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Presence of microplastics in macroinvertebrates of mangroves in the Colombian Pacific: are feeding behaviors determinant for its abundance?

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ABSTRACT

Introduction: In mangroves, microplastics (MPs), (< 0.5 mm particles) penetrate deep into the sediments and can cause adverse effects on the species that consume them by accident, affecting their development, nutrition, and life quality in general. In Colombia, studies on plastic and microplastic pollution have been focused on the Caribbean coast and its consumption by fishes, however, the Pacific has been scarcely documented.

Objective: To confirm the presence of MPs in mangrove organisms of two locations of Buenaventura Bay, Colombia, to characterize them, and to estimate their effect on trophic groups.

Methods: Two species of cockles, *Anadara similis* and *Anadara tuberculosa*, and crab *Goniopsis pulchra* were selected. MPs were extracted, counted, and classified according to shape and color.

Results: In total, 81 individuals were captured. We found MPs in 100 % of the organisms and transparent fragments were the most abundant (56.40 %). An average of 25.54 ± 23.8 particles/individual was found, and no differences were found between the cockles, but between each regarding *G. pulchra*.

Conclusion: The different feeding behaviors could explain these differences; more generalist species would ingest a more significant amount of particles. This is the first approximation to studying MPs inside invertebrate organisms in the Colombian Pacific.

Keywords: marine debris; filter feeders; cockles; Buenaventura Bay; mangrove crab.

RESUMEN

Presencia de microplásticos en macroinvertebrados en manglares del pacífico colombiano: ¿comportamientos alimenticios determinan su abundancia?

Introducción: En los manglares, los microplásticos (MPs), (partículas < 0.5 mm) penetran profundamente en los sedimentos y pueden causar efectos adversos en las especies que los consumen accidentalmente, afectando su desarrollo, nutrición y calidad de vida en general. En Colombia, los estudios sobre la contaminación por plásticos y microplásticos se han centrado en la costa del Caribe y su consumo por parte de los peces, sin embargo, el Pacífico ha sido escasamente documentado.

Objetivo: Confirmar la presencia de MPs en organismos de manglares de dos localidades de la Bahía de Buenaventura, Colombia, caracterizarlos y estimar su efecto sobre grupos tróficos.
Métodos: Se seleccionaron dos especies de berberechos, *Anadara similis* y *Anadara tuberculosa*, y el cangrejo *Goniopsis pulchra*. Los MPs se extrajeron, contaron y clasificaron según su forma y color.
Resultados: En total, 81 individuos fueron capturados. Se encontraron MPs en el 100 % de los organismos y los fragmentos transparentes fueron los más abundantes (56.40 %). Se encontró una media de 25.54 ± 23.8 partículas/individuo y no se encontraron diferencias entre los berberechos, pero sí entre cada uno de ellos respecto a *G. pulchra*.
Conclusión: Los diferentes comportamientos alimentarios podrían explicar estas diferencias, las especies más generalistas ingerirían una cantidad más significativa de partículas. Esta es la primera aproximación al estudio de MPs dentro de organismos invertebrados en el Pacífico colombiano.

Palabras clave: desechos marinos; filtradores; berberechos; bahía de Buenaventura; cangrejo de manglar.

INTRODUCTION

Marine litter, defined as "any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment" (Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2019), has been identified as one of the most significant problems for marine ecosystems and a threat to biodiversity (Maghsodian et al., 2022). Plastic litter is of particular concern due to its abundance and persistence in the environment (United Nations Environment Programme, 2021), it is present from the poles to the equator and from the coast to the deep sea (Secretariat of the Convention on Biological Diversity, 2016). In recent decades, minute fragments of plastic litter, called microplastics (MPs), (particles < 0.5 mm; Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2019) have also been found in oceans around the world as a ubiquitous component of marine litter (Andrady, 2011). These can be of primary origin (manufactured to have a microscopic size) or secondary (derived from the fragmentation of macroplastic elements) (Wright et al., 2013).

