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Research article

Macro and microplastic intake in seafood variates by the marine organism's feeding behaviour: Is it a concern to human health?

Lenin Cáceres-Farias [a,b], María Mercedes Espinoza-Vera [a,b], Jorge Orós [c], Miguel Angel Garcia-Bereguiain [d,e], Alonzo Alfaro-Núñez [f,g,*]

[a] *AquaCEAL Corporation, Urb. Las Palmeras, Ave. Capitán Byron Palacios & General Quisquis, #8 EC230101, Santo Domingo de los Colorados, Ecuador*

[b] *Grupo de Investigación en Biología y Cultivo de Moluscos, Departamento de Acuicultura, Pesca y Recursos Naturales Renovables, Facultad de Acuicultura y Ciencias del Mar, Universidad Técnica de Manabí, Bahía de Caráquez, Manabí, 131101, Ecuador*

[c] *Department of Morphology, Veterinary Faculty, University of Las Palmas de Gran Canaria, Las Palmas, Spain*

[d] *One Health Research Group, Universidad de las Américas, Quito, Ecuador*

[e] *Facultad de Ciencias de la Salud, Universidad Latina de Costa Rica, San José, Costa Rica*

[f] *Department of Clinical Biochemistry, Naestved Hospital, Ringstedgade 57a, 4700, Naestved, Denmark*

[g] *Section for Evolutionary Genomics, GLOBE Institute, University of Copenhagen, Øster Farimagsgade 5, 1353, Copenhagen K, Denmark*

A R T I C L E I N F O

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A B S T R A C T

Seafood is considered one of the healthiest sources of food intake for humans, mainly because of its high protein content. However, oceans are among the most polluted environments, and microplastics have been widely reported to be ingested, absorbed or bioaccumulated by marine organisms. The different feeding behaviour may contribute to infer the amounts of microplastic particles accidently intake by marine organisms. We investigated the putative levels of microplastics in different edible species of fish, molluscs, and crustaceans. Plastic fragments larger than 200 μm were detected in the digestive tract of 277 out of 390 specimens (71.5 ± 22.2%) of the 26 different species analysed. There was no evidence of microplastic translocation or bioaccumulation in the muscle tissue of fish, molluscs, and crustaceans. Organisms with carnivorous feeding habits had the highest prevalence of plastic ingestion (79 ± 9.4%), followed by planktivorous species (74 ± 15.5%), and detritivorous species (38 ± 36.9%), suggesting a transfer through the food chain. Moreover, we found evidence that species with less selective feeding habits may be the most affected by the ingestion of large microplastic particles. Our results provide further evidence to the ubiquitous presence of microplastics in marine organisms representing a direct threat to marine wildlife, and to human health with potential consequences for future generations according to the One Health initiatives approach.

1. Introduction

Preserving ocean health, as an intrinsic part of the One Health framework, is beneficial not only for human health but also for productive activities related to sustainable fisheries, aquaculture, and the conservation of marine ecosystems [1]. Seafood has long been considered one of the healthiest proteins sources in the human diet as well as the most sustainable food system for minimising the

* Corresponding author. Department of Clinical Biochemistry, Naestved Hospital, Ringstedgade 57a, 4700, Naestved, Denmark.
E-mail address: alonzoalfaro@gmail.com (A. Alfaro-Núñez).

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effects of excessive emissions of greenhouse gases into the atmosphere [2,3]. However, awareness of the levels of microplastic contaminants detected in seafood and marine ecosystems has increased in recent decades [4–6]. Discarded microplastic particles can be found in different shapes and sizes in all oceans and in countless marine species [5–7]. The problem of plastic pollution is expected to increase in the coming years, with estimates of between 23 and 53 million tonnes of plastic discarded into the oceans each year by 2030 [8].

