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# Prevalence of microplastics in Peruvian mangrove sediments and edible mangrove species



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#### ABSTRACT

Mangrove ecosystems have been hypothesised as a potential sink of microplastic debris, which could pose a threat to mangrove biota and ecological function. In this field-study we establish the prevalence of microplastics in sediments and commercially-exploited *Anadara tuberculosa* (black ark) and *Ucides occidentalis* (mangrove crab) from five different zones in the mangrove ecosystem of Tumbes, Peru. Microplastic were evident in all samples, with an average of 726  $\pm$  396 microplastics/kg for the sediment, although no differences between the different zones of the mangrove ecosystem were observed. Microplastic concentrations were  $1.6 \pm 1.1$  items/g for the black ark and  $1.9 \pm 0.9$  microplastics/g for the mangrove crab, with a difference in the microplastic abundance between species (p < 0.05), and between the gills and stomachs of the crab (p < 0.01). Human intake of microplastics from these species, for the population in Tumbes, is estimated at 431 items per capita per year. The outcomes of this work highlight that the mangrove ecosystem is widely contaminated with microplastics, presenting a concern for the marine food web and food security.

#### 1. Introduction

Marine plastic is a global pollutant that can have negative impacts on ecosystems, ecosystem services, the economy and society (Clark et al., 2016; Iroegbu et al., 2021; Napper and Thompson, 2019). Of widespread environmental concern are microplastics, describing small plastic particles (0.1 um - 5 mm diameter) that are either intentionally manufactured to be microscopic in size, or derive from physical, chemical and biological fragmentation of macroplastics (Cole et al., 2011; John et al., 2022; Maghsodian et al., 2022). Given their small size, microplastics can be consumed by an array of marine biota, exposing them to plastic-associated chemical compounds such as UV stabilizers, pigments, antioxidants, plasticizers and flame retardants (Crawford and Quinn, 2017; Hamilton et al., 2022; Kühn et al., 2020). There is widespread evidence that microplastics can cause adverse health effects in exposed organisms (Doyle et al., 2022).

Coastal mangroves are "blue carbon" ecosystems valued for their high productivity, biodiversity and their role in carbon sequestration (Alongi, 2020; Hilmi et al., 2021; Rovai et al., 2022; Taillardat et al., 2018). However, the ecosystem services provided by mangroves are at risk from anthropogenic activities and pollution, such as plastic (Deng et al., 2021). Field studies have demonstrated that macroplastic debris can become entrapped in aerial roots or the interior scrub zone of the mangrove (Garcés Ordóñez et al., 2019; Meera et al., 2022). Further, there is some indication that microplastics can accumulate in underlying sediments and be ingested by or adhere to the gills or appendages of a wide range of aquatic organisms, including commercially exploited invertebrate species (Cordova et al., 2021; Maghsodian et al., 2022).

In Peru, the Tumbes mangroves span 5974 ha from the Ecuadorian border (International Channel) to Playa Hermosa (Tumbes) providing refuge to an array of commercially exploited species (INRENA, 2007). In the Rural and Tourist Central Zone, mangroves have been removed to allow for shrimp farms and rural urbanisation, with beaches and tourism in the crocodile (*Crocodylus acutus*) vivarium being major anthropogenic influences of plastic (INRENA, 2007). The mouth of Tumbes river is located in the Lower Zone, (Fig. 1); here the river water is highly contaminated by heavy metals such as lead, exceeding water quality

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Fig. 1. a. Location of field sampling sites for the collection of sediments, mangrove crab (*Ucides occidentalis*) and black ark (*Anadara tuberculosa*). b. (A) The Tumbes mangrove ecosystem and their principal commercial species: (B) the black ark (*Anadara tuberculosa*) and (C) the mangrove crab (*Ucides occidentalis*).

standards (from 0.05 mg/L to 0.3174 mg/L), adversely affecting the inhabitants and the native fauna in this area (Gavilanez Garcia, 2016). Anthropogenic activities (e.g. aquaculture, tourism) can adversely impact on the mangroves (Dioses Puelles, 2020). As such, inside the Channel Upper Zone and Coastal Upper Zone (Fig. 1), the National Sanctuary Los Manglares (SNLMT) was created, under the RAMSAR Convention on Wetlands of International Importance Especially as Waterfowl Habitat 1997 (Angulo, 2014; INRENA, 2007; Martínez, 2021).

The edible invertebrates Anadara tuberculosa (black ark) and Ucides occidentalis (mangrove crab) are of highest commercial value in Tumbes mangroves and support the nutrition and socio-economic wellbeing of inhabitants in the region (INRENA, 2007). A. tuberculosa are filterfeeding bivalve shellfish with distinctive dark hairy periostracum that live buried at a depth of 1-20 cm in muddy-silty sediments in the low intertidal zone associated with the red mangrove Rhizophora mangle (Lazarich Gener et al., 2008) (INRENA, 2007) (Fig. 1b.B). U. occidentalis is a detritivorous crab that feed on R. mangle leaves and organic matter in the mangrove sediment, which burrow in muddy-silty sediments below red mangrove roots in the high intertidal zone (Alemán and Ordinola, 2017; Cabanillas et al., 2016; Ordinola et al., 2010; Zambrano and Meiners, 2018) (Fig. 1b.C). The black ark and mangrove crabs are manually extracted by fishermen, commonly called "concheros" and "cangrejeros", in a sustainable manner which is recognised as part of the 'Cultural Heritage of the Nation' in Peru since 2022 (N° 000036-2022-VMPCIC/MC) (Ordinola et al., 2019; Martínez, 2021). These mangrove species are typically eaten whole (i.e. gills, digestive tract is consumed) in traditional dishes such as black ark ceviche, crab carapace, crab chupe and Tumbesian majarisco (Martínez, 2021). Aguirre-Sanchez et al. (2022) evidenced the presence of microplastics in the gills and digestive tract of 30 mangrove crabs collected from local markets in the city of Tumbes, noting this may pose a risk to food security (Caruso, 2019). For this reason, the primary objective of this field-study is to establish the prevalence of microplastics in sediments and commercially-exploited A. tuberculosa and U. occidentalis of the mangrove ecosystem of Tumbes, Peru, and estimate dietary exposure to microplastics in local populations.

