for *Parasesarma bidens* and *M. frontalis*, respectively, PERMANOVA post hoc tests). The predator *T. crenata* had a lower amount of ingested microparticles compared to the omnivorous *Parasesarma bidens* ($t = 2.50$, $p < 0.03$, PERMANOVA post hoc test), but a higher amount than the microphytobenthos feeder *Paraleptuca splendida* ($t = 2.26$, $p < 0.05$, PERMANOVA post hoc test). These results can be easily explained by the food preferences and the feeding habits of these species. The highest rates of microparticles were recorded in the stomachs of the detritivore *Parasesarma bidens*, known to actively pick up plant materials and particulate organic matter (POM) from the sediment (Lee 2000; Poon et al. 2010), and of the omnivorous *M. frontalis*, known to ingest similar rates of animal, plant, and POM from the sediment (Poon et al. 2010). *T. crenata* is also a generalist feeder, but this crab avoids feeding on sediment and exerts a strong preference for predation on crabs and molluscs (Cannicci et al. 1996). Finally, out of the four species, the lowest content of microparticles was found in the stomachs of *Paraleptuca splendida*, which, like other fiddler crabs, filters pellets of mud to feed on microphytobenthos, using water stored in its gill chambers (Icely and Jones 1978; Dye and Lasiak 1987; Kawaida et al. 2019). Such active filtering could prevent this species from ingesting the heaviest microparticles, which are instead discharged into the pseudo-pellets contained within inorganic components of the mud.

PCA ordination results show clear patterns regarding the types of microparticles found in stomachs and gills across the different species (Fig. 3A). CAP showed that stomachs contained more fragments and beads, whereas gills contained mainly fibers (Fig. 3A,B). These results confirm that both dietary and ventilation processes are responsible for microparticle intake by crabs (Watts et al. 2015, 2016). The preferential presence of fibers during the ventilation process is consistent with microparticles found in filtering organisms such as oysters and mussels (Li et al. 2016, 2018). CAP also confirmed that *Parasesarma bidens* and *M. frontalis* preferentially ingested fragments (~ 80%) and beads (~ 13%), whereas *T. crenata* ingested a mixture of fragments, beads, and fibers and *Paraleptuca splendida* ingested mostly fibers (Fig. 3B). Based

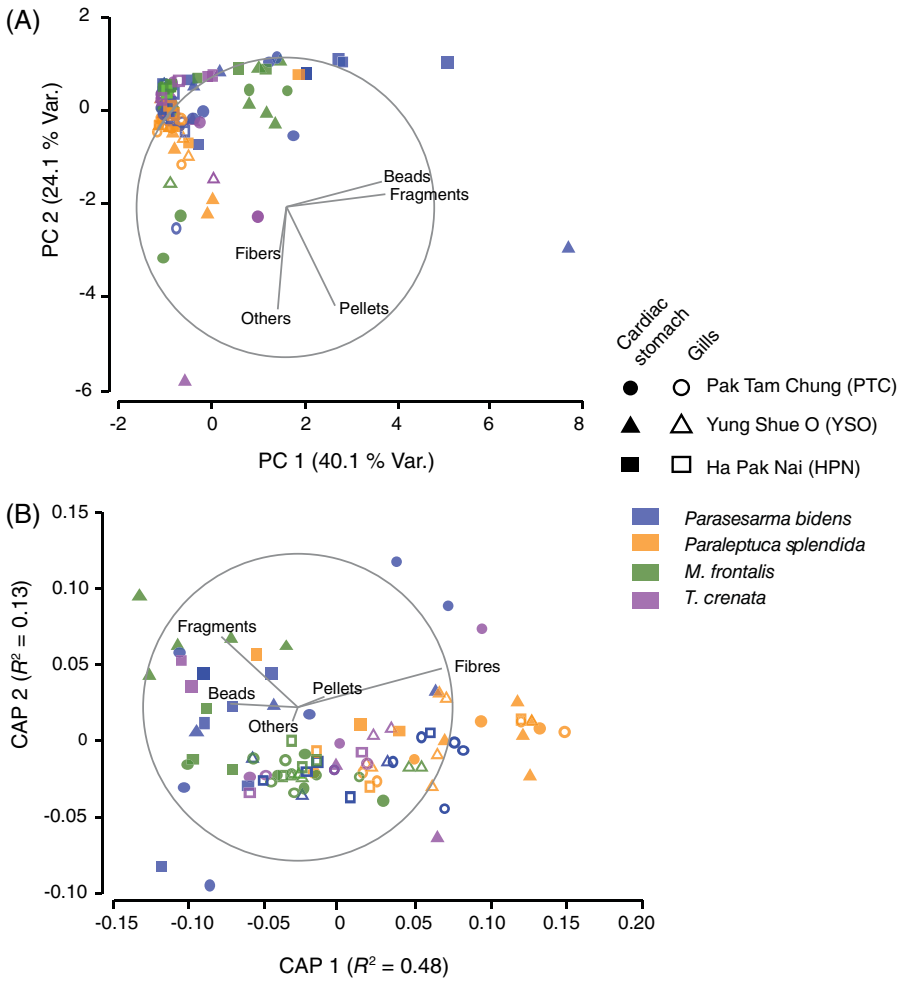

Fig. 3. Two-dimensional scatter plots of the first and second principal components (**A**) and of the first and second canonical axes (**B**) of microparticle type compositions found in the stomach and gill chambers of four species of mangrove crab from Hong Kong. Open symbols represent the data collected from the gills, closed ones the data from the gut. Triangles represent data from YSO, dots represent PTC, and squares represent HPN. Vectors of the linear correlations between individual variables are superimposed on the graph.

on these observations, we suggest that both the abundance and type of microparticles ingested in the cardiac stomach depend on the feeding habits of the crabs. For example, *Parasesarma bidens* as a detritivore ingested predominantly fragments whereas *Paraleptuca splendida* ingested less fragments but more fibers, which have lower sinking velocity (Khatmullina and Isachenko 2017; Hoellein et al. 2019) and could be ingested together with microphytoplankton during the filtering process carried out by this species.

Conclusion

Hong Kong mangroves are highly polluted by human activities, and mangrove crabs, one of the keystone taxa in the mangrove environment, appear to be heavily impacted by such anthropogenic debris. All of the sampled specimens in this study, belonging to four dominant species and located in three different mangroves throughout Hong Kong, present intake of microparticles. We have shown here that the abundances and types of microparticles found in crab stomachs are related to the crab's role in the food web and their feeding habits, with crabs that employ generalist feeding strategies ingesting a higher abundance and more diverse range of types of microparticles. Although we could confirm that crabs absorbed microplastics via both their ingestion and ventilation efforts, we clearly showed that most of the intake happens via feeding.

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