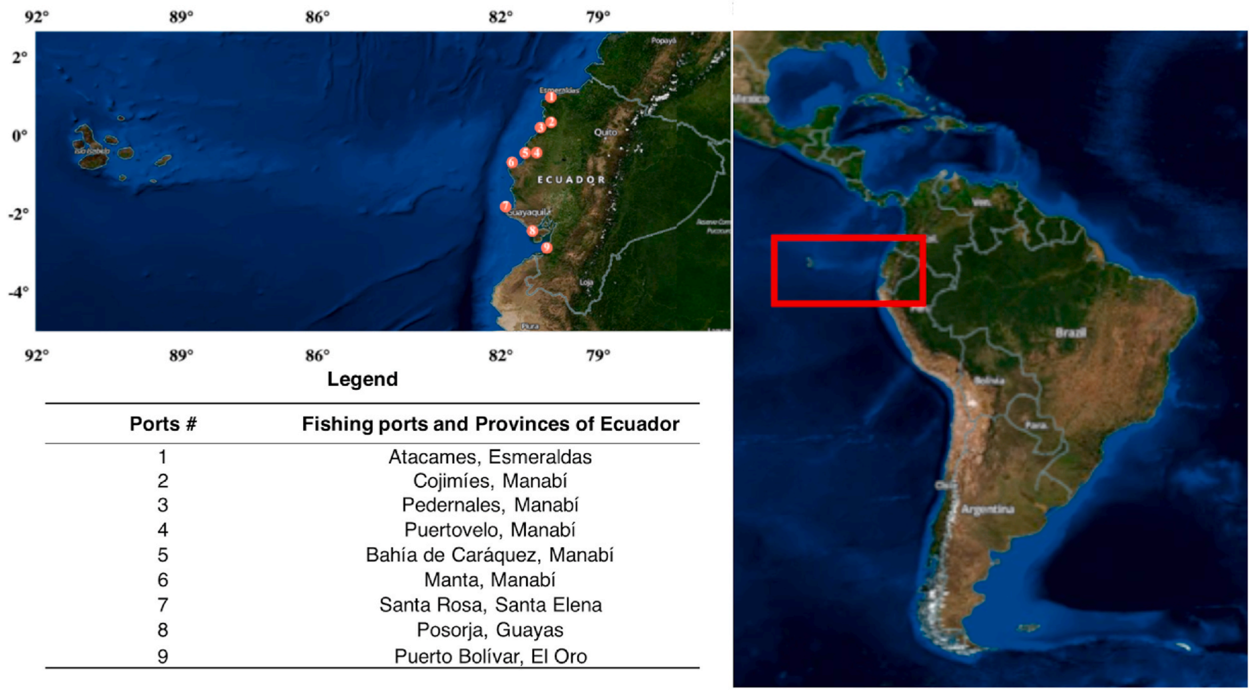
The ingestion process of microplastic particles by marine organisms (including seafood) can be broadly summarised as ingestion by active and passive pathways. The active ones are related to direct ingestion by mistaking plastics for food, and the passive ones are related to feeding preferences, habitat, or genetic background of the fish [9]. Aquaculture species (e.g., fish, oyster, and sea cucumber) have also been widely reported with microplastics [10–12]. It should be noted that aquaculture organisms are usually fed with feed (fishmeal and fish oil), which is typically sourced from 25% of the world's marine fisheries, especially several species of anchovies [13–15]. Therefore, a systematic assessment of the presence of macro- and microplastic particles in marine organisms intended for human consumption, as well as in their derivatives (e.g., fishmeal) used as feed for poultry, pigs, and aquaculture, is of unprecedented relevance for human health [5,16,17].

Marine animals can incorporate plastics into their bodies through their digestive tracts or gills [18–20]. Ingestion could occur due to the inability to identify plastics from prey or ingestion of lower trophic level organisms containing these particles [18]. Microplastics are potentially accessible to a wide range of organisms by ingestion due to their small size, which overlaps with the size range of their prey [21]. Plastics can also be ingested through depositional feeding and active filtration systems [18,22].

Several studies have shown that microplastics, alone or in combination with other marine contaminants such as metals and organic pollutants (e.g., DDT, industrial chemicals, polychlorinated biphenyls PCB and dioxins), pose a significant health risk to many marine biota [23,24]. Once ingested, microplastics are absorbed, distributed throughout the cardiovascular system and into different tissues, where they can have potentially harmful consequences [25–27]. Plastic particles and other toxic compounds present in prey are passed on to marine predators [28].