#### 2. Materials and methods

#### 2.1. Sampling design

In November 2021, mangrove crabs (*U. occidentalis*), black arks (*A. tuberculosa*), and mangrove sediment were collected from 22 sampling sites located throughout the mangrove ecosystem of Tumbes (Fig. 1; S3). The sampling sites were located within five different geographical zones: 1) The Channel Upper Zone, located in the National Sanctuary and close to the International Channel between Peru and Ecuador; 2) the Coastal Upper Zone, located between the shore and the National Sanctuary with shrimp farms in the area; 3) the Rural Central Zone, located in an area with only shrimp farms in it; 4) the Tourist Central Zone, located in Puerto Pizarro bay near the crocodile vivarium being the most touristic zone in the Peruvian mangrove ecosystem; and 5) the Lower Zone which is located in the mouth of Tumbes river.

Invertebrates were collected using the Cultural Heritage of the Nation technique (Peruvian article No. 000036-2022-VMPCIC/MIC). At each site 3 specimens of each species were collected between the tidal channel and mangrove canopy. Mangrove crabs (45 individuals across 15 sites) and black arks (42 individuals across 14 sites) were stored in a cooler covered with aluminium foil, and transported to the IMARPE coastal laboratory for further processing.

Discrete sediment samples (750 cm<sup>3</sup>) were collected from the top 5 cm of intertidal sediment between the shore and canopy (n = 22) using a stainless-steel shovel. Sediment samples were stored in a handmade aluminium box (R. Li et al., 2020), and transported to the IMARPE coastal laboratory where the pH of the samples was evaluated using a

portable potentiometer (HI99121/Hanna) at a controlled temperature (22 °C). The average pH of the sediment from every zone was 6.5  $\pm$  0.6. Sediments were stored at 0 °C until further treatment.

## 2.2. Digestion of the soft tissue and isolation of microplastics for the mangrove crab (Ucides occidentalis) and the black ark (Anadara tuberculosa)

In the laboratory, soft tissues were excised using stainless-steel tweezers, scissors and scalpel and their wet-weight recorded (Ohaus Adventurer AR3130 analytical balance). The gills and stomachs of the mangrove crabs and the soft tissue of the black arks were individually wrapped in aluminium foil and stored at 0 °C prior to microplastic extraction (Naji et al., 2018). Potassium hydroxide (KOH) is a strong base that can effectively remove biological material without damaging microplastics (Dehaut et al., 2016; Kühn et al., 2017; Lusher et al., 2017). In this study, soft tissues were placed in 50 mL Falcon tubes with 50 mL of 10 % KOH, maintained in an incubator (INCUCELL 111/MMM Medicenter) at 60° for 48 h, intermittently manually shaking the tubes to facilitate digestion (Aguirre-Sanchez et al., 2022). Subsequently, the supernatant was filtered through a 45 µm nylon mesh filter (47 mm diameter). Owing to the muddy habitat in which the invertebrates live. sediment was found in all samples. Therefore, digests underwent density-separation, whereby digests were rinsed back into their original Falcon tube with 25 mL of 1.2 g/mL sodium chloride (NaCl), shaken manually and then left to rest for 20 min. Finally, the supernatant was filtered through a nylon mesh filter with 45 µm The filter was immediately placed in a labelled Petri dish and sealed with Parafilm (Coppock et al., 2017).

#### 2.3. Processing the matrix: Sediment

To separate microplastic from mangrove sediment, a multi-step protocol was applied. First, sediment samples were dried at 50 °C for 72 h (Coppock et al., 2017), due to the presence of clay and silt in the sediment, it had to be manually ground using a mortar and pestle to homogenise the sample. Next, 40 g of dry sediment was digested in 50 mL of 20 % KOH at 60′C for 48 h. Then the supernatant was filtered through a 45 m nylon mesh filter. Per the density-separation protocol of Coppock et al. (2017), the digested sediment was added to a Sediment-Microplastic Isolation (SMI) unit with 700 mL of brine. For this study, the brine was hypersaline NaCl solution (1.2 g/cm3), which is both cost-effective and environmentally benign, with the capacity to float-out commonly used polymers including polypropylene, polyethylene and polystyrene. Following density-separation, the upper layer of the brine was filtered sequentially through 300  $\mu$ m and 45  $\mu$ m nylon mesh filters, and filters then stored in a Petri dish sealed with Parafilm.

#### 2.4. Visual identification

A stereomicroscope (Olympus SZX16) was used to visualise putative microplastics on each mesh filter. Putative microplastics were identified based on their shape, colour and size. For each particle, their shape, size and optical properties (colour) was recorded (Lusher et al., 2020). Particle shape were classified as foam, film, fibre, pellet, microbead and fragment (Kovač Viršek et al., 2016). Particle size (longest dimension) was measured using CellSens(R) (Olympus). Given weather and KOH digestions can result in colour leaching (Bråte et al., 2018), particle colours were categorised broadly (e.g pink, violet, lilac as red) (Lusher et al., 2020).

