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# **Original article**

# MICROPLASTIC ABUNDANCE IN THREE COMMERCIAL FISH FROM THE COAST OF LIMA, PERU

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# Informações do artigo

Abstract

Palavras Chave: Microplastic, Peru, fish

Microplastics (< 5 mm) are widespread within the marine environment, posing a major threat to marine biota. The aim of the present study was to investigate microplastic contamination in three widespread and highly commercial fish from the coast of Lima, Peru. Peruvian silverside (Odontesthes regia), Peruvian morwong (Cheilodactylus variegatus), and Peruvian grunt (Anisotremus scapularis) specimens were caught off the coast of Lima. Fish stomach and intestines were extracted and submerged in 10% (w/v) KOH, followed by 24 h incubation at 60 °C. The resulting supernatant solution was vacuum filtrated and filters were then observed under an optical microscope and stereomicroscope. Strict quality control and external contamination prevention measures were taken. Microplastic abundance, type and color were recorded. Quality control measures resulted in reduced external contamination. C. variegatus was the most contaminated fish (5.13  $\pm$  0.81 MP/individual), similar to *A. scapularis* (5.00  $\pm$  0.46 MP/individual), but significantly different to O. regia (0.43  $\pm$  0.11 MP/individual). Fibers were the overall most abundant microplastic type, while blue the dominant color. Results indicated highly contaminated fish, compared to those from other parts of the SE Pacific. Microplastic ingestion by C. variegatus and A. scapularis may be subject to trophic transfer from their natural prey. Highly populated cities and poor solid waste management may contribute to worsen microplastic ingestion by native marine species. The need for further research and a marine microplastic monitoring program was discussed.

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# Introduction

Microplastics are plastic particles smaller than 5 mm in diameter. They have reached global concern due to the enormous amount and ubiquity in the marine environment (1), as their presence have been evidenced in various water bodies around the world (2,3). Primary microplastics are manufactured to be of micro-size (4), like most preproduction resin pellets, microbeads in cosmetics, toothpaste and blasting, microsized powders for textile coatings, and drug delivery media (5). Secondary microplastics are a result of fragmentation of larger plastic materials due to photo-oxidation, mechanical degradation and biodegradation (6). Once in the environment, microplastics can easily adsorb persistent organic pollutants, heavy metals and other xenobiotics (7-10), consequently working as a vector for environmental contaminants.

Microplastic ingestion have been reported in a wide range of organisms (11–14). Ingested microplastics could be excreted or bioaccumulate in the gastrointestinal tract (15). Thus, posing a threat to the survival of several marine species. Moreover, trophic transfer of microplastics could pose a major pathway for microplastics to reach top predators (16) and, ultimately, humans through contaminated seafood consumption (17).

Research regarding microplastic ingestion by marine fish from the South Pacific and, more specifically, in Peru is still scarce. The aim of the present study was to investigate microplastic contamination in three commercial fish from the coast of Lima, Peru. The selected fish species for this study were the Peruvian silverside (*Odontesthes regia*), Peruvian morwong (*Cheilodactylus variegatus*), and Peruvian grunt (*Anisotremus scapularis*), as these species are widespread and highly commercial.

## **Materials and Methods**

#### Study area and fish collection

The research was conducted off the coast of Lima region. This city is the most populated in Peru, strongly influenced by beachgoers and solid waste marine pollution (18). It is also ground to small scale artisanal fishing activity and a wastewater treatment plant (WWTP) discharge point. Previous research have evidenced the presence of microplastic pollution in sandy beaches along the Peruvian coast (19).

During August 2019, specimens of *O. regia* (n = 40), *C. variegatus* (n = 8), and *A. scapularis* (n = 8) were captured off the coast of Lima. Fish were captured by either throw net or gillnet and acquired from local fishermen. Sampled fish were immediately stored in clean cooler boxes with ice, transported and chilled at -20 °C until further laboratory analysis.