Plastic litter is more evident on the coasts, where it accumulates due to the action of currents, waves, winds, river drainages, and garbage tossed directly on the beaches. Mangrove forests are significantly different from other coastal areas because they act as a trap and filter for marine litter; large fragments get trapped at forest margins, while smaller traces penetrate deeper into the sediments (Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2019).

Mangroves influence the carbon cycle at the global scale (Kuwae et al., 2015), however, this could be altered due to the effect of plastic litter on plankton and primary production in these ecosystems (United Nations Environment Programme, 2021). According to recent studies, the presence of plastic could cause stress, suffocation, and even the death of mangrove roots (van Bijsterveldt et al., 2021), in addition to this, its presence can lead to death or weakening of fauna, reduction of quality of life and reproductive performance and, malnutrition of organisms that are susceptible to consuming them by accident (Gregory, 2009). The risks increase when talking about invertebrate benthic organisms such as crustaceans, mussels, and bivalves since, due to their feeding styles, they are more likely to ingest MPs in mangrove sediments (John et al., 2021).

Colombia, due to its location, coastal landforms, variety of climates, and different annual precipitation rates, among other factors, can be described as a unique country of global importance for the presence of mangrove ecosystems (Blanco-Libreros & Álvarez-León, 2019). Likewise, the heterogeneous landscapes that surround the Colombian mangroves provide biological corridors for native and migratory fauna, and strategic habitats for reproduction, feeding, refuge, and transit. Mangrove forests support the life of invertebrates such as polychaetes, mollusks, arthropods, fish, reptiles, amphibians, birds, and mammals (Rodríguez-Rodríguez et al., 2018).

At a global level, most of the studies on the impact of plastics and MPs on marine organisms have focused on the entanglement of marine mammals, and other species in net fragment debris abandoned in the benthos, plastic ingestion by birds and turtles is also widely documented around the world (Andrady, 2011). However, the ingestion of MPs by invertebrates of gastronomic importance, such as shellfish, is of special concern, since these are consumed whole and would be a direct route for the consumption of MPs by humans (Smith et al., 2018).

In Colombia, studies on pollution by plastics and MPs have focused on the Caribbean coast (Acosta-Coley et al., 2019; Garcés-Ordóñez, Espinosa et al., 2020; Garcés-Ordóñez et al., 2019; Portz et al. al., 2020), in fish (Garcés-Ordóñez et al., 2022; Garcés-Ordóñez, Mejía-Esquivia et al., 2020; Ory et al., 2018) and, in the impact on the tourism sector (Garcés-Ordóñez, Espinosa-Díaz et al., 2020; Rangel-Buitrago et al., 2018; Williams et al., 2016).

Taking into account that contamination by MPs can generate immediate and long-term impacts on the diversity of species in the trophic chain, this research aimed to confirm the presence of MPs in mangrove invertebrates from two locations in the Colombian Pacific, to characterize them, and estimate their effect on trophic groups.

MATERIAL AND METHODS

Study area: Buenaventura Bay is located on the Central Pacific coast of Colombia (between 3°44’ N & 3°56› N & 77°01’ W and 77°20’ W) (Gamboa-García et al., 2018). It is home to mangrove forests dominated by *Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia germinans*, and *Pelliciera rhizophorae* (Cantera & Blanco, 2001). Two mangrove zones were chosen in this bay (Fig. 1), San Pedro (SP), in the outer part of the Northern end of the bay, (3°49’53.057” N & 77°15’17.817” W) which is used for tourism purposes and is located close to one of the most recognized hotels in the area. And, Punta Soldado (PS), located on the Southern outer edge of the bay (3°48’30.834” N & 77°10’37.002” W), close to the Colombian National Army station.

Sample collection: The samplings in both locations were carried out in March (M) 2022 and August (A) 2022 during low tides. Two species of cockles, *Anadara similis*, and *Anadara tuberculosa*, which are filter feeders, and a species of crab, *Goniopsis pulchra*, which is a detritivore and very abundant in these locations, were selected as study organisms.

