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RESEARCH NOTE

Microplastic ingestion and feeding ecology in three intertidal mollusk species from Lima, Peru

Ingestión de microplásticos y ecología alimentaria en tres especies de moluscos intermareales de Lima, Perú

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Abstract. Microplastics (< 5 mm) are ubiquitous contaminants in the marine environment. The aims of the present study were to report the incidence of microplastic pollution in three mollusk species from the coast of Lima and to investigate the relationship between microplastic ingestion and feeding ecology. Specimens of three mollusk species *Semimytilus algosus*, *Tegula atra* and *Chiton granosus* were sampled from the intertidal rocky zone. For microplastic isolation, soft tissues were digested in 10% KOH, vacuum filtrated and analyzed under a microscope. Rigorous contamination prevention measures were taken into account. *C. granosus* was the most contaminated mollusk (6.92 ± 2.13 particles g^{-1}). Red fibers were the overall most abundant microplastic. The feeding behavior of *T. atra* promotes microplastic exposure to *C. granosus*. More research is needed to fully understand the microplastic effects on mollusk species.

Key words: Microplastic, mollusk, plastic debris, Peru

INTRODUCTION

In 2017, annual global production of plastic reached close to 350 million tons (PlasticsEurope 2018)¹, demonstrating its importance within our lifestyle. Plastics are synthetic organic polymers derived from petroleum that are versatile, lightweight, strong and durable, thus making ideally suited for a variety of applications and highly resistant to degradation (Derraik 2002, Rios *et al.* 2007, Andrady 2011). Plastics are a global issue and is perceived as one of the most severe forms of pollution in shorelines, oceans and freshwater bodies (Li *et al.* 2016a).

Whilst there is not a scientific standard, microplastics have been attributed with different size-ranges (Cole *et al.* 2011). The present study refers to microplastics as anthropogenic particles ranging below 5 mm in diameter (Barnes *et al.* 2009). These are subdivided in two categories: primary microplastics, which are plastics that are manufactured to be of microscopic size (Cole *et al.* 2011) such as plastic scrubbers in skin cleaners as reported by Lei *et al.* (2017) and secondary microplastics, as consequence of the breakdown of larger plastic debris by degradation (Cole *et al.* 2011). The distribution of plastic debris in the ocean is irregular due to local wind and current conditions; it is apparent that microplastics have become both wide-spread and ubiquitous (Barnes *et al.* 2009, Cole *et al.* 2011). Due to their size, microplastics are prone to be ingested by marine biota; in addition to potential adverse effects from ingestion, toxic responses could also result from inherent contaminants leaching from

the microplastics and extraneous hydrophobic pollutants adhered to the microplastics (Cole *et al.* 2011). Moreover, it is suggested that microplastics can be transferred within different food webs (De-la-Torre 2020), raising concerns regarding microplastic bioaccumulation and biomagnification in marine biota (Barboza *et al.* 2018).

Filter feeders are between the most vulnerable species to microplastic pollution in the marine environment. Bivalves are widely used as biomonitors in marine ecosystems due to their global distribution, accessibility and high tolerance to salinity (Li *et al.* 2016b). More specifically, mussels have been previously proposed by Li *et al.* (2019) as a global bioindicator of microplastic pollution. On the other hand, Polyplacophora and Gastropoda species are poorly studied. Rock grazers and detritivore species, like most marine chitons and snails, are also exposed to microplastics from the marine environment, therefore compromising their survival. Little is known about the relationship between microplastic ingestion and feeding ecology of coastal marine mollusks.

There is still scarce information assessing microplastic pollution in the marine environments of Peru. Thus, the objectives of the present study were (1) to report the incidence and characteristics of microplastic pollution in three mollusks from the intertidal rocky zone from Lima, Peru; and (2) investigate the relationship between microplastic content and feeding behavior in three different mollusk species.

MATERIALS AND METHODS

Specimens of three mollusk species were collected from the intertidal rocky zone in Los Yuyos (12°09'11.7"S; 77°01'31.5"W) and Las Sombrillas (12°09'25.4"S; 77°01'35.1"W) beaches of Lima, Peru, both highly polluted with marine litter (De-la-Torre & Laura 2019) and microplastics (De-la-Torre *et al.* 2020). Importantly, these sites are considered unhealthy by local authorities due to anthropogenic pollution. Two sampling points were considered in the intertidal rocky shore of these locations. Specimens of filter feeder *Semimytilus algosus* (n= 45), and grazers *Chiton granosus* (n= 15) and *Tegula atra* (n= 15) were collected in three sampling campaigns throughout February 2019. Collected specimens were placed in glass containers and stored at -20 °C until further laboratory analysis.