Intake of plastic particles by different marine organisms may differ according to habitat and/or feeding behaviour [29,30]. One of the main reasons for this difference in the intake of plastic quantities is that microplastic particles are found in a wide range of sizes like those of zooplankton. Thus, carnivorous organisms may mistake microplastics for their natural prey, whereas and due to the intrinsic feeding effect of planktivores, they should appear less contaminated with plastic particles. Finally, it is to be expected that detritivores that feed mainly on sediment filtration have a higher intake of microplastic particles, as all micro and nanoparticles are likely to sink to the seabed [9,31,32]. However, despite the rapidly growing of literature on this topic, only a few studies have been focused on the relationship between the feeding behaviour of marine species and their microplastic content [9,33,34].

As indicated above, most of the intake of microplastics in marine organisms for regular human consumption occurs through the ingestion process. Therefore, the type of feeding behaviour expressed by a group of marine organisms can potentially elucidate the total amount of contaminating microplastic particles present in their bodies. In this study, marine organism's species were classified



Ports #	Fishing ports and Provinces of Ecuador
1	Atacames, Esmeraldas
2	Cojimíes, Manabí
3	Pedernales, Manabí
4	Puertovelo, Manabí
5	Bahía de Caráquez, Manabí
6	Manta, Manabí
7	Santa Rosa, Santa Elena
8	Posorja, Guayas
9	Puerto Bolívar, El Oro

Fig. 1. Locations of the fishing ports at their respective provinces along the Pacific coast of Ecuador, where the marine organisms were obtained (Map source: https://podaac-tools.jpl.nasa.gov/soto).

according to their feeding behaviour to determine the quantification levels of putative macro- and microplastic particles in their bodies.

2. Materials and methods

2.1. Sampling and processing of marine organisms

In between 2019 and 2022, 150 specimens of 10 different species of fish, molluscs, and crustaceans were collected (15 specimens per unique species). All marine specimens were collected by local fishermen using artisanal fishing equipment and gear. Except for *Penaeus vannamei* (crustacean), *Dormitator latifrons* (fish) and *Crassostrea* cf. *corteziensis* (mollusc) that were collected in rocky and estuarine areas, all other marine organisms were collected between 5 and 50 km from the Pacific coastline along the Provinces of El Oro, Santa Elena, Manabí, and Esmeraldas in Ecuador (Fig. 1). In addition, previously published data from other 16 species of fish and shellfish from the same area, with the same set up (15 specimens per species), were also combined [5]. A total of 390 marine organisms of habitual human consumption of 26 different species were analysed and grouped according to their feeding behaviour. This research in collaboration with the National Institute of Biodiversity was granted by permit # MAE-DNB-CM-2016-0045 from the Ministry of Environment, Water and Ecological Transition of Ecuador. After collection, the organism samples were stored at −20 °C in glass containers in the lab.

The animals were dissected, and microplastic particles were investigated in the digestive tract, cephalothorax (or main body for molluscs), and muscle tissue of all organisms, including 19 fish species (total of 285 specimens), four molluscs species (60 specimens in total) and three crustacean species (total of 45 specimens) (Table 1 and Fig. 2). In addition, the soft tissues of the dissected animals, the stomach in the case of fish, the intestines in shrimp, and the gills in crabs, were removed under sterile conditions in a laminar flow hood. Samples were digested in 20% KOH at 60 °C for 48 h with shaking every 24 h. Later, samples were filtered at 40 °C. Samples were then filtered through 10 μm polycarbonate filters (Whatman Nucleopore hydrophilic membrane). Open Petri dishes with filters were set up as atmospheric controls.

For muscle inspection, 0.5 cm^3-muscle tissue fragments were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm for light microscopy and stained with hematoxylin and eosin (H–E) [35]. Collected histological slides were inspected and analysed for microplastic presence under an Olympus BX53 microscope coupled to a microscale to visually quantify the presence of microplastic particles larger than 200 μm following the same protocol described by Ref. [5]. The figure presenting the concentrations of microplastic particles in marine organisms was made on Adobe Acrobat DC Pro software (https://acrobat.adobe.com). Organisms' illustrations were obtained at www.pexels.com (free access and use) and manually adjusted to the figure.

Table 1
Abundance of microplastics (MP) across species of marine organisms combined and analysed for the presence of MP in this study and from a previous work. 15 specimens per each species were analysed and the total number of MP particles is presented as abundance and average intensity, respectively (See Supplementary file 1).