We performed manual captures in muddy areas, among the mangrove roots. Handmade traps made from plastic bottles and tuna as bait were also used for crabs. The sacrifice of the individuals was done by freezing, for this, they were wrapped in foil and placed in clean, sealed plastic bags. Crabs were stored in the freezer for 5 hours in covered containers, before wrapping them in foil.

Cross-contamination avoidance: To avoid contamination by external particles of MPs in the laboratory, cotton gowns and nitrile gloves, metal and glass instruments, and containers were used. Also, instruments were washed with distilled water before starting the extraction procedure.

Microplastic isolation and identification: The isolation, identification, and characterization of MPs were performed according to the methodologies mentioned in Lusher et al. (2017) with some modifications. The organisms were washed with distilled water, the length and width of the shells and carapace of the crabs were measured, and the weight of the whole animal and the soft weight were recorded. The individuals were opened with a scalpel and all soft tissues were removed, each tissue was weighed and placed in 250 ml glass

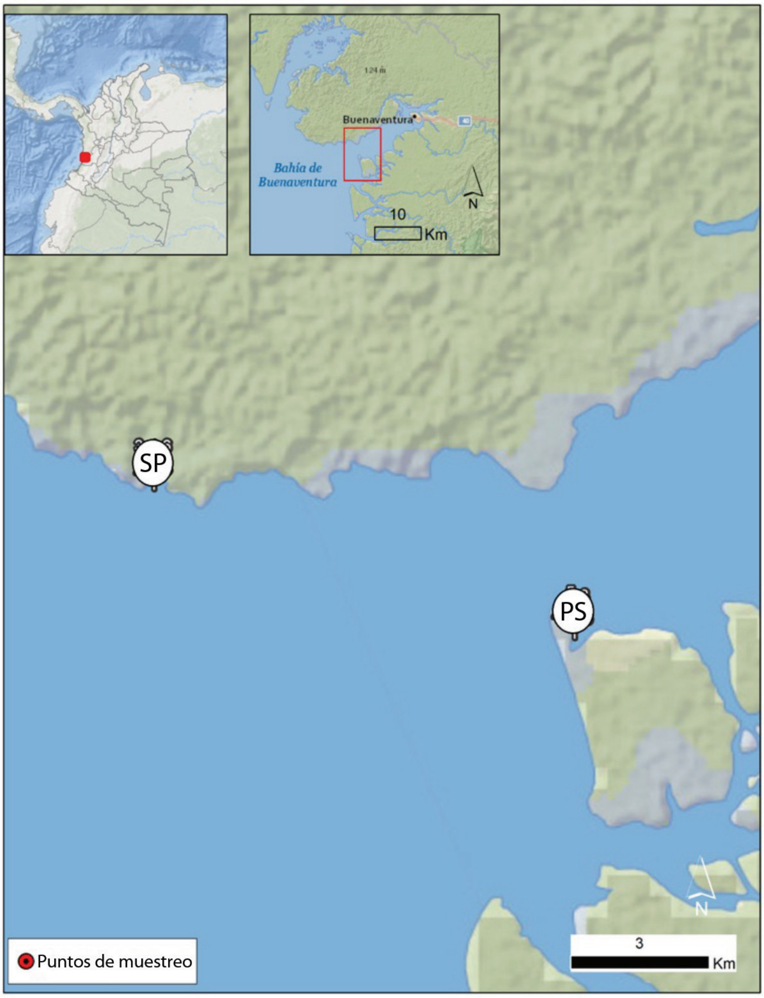

Fig. 1. Map of sites collection within Buenaventura Bay, Colombia. PS = Punta Soldado; SP = San Pedro.

containers. Between 100 and 150 ml of 10 % potassium hydroxide (KOH) was added to each container, depending on the size of the animal, the containers were sealed with foil, and the samples were incubated in an oven at 75 °C for 5 hours.

The digested solution was filtered through Whatman #1 paper (pore size: 11 µm), using a vacuum pump. The filters were placed individually into glass Petri dishes and a visual identification of MPs was made in an optical stereoscope. The MPs were counted, photographed, and classified according to their shape (fibers, spheres, fragments) and color. The maximum length of the particles was also measured.