#### 2.5. FT-IR analysis

Following morphological and optical classification, a randomly selected subsample (N = 213) of the putative particles were selected for polymeric analysis. Per Cole et al. (2023), putative microplastics were

placed on pre-labelled divots on aluminium-coated slides, and analysed using Fourtier-transform infrared (FT-IR) spectroscopy (Spotlight 400, PerkinElmer) at Plymouth Marine Laboratory (UK). Scans were performed in reflectance mode (4000–600 cm-<sup>1</sup>; 10 scans). Resultant spectra were compared with in-house and commerically-available polymer libraries with matches of  $\geq$ 65 % considered acceptable (Fu et al., 2021). Owing to the challenges of differentiating natural and anthropogenic cellulose, particles with spectra matches to cellulosic materials were considered plastic if their colour was red or blue. The percentage of putative microplastics confirmed to be anthropogenic polymers was 60 %, and this value was used as a conversion rate to calculate microplastic concentrations based on the number of putative microplastics identified in each sample.

#### 2.6. Quality assurance (QA) and quality control (QC)

Microplastics contamination can stem from airborne deposition, clothing and consumer products (Henry et al., 2019; Jarosz et al., 2022; van Wezel et al., 2016). To minimise potential contamination of samples, and account for any contamination that may occur during sampling and processing a number of steps were taken to limit contamination during sampling, use of protective equipment made of cotton was encouraged. In the laboratory and field, the majority of apparatus and storage vessels were replaced with glass or metal, and samples covered with aluminium foil (Prata et al., 2021). To prevent airborne contamination, all laboratory equipment was washed, rinsed with 70 % alcohol and stored in a laminar flow hood, and rinsed three times with filtered distilled water before used and between the samples (Li et al., 2022; Prata et al., 2021). All solutions (e.g. KOH, NaCl) were filtered through a 0.5 um glass fibre filter and stored in clean glass bottles prior to use (Prata et al., 2021). Lastly, procedural blanks and open filters (comprising 45 µm nylon mesh filter) were used to account for contamination. Analysis of blanks showed an average of 2±2 microplastics per sample, with a detection limit of the method of the minimum size of plastic that can be detected is 1.1  $\mu$ m, and this metric was used to adjust microplastics concentrations in samples accordingly.

## 2.7. Human exposure to microplastics assessed by shellfish consumption in Tumbes

The annual intake of mangrove crab and black ark per capita in Tumbes region was obtained using the following formula (Eq. (1)), with a regional population of 179,900 inhabitants aged 10 years (INEI, 2018) and an annual extraction for direct human consumption of 31.4 tons for mangrove crab and 12.7 tons for black ark (Ordinola Zapata, 2022). Subsequently, the average number of microplastics in soft tissue was used to estimate the approximation of human intake of microplastics from *U. occidentalis* and *A. tuberculosa* (Eq. (2)).

Intake of shellfish per year per capita (g/inhabitant)

$$=\frac{annual \ production \ of \ shellfish \ (g)}{number \ of \ inhabitant \ in \ the \ region \ (inhabitants)}$$
(1)

Micropastic intake by human per year per capita (MP items/year/capita) = average of MP items in the soft tissue of the organism (MP items/g) x intake of shellfish per year per capita (g)

#### 2.8. Statistics

The normal distribution of the data and the homogeneity of variances were verified using Shapiro-Wilk and Levene's test. Non- parametric data was transformed using log. The non-parametric data of the microplastic concentration between species and tissues in the mangrove crab was compared using Mann-Whitney-Wilcoxon test. Two-way ANOVA and post hoc Tukey test was used to compare the average number of microplastics per species among the different zones of the Tumbes mangrove and one-way ANOVA and Kruskal-Wallis tests were used to compare microplastic abundance in the sediment, black ark and mangrove crab among the zones. The Pearson correlation analysis was used to calculate the correlations between variables. Statistics were executed in RStudio program (R Core Team, 2020).

#### 3. Results

#### 3.1. Microplastic abundance in the mangrove crab and the black ark

In the black ark, microplastic concentrations were  $1.56 \pm 1.12$  items/ g (4.9±2.18 items/ind). In the mangrove crab, microplastic concentrations were  $1.94 \pm 0.87$  items/g (7.8  $\pm$  3.14 items/ind). Across all sites the mangrove crabs showed significantly greater microplastic concentrations in their gills and stomach than observed in the soft tissues of the black ark (Mann-Whitney-Wilcoxon, *p*-value = 0.02; Fig. 2A). Microplastic averages differ significantly between species (two-way ANOVA, *p*-value = 0.01; Tukey post hoc, *p*-value = 0.01) but not between zones (*p*-value = 0.19) or in the interaction between species and zones (*p*value = 0.3362). Microplastic concentrations in the crab gills were 4.1  $\pm$ 2.41 items/g, which is significantly greater than the 0.8  $\pm$  0.7 items/g



**Fig. 2.** (A) Microplastic abundance (items/g) between the mangrove crab and the black ark differ significantly (Mann-Whitney-Wilcoxon, p-value = 0.02). (B) Microplastic abundance between the tissues of the mangrove crab differ significantly (Mann-Whitney-Wilcoxon, p-value =  $8.47e^{-12}$ ).

(2)

observed in the crab stomach (Mann-Whitney-Wilcoxon test, *p*-value =  $8.47e^{-12}$ , S1, S2, Fig. 2B). No significant difference was evident between the abundance of microplastic particles (items/g) for the black ark among different zones of the Tumbes mangrove ecosystem (Kruskal-Wallis, p-value = 0.18)(Fig. 3A) or for the mangrove crab among different zones (Kruskal-Wallis, p-value = 0.97) (Fig. 3B).