Taxonomic group	Species	Abundance	Average intensity	Reference
Fish	*Alopias pelagicus*	2.87	3.31	[5]
	Alopias superciliosus	2.67	3.08	This study
	Centropomus robalito	1.27	2.11	[5]
	Cetengraulis mysticetus	1.80	2.08	This study
	Coryphaena hippurus	2.33	2.69	[5]
	Chloroscombrus orqueta	1.47	2.44	[5]
	Cynoscion stolzmanni	1.87	2.55	[5]
	Cynoscion analis	2.20	3.00	[5]
	Diapterus brevirostris	1.33	1.67	[5]
	Diplectrum maximun	1.20	1.80	[5]
	Dormitator latifrons	1.87	2.00	This study
	Hemanthias peruanus	1.27	2.11	[5]
	Larimus argenteus	1.73	2.17	[5]
	Lutjanus argentiventris	2.07	2.58	This study
	Mugil cephalus	1.53	2.56	[5]
	Peprilus medius	1.07	2.00	[5]
	Selene peruviana	1.33	1.82	[5]
	Sphyrna lewini	2.47	2.85	This study
	Urotrygon chilensis	1.93	2.42	[5]
Molluscs	*Crassostrea* cf. *corteziensis*	0.93	1.00	This study
	Dosidicus gigas	3.00	3.21	[5]
	Mytella charruana	0.80	0.92	This study
	Striostrea prismatica	1.20	1.38	This study
Crustaceans	*Penaeus occidentalis*	0.40	2.00	[5]
	Penaeus vannamei	0.47	1.75	This study
	Menippe frontalis	0.13	1.00	This study

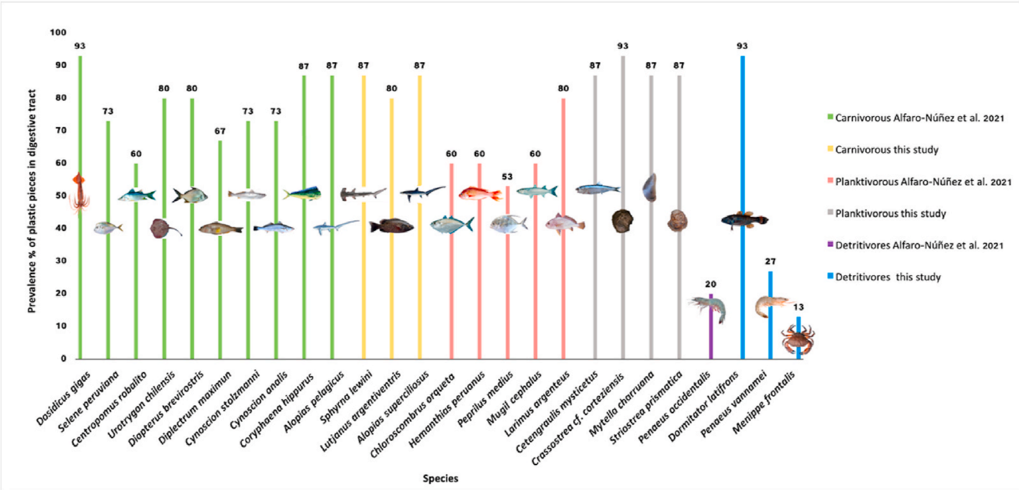


Fig. 2. Prevalence of microplastic particles in the digestive track of marine species. Marine organisms were categorized by their feeding behaviour: carnivorous, planktivorous, and detritivores. 15 specimens (n = 15) were taken for each of the 26 species analysed.

2.2. QA/QC

Quality assurance and quality control procedures were carried out following as described by Refs. [5,6]; by collecting negative blank controls, atmospheric blanks, and filter water blanks to measure and detect potential cross-contamination. A wet filter paper, placed in a Petri dish, was kept at the height of the net opening in front of the net to also monitor for ongoing atmospheric contamination.