Data analysis: The percentage of individuals with MPs presence was determined, the number of particles per individual (particles/indiv) and the number of particles per soft weight of the animal (particles/gram) were recorded. Generalized Linear Models (GLM) with a negative binomial distribution were used to determine if these variables differ between species, localities (SP, PS), and sampling

Table 1
Measures and average weights of organisms collected in mangroves from Punta Soldado and San Pedro, Buenaventura.

Species	N	Width (cm)	Lenght (cm)	Total weight (g)	Soft weight (g)
A. similis	25	3.74 ± 0.43	2.70 ± 0.53	12.71 ± 5.83	4.91 ± 2.11
A. tuberculosa	43	4.60 ± 1.12	3.36 ± 0.87	35.64 ± 39.90	11.68 ± 10.60
G. pulchra	13	2.99 ± 0.46	2.53 ± 0.34	11.26 ± 4.06	2.06 ± 0.83
Total	**81**	**4.07 ± 1.06**	**3.03 ± 0.80**	**24.65 ± 31.42**	**8.05 ± 8.75**

cm: Centimeters, g: Grams.

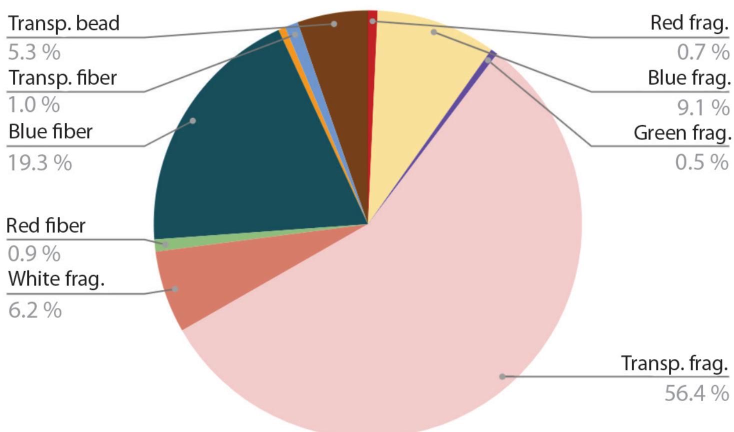

Fig. 2. Abundance and percentages of MPs types by color and form found in collected invertebrates of Punta Soldado and San Pedro, Buenaventura.

months (M, A). The analyzes were carried out in the statistical program R v4.2.1 using the arm, emmeans, and ggplot2 packages (R Core Team, 2021).

RESULTS

In total, 81 individuals were captured. In PS, 66 individuals were collected, and 15 in SP. Table 1 shows the measurements and average weights of each species in both locations.

MPs were found in 100 % of the organisms and a total of 2 085 particles were counted. Transparent fragments were the most abundant (56.40 %), followed by blue fibers (19.3 %). The rest of the types of MPs differentiated by color and shape represent less than 10 % each (Fig. 2). Red and blue fibers, and transparent fragments were the largest types at around 2.8 mm in length, with fragments measuring between 2.45 mm -0.063 mm, fibers between 2.83 mm -0.09 mm and the beads between 0.212 ± 0.094 mm. On average for the total number of individuals, 25.54 ± 23.8 particles/indiv and 4.57 ± 4.1 particles/gram were found. The differences by species in terms of particles/indiv and particles/gram are listed in Table 2. An example of the different MPs found is shown in Fig. 3.

Table 2
Average number of MPs particles present on collected species from mangroves of Punta Soldado and San Pedro, Buenaventura.

