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## Microplastic-like particles in the black sea urchin *Arbacia nigra* (Molina, 1782) (Echinoidea: Arbaciidae) from three beaches on the central coast of Peru

Partículas tipo microplástico en el erizo negro *Arbacia nigra* (Molina, 1782) (Echinoidea: Arbaciidae) en tres playas de la costa central del Perú

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### Highlights:

- Yellow, blue, and red were the predominant colors of microplastic-like particles in *Arbacia nigra*.
- Fragments and fibers were the predominant microplastic-like particle shapes in *Arbacia nigra*.
- The occurrence of microplastic-like particles in *A. nigra* highlights the need to strengthen plastic waste management and environmental monitoring in coastal marine areas.

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## ABSTRACT

**Introduction.** The black sea urchin *Arbacia nigra* (Molina, 1782) (Echinoidea: Arbaciidae) is part of Peru's marine biodiversity. **Objective.** To characterize and quantify microplastic-like particles (MLPs) in *A. nigra* from three beaches on the central coast of Peru. **Materials and methods.** A total of 77 individuals of *A. nigra* were collected from El Boquerón (Pucusana), La Arenilla (La Punta), and Bahía Blanca (Ventanilla). Morphometric parameters were evaluated, and procedures for extraction, physical identification, and morphological characterization of MLPs were applied. **Results.** Significant differences among beaches were detected in test diameter (TD), total weight (TW), and gonad weight, with the highest values recorded at La Punta. A total of 905 MLPs were recovered, equivalent to 11.75 MLPs per individual. In general, no significant correlations were observed between TD or TW and MLP abundance; however, at La Punta, TW was positively correlated with MLP abundance in the intestine and in the whole individual. Sea urchins from Ventanilla showed lower MLP abundance per individual than those from Pucusana and La Punta. At La Punta and Ventanilla, gonads contained a higher abundance of MLPs than coelomic fluid. Fragments and fibers predominated, mainly yellow, blue, and red. MLPs <500 µm were the most abundant. **Conclusions.** Sea urchins from Ventanilla showed lower MLP abundance than those from Pucusana and La Punta. Fragments and fibers predominated, mainly yellow, blue, and red, and MLP abundance decreased as particle size increased. These findings highlight the need to strengthen plastic waste management in marine-coastal areas and to expand studies involving organisms from different trophic levels to better understand the associated ecological risks.

**Keywords:** coastal marine environment; biomonitoring; marine pollution; bioindicators; plastic waste; invertebrates.

## RESUMEN

**Introducción.** El erizo negro *Arbacia nigra* (Molina, 1782) (Echinoidea: Arbaciidae) forma parte de la diversidad marina peruana. **Objetivo.** Caracterizar y cuantificar partículas tipo microplásticos (PMPs) en *A. nigra* en tres playas de la costa central del Perú. **Materiales y métodos.** Se recolectaron 77 individuos de *A. nigra* en El Boquerón (Pucusana), La Arenilla (La Punta) y Bahía Blanca (Ventanilla). Se evaluaron parámetros morfométricos y se aplicaron procedimientos de extracción, identificación física y caracterización morfológica de PMPs. **Resultados.** Se detectaron diferencias significativas entre playas en el diámetro de la testa (DT), peso total (PT) y peso gonadal, con los valores más altos en La Punta. Se recuperaron 905 PMPs, equivalentes a 11.75 PMPs por individuo. En general, no se observaron correlaciones significativas entre DT o PT y la abundancia de PMPs; sin embargo, en La Punta el PT se correlacionó positivamente con la abundancia de PMPs en el intestino y en el individuo completo. Ventanilla presentó una menor abundancia de PMPs por individuo que Pucusana y La Punta. En La Punta y Ventanilla, las gónadas presentaron mayor abundancia de PMPs que el fluido celómico. Predominaron los fragmentos y las fibras, principalmente de color amarillo, azul y rojo. Las PMPs <500 µm fueron las más abundantes. **Conclusiones.** Los erizos de Ventanilla presentaron una menor abundancia de PMPs que los de Pucusana y La Punta. Predominaron los fragmentos y las fibras, principalmente de color amarillo, azul y rojo, y la abundancia de PMPs disminuyó conforme aumentó el tamaño de las partículas. Estos hallazgos resaltan la necesidad de fortalecer la gestión de residuos plásticos en zonas marino-costeras y ampliar los estudios en organismos de diferentes niveles tróficos para comprender mejor los riesgos ecológicos asociados.

**Palabras clave:** ambiente marino-costero; biomonitoreo; contaminación marina; bioindicadores; residuos plásticos; invertebrados.

## INTRODUCTION

Over the past seven decades, global plastic production has increased significantly. In 1950, approximately 2 million metric tons (Mt) of plastic were produced worldwide, whereas by 2019, production had reached 368 Mt <sup>(1-2)</sup>. This increase has led to estimates suggesting that current amounts could triple by 2060 <sup>(3)</sup>. A considerable proportion of this plastic waste ultimately enters aquatic environments <sup>(4)</sup>.

Much of this plastic waste is transformed into plastic particles or microplastic-like particles (MLPs), which have been identified in both terrestrial and aquatic environments and represent a modern and emerging form of pollution <sup>(5)</sup>. This type of pollution is ubiquitous worldwide and has expanded rapidly in recent years <sup>(6)</sup>. The abundance of MLPs is a matter of concern because of their small size (<5 mm) and their capacity to disperse through marine and river currents, wind, surface runoff, wastewater, living organisms, and human activities <sup>(7-9)</sup>. This growing concern has prompted studies of MLPs in different environmental matrices <sup>(10)</sup>.