The length and wet weight of each mollusk were recorded (Table 1). The soft tissues were extracted by dissecting the mollusks using a scalpel. For *S. algosus* three specimens were pooled. Then, the soft organic material was digested using Protocol 1b as described by Dehaut *et al.* (2016) with minor changes. In brief, the soft tissues were submerged in 15 ml of 10% (w/v) potassium hydroxide (KOH) in Pyrex screw cap test tubes and incubated over night at 60 °C. Digestion was followed by vacuum filtration of the supernatant solution through 20 µm pore glass fiber filter paper (Whatman PLC 122 United Kingdom). Filter papers were placed in closed glass petri dishes until further analysis.

Optical identification of microplastics was performed using an optical microscope (Krüss MBL2000) under 10-40 × magnification. To avoid false positives and negatives, microplastics were identified according to their physical characteristics, structure, color, morphology, and such (Desforges *et al.* 2014). Glass fibers were identified and discarded according to its description by Davidson & Dudas (2016). Microplastic abundance, type (fibers, beads, fragments and films) and color were recorded. All confirmed microplastics were photographed.

Following Hernandez-Milian *et al.* (2019), the term microplastic was used for anthropogenic particles smaller than 5 mm. The aim of this study was to investigate the relationship between anthropogenic particles content and feeding behavior in three mollusk types, therefore polymer identification by infrared spectrometry analysis was not necessary (Lusher *et al.* 2014, Hernandez-Milian *et al.* 2019).

To reduce external contamination, the protocol described by Dioses-Salinas *et al.* (2020) was followed. In brief, cotton lab coats and latex gloves were worn at all times. All the equipment was rinsed with distilled water and all surfaces

Table 1. Shell length and soft tissue wet weight of three rocky intertidal mollusks from Lima, Peru / Longitud de la concha y peso húmedo de los tejidos blandos de tres moluscos del intermareal rocoso en Lima, Perú

Species	Number	Shell length (cm)	Soft tissue wet weight (g)
<i>Semimytilus algosus</i>	45	3.90 ± 0.05	1.21 ± 0.02
<i>Chiton granosus</i>	15	3.84 ± 0.08	1.28 ± 0.10
<i>Tegula atra</i>	15	2.22 ± 0.05	1.75 ± 0.09

were wiped clean. Glass and metal materials were used and plastic materials were avoided completely. Sampled organisms were stored in glass containers. For every batch of organisms treated, a blank control (distilled water) and a 10% KOH blank were prepared, vacuum filtrated and analyzed under the microscope.

Results were expressed in particles ind.⁻¹ and particles g⁻¹ (wet weight) ± standard error of the mean (SEM). Kolmogorov-Smirnov and Shapiro-Wilk tests invalidated the normal distribution of the data, thus non-parametric tests were used. Kruskal-Wallis test followed by Dunn's multiple comparisons test were conducted to compare microplastic abundance between mollusk species. Significance level was set to 0.05 for all the analyses. Statistical analysis was performed and graphs were created using GraphPad Prism (version 7.0 for Windows).

RESULTS AND DISCUSSION

All three mollusk species were contaminated with microplastics. Distilled water and 10% KOH blanks had a mean microplastic concentration of 0.50 ± 0.22 particles blank⁻¹, similar to Li *et al.* (2015). All particles in the blanks were identified as microfibrils.

The overall abundance in the three mollusk species was 3.79 ± 0.85 particles ind.⁻¹ and 3.15 ± 0.81 particles g⁻¹. *C. granosus* showed to be the most contaminated mollusk, containing 6.92 ± 2.13 particles g⁻¹, followed by *S. algosus* (1.65 ± 0.22 particles g⁻¹) and *T. atra* (0.88 ± 0.20 particles g⁻¹). Kruskal-Wallis test ($P < 0.01$) indicated significant differences between microplastic concentration in the three mollusk species in terms of particles ind.⁻¹ and particles g⁻¹. Dunn's multiple comparison post-hoc test indicated in both particles ind.⁻¹ and particles g⁻¹ that microplastic concentration in *S. algosus* and *T. atra* were not significantly different ($P > 0.99$), while *C. granosus* differed significantly ($P < 0.05$) from both *S. algosus* and *T. atra* (Fig. 1a). Fibers were the most common microplastics in all three species, followed by fragments, spheres and films (Table 2) (Fig. 1c).