	A. similis	*A. tuberculosa*	*G. pulchra*	Total
Aver. particles/indiv	17.8 ± 12.98	35.39 ± 27.56	9.07 ± 37	25.74 ± 23.80
Aver. particles/gram	4.29 ± 4.09	4.65 ± 4.64	4.83 ± 2.26	4.57 ± 4.14

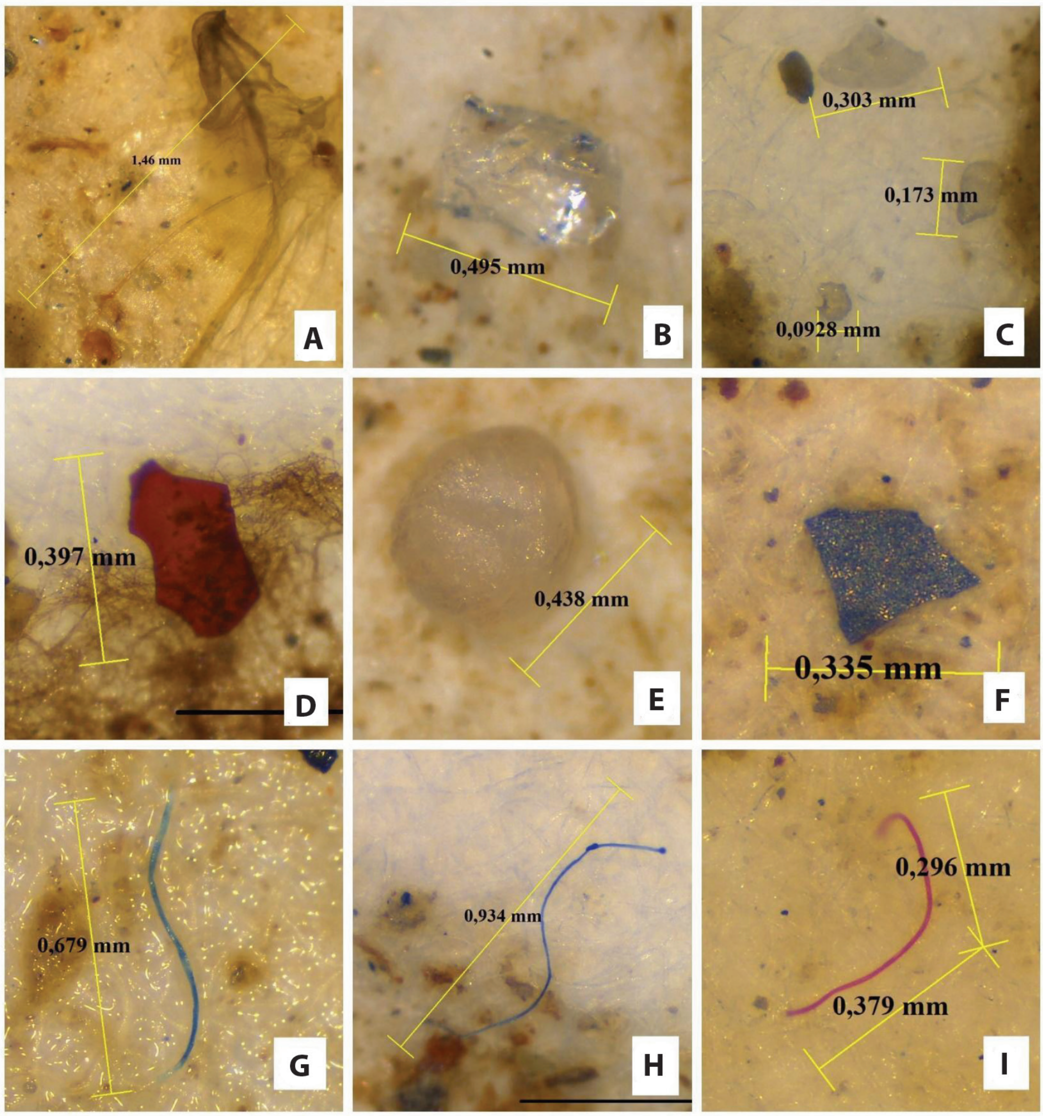

Fig. 3. Microplastics found in organisms collected in mangroves of the Colombian Pacific. Photographs were taken with an optical stereoscope at 4X. **A-C.** Transparent fragments, **D.** Red fragment, **E.** Transparent bead, **F.** Blue fragment, **G-H.** Blue fiber, **I.** Red fiber.