#### 3.2. Microplastic abundance in mangrove sediments

Microplastics were identified in all sediment samples tested with concentrations ranging  $480 \pm 342.5$  to  $870 \pm 591.9$  items/kg. No significant differences between the abundance of microplastic particles (items/kg) was evident among different zones of the Tumbes mangrove ecosystem (one-way ANOVA, p-value =1.02) (Fig. 3C).

### 3.3. Relationship between microplastics in the sediments and in the invertebrates

No significant linear relationship were detected between the abundances of microplastics in the sediment and in the mangrove crab (Pearson: R = -0.02; p-value >0.05) and in the black ark (Pearson: R = 0.03; p-value>0.05). The abundances of microplastics in the in the black ark also demonstrate no significant relationship with the abundances in the mangrove crab (Pearson: R = 0.07; p-value >0.05).

#### 3.4. Putative microplastic characteristics

Putative microplastics comprised microbeads, fragments, fibres and films (Fig. 4a). Fibres and films were the most common categories of microplastics in the black ark (80.6 %, 11.1 %), mangrove crab (82.3 %, 6.6 %) and sediment (33 %, 63 %) (Fig. 4b).

The particle sizes were binned into four size classes: 1-249 m, 250–499 m, 500–999 m and 1000–5000 m. Putative microplastics in the size class of 1-250 m were most prevalent in the black ark (36.6 %), mangrove crab (30.9 %) and sediment (85.6 %). Larger microplastics (>500 m) were the least prevalent size classes in biota and sediments (Fig. 4c).

Putative microplastics in both invertebrates and the sediment were predominantly transparent (black ark: 45.7 %, mangrove crab: 55.7 %, sediment: 22.7 %), blue (black ark: 32 %, mangrove crab:27.1 %, sediment:8.1 %) and red (black ark: 12.6 %, mangrove crab:8.5 %, and sediment:10.9 %) (Fig. 4d).

The polymers found in the subsamples were Cellophane, Polyethylene (PE), Polyester (PL), Polyvinyl chloride (PVC), Polystyrene (PS), Polyacrylamide (PAM), Polypropylene (PP), Enzacryl polyacetal, Phenol resin, Nylon (NY-Poly caprolactam), Polyacrylic acid (PAA) and Polyacrylonitrile (PAN) (Fig. 5).

#### 3.5. Estimated intake of microplastics by Tumbes inhabitants

The ingestion of microplastics by local inhabitants from consuming black ark and mangrove crab was estimated as 431 microplastics/capita/year (Table 1).

#### 4. Discussion

#### 4.1. Sediment

This study provides the first data relating to microplastics in Peruvian mangrove ecosystems, with microplastic concentrations of 726±395.9 items/kg sediment. In Peru, few studies have addressed the presence of microplastics in coastal sediments (Canchari Madueño and Iannacone, 2022; Dávila and Vasquez, 2021; De-la-Torre et al., 2020; Iannacone et al., 2020; Losno Prado, 2020; Manrique Muñante, 2019; Purca and Henostroza, 2017; Contreras et al., 2022; Zárate and Iannacone, 2021). On sandy beaches, the highest abundance of microplastics

per square meter was found in the Lima region (between 43 and 522 items m<sup>2</sup>) (Purca and Henostroza, 2017, 2017; Contreras et al., 2022; De-la-Torre et al., 2020), and the highest abundance of microplastics per kilogram of sediment was found in Carpayo beach with 202.02  $\pm$  10.28 particles/kg (Zárate and Iannacone, 2021). Elsewhere, Canchari Madueño and Iannacone (2022) found microplastic concentrations of 567 microplastics/kg in the irrigation channel of Yuncaypara, Manrique Muñante (2019) identified 90 items/kg from the sediments located in the mouth of the river Jequetepeque, and Losno Prado (2020) found 64 items/kg in Los Pantanos de Villa wetland mud. As such, the concentrations found in the mangroves is higher than observed elsewhere in Peru, suggesting the mangroves are accumulating microplastics. Mangrove roots and pneumatophores promote sedimentation of suspended particles without resuspension (Cozzolino et al., 2020), and we hypothesise this effectively supports the deposition and degradation of plastic particles in the sediment (Komiyama et al., 2008; Maghsodian et al., 2022; Martin et al., 2019).

The microplastic abundance in this study (726 $\pm$ 395.9 items/kg) was below those reported in mangrove sediments collected from Indonesia - 2358 items/kg (Hastuti et al., 2019), Malaysia - 3500 items/kg and 4000

 $\pm$  29.174 items/kg (Hamid et al., 2020; Tan and Mohd Zanuri, 2023), China with the highest abundance of 7900 items  $kg^1$  (Zuo et al., 2020), Colombia with a maximum concentration of 2745  $\pm$  1978 items/kg (Garcés Ordóñez et al., 2019) and Brazil with 10,782  $\pm$  7671 items/ kg (da Paes et al., 2022). However, our microplastics concentrations were greater than reported for mangrove ecosystems in Iran (34.5 items/kg) (Naji et al., 2019) and Singapore (62.7  $\pm$  27.2 items/kg) (Mohamed Nor and Obbard, 2014). Differences in microplastic concentrations may stem from differing levels of human activities in the study area, riverine catchment and connected via oceanic currents, the ubiquity of plastics used in the local area, as well as the methods used to isolate and identify microplastics. Sampling within mangroves is challenging owing to the protected status of such ecosystems, difficulties in accessing sampling sites and the complexity of isolating microplastics from fine sediments (Garcés Ordóñez et al., 2019; Duan et al., 2021; Maghsodian et al., 2022).