All chemicals were filtered before use and all field equipment was rinsed in filtered distilled water before use. Procedural tests were conducted for all types of samples processed, including water, vertebrates, and invertebrate samples. The same chemicals and plastic laboratory consumables were used to carry out these blank tests to control potential contamination. Nitrile gloves and cotton clothing were used in the field and in the laboratory. In the laboratory, all surfaces and equipment were thoroughly cleaned with ethanol (three times) or rinsed with Milli-Q (three times) before each processing step. Sterile plastic equipment was used directly from the container and metal and glass materials were used in favour of plastics where possible and feasible. All samples and equipment were covered whenever possible with aluminium foil. Potential atmospheric contamination was controlled by leaving the laboratory uncovered during all sampling and procedural steps.

2.3. Quantification, statistical analysis

The data were then exported to Minitab 21.1.0 statistical software, where the non-parametric Kruskal-Wallis one-way analysis of variance's test was performed to identify statistical differences between eating habits. To determine the plastic intake between the different feeding habits, a statistical comparison was made including all the specimens' existing data. Group mean differences were assessed using U-Mann-Whitney method with a 95% confidence interval. From the statistical analyses a series of figures were plotted using a few main concepts explained on next. In this study, *prevalence* is defined as the percentage number of individuals detected with microplastics divided by the total number of individuals examinate, which was then expressed as a percentage. *Abundance* is the total number of microplastics ingested in each species divide by the total number of individuals examined per unique species (with or without microplastic presence). The *average intensity* is the total number of microplastics in each species analysed divided by the number of individuals analysed for that species. These concept calculations were implemented from previous works [36,37] and adapted for microplastics analyses grouped accordingly by the marine organisms' feeding behaviours [5].

3. Results

3.1. Microplastics in marine organisms

From the total 390 organisms across 26 different species analysed, at least two individuals for all species were found with microplastics. Plastic fragments larger than 200 μm were detected in the digestive tract of 277 of the 390 specimens (71%) of the 26 different species analysed (see Table 1, Fig. 2, and Supplementary file 1).

The prevalence of plastic ingestion was 71 ± 22.2%. The species with the highest percentage of microplastic pieces in their digestive tract were taxa with carnivorous eating habits (79 ± 9.4%), followed by planktivorous species (74 ± 15.5%) and detritivorous species (38 ± 36.9%) (see Supplementary file 1).

On the other hand, the average abundance of microplastic particles intake was 1.35 ± 0.568. The feeding behaviour group with the highest abundance of microplastic pieces in their digestive tract were the taxa with carnivorous eating habits (2.04 ± 0.622), followed by planktivorous species (1.31 ± 0.348) and detritivorous species (0.72 ± 0.780). The Kruskal-Wallis's test (see Supplementary file 2) revealed a statistically significant difference ($p < 0.01$) between carnivorous, planktivorous and detritivore feeding habits (Fig. 3, panel A).

The average intensity of microplastic particles intake was 2.03 ± 0.562. The group with the highest of microplastic pieces in their digestive tract were the taxa with carnivorous eating behaviour (2.55 ± 0.555), followed by planktivorous species (1.85 ± 0.601) and detritivorous species (1.69 ± 0.473). Moreover, the statistical analysis (see Supplementary file 2) revealed a significant difference ($p < 0.01$) between carnivorous, planktivorous and detritivore feeding behaviours (Fig. 3, panel B).

Microplastics were found in 214 (75 ± 12.0%) out of the 285 fish for the 19 different species, in 54 (90 ± 3.5%) out of the 60 molluscs for the 4 species, and finally, only 9 (20 ± 7.0%) out of the 45 organisms for the 3 species of crustaceans (Fig. 2, and Supplementary files 1 and 3).

The taxonomic group with the highest abundance of microplastic pieces in their digestive tract were the fish (1.80 ± 0.525), followed by mollusc's species (1.48 ± 1.025) and crustacean species (0.33 ± 0.176). The Kruskal-Wallis's test statistical analysis (see Supplementary file 3) revealed a statistically significant difference ($p < 0.01$) between the three taxonomic groups (Fig. 3, panel C).

In agreement with the previous results, the taxonomic group the highest average intensity of microplastic intake were also fish (2.38 ± 0.464), followed by mollusc's species (1.63 ± 1.075) and crustacean species (1.58 ± 0.520) at last. Moreover, the statistical analysis (see Supplementary file 3) resulted also in a statistically significant difference ($p < 0.01$) between taxonomic groups (Fig. 3, panel D).

