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Microplastic contamination in wild shrimp *Litopenaeus vannamei* from the Huizache-Caimanero Coastal lagoon, SE Gulf of California

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Abstract

We identified and characterized microplastics (MPs) in the gastrointestinal tract (GT), gills (GI), and exoskeleton (EX) of *Litopenaeus vannamei* in a coastal lagoon from the SE Gulf of California. The most common MPs were fibers and fragments with an average size of 403 ± 296 µm, in which the transparent and blue colors predominated. The abundance (items/g as wet weight (ww)) in the GT, GI, and EX was 114.7 ± 33.2, 13.7 ± 5.3 and 3.0 ± 0.5, respectively. The abundance of MPs per shrimp was 13.3 ± 1.1, while the abundance per individual (ww) was 0.9 ± 0.2 MPs/g. Considering the consumption of shrimp in Mexico, MP abundance, and shrimp consumption (discarding GI and EX), we estimated MP ingestion as 280 items/person/year. The results from this study can be used as background information for future MP biomonitoring in shrimp species of ecological and commercial importance.

Keywords Fibers · Fragments · Films · Food security · Crustaceans · Sinaloa

Microplastics (MPs) are emerging contaminants of anthropogenic origin that consist of plastic particles < 5 mm in diameter. These are classified as primary, which are used for the cosmetics, the domestic industry, and drug vectors (Germanov et al. 2018), and secondary, which result from the mechanical, chemical, and/or photocatalytic degradation of MPs both on land and in the marine environment, including fibers from the textile industry (Li et al. 2016). MPs enter the oceans in very diverse ways and are subsequently distributed throughout the ocean basins by currents, resulting in their global distribution. MPs are accessible and could be ingested by a wide variety of organisms; thus they have been detected in a wide range of species such as zooplankton, fish, mollusks, crustaceans, seabirds, and marine mammals (Ugwu et al. 2021). Consequently, the ingestion of MPs has been observed in a range of animals of commercial interest that are consumed by humans as food. The high intake of seafood in some countries generates concern regarding the potential effects of MPs on human health (Barboza et al. 2018).

There are various studies regarding MPs in Mexico that include water, sediments, mollusks, and fish (Ramírez-Álvarez et al. 2020; Lozano-Hernández et al. 2021). However, no studies have been conducted to investigate their presence in wild shrimp, even though this group is considered both ecological and commercially important in the country. The average human consumption of shrimp in Mexico is 1.69 kg/capita/year (CONAPESCA 2018). Nevertheless, there is no scientific data regarding the incidence of MPs in marine products despite the relevance of this economic sector in Mexico.

According to its geographical distribution, *Litopenaeus vannamei* occurs along the coasts of the American tropical Pacific, from the coast of Sonora, Mexico, to Peru (Dall et al. 1990). The Huizache-Caimanero (HC) lagoon is located in the southeastern Gulf of California, Mexico. Here, *L.*

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vannamei represents 90% of the shrimp catches (Frías-Espericueta et al. 2004). The objective of this study is to characterize (abundance, type, shape, color, and size) MPs in the gastrointestinal tract (GT), gills (GI), and exoskeleton (EX) of *L. vannamei* from the HC lagoon, Mexico. This study is the first to investigate contamination of MPs in tissues of wild shrimp *L. vannamei* from coastal lagoons in Mexico, which can be considered as a reference for future research. We hypothesize that *L. vannamei* incorporates MPs in its tissues (mainly via the GT) and that the main source of these particles comes from plastic waste used in fishing, and agriculture activities.

Methods and materials

Huizache-Caimanero is a subtropical lagoon (area 10, 310 ha) that is connected to the Presidio River to the North, which receives a rural and agricultural discharge, and to the Baluarte River to the South, which receives agricultural, semiurban, and aquaculture discharges (Páez-Osuna et al. 2017). HC is an ecosystem characterized by highly productive developing fisheries that include shrimp, fish, and mollusks. In recent decades, the development and expansion of agriculture and aquaculture in the floodplain and margins of the lagoon have transformed this landscape causing notable environmental changes (Fig. 1).