Within this study, there was no significant difference in microplastic concentration between the different zones of the Tumbes mangrove ecosystem (Fig. 4C). This is likely due to a high degree of anthropogenic pressure throughout the entire mangrove. The inadequate management of plastic in the National Sanctuary has previously been evidenced during the cleaning of the mangrove carried out by Conservación Internacional Peru (2023), where plastic bottles and bottle caps were widely evident. The upper zone is prone to waste stemming from the international channel between Peru and Ecuador, where the currents can carry litter dumped from both sides of the border (Montaluisa Balcázar and Sánchez Cuenca, 2021). In the Coastal Upper and Rural Central Zones shrimp aquaculture has resulted in the introduction of fishing nets, foam buoys, paddlewheels and nursery cages into the area (Lusher et al., 2017). Such plastic equipment can become abraded and fragmented via chemical, physical and biological processes, leading to the release of secondary microplastics into the mangrove ecosystem (Lin et al., 2022). The Tourist Central Zone, which had the highest microplastic concentration of 933±364 items/kg hosts busy shipping ports and tourist hotels and beaches (Morán and Hidalgo, 2018). Previous studies have demonstrated that the bay of Puerto Pizarro in the Tourist Central Zone is polluted with other marine contaminants, with organophosphate levels in the sediment and water exceeding the Maximum Permissible Limits (Moran Avila, 2017). Similarly, in the Lower Zone overuse of insecticides, pesticides, and herbicides on plantain and rice farms in the river catchment have led to heightened metal and chemical concentrations in the Tumbes estuary (Tineo Nuñez and Periche Viera, 2019).

The presence and abundance of microplastics in the sediments can influence the levels of pH in the mangrove soil, increasing it



#### (A) Microplastics from the black ark (items/g) between the five different zones

**Fig. 3.** (A) Microplastic median items/g (median  $\pm$ SE) from the black ark among the zones did not differ significantly (Kruskal-Wallis, p-value = 0.18). (B) Microplastic median items/g (median  $\pm$  SE) from the mangrove crab did not differ significantly between zones (Kruskal-Wallis, p-value = 0.97) (C) Microplastic average items/kg (mean  $\pm$  SD) from the sediments among the zones did not differ significantly between zones (one-way ANOVA, p-value = 1.02).

Coastal Upper

Rural Central Zone Tourist Central

Lower

0

Channel Upper

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Fig. 4. (a) Putative microplastic type according to their shape: A. blue microbead, B. green fragment, C. blue fibre and D. green film. Proportion of microplastic according to their (b) shape, (c)size and (d)colour. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Maghsodian et al., 2022; Zhao et al., 2021). Polyethylene foams and films had a direct effect on increasing soil pH (Zhao et al., 2021). However, the presence of plants in the mangrove soil potentially mitigates the effects of microplastics on soil pH, this attributed to the increase in aeration and porosity of the substrate when these polymers were adhered; likewise, the leaching of the additives that the microplastic presents alters the microbiota and therefore an increase in pH occurs (Lozano et al., 2021; Zhao et al., 2021). Despite obtaining a total of 716  $\pm$  395.9 items/kg, the pH was 6.54  $\pm$  0.57 due to the presence of *R. mangle* roots in the sampling areas. This was also observed in the results of Maghsodian et al. (2021), where pH was inversely proportional to microplastic abundance, with higher particle abundance when the pH was 6.86 compared to when it was 10.

#### 4.2. Mangrove and estuary crabs

The pathways for microplastic uptake in crustaceans are the ventilation system (gills) and ingestion (stomach) (Daniel et al., 2021). In the present study there was a significant difference between the microplastic abundance found in both tissues with a mean of  $4.1 \pm 2.41$  items/g for the gills and  $0.8 \pm 0.7$  items/g for the stomach. The higher concentrations on the gills may be a result of prolonged retention times as compared with the intestinal tract (D'Costa, 2022; Villegas et al., 2021). In laboratory studies, the crab *Uca rapax* retained polystyrene fragments on their gills for two months (Brennecke et al., 2015), and the crab *Carcinus maenas* retained polystyrene microspheres microplastics on their gills for 21 days (Watts et al., 2014).

Feeding studies with crabs indicate microplastic abundance may be related to the feeding behaviour of the organism (D'Costa, 2022; Not et al., 2020; Villegas et al., 2021; Zhang et al., 2023). Among the commercial mangrove and estuary crabs, the omnivorous Metopograpsus quadridentatus has been reported with the highest microplastic abundance (33 items/g - 327.56 items/ind) (Mufti et al., 2020), while the herbivorous crab Chiromantes dehaani consumed the least amount of microplastic (0.74-4.96 items/ind) (Zhang et al., 2021). In Latin America, U. occidentalis has less items of microplastics in the gills and stomachs than C. angulatus and L. uruguayensis (Truchet et al., 2022). In contrast, N. granulata, C. sapidus and M. mercenaria presented lower values than U. occidentalis (Villagran et al., 2020; Capparelli et al., 2022) (Table 2). Although, there was no correlation between the number of microplastics in the mangrove crab and the sediment (Pearson: R = -0.02; *p*-value >0.05), there's a possibility that *U. occidentalis* interact with the presence of microplastics in the upper intertidal sediment from the Coastal Upper Zone, Channel Upper Zone, and Tourist Central Zone (Díaz-Jaramillo et al., 2021; Truchet et al., 2022) (S1 and S2A-C).