Table 2. Abundance percentage of microplastic types in three species of intertidal mollusks. Fibers derive from textile shedding, fragments form from the breakdown of larger solid plastics, spheres are manufactured micro-sized and are found in cosmetics, and films derive from packaging or plastic bags / Porcentaje de abundancia de cada tipo de microplásticos en tres especies de moluscos intermareales. Las fibras se desprenden de los textiles, los fragmentos de la ruptura de plásticos sólidos más grandes, las esferas son fabricadas de tamaño microscópico y se encuentran en cosméticos y los films derivan de plástico de embalaje o bolsas

Species	Fiber (%)	Fragment (%)	Sphere (%)	Film (%)
<i>Semimytilus algosus</i>	60.23%	32.95%	4.55%	2.27%
<i>Chiton granosus</i>	84.87%	15.13%	0%	0%
<i>Tegula atra</i>	59.09%	40.91%	0%	0%

Spheres and films were not observed in *C. granosus* and *T. atra*. Regarding microplastic color, red microplastics were dominant in *C. granosus* (46.22%) and *T. atra* (50%), while blue microplastics were dominant in *S. algosus* (35.23%) (Fig. 1b).

Microplastic content in *C. granosus* was similar to Naji *et al.* (2018) in *Amiantis umbonella* (6.9 ± 2.3 particles ind.⁻¹) and *Amiantis purpuratus* (6.1 ± 1.8 particles ind.⁻¹) specimens from the Persian Gulf. Similar to our results, Li *et al.* (2015) reported low microplastic concentrations in *Mytilus edulis* (2.2 particles g⁻¹) from China. Research regarding microplastic ingestion by mollusks is still scarce, thus more research is needed.

As wastewater treatment plants (WWTPs) play an important role in releasing microplastic to the marine environment (Sun *et al.* 2019), the proximity to the discharge point of La Chira, the largest WWTP in Lima, and active fishing activity may be the cause of abundant fiber particles, as fibers shed from laundering clothes (Browne *et al.* 2011) and degrade from fishing nets. Li *et al.* (2015) reported black, red and blue colors as the most frequent in bivalves, similar to Ding *et al.* (2018), indicating black, blue and green as the most popular. Indeed, our results have a similar approach to the color proportion reported in literature.