Eighty individuals of *L. vannamei* were captured (November 2020) from the HC lagoon (Fig. 1) (22° 55’ 36.52” N; 106° 04’ 22.8” W) with a trawl net used by shrimp boats. The organisms were transported to the laboratory where they were placed in aluminum foil and stored at -20 °C. Afterward, organisms were thawed in glass trays and rinsed with filtered (GF/F glass microfiber filter Whatman, 0.7 µm

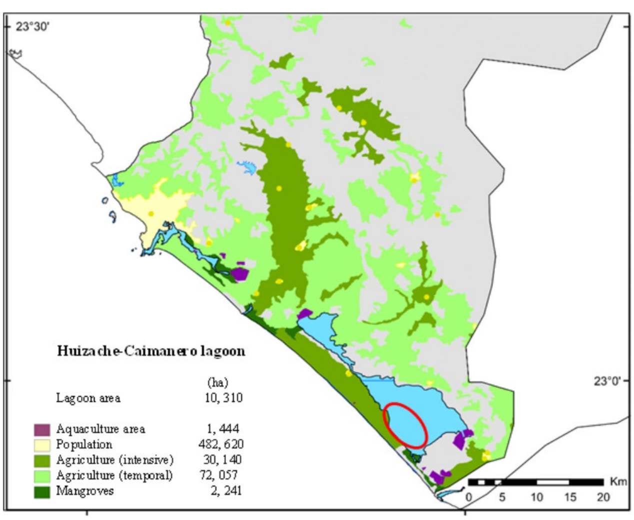

Fig. 1 Sampling site (red ellipse) and the main lower basin activities in the Huizache-Caimanero lagoon

pore size) tri-distilled water to remove sediment or other contaminants. The weight and total length of each specimen were recorded (15.1–21.8 g). The shrimp were randomly separated into groups of five organisms for dissection and digestion; the weight of the groups ranged from 75.5 to 109.2 g. The GT, GI, and EX were dissected and separated, and each tissue was weighed per group. For the analysis of MPs, the tissues of each group of shrimp were transferred into Erlenmeyer flasks for digestion using a mixture of acids (4:1; nitric acid 65%: perchloric acid 68%) according to De Witte et al. (2014) and Devriese et al. (2015). The digested solution was filtered (GF/F glass microfiber filter Whatman, 0.7 µm pore size), and the filter was transferred to a glass Petri dish for further visualization. The filters were observed under a stereoscopic microscope (VELAB model VE-S5C, VELAB Mexico) at 45x magnification, where MPs were characterized and counted. The type, shape, color, and size of MPs observed in the filter membrane were identified according to Hidalgo-Ruz et al. (2012) and Li et al. (2016). The size of the MPs was measured with an eyepiece with a micrometric scale (10-1600 µm). The most representative MPs items in tissues were verified by Nile Red (NR) staining using a stock solution (1 mg NR dissolved in 1 mL acetone (99.5%) diluted in 100 mL) (Vermeiren et al. 2020). The MPs on the filters were observed under a fluorescent light microscope (LEICA DMLS. Vienna, Austria) using blue light excitation (420–495 nm) (Shim et al. 2016). The abundance of MPs was estimated after the verification by NR staining, excluding elements with similar morphology, but not verified as MPs.

Glass, stainless steel material, and a lab coat were used to maintain quality control in the procedures and observations of the samples. All the material was washed with filtered tri-distilled water and dried in an oven (> 70 °C). In addition, filters in open Petri dishes were used detect possible air contamination during all procedures. During the sample digestion process, blank samples were prepared by adding the acid mixture solution into Erlenmeyer flasks without tissue (Wu et al. 2020). The percentage by type, shape, color, and size of MP items found in the samples of the different tissues was calculated: concentration of MPs (items/g) in the tissue (ww) and the average amount of MPs (items/g) in the tissue per individual (according to the weight of the shrimp). A one-way ANOVA (analysis of variance) and a post hoc analysis (Tukey test) were used to analyze the differences among the three analyzed tissues (GT, GI, and EX). Statistical significance was set at $p < 0.05$ (Zar 2010).

