Can fiddler crab bioturbation activity *in situ* modify the distribution of microplastics in sediments and the influence on their bioaccumulation?

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Abstract

Fiddler crabs are known as "eco-engineers" who maintain habitat health through sediment bioturbation, being able to interact with microplastics (MP) due to their daily contact with the sediment. Here, we evaluated MP contamination in mangrove substrates in Isla del Carmen, southern Gulf of Mexico, comparing MP content between burrows and pellets resulting from bioturbation and MP bioaccumulation in the soft tissues of the *Minuca rapax*. In general, MP was more abundant and diverse in burrow sediment than in pellets, however, in less urbanized sites (< amount of MP), pellets seem to concentrate more MP than burrows. The MP characteristics in the pellet and in the tissues reflected those of the burrows. Bioturbation concentrated MP in pellets and tissues, depending on how urbanized the area is, showing a strong top-down effect of MP in subtropical tidal flats. *M. rapax* is an important structuring agent of sedimentary MP in subtropical tidal flats.

Key words: *Minuca rapax,* bioturbation pellets, microplastic bioaccumulation, sediment reworking, top-down effect

Graphical Abstract
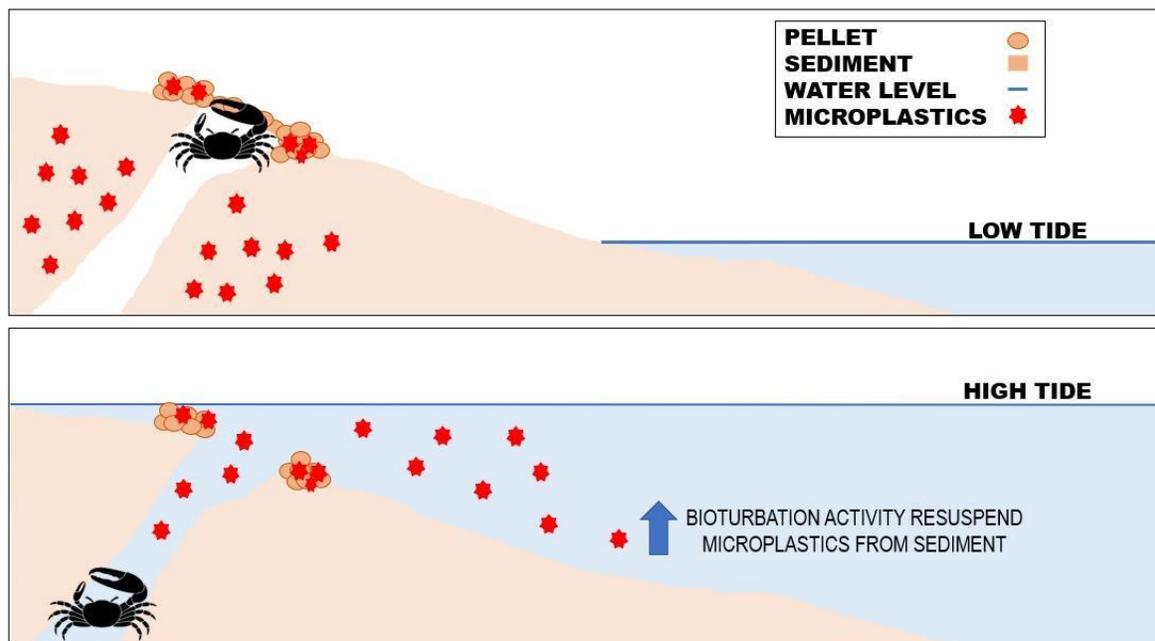
Introduction

Animals living in contact with the sediments are continuously modifying the physical and biogeochemical properties of their habitat. Burrowing, feeding, defecation, and ventilation among others (Krantzberg, G. 1985) are bioturbation mechanisms that directly or indirectly affect the sediment texture and structure due to the translocation of particles at or near the sediment-water interface (Kristensen et al., 2012). Overall, bioturbation plays an important role in global carbon and nutrient cycling, as well as in the dispersion, and burial of marine pollutants (Snelgrove, 1998; Grow et al., 2022). Among coastal invertebrates found inhabiting mangroves and saltmarshes around the fringes of tropical to temperate estuaries, the excavation and maintenance of burrows by fiddler crabs as ecosystem engineers is unparalleled (Crane, 1975). An ecological advantage of fiddler crabs is that they can turn and rework up to 48% of sediments (0–15 cm) due to their high-density populations and their intense foraging (McCraith et al., 2003). Because of their close interaction with sediments, fiddler crabs inadvertently are in close contact with pollutants such as heavy metals (Martínez-Colón et al., 2021, Capparelli et al., 2016) or microplastics (MP) (Villegas et al., 2021) among others which will open a doorway for trophic transfer mechanisms.