*U. occidentalis* is a detritivorous species that mainly ingests decaying leaves from the mangrove *Rhizophora mangle* (Ariza Gallego et al., 2023; Not et al., 2020; Zambrano and Meiners, 2018) and therefore microplastics within their stomachs likely stem from those found in the sediment and adhered to mangrove leaves. It has been shown that microplastics can be transferred through the food chain in different environments (Gutow et al., 2016; Chae et al., 2018; Chae and An, 2020). For example, in terrestrial food chain microplastics transferred from the sediments to a primary producer *Vigna radiata* and then to the snail *Achatina fulica* (Chae and An, 2020), in marine food chain from the seaweed *Fucus vesiculosus* to the periwinkle *Littorina littorea* (Gutow et al., 2016) and in freshwater food chain from microalgae *Chlamydomonas reinhardtii* to *Daphnia magna* and finally to the fish *Zacco* 



3500 2000 1000 Wavenumbers (cm<sup>-1</sup>)

500

Fig. 5. FT-IR spectra from fibres showing (A) Polypropylene, (B) Polyester and (C) Cellophane spectrum.

1500

#### Table 1

Estimated human intake of microplastics from the consumption of mangrove crab (Ucides occidentalis) and black ark (Anadara tuberculosa) from Tumbes region.

3000

2500

4000

	Tumbes Region General population ( $\geq 10$ years)
Per capita mangrove crab consumption (g/ capita/year)	174.5
Per capita black ark consumption (g/capita/ year)	70.6
Human intake from mangrove crab (MP items/capita/year)	332
Human intake from black ark (MP items/ capita/year)	99
Human intake from both species (MP items/ capita/year)	431

temminckii (Chae et al., 2018).

The presence of microplastic in the gills and stomach may have physiological and chemical effects in the organism (D'Costa, 2022). For example: exposure to polypropylene fibres and polystyrene spheres reduced food consumption, gill function and scope for growth (i.e.

energy) in the crab Carcinus maenas (Watts et al., 2014, 2015); a long term exposure to polypropylene fibres, polyethylene terephthalate fragments and microbeads led to weight loss in the crab Rhithropanopeus harrisii (Torn, 2020); polystyrene spheres increase the oxidative stress, decrease immune enzyme activity, immune-related gene expression and alteration in the intestinal microflora in the crab Eriocher sinensis (Liu et al., 2019; Yu et al., 2018); microplastics were shown to block the opening of the gastrointestinal tract preventing regurgitation and thus increase the retention of microplastics in the stomach in Eriocheir sinensis (McGoran et al., 2020); and the accumulation of microplastics in the gills of the crabs Leptuca festae and Minuca ecuadoriensis reduce gas exchange in the gill's chamber and thus respiratory rate and osmoregulatory functions (Villegas et al., 2021).

Crustaceans don't have an adaptive immune system (D'Costa, 2022; Huang et al., 2020) Nevertheless, decapods have several mechanisms that might be used to eliminate microplastics from the gills and stomach (D'Costa, 2022). In the gills, crabs can remove the particles by brushing it with their chelipeds, by epipods on the thoracic appendages that cause filament jostle, and by a behaviour called "gill grooming" commonly use to expel microbes (D'Costa, 2022; Waite et al., 2018). The removal of microplastic from the stomach is through excretion and regurgitation as a result of stressful conditions (D'Costa, 2022; McGaw and Curtis, 2013).

#### Table 2

Comparison of microplastic abundance (items/g) in commercial invertebrates from mangrove and estuary ecosystems.

	Studied area	Species	Microplastic abundance (items/ ind)	Microplastic abundance (items/g)	Most common shape	Most frequent size (m)	Most common colour	Reference
Crabs	Hong Kong, China	Parasesarma bidens	Average 60.5	-	Fragments and beads	> 10	Blue (87 %)	Not et al. (2020)
		Paraleptuca splendida		-	Fibres			
		Metopograpsus frontalis		-	Fragment and beads			
		Thalamita crenata		-	Fragments, beads and fibres.			
	Jakarta Bay, Indonesia	Metopograpsus quadridentatus	327.56	33	Fibres and Films	-	-	Mufti et al. (2020)
	Beibu Gulf, China	Chiromantes dehaani	0.74–4.96	-				Zhang et al. (2021)
	Buenaventura Bay, Colombia	Goniopsis pulchra	9.07 ± 3.7	4.57± 4.14	Fragments and fibres	>9	Transparent and blue	Ariza Gallego et al. (2023)
	Mangrove wetlands of	Uca vocans	0.22	-	Fibres	0–1 mm	Brown and	Zhang et al.
	Hainan, South China	Uca arcuata	1.16	-			blue	(2023)
		Perisesarma bidens	1.07	-				
		Uca dussuimeri	2.00	-				
		Helicana wuana	0.78	-				
	Dahia Dianaa Fatuanu	Sesarma plicata	1.36	-	Tibucc	<500 1500	Direc	Ville such at al
	Argentina	Neoneuce granulata	-	Digestive tract: $\geq$ 0.24	Fibres	<500-1500	вше	(2020)
	Bahía Blanca Estuary, Argentina	Nohelice granulata	-	Gills: 0.17 $\pm$ 0.14	Fibre	<0.5 mm	Transparent and black	Truchet et al. (2022)
		Cyrtograpsus angulatus	-	Gills: 0.11 $\pm$ 0.07	Fibre	<0.5 mm	Transparent and black	
		Leptuca uruguayensis	-	Gills: $1 \pm 1$	Fibre	<0.5 mm	Transparent and black	
	Laguna de Terminos, Mexico	Callinectes sapidus	-	37.9	Fragments and fibres	$\geq$ 0.9 mm	Red and blue	Capparelli et al. (2022).
		Menippe mercenaria	-	76	Fragments	$\geq 2 \ mm$	Blue	
	Tumbes, Peru	Ucides occidentalis	$7.8\pm3.14$	Total: $1.94 \pm 0.87$ Gills: $4\ 0.1 \pm 2.41$ Stomach: $0.8 \pm 0.7$	Fibres	500	Transparent and blue	Present study
Bivalves	Pangkal Babu, Indonesia	Anadara granosa	434± 97.05	9.8 ± 2.26	Fibres	-	-	Fitri and Patria (2019)
	Buenaventura Bay, Colombia	Anadara similis	$\textbf{17.8} \pm \textbf{12.98}$	$\textbf{4.29} \pm \textbf{4.09}$	Fragments and fibres	>9	Transparent and blue	Ariza Gallego
		Anadara tuberculosa	$\textbf{35.39} \pm \textbf{27.56}$	$\textbf{4.65} \pm \textbf{4.64}$				et al. (2023)
	Jeneponto Coast, Indonesia	Anadara granosa	-	1.455–3.806	Line, fragments and films	-	Blue	Daud et al. (2023)
	Mangrove wetlands of Hainan, South China	Vignadula atrata	0.22	-	Fibres		Brown and blue	Zhang et al. (2023)
		Geloina erosa	0.23	-				
		Saccostrea echinata	1.85	-				
	Itapessoca estuary in Pernambuco, Brazil	Anomalocardio flexuosa	$5.15\pm3.80$	$3.66\pm2.59$	Fragments	17–1057	-	Bruzaca et al. (2022)
	Situbondo, Indonesia	Geloina erosa	$15.55\pm8.51$	$1.72 \pm 1.58$	Fibre	-	-	Yona et al. (2023)
	Southwest Coast estuary, India	Perna viridis	-	Digestive gland: 5.6 Gills: 8.5	Fibre	1–2 mm	Red	Joshy et al. (2022)
	Tumbes, Peru	Anadara tuberculosa	$\textbf{4.9} \pm \textbf{2.18}$	$1.56\pm1.12$	Fibres	500	Transparent and blue	Present study