Results and discussion

The average length and weight of the examined specimens were 14.6 ± 1.3 cm and 19.2 ± 4.6 g, respectively. 1,066 plastic-like particles were verified as MPs in the 80 examined shrimp; 26.5% were found in the GT, 33.5% in the GI, and 40.0% in the EX. The number of MP items between tissues showed significant differences ($p < 0.05$), in which the GT > GI > EX (Fig. 2a), while the MPs per gram of tissue (ww) in the EX (114.7 ± 33.2) were significantly higher than those in the GI (13.7 ± 5.3) and the GT (3.0 ± 0.5) (Fig. 2b). The abundance of MPs per shrimp in the three tissues was 1.3 ± 1.1 items/shrimp, while the abundance per gram of shrimp (ww) was 0.9 ± 0.2 items/g. Gurjar et al. (2020) found 64.8 ± 24.6, 78.5 ± 48.4, and 47.5 ± 38.0 MPs/g in the GT of *Parapeneopsis stylifera*, *Metapenaeus monoceros*, and *Penaeus indicus*, respectively, from the Arabian Sea. Wang et al. (2020) observed 7.8 MPs/g in the GT and the GI of *Parapenaeopsis* h*ardwickii* from Hangzhou Bay, China. The abundance of MPs in the GT of this study is higher compared to other studies conducted in penaeid shrimp, except for those registered by Curren et al. (2020) in *Fenneropenaeus indicus* (Table 1).

The type of MPs found in our study were mostly fibers (92.3 ± 5.8%), followed by fragments (6.0 ± 5.6%), films (1.0 ± 0.6%), and granules (0.3 ± 0.0%) in the three tissues (Fig. 3a); while the predominant shape of MPs were filaments (95.9 ± 0.9%), followed by sub-angular (2.7 ± 0.3%), irregular edges (1.0 ± 0.4%), and sub-rounded (0.4 ± 0.2%) (Fig. 3b). Fragments and fibers are the most common types of MPs found in marine ecosystems, in which fragments are generally more abundant than fibers (Barrows et al. 2018; Capparelli et al. 2021). MPs fragments could originate from plastic disposal items associated with touristic activities as discarded plastic that breaks down into smaller pieces, while the presence of fibers could be associated with untreated wastewaters that are discharged into coastal areas and waste from fishing.

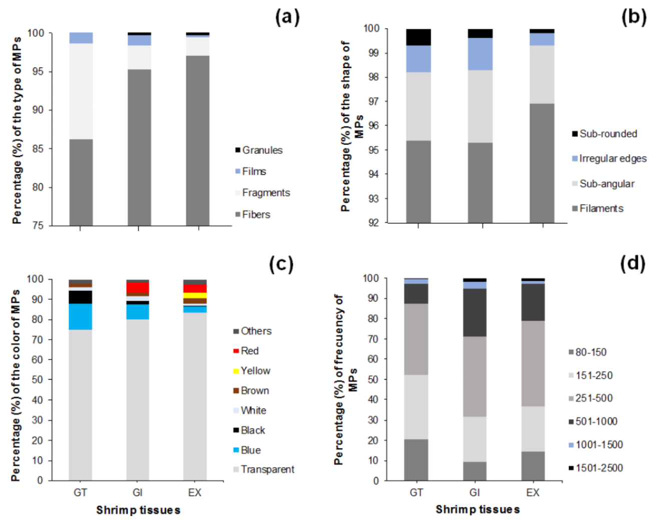

Fig. 3 Type (a), shape (b), color (c), and size (d) of MPs in the GT, GI, and EX of the shrimp *L. vannamei* from the HC coastal lagoon