Histological analyses did not detect plastic in the muscle tissues of fish and molluscs. However, plastic was evident in the cephalothorax of the crabs, which allows us to suspect the possibility of microplastic translocation in the tissue in this group of organisms.

Finally, as for the blank negative controls (see Supplementary file 1), each of the procedural and atmospheric blanks underwent the same processing steps as the environmental samples. Contamination was low but measurable: in the 12 atmospheric blanks had one black cellulose fibre and 1 of the 12 had two fibres, one green and one blue fibre. These particles of control blank (3 particles) were subtracted from all the positive samples with microplastics to carry out the abundance analyses of both the vertebrates and the invertebrates studied.

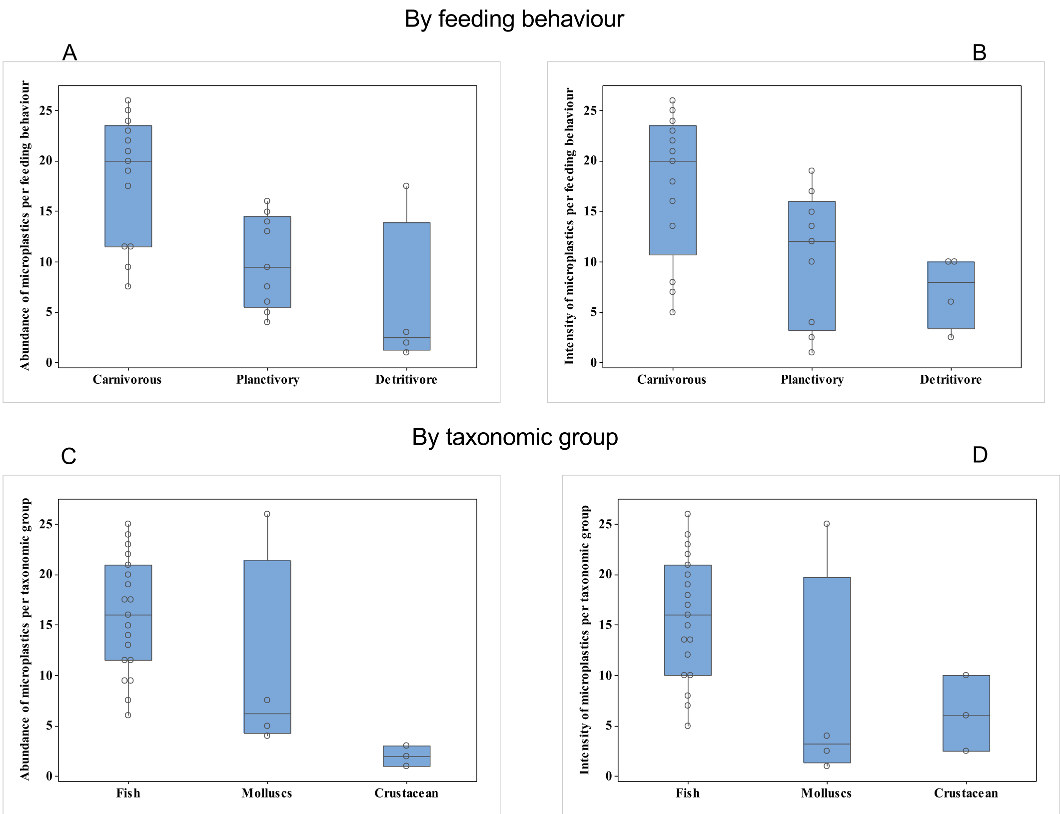


Fig. 3. Kruskal-Wallis one-way analysis of the variance by comparison of groups. Feeding behaviours in panels A and B with carnivorous (n = 13 species), planktivorous (n = 9 species), detritivorous (n = 4 species). Taxonomic groups in panels C and D with fish (n = 19 species), molluscs (n = 4 species) and crustaceans (n = 3 species). Panels: (A) abundance between feeding behaviour of microplastic particles per individual, (B) average intensity between feeding behaviour of microplastic particles per individual, (C) abundance between taxonomic groups, and (D) average intensity between taxonomic groups. Vertical lines indicate data variance.