With the GLM it was found that the species and the month (Fig. 4), but not the location, had a significant effect in terms of the number of particles/indiv. Between species, no differences were found between the cockles ($p = 0.0546$), but between each of them with respect to *G. pulchra* ($p = 0.0079$ and $p < .0001$), species with fewer amounts of MPs particles (Fig 5). Regarding the measurements, only the soft weight had a significant effect ($p = 0.00315$) the higher the soft weight, the more MPs particles (Fig. 4). No differences were found between the species or the location in terms of the number of particles/gram (Fig. 6), but in the month

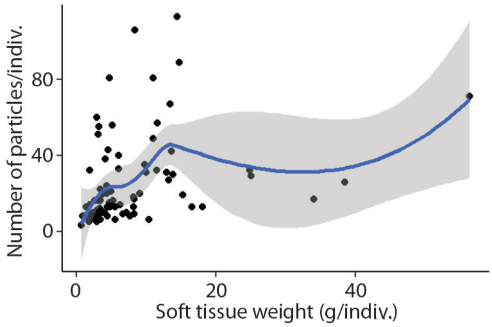

Fig. 4. Lineal regression between soft tissue weight of each individual (g/indiv) and the number of particles/indiv.

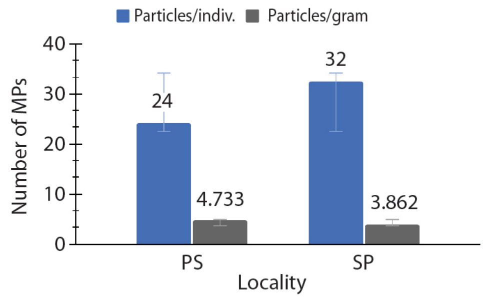

Fig. 6. Average MPs particles found by location presented in particles/indiv and particles/gram of soft weight. PS = Punta Soldado; SP = San Pedro.

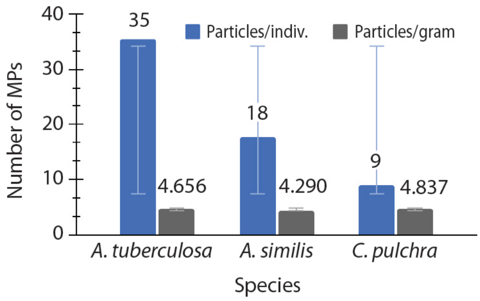

Fig. 5. Average MPs particles found by species presented in particles/indiv and particles/gram of soft weight.

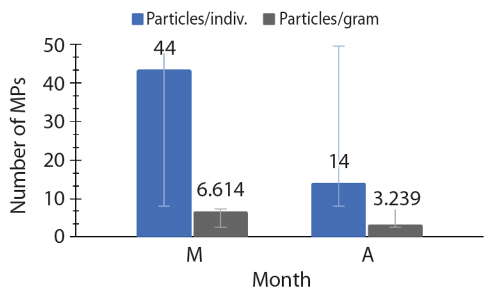

Fig. 7. Average MPs particles found by month presented in particles/indiv and particles/gram of soft weight. M = March; A = August.

(p = 4.96e-07) being M the month in which more particles/indiv and particles/gram was recorded (Fig. 7).

DISCUSSION

This would be a first approximation to the study of MPs inside invertebrate organisms in the Colombian Pacific. Regarding the sizes of the individuals collected, only 12 of the *A. tuberculosa* had a size equal to or greater than the minimum capture size established in Colombia (5 cm) (Díaz et al., 2011).

The highest number of particles/indiv was found in *A. tuberculosa* and, in general, fragments were the most common type of MP. These fragments were mostly transparent, with a smooth surface, the largest (> 1mm) with the appearance of a thin film (Fig. 3A), and the smallest with a solid appearance, and irregular edges. These findings differ from what has been reported for the MPs present in the sediments of Buenaventura (Vásquez-Molano et al., 2021), with what has been found in other *Anadara* species such as *A. antiquata* in Tanzania (Mayoma et al., 2020) and *A. granosa* in Indonesia (Fitri & Patria, 2019; Ukhrowi et al., 2021) and Thailand (Goh et al., 2021) and for other mangrove crab species such as *Chiromantes dehaani* in China (Zhang et al., 2021) and *Metopograpsus quadridentata* (Patria et al., 2020) in Indonesia where the predominant type of MPs were fibers. Most of the particles found correspond to secondary and not primary MPs, as is the case of the beads, which represented only 5.3 % of the total particles (Fig. 2).