Given that MP is an emergent pollutant found in virtually all ecosystems, estuaries and their associated habitats are highly vulnerable (Alvez & Figueredo et al., 2019). These ecotones are subjected to numerous point- and nonpoint sources of pollution, and they serve as a sink of MP in particular in mangrove sediments as well as in the water column. Because of their small size (< 5 mm) (Frias & Nash, 2019), MP can be ingested by organisms (e.g., small fish, crustaceans) at the base of food webs and can potentially be biotransferred to higher trophic levels (Lavezzo et al., 2020), causing deleterious effects that range from the subcellular to the ecosystem level (Anbumani & Kakkar, 2018; Guzzetti et al., 2018). For example, MP bioaccumulation can cause internal and external injuries, ulcers, digestive tract blockage, among other lethal and sublethal effects (Ogonowski et al., 2016; Islam et al., 2021; Villegas et al., 2021, 2022). As there is already evidence of MP occurring in benthic food webs (Cauwenberghe et al., 2015; Taylor et al., 2016), their accumulation in sediments raises concerns about their current and future effects on these habitats, as well as the ecological implications for benthic species.

Fiddler crabs (Crustacea, Decapoda) are also known as "eco-engineers" that maintain habitat health through sediment bioturbation (Wang et al., 2020), feeding avidly on the detritus found in sediment by using their smaller chela (claw) (Christy, 1978; Caravello & Cameron, 1987). It has been found that certain species of fiddler crabs can differentiate sediments based on particle size after it is brought to the mouth (Colpo & Negreiros-Fransozo, 2011). This allows for better removal of food particles (e.g., detritus, algae, small organisms, and bacteria) by the buccal appendages including the accidental ingestion of inorganic particles (Takeda & Murai, 2003; Sayão-Aguiar et al., 2012; Booth et al., 2019). The bioturbation-mediated influence of fiddler crabs on sediment biogeochemistry includes altering carbon and nitrogen distribution (Wang et al., 2010), decreasing organic matter accumulation (Thomas & Blum, 2010), and altering rates and pathways of various microbial metabolic processes (Gribsholt et al., 2003; Kostka et al., 2002; Dollhopf et al., 2005). Several studies indicate that fiddler crabs can be considered as bioindicators of ecosystem health (Azpeitia et al., 2013; Capparelli et al., 2017, 2019; Yáñez-Rivera et al., 2019). For example, heavy metal concentrations have been found bioaccumulated in their soft tissues with several orders of magnitude higher than the surrounding sediments. (Capparelli et al., 2016, 2019; Martínez-Colón et al., 2021). Most recently, MP has been reported in the soft

tissues (e.g., gills and digestive tract) of fiddler crabs (Brennecke et al., 2015; Villegas et al., 2021, 2022) which is direct evidence of bioaccumulation.

Benthic organisms play an indirect role in the spatial distribution as well as the vertical migration of MP because of bioturbation, regardless of wheter the particles are ingested or not. Results from microcosm experiments have demonstrated how the mollusk *Macoma balthica* (Näkki et al., 2017) and the worm *Arenicola marina* (Gebhardt & Forster, 2018) have translocated MP towards deeper sediment layers during bioturbation and associated burrowing. Unlike the top-to-bottom translocation of MP by *M. balthica* and *A. marina*, fiddler crabs have a bottom-to-top mechanism during burrowing and bioturbation (Gardner et al., 1987) in which they are constantly "regenerating" translocated sediments (Kristensen et al., 2012). However, it is uncertain what is the influence of fiddler crab constant bioturbation on the MP vertical distribution and the potential bioaccumulative toxic effects of these pollutants on their biology.

Here we assess the presence of MP from mangrove sediments directly associated with *Minuca rapax* burrows and pellets (feeding and burrowing) and their concentration and bioaccumulation in their soft tissue (whole-body) from areas with different degrees of anthropogenic impact (urbanization) in Isla del Carmen, Mexico. The analyses we present address three major questions: 1) Can the presence and characteristics of MP in sediments be modified by M. rapax bioturbation? 2) Can *M. rapax b*ioaccumulate MP based on their characteristics (size, shape, and color)? 3) Does the degree of urbanization affect MP bioaccumulation?

Methodology

Study organisms

Minuca rapax is distributed from Florida to southeastern Brazil, and is a generalist species, inhabiting sandy to muddy substrates (Thurman et al., 2013). It tolerates a wide range of salinities and exhibits extensive hyper-/hypo-osmoregulatory capability (Thurman et al., 2013; Zanders and Rojas, 1996), including contamination tolerance (Capparelli et al., 2017). This species has a wide distribution and abundance in the study area (Figure 1), inhabiting from areas with vegetation cover to degraded areas, but mostly it's an intertidal species.

Study area and collection

Isla del Carmen is located on the southwestern Yucatán Peninsula (Figure 1), bordering the southern Gulf of Mexico, Campeche State, Mexico, and lies within the protected natural area of Laguna de Términos, recognized as a Ramsar site (Ramsar, 2017). Although Isla del Carmen falls within this protected area, it has suffered from poorly planned, rapid urban growth over the last 40 years (Pérez-Ceballos et al., 2013), leading to mangrove deforestation and the filling and draining of wetlands (Ramos-Miranda & Villalobos-Zapata, 2015). The island has housed various oil extraction activities since the mid-1900s and has been exposed to pollutants such as heavy metals (Celis-Hernandez et al., 2020, 2022), organic pollutants (Carvalho et al., 2009), and MP (Celis-Hernandez et al., 2021).