activities (Valencia-Castañeda et al. 2022). This agrees with the dominance of fibers found in *L. vannamei* from the HC lagoon, as well as with other studies on penaeid shrimp such as *M. monocerous*, *Penaeus monodon*, *F. indicus*, *P. hardwickii*, *Metapenaeus affinis* (Hossain et al. 2020; Daniel et al. 2020; Wu et al. 2020; Keshavarzifard et al. 2021), and with farmed *L. vannamei* (Valencia-Castañeda et al. 2022). Larger plastic pieces in the environment are unable to break down into smaller pieces in a short period (Weinstein et al. 2016) and are unlikely to be ingested by shrimps due to their larger sizes, so we may not be able to find a lot of pellets, films, and fragments in shrimp tissues. Conversely, fibers are relatively smaller and may be easier to ingest.

Seven colors (transparent, blue, red, black, brown, yellow, and white) were observed in MP.

items from the analyzed tissues of *L. vannamei*. The predominant color was transparent (79.4 ± 4.2%), followed by blue (8.0 ± 5.0%), red (5.3 ± 0.9%), black (2.9 ± 3.0), brown (2.0 ± 0.5%),

Fig. 2 Mean (± SD; n = 80) number of MPs per gram of tissue expressed as wet weight (a) number of MPs per tissue (b) of *L. vannamei* from the HC coastal lagoon. Different letters indicate significant differences ($p < 0.05$) among tissues.

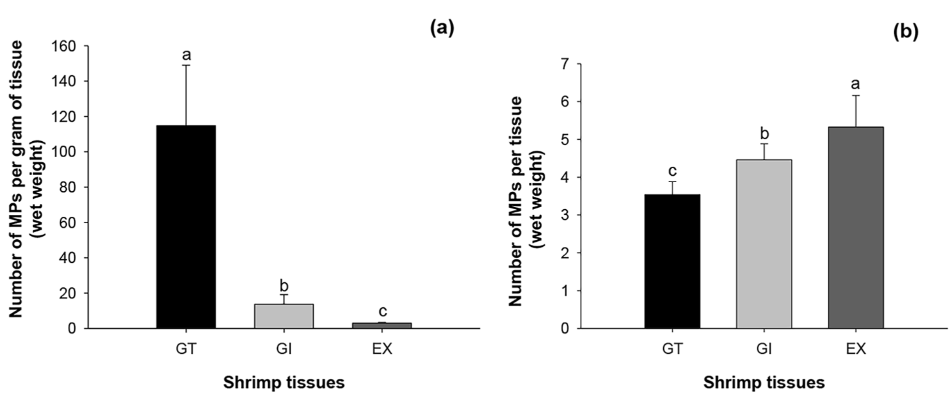

Table 1 Abundance and predominant microplastics in shrimp penaeid species (MU, muscle; EX, exoskeleton; GT, gastrointestinal tract; GI, gills; ---, not available)