A high variety in the colors and shapes of the MPs was found, which could mean a wide range of sources of MPs (Group of Experts on the Scientific Aspects of Marine Environmental

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Protection, 2019). However, to correctly identify the type and origin of the plastic present in the samples, polymer verification analyses should be carried out, which are usually expensive, such as Fourier Transformed Infrared Spectrometry (FT-IR); the Attenuated Total Reflectance (ATR); Raman spectrometry for color pigment spectra; or pyrolysis-gas chromatography combined with mass spectroscopy (Pyr-GC-MS), techniques that analyze particles using their thermal degradation properties (Lusher et al., 2017).

The number of particles/indiv (Table 2) varied greatly but turned out to be one order of magnitude lower than what was found in *A. granosa* (618.8 ± 121.4) (Ukhrowi et al., 2021) (434 ± 97.05) (Fitri & Patria, 2019) (434 ± 97.05). It should be noted that the study of MPs is a relatively new field and the lack of standardized methods is a recurring theme in their research (Nunes et al., 2023), this lack of consistency both in methods and in the use of units to adopt, makes it difficult to compare studies conducted by unrelated researchers and could underestimate or overestimate the apparent impact of MPs on a species or location (Lusher et al., 2017). However, it has been found that, in general, benthic species have higher MPs values compared to pelagic species, which could be related to direct contact with sediments, which are considered MPs sinks (Nunes et al., 2023). Due to the above, it would be expected to find lower amounts of MPs in species that do not inhabit the mangroves.

Regarding the differences between cockles and the crab (*G. pulchra*), they could be explained by the different modes of feeding, less selective species, such as cockles that are filter feeders, ingest a greater amount of particles, which agrees with what was described by, Not et al. (2020), they found that the abundance of MPs in stomachs of mangrove crabs was related to its role in the trophic web and their eating habits, the most generalist species being those that present a greater abundance and diversity of MPs.

The size of the MPs depends mainly on those feeding behaviors, the MPs are transmitted directly or indirectly by consuming prey contaminated with them. It is important to mention that MPs could be expelled through excretion or remain in the organism for a long time (Maghsodian et al., 2022), which is unknown in the species studied here. In this case, the prevalence of MPs for the three species was 100 %, which could indicate inadequate management of wastes in seawater, wastewater discharge, coastal tourism, and fishing in this region. Precisely, Riascos et al. (2019) reported the mangrove forests that surround the city of Buenaventura as one of the most polluted coastal areas in the world.

Speaking particularly of the cockles, these are of great cultural and gastronomic importance for the Pacific region of Colombia. They are traditionally exploited by Afro-descendant communities settled in the vicinity of the mangroves, being an important source of protein in their diet and for their livelihood through their commercialization (Díaz et al., 2011). The presence of MPs could pose a risk to the food security of these communities since they are a threat not only to the survival of the species but also due to their ability to absorb organic pollutants. In fact, precisely because their high capacity to absorb MPs is well known, bivalves have been frequently used as indicators of contamination by MPs (Li et al., 2015).

This study represents the first contribution to the characterization of microplastics within invertebrate organisms in the Colombian Pacific. The results indicate that the studied organisms are consuming the plastic debris that accumulates in the mangroves of Punta Soldado and San Pedro, in Buenaventura Bay, since all the individuals had particles inside. Statistical analyzes showed interspecific differences in the number of MPs within the individuals, with the most generalist species being the ones that presented a greater number of particles, which could be due to feeding habits. Studies such as this allow for establishing the basis for designing waste management plans in the region, with species such as cockles as the main focus for being an essential resource for the economy and gastronomic culture in the Colombian Pacific.

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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