Figure 1. a) Isla del Carmen is located on the northwestern Yucatán Peninsula (18° 41' N, 91° 40' W - red square in upper right side panel). Sampling stations (1 to 7) for b) *Minuca rapax* (white line scale 1cm), c) sediment from the burrow and pellets (burrowing + feeding). Land use composition pie charts are displayed for each collection site.

A total of approximately 70 adult specimens of *M. rapax* (≈13 mm carapace width), of either sex, were collected from different localities in August 2021. These locations were chosen based on the degree of urbanization and the presence of *M. rapax*. Using land use compositions (UA = urbanized area, MG = mangrove, SV = secondary vegetation) determined in QGIS 3.12.1 for each collection site. Sites 1, 2 and 3 were categorized as urbanized (<80% UA) while sites 4–7 as highly urbanized (>80% UA) (see Figure 1) following Capparelli et al (2021) urban development classification. Land use class proportions were calculated excluding areas covered by water and by clipping a national scale land use map of Mexico with a 2 km-buffer around each site (INEGI, 2016). Misclassification of the agricultural area between sites 3–4 was corrected to an urban area, due to the absence of agriculture activities on the island and the verification with the Landsat 8 image composite from 2015 to 2021 used as a background (Figure 1a). The proportion of land use classes can be found in Table 1.

In addition to *M. rapax* collection, samples consisting of 500 g of sediments from the burrows (15 cm deep) and 100 g from surface pellets (excavation + feeding) were collected for MP analysis (150 g in pre-cleaned plastic bags) from each site. The sediments were kept refrigerated at 4 °C until analysis. After collection (1–2 h), the crabs were transported to the Estacíon El Carmen del Instituto de Ciencias del Mar y Limnología of the Universidad Nacional Autónoma de México in pre-cleaned plastic boxes containing water from the collecting sites with small stones as a refuge. Immediately upon arrival at the laboratory, the crabs were cryo-anesthetized in crushed ice for 10 min, after which all soft tissues were dissected for MP quantification and subsequently stored at 20 °C until analysis.

Microplastic quantification

Following the chemical digestion techniques of Li et al. (2015) and NOAA (Masura et al., 2015) a pool of soft tissues from 10 *M. rapax* per sampling site was dissected and placed in separate Erlenmeyer flasks. Then, 30% hydrogen peroxide (H_2O_2) was added to each flask at a 40:1 ratio with 200 mL of H_2O_2 for every 5 g of organic tissue for 72 h at room temperature (25 °C), followed by vacuum filtration (45μm pore size filters). For the sediments (burrow + pellet), the NOAA (2015) methodology was implemented.

To avoid background plastic contamination during sample (tissue and sediment) processing and analysis, all laboratory materials were rinsed first with Milli-Q water and pre-cleaned filter papers were placed in Petri dishes and exposed to the air in the laboratory during the processing time to account for atmospheric contamination. For tissue samples, three blanks were prepared using H_2O_2 (30%) following the protocol described above. For the sediments, since they were wet sieved, three blanks were prepared individually with 1L of filtered Milli-Q water following the same Gimiliani et al. (2020) methodology. If any MP were present, then a blank average was calculated and subtracted from the total counts from each sediment or tissue sample.

Quantification and isolation of MP from sediment and pellet samples were carried out based on the methodology proposed by Gimiliani et al. (2020). In summary, both types of sediment substrates were homogenized and dried (50 °C) for 24–48 h or until it is completely dry then weighed. Subsequently, subsamples (100 g) were wet sieved in order to separate the MP into size fractions (2 mm, 0.5 mm, 0.25 mm, 0.125 mm, and 0.063 mm). The wet sediments retained in each sieve were transferred to Petri dishes (pre-weighed), dried (50 ℃), and weighed again. For tissue samples, filters were divided into four sections to facilitate an accurate manual counting of the MP.

MP in Petri dishes (for sediments) and filters (for tissues) were counted using a stereomicroscope with a magnification of 20X. The patterns and shapes used to identify MP were based on the descriptions provided by Mohamed Nor and Obbard (2014) and Frias et al. (2018), as well as on visual inspection. Two observers used stainless steel tweezers to actively search for MP and to separate them from the tissue and sediment samples. MP was categorized by color (blue, transparent, red, yellow, white, orange, light blue, light brown, violet, black and mixed) and shape (fibers and fragments). To distinguish between organic material and MP, if the material crumbled or was easily crushed, they were not considered as plastic compounds. If the particles kept their shape, they were included as an MP in the counting (Mohamed Nor & Obbard, 2014).