4. Discussion

The ingestion of microplastics had been confirmed in marine organisms of different feeding habits in the Eastern Tropical Pacific region, concluding a greater prevalence in carnivorous marine organisms, followed by planktivorous and finally detritivorous organisms [5]. However, the pooling of previously published data confirms that microplastic residues are part of fish food and can accumulate over time, space, and now also due to their feeding habits.

In our study, carnivorous species were found with the greatest amount of microplastic pieces in their digestive tract (79%), followed by planktivorous (74%) and detritivores (38%). However, the high prevalence (93%) found in the lower of the food chain (Fig. 2), such as bivalve molluscs, may lead to accumulation processes in high trophic levels. Moreover, our statistical analyses results show that pollution may not only be caused by accumulation in the food chain, but also that planktivorous species, due to their less differentiated feeding as filter feeders, may unintentionally ingest plastic particles. This is by mistaking them for natural prey during foraging, as well as by direct ingestion of animals containing microplastics [38–40].

Several studies on the uptake of microplastics by commercially important marine species have been conducted worldwide, resulting in different prevalence: 36.5–63.5% on the Portuguese coast [41], 14.6% in the Red Sea coast [42], 34% in the Yellow Sea [43], 19.7–23.3% in the Mediterranean Sea [44], 37%–43% in the northern Bay of Bengal [45], 83.4–86.3% in the Lebanese coast [46], 56.6–83.3% in Irish waters [47], 1.8%–21% in the North Sea [48], 94.1% in the southern Tyrrhenian Sea [49], 34.6–57.7% in the Gulf of Beibu, South China Sea [50],

85.4% in the Bohai Sea [51], and 20–93% in the tropical eastern Pacific and Galapagos islands [5].

Microplastic fragments were detected in 111 (74%) of the total 150 fish specimens analysed in our study (Fig. 2 and Supplementary file 1). *Cetengraulis mysticetus* has been included in the list of microplastic-contaminated species, which is relevant due to the ecological and aquacultural importance of anchovy species [52]. Thus, it is also remarkable the vulnerability of the traceability and safety of fishery products such as feed for aquaculture [14].

Different mussels' species with contamination by microplastic particles in their soft bodies have previously been reported with a prevalence of 97% [53]. In our survey, the mussel species *Mytella charruana* was assessed and a prevalence of 87% of microplastic particles was detected in its soft body. In other recent studies, other molluscs such as the Pacific oyster *Crassostrea gigas*, and the palmate oyster *Saccostrea palmula* showed a prevalence of 83% and 47% respectively (Hamilton et al., 2021; [54]. In our study, the prevalence of microplastic particles in the species *Crassostrea* cf. *corteziensis* was 93% being one of the species with the highest plastic contamination (Fig. 2). Another oyster species, *Striostrea prismatica*, had a prevalence of 87% of plastic particles, adding to the growing list of plastic-contaminated organisms. Bivalve molluscs represent an economical interest, and their high prevalence of plastic ingestion is a food safety alert, due to the consumption of their soft body where these microplastics are found. However, at an ecological level, the ingestion of microplastics in bivalve has revealed several negative effects in molluscs, including immunological response, oxidative damage, and cytotoxicity [55].

Molluscs are a group of filter feeders that are vital for both the environment and the economy. These sessile animals filter a considerable amount of water, which is absorbed and deposits a wide range of marine toxins that humans can tolerate to some level in their tissues [56]. Bivalves such as *Mytilus galloprovincialis* and *Mytilus edulis* are widely used in the food industry and serve as bio-indicators of pollution [57–59]. *Ostrea edulis* has been contaminated by plastic particles, both conventional and biodegradable [60]. As bivalves filter their food, they can represent an important source of toxins accumulated by microplastics for humans [61,62].

Microplastics have been reported in crustaceans with a prevalence of 20% in penaeid shrimps in the Tropical Pacific [5], and in 53% of crustaceans in the North Pacific zone (Hamilton et al., 2021). Crabs pump water over their gills to get oxygen, and plastics can become trapped during this process [63]. Crabs can continue to expel microplastics from their gills and digestive tract for up to 21 days in the laboratory [32].