-: Not found.

Depending on the species and its gut physiology, there would be differences in the rates of microplastic elimination, which was shown in the crab *Carcinus maenas* who took over six times longer to expel microplastics from the body compared to the typical excretory period (Watts et al., 2014).

#### 4.3. Mangrove and estuary bivalves

In the present study the microplastic abundance in the soft tissue of *A. tuberculosa* was 1.56  $\pm$ 1.12 items/g or 4.9  $\pm$  2.18 items/ind, which is lower than observed in *A. tuberculosa* (4.65  $\pm$  4.64 items/g or 35.39  $\pm$  27.56 items/ind) from Buenaventura Bay, Colombia, *A. flexuosa, G. erosa* y *P. viridis* from Brazil, Indonesia and India (Table 2) (Ariza Gallego

et al., 2023; Bruzaca et al., 2022; Yona et al., 2023; Joshy et al., 2022). Other Anadara species have also been shown to have high levels of microplastic contamination (Table 2), including *Anadara granosa* ( $9.8 \pm 2.26$  items/g -  $434 \pm 97.05$  items/ind) and *Anadara similis* ( $4.29 \pm 4.09$  items/g -  $17.8 \pm 12.98$  items/ind) from Indonesia and Colombia, respectively (Ariza Gallego et al., 2023; Fitri and Patria, 2019). The main pathway of microplastic uptake in mollusc is ingestion (Wang et al., 2021) with microplastics potentially accumulating in the gills and digestive glands (Green et al., 2019). Microplastics can also adhere to bivalves: pervading the shells, mantle and foot for a long period (Kolandhasamy et al., 2018).

The abundance of microplastics in bivalves tend to vary following the variation of the microplastic number in the associated near-by matrix (Joshy et al., 2022), however, in the present study there was no significant correlation between the abundances of microplastics in sediment and in the black ark. The main sources of microplastic pollution in bivalves are associated with anthropogenic activities such as tourism, aquaculture and artisanal fishing (Bruzaca et al., 2022). Notably the greatest abundance of microplastics in A. tuberculosa was in the Rural Central Zone and Tourist Central Zone (S1, S2B), which are surrounded by shrimp farms (Fig. 1). Microplastic exposure can cause an ecotoxicological risk for molluscs (Sussarellu et al., 2016). Studies under laboratory conditions, demonstrate an interference in the reproductive performance reducing sperm velocity and possibly lowering their ability to fertilize oocytes in the oyster Crassotrea gigas (Sussarellu et al., 2016); significantly decreases the growth rate of Mytilus spp. (Walkinshaw et al., 2023); presents inflammatory responses, lysosomal destabilization and the formation of granulocytomas in Mytilus edulis (von Moos et al., 2012); in addition to cellular effects altering immunological responses, changes gene expression profile and genotoxicity in Mytilus galloprovincialis y Mytilus edulis (Avio et al., 2015; Cole et al., 2020). In recent years, there has been notable a decrease in the population of A. tuberculosa observed in Tumbes (Ordinola Zapata, 2022), which may be attributed to pollution, potentially including microplastics.

Thus, Bivalves have an important role as environmental bioindicators to monitor microplastic pollution (Ding et al., 2021; Staichak et al., 2021). Due to their filter-feeding behaviour allowing the capture of significant amounts of microplastics in the wild and in laboratory environment (Staichak et al., 2021; Wesch et al., 2016). In addition, their easy with which they can be collected, the fact they are ubiquitous and that they interact with the surrounding environment (Ward et al., 2019).

#### 4.4. Food security and human health

The presence of microplastic in commercial species is a concern for human health (De-la-Torre, 2020). Consumers that eat microplasticcontaminated food, especially organisms that are consumed whole (i. e. including the gills and digestive tract where microplastics are prevalent) are at high risk of consuming microplastics (Mercogliano et al., 2020; Smith et al., 2018). Microplastics have been identified in a wide array of beverages and foodstuffs including water, beer, salt, sugar, honey, marine finfish and shellfish (Afrin et al., 2022; Al Naggar et al., 2023; Baechler et al., 2020; Lundebye et al., 2022; Senathirajah et al., 2021). In Peru, both species can be consumed whole in traditional dishes (Azabache Cobeña, 2016; Aguirre-Sanchez et al., 2022).