The total concentration of MP in each sample was calculated by dividing the total number of MP by the dry mass weight (prior to wet sieving) for sediments (burrows or pellets) or wet mass weight (prior to chemical digestion) for *M. rapax* soft tissue. The unit

for MP concentration is MP g^{-1} of dry sediment for burrows and pellets, and for tissues MP g^{-1} of wet tissue.

Data Analysis

We used Generalized linear models (GLM) with Poisson distribution to test whether there are differences in MP concentration between sample types (tissues, pellets, and sediments). MP concentration was the dependent variable, whereas sample types were used as independent variables. Also, the same GLM models were used to test whether there were differences in MP shapes, colors, and sizes between sample types.

To test whether the MP concentration in the sediments affected the bioturbation, the sampling sites were classified into "High" and "Low" groups according to their concentration. For that, we first ranked the seven sampling sites and then used the median to separate the groups (min = 0.5 MP g^{-1}, max = 35.71 MP g^{-1}, median = 3.63 MP g^{-1}). We also tested whether bioturbation could be due to the degree of urbanization, as shown in Figure 1, by classifying sites in two groups according to the degree of urbanization, setting the limit as 80% of the urban area and the low presence of mangroves (*sensu* Capparelli et al., 2021). Then we used two independent GLMs with Poisson distribution, where MP abundance was the dependent variable, and site classification, either per degree of urbanization or by MP concentration were used as independent variables.

For each GLM, ANOVA tables were created, and multiple comparisons were done with the Tukey test. Mean differences were considered significant at P < 0.05. All statistical analyses were performed with R (R Core Team, 2020), using functions of the MASS package (Ripley et al., 2021), *Anova* function of the car package (Fox & Weisberg, 2019) and *pairs* and *emmeans* functions of the emmeans package (Lenth et al., 2018).

Results

Presence and concentration of MP in sediments and pellets.

We found evidence of MP concentration differences (Fig. 2A) between sediments and pellets (P = 0.045). The overall spatial distribution of MP concentrations in sediments increased from following the urbanization gradient, from east to west. The two pristine sites have 100% vegetation cover, although the concentrations of MP had a noticeable variation.

Station 2 with 99.6% mangrove cover (MG) had a concentration of two MP g^{-1} while further west towards the urban development areas, the MP concentration in station 1 (42.9% MG and 57.1% secondary vegetation [SV] cover) was six times higher (Table 1). Based on urban development areas (UA) (Figure 1), station 3 (54.2% UA and 43.7% SV) is located in a buffering or transition zone with only one MP g^{-1}. It came to be a surprise that the rest of the stations with >54% UA did not show a constant increase in MP concentrations, although they were significantly higher when compared to the pristine sites. Stations 5 (96.6% UA) and 6 (100% UA) had the highest MP concentrations with 36 and 11 MP g^{-1} respectively. On the other hand, stations 4 (86.9% UA) and 7 (100% UA) had the lowest concentrations with 4 and 3 MP g^{-1} given the island's highly developed western portion. A somewhat similar distribution was observed with MP found in the pellets (burrow + feeding), especially in the pristine stations. However, of all the sampled sites, buffer station 3 had the highest concentration with 18 MP g^{-1}. Stations 6 and 7 with 100% UA had low MP concentrations (Table 1).

Although MP fibers were the predominant shape in all sample types (sediment- 85% and pellets- 75%) when compared to fragments (Figure 3A) no significant statistical difference ($P = 0.220$) was found between the two substrates (Figure 2B). However, it is
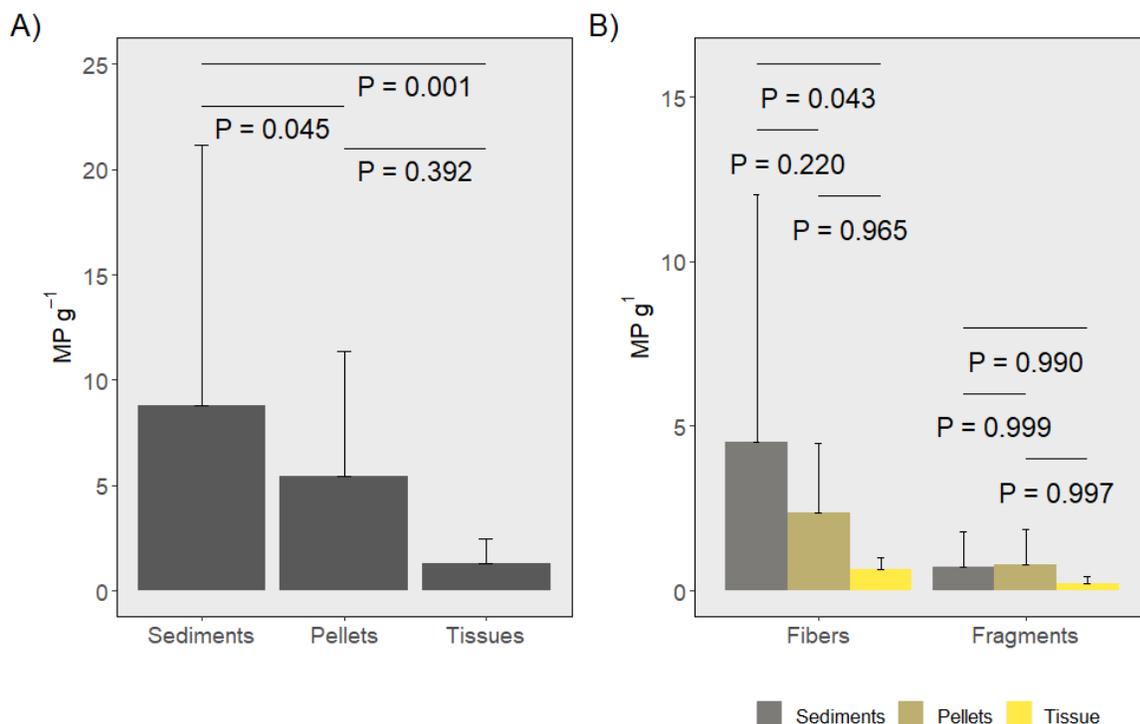
important to highlight that in the sediment substrate by far the two sites with the highest combined number of fiber (194) and fragment (114) counts were 6 and 7 with 100% UA followed closely by the two pristine sites 1 and 2 (fiber= 112; fragment= 17) with 0% UA. With respect to the pellets, stations 6 and 7 had the highest combined values (fiber= 257 and fragment= 37) but the pristine sites (1 and 2) had the lowest (fiber= 25 and fragment= 8).