The ability of microplastics to bind and adsorb metals and POPs (e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, dichloro-diphenyl-trichloroethane and polybrominated diphenyl ethers) from the ecosystem is of concern [64,65]. Microplastics can adsorb POPs in greater quantities than larger plastics due to their greater surface area to volume ratio [66], which is detrimental to the ecosystem [65].

Unfortunately, our study did not fulfil the quantification of POPs and metals associated with the plastic particles, as originally intended. Molecular oil residues were detected during the first analytical chemistry quantifications to cause contamination in the samples, which unable this analysis to be completed to confirm the polymers characterization and POPs.

Although our study did not evaluate the potential translocation of microplastic particles from the studied marine organisms to humans, we considered it pertinent to highlight the mechanisms and unpredictable consequences. Humans are vulnerable to the hazardous effects of microplastics, which have been shown to contain toxins, neurotoxic chemicals, and endocrine disruptors [67–70]. Ingestion, dermal absorption, and inhalation are the three ways by which plastics and their compounds can enter the human body. One of the most common pathways for microplastic to enter the human body is through the direct consumption in seafood and other foods [4,5,69]. Consequently, plastic particles act as a conduit for toxic contaminants from marine organisms into the human body and pose a serious threat to human well-being [4].

The consumption of seafood containing microplastic poses a bioaccumulative risk to human health. Consumers in European countries with high seafood consumption ingest up to 11,000 plastic particles per year. Consumers in countries with low-seafood-consumption ingest an average of 1800 plastic particles/year, which is still a significant amount [61]. Estimates suggest that each person in Europe's coastal areas ingests around 175 microplastic particles per year from shrimp consumption alone [71]. Microplastics have also been detected in two commercial species of mussels *Mytilus edulis* and *M. galloprovincialis* from five European countries [72].

The presence of plastics in the gastrointestinal tract of fish does not necessary mean that humans are directly exposed, as these organs are rarely consumed [5,69]. Whole seafood (such as molluscs, crustaceans, and juvenile fish) has a higher risk of contamination than gutted fish or sliced shrimp. However, microplastics were found in the gutted meat of two families of commercial fish at much higher concentrations than in the removed organs, suggesting that gutting does not always minimize the risk of plastic ingestion by humans [73]. Macro and microplastics have also been found in the muscle of commercially valuable fish species and a crustacean [5,74]. These findings highlight the implications for human consumers and their health.

In the present study, there was no evidence of bioaccumulation in muscle tissue of fish, molluscs, and crustaceans through histology, coinciding with the only previous evaluation using this technique [5].

In conclusion, although species with less selective feeding habits, such as planktivorous, may ingest many plastic particles, or detritivores because they live in the benthos, our results confirm that carnivorous species are the most affected by the ingestion of microplastics. It also includes species never reported to the list of marine organisms destined for human consumption in the Eastern Tropical Pacific region and around the Galapagos archipelago. This is of special interest since knowing the plastic pollution in organisms of fishery and aquaculture interest in this region will allow us to have a clear picture to generate transnational public policies since plastic pollution is a problem to be solved by all nations. In this study, we provided enough data to raise a global alarm to the fact that plastic pollution can be transboundary ingested through seafood.

Humans and all living organisms require a large amount of protein-rich foods to sustain life, and seafood is an excellent source of it [2]. Therefore, the recent and increasing detection and characterization of microplastics in seafood deserve special attention [75] as it may represent a direct threat not only to marine ecosystems, but also to human health, with potential consequences for future generations, in line with the One Health initiative acts [17,76]. Therefore, our results endorse the importance of the One Health approach, where the health of humans is linked to animal and environmental health. Seafood has historically been considered one of the healthiest foods, but the vast microplastic pollution of our oceans can change for ever this perspective and transform seafood as an unhealthy food source and then less appealing.

Author contribution statement

Lenin Cáceres-Farias: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data.

María Mercedes Espinoza-Vera: Performed the experiments.

Jorge Orós and Miguel Angel Garcia-Bereguiain: Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Alonzo Alfaro-Núñez: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

Authors Lenin Cáceres-Farias and María Mercedes Espinoza-Vera were employed by AquaCEAL Corporation during the study. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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