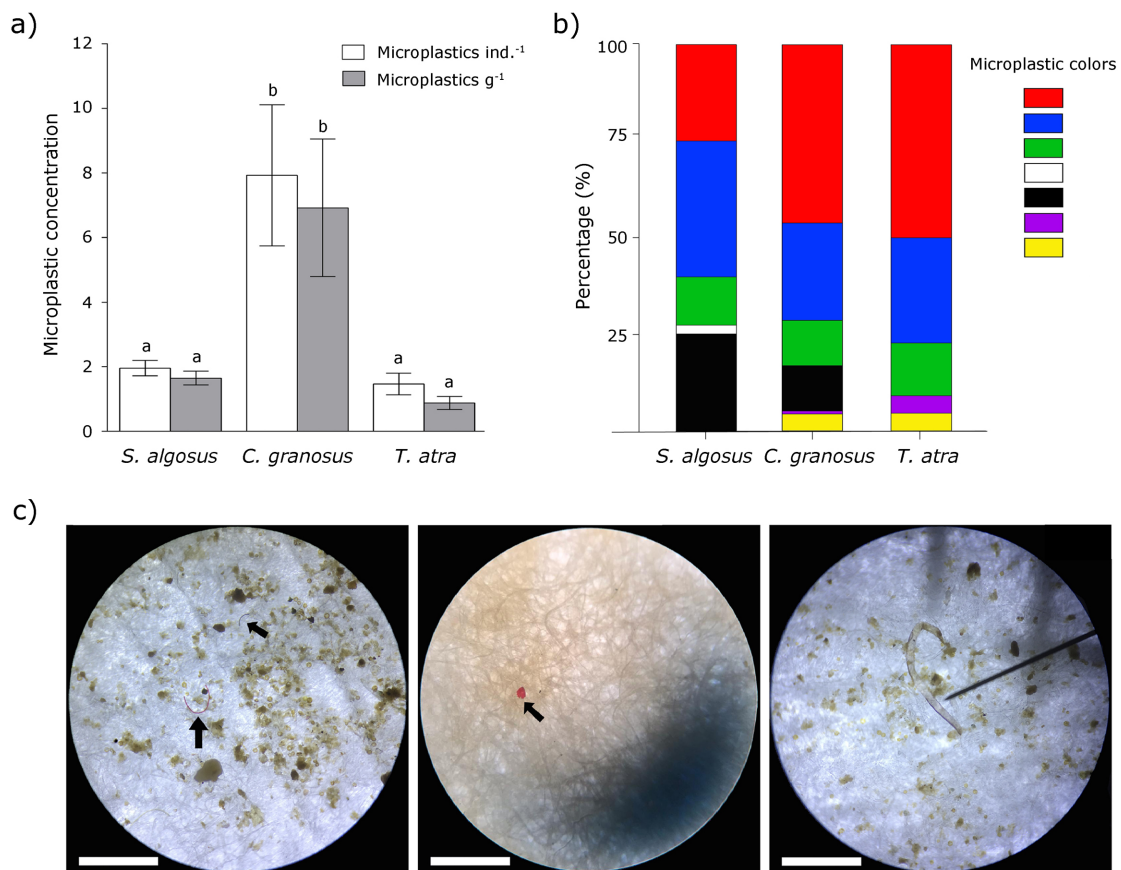


Figure 1. a) Microplastic concentrations in three intertidal mollusks from Lima, Peru. Error bars indicate SEM. Letters indicate significant differences; b) Composition of different microplastic colors in three rocky intertidal mollusks from Lima, Peru; c) Photographs of three different microplastics (fibers, fragment and film) extracted from molluskan soft tissue. Scale bar indicates 1 mm. Arrows point the identified microplastic / a) Concentración de microplásticos en tres moluscos del intermareal rocoso en Lima, Perú. Las barras de error indican el error estándar de la media. Las letras indican diferencias significativas; b) Composición de diferentes colores de microplásticos en tres moluscos del intermareal rocoso en Lima, Perú; c) Fotografías de tres microplásticos diferentes (fibras, fragmento y film) extraídos de tejidos blandos de moluscos. La barra de escala indica 1 mm. Las flechas señalan los microplásticos identificados

In both sampling sites, *T. atra* specimens were found gliding on the surfaces of rocks and big boulders, while *C. granosus* inhabiting rock crevices. In numerous occasions both species were located close to each other or resting on the same boulder. On the contrary, *S. algosus* beds were separated by a few meters, colonizing isolated boulders partially buried in the sediment. *C. granosus* carry out foraging excursions of about 30-40 cm twice a day and has a tendency to come back into the rock crevices (Aguilera & Navarrete 2007), thus indicating a direct interaction with *T. atra* individuals. *In situ* observations determined that the foraging excursions by the sampled *C. granosus* specimens were carried out across *T. atra* gliding trace. Previous research (Gutow *et al.* 2019) revealed that gastropod pedal mucus retains suspended microplastics and foraging on the contaminated mucus promotes microplastic ingestion by marine grazers. Consequently, Polyplacophora and Gastropoda species from the intertidal rocky shore are exposed to microplastics adhered to pedal mucus. However, *S. algosus* are not subject to species interactions that may promote microplastic exposure. Once ingested, microplastics in marine mollusks are expected to scale along the food chain. De-la-Torre *et al.* (2019) suggested that high microplastic abundance in the gastrointestinal tracts of carnivorous fish were due to ingestion of contaminated mollusks.

It has been observed in other marine gastropod species (*Littorina littorea*) with similar feeding activity to *T. atra* that ingested microplastics through contaminated seaweed were mostly released through the feces and do not bioaccumulate rapidly (Gutow *et al.* 2015). This suggests *T. atra* may self-depurate from ingested microplastics and provoke a higher microplastic exposure to *C. granosus* when foraging, thus explaining why results indicated *C. granosus* as the most contaminated mollusk (Fig. 1a) and the microplastic type (Table 2) and color proportion similarities (Fig. 1b).

In the present study, the first evidence of microplastic ingestion by mollusks from Peru was presented. Microplastic concentrations in three mollusk species and physical characteristics of ingested microplastics were determined. The relationship between feeding ecology and microplastic exposure in three mollusk species was investigated and discussed. Further research must focus on determining the toxicity, chronic effects and physical impacts of microplastics in mollusks.

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