Figure 2. Results of GLM models, indicating the comparison of MP abundances (mean ± standard deviation, MP g-1) between (A) MP sample type, and (B) MP shapes by sample type.

Regarding color (Figure 3B), the two most predominant, in both sediments and pellets, were transparent (74.6% and 62.8%, respectively) followed by blue (9.7% and 24.5%, respectively). As for size (Figure 3C), the most abundant MP size was in the 125 micron (0.125 mm < MP < 0.25 mm) in both sediments (37%) and pellets (44%). These were closely followed by the 250 micron (0.25 mm < MP < 0.5 mm) in the sediments (32%) and in the >2 mm in the pellets (24%). We found no significant statistical differences in the concentration of MP between sediments and pellets of different sizes.

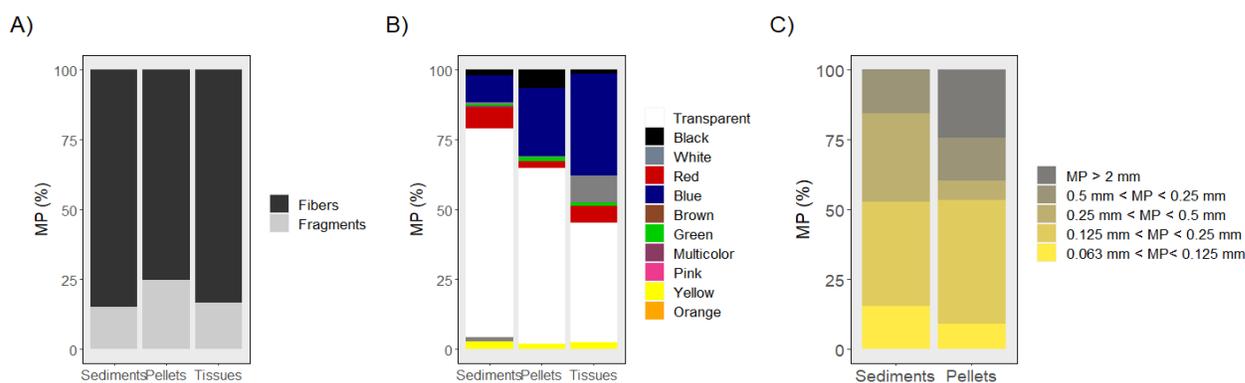Figure 3. Proportion of MP A) shapes, B) colors and C) size in pellets, sediments and tissues from *M. rapax*. All microplastics found in tissues (not shown in C) have sizes < 0.45 mm.

Presence and concentration of MP in tissues.

We found significant statistical differences in MP concentration between sediments and tissues (P = 0.001), but none between pellets and tissues (Figure 2A). Regarding MP

shape, we found evidence of differences (Figure 2B) in fiber concentrations between sediments and tissues (P = 0.043) but no statistical difference with pellets (P = 0.965). On the other hand, MP shapes show no statistical correlation between the matrices. As expected, sites 6 and 7 with 100% UA had the highest combined number of fiber (14) and fragment (6) counts in the *M. rapax* tissues where the pristine sites (0% UA) 1 and 2 had the second-highest values (fiber= 6; fragment= 1).

Regarding MP color, no differences were found between pellet and tissue samples, (Figure 3B). Similar to the colors found in the sediments and pellets, transparent (42.9%) and blue (36.3%) were the two most dominant ones in the tissues.