#### Microplastic extraction

Prior to microplastic extraction, fish samples were measured and gut content (stomach and intestine) was removed using a scalpel and placed in clean glass petri dishes. Microplastic extraction from fish guts was conducted following a benchmark protocol for biological samples (20) and previous research (21) with some changes. In brief, stomach and intestines were placed in 25 ml glass screw cab test tubes and filled with 10% (w/v) potassium hydroxide (KOH), shaken for a few seconds and heated at 60 °C over 24 h. Four O. regia individuals were pooled per test tube. Following digestion, the supernatant solution was vacuum filtrated through a 20 – 25  $\mu m$  pore glass fiber filter paper (Whatman) in an 8 cm in diameter porcelain Büchner funnel. In case of an incomplete digestion or solid material in the tube, the digested solution was diluted in a 100 ml saline solution (120 g/L NaCl), stirred with a glass rod and left to precipitate for 10 minutes, followed by vacuum filtration. Procedural steps are summarized in Figure 1.

# Microplastic identification

Every filter was observed by two people in detail under both stereomicroscope (HUND WETZLAR\*) and optical microscope (Krüss MBL2000) under 10 –  $40 \times$  magnification immediately after filtration. To avoid false positives, microplastics were identified for their physical characteristics, color, structure, geometry and missing biological features (22) and glass fibers were identified and discarded according to its description (23). All confirmed particles, along with their physical characteristics (type and color) were recorded and photographed.

#### Quality control

To reduce the potential external contamination of



Figure 1: Procedural steps for extracting microplastics from fish guts.

the samples, cotton lab coats and cleaned latex gloves were worn at all times; all materials were rinsed with distillated water prior to usage; all surfaces were wiped clean and glass and metal equipment and containers were preferred over plastic materials (24). For every sample batch a distillated water and 10% KOH solution blanks were prepared by filling and incubating two glass test tubes. A wet filter paper was placed close to the working table for the time the laboratory analysis lasted and later observed under the stereomicroscope to determine airborne external contamination (24).

#### Data analysis

Microplastic abundance was expressed in MP/individual  $\pm$  standard error of the mean (SEM). Statistical analyses were conducted and graphs were created using GraphPad Prism (version 7.00 for Windows). Kolmogorov-Smirnov normality test and D'Agostino & Pearson omnibus normality test validated the normal distribution of the data (P > 0.05), thus parametric analyses were carried out. To determine significant differences in microplastic abundance between the three fish species, a one-way ANOVA followed by Tukey's multiple comparison test were conducted. Significance level was set to 0.05 for all the analyses.

### **Results and discussion**

The three species, *O. regia*, *C. variegatus*, and *A. scapularis*, were contaminated with microplastics. Quality control measures reduced external airborne contamination. Airborne and 10% KOH procedural blanks presented a reduced mean microplastic contamination of  $0.33 \pm 0.21$  MP/blank, ranging from 0 to 1 MP/blank individually. Fish biometrics are shown in Table 1.

Species	n	Body length (cm)		
O. regia	40	$16.38\pm0.18$		
C. variegatus	8	$24.63 \pm 1.06$		
A. scapularis	8	$24.80 \pm 0.25$		

**Table 1:** Body length of the three fish species.

*C. variegatus* was the most contaminated fish (5.13  $\pm$  0.81 MP/individual), although very similar results were found in *A. scapularis* (5.00  $\pm$  0.46 MP/individ-

ual). Importantly, *O. regia* specimens presented low microplastic occurrence  $(0.43 \pm 0.11 \text{ MP/individual})$ . One-way ANOVA [F(2, 23) = 30.53, *P* < 0.0001] test determined significant differences of microplastic content in the three species. Post hoc Tukey's multiple comparison test indicated that the mean score for *O. regia* (M = 0.43, SD = 0.33) was significantly different than *C. variegatus* (M = 5.13, SD = 2.30) and *A. scapularis* (M = 5.00, SD = 1.31). However, *C. variegatus* did not differ significantly from *A. scapularis* (Figure 2).