Species	Location	Tissues	Abundance		Dominant MPs			References
			Individual	Items/g (ww)	Type	Color	Size (µm)	
Penaeus semisulcatus	Musa estuary, Persian Gulf	EX and MU	7.8	1.5	Filamentous fragments	Black or grey	100–250	Abbasi et al. (2018)
Penaeus semisulcatus	Persian Gulf	MU	---	0.360	Fibers	Black	500–1000	Akhbarizadeh et al. (2019)
Penaeus semisulcatus	Persian Gulf	GI	---	0.773	Fibers	Black	50–100	Akhbarizadeh et al. (2019)
P. monodon	Bay of Bengal,	GT	6.6 ± 2.0	3.40 ± 1.23	Fibers	Black	1000–5000	Hossain et al. (2020)
M. monocerous	Bay of Bengal	GT	7.8 ± 2.0	3.87 ± 1.05	Particle	Black	250–500	Hossain et al. (2020)
F. indicus	Coastal waters off Cochin, India	GT	0.39 ± 0.6	0.04 ± 0.07	Fibers	Red and Blue	500–600	Daniel et al. (2020)
P. hardwickii	Xiangshan Bay, China	MU	0.95 ± 0.28	0.25 ± 0.08	Fibers	---	500 – 100	Wu et al. (2020)
M. monoceros	North eastern Arabian Sea	GT	7.2 ± 2.6	78.5 ± 48.4	Fibers	Black	100–250	Gurjar et al. (2021)
P. stylifera	North eastern Arabian Sea	GT	5.4 ± 2.8	64.8 ± 24.6	Fibers	Black	100–250	Gurjar et al. (2021)
P. indicus	North eastern Arabian Sea	GT	7.4 ± 2.6	47.5 ± 38.0	Fibers	Black	100–250	Gurjar et al. (2021)
P. hardwickii	Hangzhou Bay, China	GT, GI	2	7.8	Fibers	---	500–1000	Wang et al. (2020)
M. affinis	Northwest Persian Gulf	GT	---	1.02	Fibers and film	White or transp.	500–1000	Keshavarzifard et al. (2021)
F. indicus	Indonesia, Eastern Indian ocean	GT	---	5570 ± 100	Spheres	Opaque	10–20	Curren et al. (2020)
L. vannamei	Ecuador	GT	---	13 ± 1	Films	Transp.	---	Curren et al. (2020)
L. vannamei	Malaysia	GT	---	21 ± 4	Films	Transp.	----	Curren et al. (2020)
L. vannamei	Gorgan Bay, Caspian Sea	GT	---	5.7	Fibers	Black	100–500	Bagheri et al. (2020)
L. vannamei	Shrimp farm, Guangdong Province, China	GT	6.3 ± 2.4	14.1 ± 5.7	Fibers	Blue	< 500	Yan et al. (2021)
L. vannamei	HC lagoon, Mexico	GT	3.5 ± 0.3	114.7 ± 33.2	Fibers	Transp.	251–500	This study
L. vannamei	HC lagoon, Mexico	GI	4.5 ± 0.4	13.7 ± 5.3	Fibers	Transp.	251–500	This study
L. vannamei	HC lagoon, Mexico	EX	5.3 ± 0.8	3.0 ± 0.5	Fibers	Transp.	251–500	This study

yellow (1.4 ± 1.7%), and white (1.7 ± 0.6%) (Fig. 3c). The most representative colors in the tissues were transparent (74.8–83.2%) and blue (3.2–13.1%) (Fig. 3c). These colors and the predominance of fibers in environmental samples is a common result in MPs studies, which have been related to fishing nets, ropes, lines, damaged clothing (laundry), and urban wastes. Transparent color in MPs has been reported as the predominant color in shrimp *L. vannamei* from Ecuador and Malaysia (Curren et al. 2020) and in *M. affinis* from the Northwest Persian Gulf (Keshavarzifard et al. 2021). Moreover, MP content has been reported in various organisms including decapod crustaceans. An overview including the uptake of MPs by shrimp penaeid species from different localities is shown in Table 1. In other localities and shrimp species, the color black has been the most recorded, followed by blue and red (Table 1).