Presence and concentration of MP based on the degree of urbanization

Statistical significant differences were found only between sediments and pellets (P < 0.001) and pellets and tissues (P < 0.001) in sites classified as "High" MP concentration in the sediments (Figure 4A). In areas classified as "Low", we found evidence of significant differences only between sediments and pellets (P < 0.026) and sediments and tissues (P < 0.020). Regarding urbanization level (Figure 4B), statistically significant differences were found only between sediments and pellets (P < 0.001) and pellets and tissues (P < 0.001) in sites with less than 80 % of urbanization.
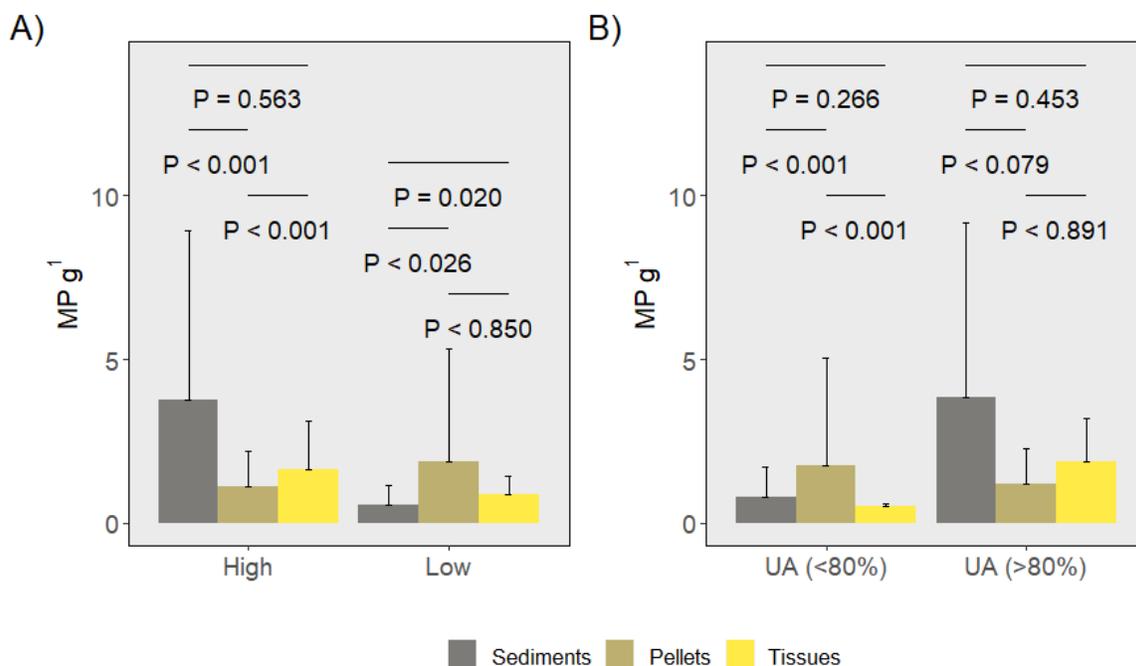
Figure 4. Results of GLM models, indicating the comparison of MP abundances (mean ± standard deviation, MP g^{-1}) between (A) MP sample type (sediments, pellets, and tissues) in areas classified as "High" and "Low", and (B) MP sample type in areas classified according to the Urbanization level, as defined by more than 80 % of Urban area in the land use map (Figure 1).

Table 1. Characteristics of the study area. Mean MP concentration in each site is represented as MP g^{-1}. Land use classes were: urban area (UA), mangrove (MG), and secondary vegetation (SV).

Site	Sediment	Pellets	Tissues	Land use	Sediment concentration
1	6	2	2	42.9% MG 57.1% SV	High
2	2	1	2	99.6% MG 0.4% SV	Low
3	1	18	2	54.2% UA	Low

				43.7% MG	
4	4	12	2	86.9% UA 12.9% MG 0.2% SV	High
5	36	7	11	96.6% UA 3.4% MG	High
6	11	7	5	100% UA	High
7	3	2	5	100% UA	Low

Discussion

1) Can the presence and characteristics of MP in sediments be modified by M. rapax bioturbation?

Fiddler crabs are one of the main agents of bioturbation in intertidal flats (Kristensen, 2008; Wang et al., 2010). In line with our expectations, the results of the present study show that the bioengineering effects of fiddler crab foraging, as well as the construction and maintenance of burrows, have modulated the distribution of MP in sediment. Their burrowing and feeding activities not only result in short-distance translocation of sediment particles near the surface, but they also redistribute sediment within their burrows (Kristensen et al., 2012; Atkinson & Eastman, 2015) to strengthen the walls "foundations" since the burrows serve as a way to escape predators, avoid natural environmental changes (e.g., high tide), provide sustenance at low tide (e.g., water), and are used for physiological needs when vulnerable (e.g., molting and reproduction) (e.g., Crane et al., 1975). It is not clear during burrowing if *M. rapax* discards and leaves behind particles in the burrow itself. It is however speculated in this study that while bioengineering their burrows, *M. rapax* does "discriminate" between particles, as evidenced by the noticeable difference in the concentration of MP found in the sediments and pellets. This can be explained by the volume of sediment being processed by fiddler crabs. For example, M. *rapax* intense foraging in the immediate vicinity of their burrow (10-15 cm) and during feeding manipulate portions of sediment by sorting different particle sizes and scraping them of organic matter (Sayão-Aguiar et al., 2012). The burrows of some fiddler crabs, depending on the type of substrate