Figure 2: Column bar graph of the comparison

between mean microplastic concentration among three fish species. Error bars indicated SEM; asterisks indicated significant differences.

Fibers were the most abundant microplastic type in all three species, followed by fragments (Table 2; Figure 3). Only one film was found in a *O. regia* sample. Similar proportions of microplastic types were found in the three species.

Species	Fiber	Fragment	Film
O. regia	88.24%	5.88%	5.88%
C. variegatus	95.12%	4.88%	0.00%
A. scapularis	75.00%	25.00%	0.00%

**Table 2:** Percentage of microplastic types in thethree fish species.

Regarding color, blue microplastics were the overall most abundant (52.53%), followed by red (26.26%), black (17.17%), green (1.01%), brown (1.01%), purple (1.01%) and transparent (1.01%). Specific color distribution per species were showed in Table 3. Microplastic color proportions were similar in *C. variegatus* and *A. scapularis*, but distinct to that of *O. regia*.

Species	Red	Blue	Black	Purple	Brown	Green	Transparent
O. regia	5.88%	41.18%	47.06%	0.00%	0.00%	0.00%	5.88%
C. variegatus	33.33%	47.62%	14.29%	0.00%	2.38%	2.38%	0.00%
A. scapularis	27.50%	62.50%	7.50%	2.50%	0.00%	0.00%	0.00%

Table 3: Percentage of microplastic colors in the three fish species.



**Figure 3:** A-Photograph of blue fibers found in fish guts. B-Photograph of a blue fragment found in fish guts. C-Photograph of a red fiber found in fish guts.

In a previous study (25), out of 292 planktivorous fish captured along the SE Pacific coast, only 6 individuals had ingested microplastics. However, results may be subject to the omission of fibers as plastic particles due to airborne contamination (25). Importantly, in the present study fish were caught off the coast of Lima, the most populated city in Peru. The proximity to highly populated urban areas promotes microplastic ingestion by marine fish (26). In tropical fish from Moorea Island, French Polynesia, low microplastic occurrence was found in Siganus spp.  $(0.15 \pm$ 0.10 MP/individual), Epinephelus merra (0.39  $\pm$  0.14 MP/individual) and Cheilopogon simus (0.24  $\pm$  0.13 MP/individual) (27), similar to O. regia results in the present study. Microplastic abundance in six planktivorous fish from the North pacific Central Gyre accounted an overall mean of 2.1 MP/individual (28). In general terms, C. variegatus and A. scapularis showed high microplastic pollution compared to literature.

Similar to the results of the present study, fibers have been found dominants in fish in many parts of the world (29–31). In spite of this, some researches have determined other type of microplastics as majority (1). The proximity to a WWTP discharge point may be subject to important micro-fiber emissions, as microplastics shed from laundering clothes (32).

High microplastic abundance in *C. variegatus* and *A. scapularis* may be subject to trophic transfer from their natural prey. Both fish species are considered carnivores, as they feed from benthonic mollusks and crustaceans. Previous unpublished data have demonstrated microplastic pollution in Peruvian coastal molluscs, thus indicating a potential pathway for microplastic transferring to higher trophic levels. On the contrary, *O. regia* is a pelagic planktivorous fish, thereby prone to a lesser exposure.

The present study represents a first report of microplastic contamination in commercial fish from the coast of Lima, Peru. The occurrence of microplastics in the stomach and intestines of the Peruvian silverside (*O. regia*), Peruvian morwong (*C. variegatus*), and Peruvian grunt (*A. scapularis*) was reported. *C. variegatus* and *A. scapularis* were highly contaminated, probably due to trophic transfer from natural prey. Fibers were the most abundant microplastic type, and blue the dominant color. The proximity to a highly populated urban area may promote microplastic emissions and ingestion by marine fish. Further research must focus on implementing microplastic contamination monitoring and prevention along the Peruvian coast.

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