The size of MPs varied widely in the three tissues, ranging from 80 to 2500 µm, with an average of 403.3 ± 295.8 µm. The mean size per tissue was 332.4 ± 240.7 µm in the GT, 455.5 ± 316.1 µm in the GI, and 407.5 ± 30.2 µm in the EX (Fig. 2c). However, the highest frequency of MP items for the three tissues was found within the interval 251–500 µm (39.0 ± 3.5%), followed by 151–250 µm (25.4 ± 5.5%) and 501–1000 µm (17.0 ± 7.0%) (Fig. 3d). The shrimp *L. vannamei* is an omnivorous species that can ingest pellets of 700–3000 µm, though the preferred size of juveniles appears to be 124–210 µm (Valencia-Castañeda et al. 2022). Hence, the adult shrimp examined may have predominantly accumulated MP particles that ranged from 251 to 500 µm. Shrimp are bottom feeders, which can increase their exposure to MPs found in the sediment. The detect food using a wide group of chemoreceptors (antennules) (Dall et al. 1990). However, future studies are required to understand the mechanism of capture and selection of MPs (and food) by shrimp in different environments.

The predominant of sizes found in the different tissues of *L. vannamei* from the HC lagoon are similar to those reported in *M. monocerous* (250–500 µm) from the Bay of Bengal (Hossain et al. 2020), as well as farmed shrimp *L. vannamei* (< 500 µm) from Guangdong Province, China (Yan et al. 2021). The present study concludes that the shrimp captured in the HC lagoon exhibited MPs in the three analyzed tissues (GT, GI, and EX), in which the GT presented the highest abundance (114.7 ± 33.2 items/ g) with sizes from 80 to 2500 µm (mean 403.3 ± 295.8 µm). The HC lagoon receives rural, semi-urban, agricultural, and aquaculture discharges that could contribute to the presence and predominance of fibers (92.3 ± 5.8%) and fragments (6.0 ± 5.6%) found in the three tissues, while the predominant colors were transparent and blue.

Crustaceans have an exoskeleton that must be shed to grow. During proecdysis a new cuticle is produced underneath the old one, which is subsequently shed (ecdysis). Calcium is reabsorbed from the exoskeleton just prior to molting, which softens the carapace for it to shed. After ecdysis has occurred, the stored calcium is utilized to harden the new cuticle. Metals can be bound to chitin in the exoskeleton of crustaceans (Keteles and Fleeger 2001). These metals become associated with the calcium in the exoskeleton matrix and may be absorbed into the surface of the exoskeleton or bind to the inner exoskeleton matrix after uptake (Keteles and Fleeger 2001). We hypothesized that MPs could calcify the still soft carapace of the post-molten animal, and remain until the next ecdysis, similarly to what occurs with metals. However, this mechanism needs to be investigated for MPs. Information regarding the effects of MPs on shrimp is scarce. However, Duan et al. (2021) found that in *L. vannamei*, MPs affect the intestinal microbiota and the host´s immunity, while Hsieh et al. (2021) observed that polyethylene MPs can affect antioxidant enzymes, increase lipid peroxide content, and cause tissue (midgut gland and gills) damage.

The ingestion of MPs in humans has been estimated in different countries based on the consumption of some species of fish and shellfish that have documented the presence of MPs. In our study, four conditions were considered to estimate MP intake in humans: (i) the average Mexican consumption of shrimp (1.69 shrimp/capita/year; CONAPESCA 2018); (ii) MP abundance found in our study, which is representative of the shrimp consumed in Mexico; (iii) the effect of cooked on the MPs content is insignificant; and (iv) considering account the least (shrimp is consumed whole) and more (when the GI and EX are discarded) common scenarios. The estimated intake was 1170 and 280 MPs/capita/year for the least and more common scenarios, respectively. This information warns about the possible effects on human health due to the ingestion and exposure to MPs. However, the effects of MPs on human health through the consumption of seafood are poorly understood and require further investigation regarding the implications for human food security, food safety, and health (Barboza et al. 2018).

Detailed quantitative information of MP contamination can aid in conservation actions toward the control of plastic disposal in coastal areas. Considering that, the HC lagoon area is associated to an agricultural land and several villages that border the system, residues are carried into the basin via wind and land runoff becoming an important source of MPs for the lagoon. This first study provides an MP background of contamination in the wild whiteleg shrimp *L. vannamei*, which is useful for future biomonitoring of MPs in a species of great ecological and commercial importance.

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