(e.g., sediment, vegetation; Wang et al., 2014) can reach depths greater than or up to 10 cm (Penha-Lopes et al., 2009; Egawa et al., 2021). In fact, mesocosm experiments have demonstrated an eight-day turnover excavation rate of ~10 g (volume= 40 cm3) per burrow (Penha-Lopes et al., 2009).

Given that the pellets consist of burrowed sediments, it is surprising that the MP concentrations is so different, especially since it was expected that the portion that consists of feeding pellets to have higher concentrations, but since they are from the surface layer, what has been recorded is what has been "recently" deposited. An explanation for the difference could be based on the sedimentary environment of the sampled sites and for how long the MP's have been concentrating on the sediments at depth. This is hard to answer given that to our knowledge there is no published work on sediment accretion rates from the mangrove sites. However, a compelling assumption of why the pellets have lower MP concentrations could be that the volume of pellets excavated is minuscule when compared to the bulk sediments from which *M. rapax* makes its burrows. It is speculative that *M. rapax* preferentially selected the two most common colors (transparent and blue) to be found in the pellets, since they are also the most abundant in the sediments. In addition, size does not seem to be a selective parameter to explain the differences in MP concentrations.

It is important to remember that the dynamics of sediment mixing during bioturbation is species-dependent as it will vary in intensity, depth of penetration, and can substantially alter the vertical profiles of sediment constituents from those that would otherwise exist. For example, worms ingest sediments from the surface and excrete them in deeper zones (Robbins et al., 1979), and for these group of species, the displacement and accumulation of MP in the lower layers of the sedimentary column can result in a variation in environmental concerns, such as MP preservation. In contrast, while digging their burrows, fiddler crabs move sediment parcels at depth to the surface (bottom-to-top effect), where they have washed away during high tides (Wang et al., 2010) and inadvertently lower their concentration. This transport mechanism has already been proven when related to carbon stocks (Grow et al., 2022) and oil contamination (Franco el al., 2017) associated with fiddler crab sediment translocation. This could explain that, indeed, *M. rapax*'s bioturbation activity has led to a disproportionate amount of MP concentrations due to bottom-up translocation. However, MP

could be recycled back to the surface by the same tidal cycling since Celis-Hernandez et al. (2021) reported 287 particles L^{-1} (close to sites 5 and 6) and 130 particles L^{-1}(close to site 2) although they did not find a significant variation between the MP in the water and sediment samples. Another possibility could be the very nature of the fiddler crab burrow. Kristensen et al. (2012) classified fiddler crabs as a sediment "regenerator" and explained that the excavated sediments found at the surface can be returned to the bottom by inadvertently burrow collapse or by infilling during by currents (e.g., tides). Salmon (1984) documented that fiddler crabs "harvest" sediments from the surface and move into the burrow as a food supplement for later usage (Zeil and Hemmi, 2006) which could augment this MP concentration difference.

2) Can M. rapax bioaccumulate MP based on their characteristics (size, shape, and color)?

Fiddler crab bioturbation is performed by two main activities, burrow construction and maintenance, coupled with foraging. The burrowing activities produce not only the burrow but also excavation pellets (the manipulated sediment from digging or maintenance). During the feeding process, which usually occurs next to the burrow, the crab transfers particles from the sediment to the mouth with the aid of the cheliped (Takeda et al., 2004). In the buccal cavity, the edible particles are separated by flotation feeding– organic matter floats in the water from the branchial chamber and is then ingested (Kristensen, 2008). Inorganic and heavy particles are deposited on the bottom of the buccal cavity and dropped as feeding pellets (Takeda et al., 2004; Wolfrath, 1992).

Although there are several studies that demonstrate the ability of fiddler crabs to recognize colors for mating and escaping predators (Hemmi et al.,2006; Detto et al., 2006), feeding seems to be an elusive parameter. In this study, no difference was observed between the ingested colored MP with those found in the sediment and pellets by *M. rapax.* Similarly, the ability to select particles by size has also been studied, but so far it has been addressed to meiofauna organisms and different types of food (Citadin et al., 2016), never with MP. Due to this capacity, we would expect that there would be some sort of color, shape, and size selection, however, what was possible to observe is that the colors and shapes present in the tissues were the most abundant found in the sediment and pellets.