Various predictions have been made of how many microplastics might be consumed by humans through dietary means, European shellfish consumers are estimated to have an annual exposure of 11,000 MPs/person/year (Li et al., 2021; Van Cauwenberghe and Janssen, 2014) while the global average rate of microplastic intake through shellfish consumption is estimated at 2602–16288 items/capita/year (Senathirajah et al., 2021). In Tumbes, the estimated human intake of microplastics is 332 items/capita/year from the mangrove crab, 99 items/capita/year from the black ark and 431 items/capita/year from both species (Table 1). However, food is just one route by which humans can be exposed to microplastics. For example, a number of studies have demonstrated the ubiquity with which microplastics are found in the air we breathe (Amato-Lourenço et al., 2021; Gasperi et al., 2018; Sridharan et al., 2021), with an estimated between 13.731 and 68.415 microplastics per year per capita expected to settle on a plate during a meal exceeding those likely ingested from consuming the food on the plate (Catarino et al., 2018). The risks posed to humans by inhaling or ingesting microplastics may include oxidative stress (Deng & Zhang, 2019), affectation of gene expression and cell morphology (Forte et al., 2016), aggravating allergic diseases (Lu et al., 2022), genotoxic and neurotoxic effects (Oliveira & Almeida, 2019). Of additional concern is that microplastics may act as vectors of pathogenic microbes, metals or persistent organic pollutants (Caruso, 2019), human pathogens, such as bacteria (Ghosh et al., 2021), can colonize plastic surfaces (biofouling) when they come into contact with wastewater treatment plants (Kaiser et al., 2017; Oberbeckmann et al., 2015; Vethaak and Leslie, 2016).

Additionally, these discarded polymers can retain stagnant water in the sediment, creating habitats for parasites and viruses like Dengue and Zika, posing a risk to densely populated and flood-prone areas (Vethaak and Leslie, 2016). The Tumbes region is vulnerable to the persistence of autochthonous Zika virus cases in infants, children, adults, and pregnant women since 2016 (Solis et al., 2018). Furthermore, the presence of total coliforms, thermotolerant coliforms, and *Vibrio* sp. in *A. tuberculosa* (Pernía et al., 2019; Zapata Vidaurre, 2017), as well as *Escherichia coli, Salmonella* spp., and *Pseudomonas* spp. in the meat of *U. occidentalis* (Guamán Quishpi, 2012), poses a risk to the health and food security of the inhabitants of Tumbes (Mercogliano et al., 2020).

Further, microplastics may be trophically-transferred to natural predators in the food web (Maghsodian et al., 2022). The natural predators from *U. occidentalis* and *A. tuberculosa* include the great egret (*Ardea alba*), the tiger heron (*Trigrisoma mexicanum*), the little blue heron (*Egretta caerulea*) and the crab racoon (*Procyon cancrivorus*) which may ingest microplastics via consumption of the mangrove invertebrates (Diele and Koch, 2010; Maghsodian et al., 2022).

#### 4.5. Physicochemical properties

The bioavailability of microplastics in mangrove species depends on their size, colour, and shape (Browne et al., 2008). The small sizes found in the crab (Fig. 4d) were due to the ability to fractionate their food in the cardiac stomach and prevent the passage of large particles through filtering silks in the pyloric stomach (Córdova-Ortiz, 2015). The buoyancy of the fibres and the difficulty to remove them from the soft tissue (Zhang et al., 2020; Ziajahromi et al., 2017; Maghsodian et al., 2022) made them the most common shape of microplastic found in both commercial species (Table 2).

The predominant microplastics in living organisms associated to the mangrove ecosystem have a bright colour (Fig. 4d), however, despite not being attractive to the biota, transparent microplastics can blend in with their environment and eventually be ingested (Ma et al., 2020a,Ma et al., 2020b). The polymers identified may stem from a wide range of sources, including packaging, abandoned, lost and discarded fishing gears (ALDFG) or wastewater very common in tourist zones as Puerto Pizarro Bay, which may be particularly problematic given the lack of wastewater treatment in Tumbes (SUNASS (Superintendencia Nacional de Servicios de Saneamiento), 2010).

#### 5. Conclusion

Widespread anthropogenic activity, such as improper waste disposal, shrimp farms, tourism, agriculture and border trade, generate a homogeneous distribution of microplastics abundance throughout the region, which is accumulating in sediments throughout the Tumbes mangrove. Notably, microplastic concentrations within the mangroves represent some of the highest microplastic concentrations in Peru, indicating that mangroves are accumulating microplastics more readily than unvegetated habitats. Microplastics were identified in the tissues of commercially exploited mangrove species. In the mangrove crab microplastics were more prevalent on the gills, indicating they are the main pathway of microplastic uptake and/or retained more readily on the gills than the digestive tract. The presence of microplastics in commercially exploited species poses a risk to consumers, including marine predators and humans. With the local population likely to ingest 431 microplastics/capita/year from consuming black arc and mangrove crab. The outcomes of this work highlight that the mangrove ecosystem is widely contaminated with microplastics, and this may pose a risk to the marine food web and food security.

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#### CRediT authorship contribution statement

Angelica Aguirre-Sanchez: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. Sara Purca: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. Matthew Cole: Conceptualization, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing. Aldo G. Indacochea: Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – review & editing. Penelope K. Lindeque: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Conflicting Interests.

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

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