Although Not et al. (2020) reported that the gill water-storing capacity of fiddler crabs could be a potential depuration mechanism of MP ending in the feeding pellets and not in the stomach, we speculate that the ability to select and even avoid eating MP works up to a certain environmental concentration in the sediments, but this pattern would need to be confirmed in laboratory experiments. In experiments, Brennecke et al. (2015) and Villegas et al. (2022) reported MP bioaccumulation in fiddler crabs and noted a positive correlation between particle abundance in the treatment and in the organs (e.g., gills, hepatopancreas). The enrichment of the pellets with MP is due to the feeding strategy of fiddler crabs. Like the specialized mouthparts that are used to separate edible matter from indigestible material, fiddler crabs are thought to be selective deposit feeders, eating bacteria, protozoa, microalgae, meiofauna, and sedimentary debris (Dye & Lasiak, 1986; Meziane et al. 2002; Reinsel 2004) selected primarily by size (Brennechke et al., 2015) and probably by density. However, fiddler crabs may exhibit generalist feeding behavior, consuming small MP particles (< 0.45 mm). Thus, it is likely that the rejected material (edible or not) present in the feed pellets depends on the characteristics of the site, or the material handled and the selection can even be efficient, but up to a certain MP concentration in the environment. Nevertheless, due to the fact that we find MP in their tissues, this ability to select desirable particles or not, may not be efficient for MP.

3) Does the degree of urbanization affect MP bioaccumulation?

The degree of urban development, and clearly, the amount of MP in the burrows' sediment, did play a role in the MP bioaccumulation in soft tissues of *M. rapax*. MP concentration in tissues was higher in areas with more than 80% of urbanization areas. However, no significant differences between burrows and tissues were found in sites with high urbanization levels (UA >80%), which would indicate more bioaccumulation in these areas. (Figure 4AB). This could be due to the fact that MP in the sediments from burrows was ubiquitous in all sampled sites. Except for heavily urbanized areas, such as sites 5 (36 MP g^{-1}) and 6 (11 MP g^{-1}), a basal amount (ranging from 1 to 6 particles MP g^{-1}) was observed in the burrows' sediments. It has been shown that fiddler crabs reflect the conditions of their surroundings. Capparelli et al. (2019) reported that the degree of contaminant bioaccumulation can be proportional to those concentrations found in the environment and,

most importantly, that the bioaccumulation capacity seems to exceed some minimum threshold of pollution. This pattern has already been observed for metals (Capparelli et al., 2016), however, with other contaminants such as pesticides or MP (Villegas et al., 2021; 2022) this relationship does not appear to be linear, which may be related to the species ability to excrete and eliminate some contaminants, or even to the ability to select and avoid compound incorporation.

During bioturbation-related activities, *M. rapax* can concentrate MP in the pellets, however, this dynamic can be affected by how contaminated the area is. In sites with high amounts of MP, there is a greater amount of MP in the sediment than in the pellets, and there is also high bioaccumulation. This leads us to hypothesize that *M. rapax* has the ability to select particles, and there is a relationship between this capacity and how contaminated the area is. The more urban areas, characterized by mangroves impacted by untreated urban water discharges, presented a high amount of MP in sediments. In sites characterized by more pristine mangroves, the amounts found in the tissues are also higher than the environmental sediment quantities. Plus, it is interesting to note the trend (Figure 4) that the amount of MP in the tissues added to that of the pellets, is close to the amount of MP in the sediment of the burrows (MP sediment = MP in the pellets + MP in tissues). This leads us to conclude that the difference between MP from the surface sediment of pellets and sediment from the burrows is the one ingested and accumulated in the tissues of *M. rapax.* In a way, not incorporated MP can be discarded by *M. rapax* into the pellets and returned to the aquatic environment in a top-down effect.

The MP contamination in coastal sediments is not necessarily brought from the nearest continental contamination source, as MP particles may be transported from other continental or offshore regions (Lusher, 2015). The process of transport and deposition of floating plastics in the oceans is determined by marine and estuarine dynamic conditions, such as wind strength and geostrophic circulation (Li, 2019). Thus, even non-urban areas or areas with fewer sources of contamination can receive MP that will accumulate in estuarine sediments and mangroves, affecting several ecological processes, such as bioturbation. Fiddler crabs are also an important prey item for commercially important species, such as other crabs, and a wide variety of fish, birds, and mammals. Consequently, MP impacts on

fiddler crabs are likely to have a cascade of effects, with negative consequences on many members of coastal ecosystems.

Conclusion

In summary, the general engineering effects of the construction and maintenance of burrows and foraging of fiddler crabs are multifaceted, modulate the amount of MP in the sediment, and influence MP bioaccumulation, reflecting the degree of MP contamination. On the other hand, foraging engineering structures of fiddler crabs can concentrate MP in pellets and tissues, showing a strong top-down effect of MP in subtropical tidal flats. Furthermore, our results do not show an ability to choose or select colors and sizes by *M. rapax*. The pellets and tissues reflected the characteristics of the MP found in the burrows. The present study demonstrates that different engineering effects of fiddler crabs are important MP sources and structuring drivers of sediments in subtropical tidal flats and that this effect occurs both in areas with high concentrations of MP and in less contaminated areas.

Acknowledgments

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that might influence the present study.

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