The presence of MLPs in marine debris represents a global environmental problem affecting biota. A considerable proportion of these particles reaches marine environments <sup>(11)</sup>, and their distribution is influenced by environmental factors such as sea level, wind speed, precipitation, and marine currents, among others <sup>(3,12-13)</sup>. Increased exposure to sunlight may also accelerate the degradation of MLPs <sup>(14)</sup>. These particles have been reported even in marine protected areas <sup>(15)</sup> and Ramsar sites <sup>(16)</sup>.

MLPs can affect biodiversity in several ways <sup>(8, 16-17)</sup>. These effects may occur directly when organisms are small enough to mistake MLPs for food, or indirectly through the food web, particularly in fish and crustaceans <sup>(18-20)</sup>. In marine environments, the shape, size, and color of MLPs are relevant characteristics because these particles can cause direct physical damage to marine organisms, release toxic substances, act as vectors for toxic chemicals and microorganisms, and accumulate in organisms and throughout the food chain <sup>(21-24)</sup>. In Peru, studies addressing MLPs in the marine environment remain limited, and those available have mainly evaluated water, sediments, and a relatively small number of species <sup>(25-33)</sup>.

In marine environments, sea urchins (Echinoidea) are considered suitable bioindicators for evaluating the presence of MLPs because of their marked sensitivity to contaminants and their important ecological role, often acting as primary or secondary consumers within benthic communities <sup>(34-37)</sup>. Their feeding habits in these environments favor the ingestion of MLPs <sup>(38-40)</sup>. Several studies have shown that MLPs can affect larval growth, normal development, and the immune response of adult individuals <sup>(41)</sup>. Sea urchins can also act as indicators of terrestrial impacts because their spines commonly retain marine debris <sup>(42)</sup>, reflecting the pressure exerted by waste derived from human activities. In addition, they are widely used as model organisms to investigate the bioaccumulation of contaminants in tissues and cells <sup>(43-45)</sup>. Their larvae, which are particularly sensitive to physical and chemical agents, are used in ecotoxicological studies to assess the effects of pollution during early life stages <sup>(46)</sup>.

The black sea urchin *Arbacia nigra* (Molina, 1782) (Echinoidea: Arbaciidae) plays an important ecological role in regulating algal populations in rocky ecosystems, thereby contributing to the balance and dynamics of benthic communities <sup>(47-48)</sup>. It is also

economically relevant to artisanal fisheries, where it is valued for its nutritional and gastronomic importance, and has considerable potential as a model organism for biological research along the coastal Pacific of Peru and Chile <sup>(49-50)</sup>.

The beaches of El Boquerón in Pucusana, Lima; La Arenilla in La Punta, Constitutional Province of Callao; and Bahía Blanca in Ventanilla, Constitutional Province of Callao, Peru, contain rocky habitats where the black sea urchin *A. nigra* occurs. These locations share problems related to the accumulation of solid waste, including plastics, construction debris, and household waste, as well as deficiencies in municipal cleaning services and direct impacts on sensitive ecosystems. In addition, they receive waste transported by marine currents and river discharges.

Based on the above, the following hypothesis was proposed: Is there significant variation in the abundance, shape, size, and color of microplastic-like particles (MLPs) associated with the black sea urchin *A. nigra* among three beaches in Lima and Callao, Peru, influenced by human activities? Therefore, the objective of this study was to characterize and quantify MLPs in *A. nigra* according to their shape, size, and color at three beaches in Lima and Callao, Peru.

## MATERIALS AND METHODS

### Description of the sampling area and sample collection

Sea urchins were collected during spring, between November and December 2024, from three beaches in Lima and the Constitutional Province of Callao, Peru: El Boquerón in Pucusana (12°28.9179' S, 76°48.0439' W), Lima, on November 24, 2024; La Arenilla in La Punta (12°04'04" S, 77°09'23" W), Constitutional Province of Callao, on December 8, 2024; and Bahía Blanca in Ventanilla (11°50'14.7" S, 77°11'08.1" W), Constitutional Province of Callao, on December 15, 2024 (**Figure. 1** and **2**).

El Boquerón (Pucusana) exhibits the highest degree of environmental contamination, particularly microbiological contamination (fecal coliforms), solid waste accumulation, and wastewater discharges, and is frequently classified as an unhealthy beach <sup>(51)</sup>. La Arenilla (La Punta) shows an intermediate condition, with good microbiological quality in many assessments; however, the high number of visitors favors the temporary accumulation of solid waste and may affect its sanitary classification. This beach frequently alternates between healthy and unhealthy classifications according to weekly inspections. Finally, Bahía Blanca (Ventanilla) generally exhibits the best sanitary conditions among the three beaches, with microbiological levels that are usually acceptable and lower accumulation of solid waste, although it remains exposed to the urban and industrial pressures characteristic of the Callao coastline. This beach is more frequently classified as healthy.

Sea urchins were collected manually during low tide using resistant gloves, spatulas, and forceps to detach them from the rocks <sup>(52)</sup>. A total of 77 individuals were collected: 26 from El Boquerón, 25 from La Arenilla, and 26 from Bahía Blanca. Each individual was considered a biological replicate of its respective population. The organisms were subsequently transported individually in containers with cooling gel packs to maintain a low temperature until arrival at the laboratory and subsequent evaluation <sup>(53)</sup>. Laboratory analyses were conducted during November and December 2024.

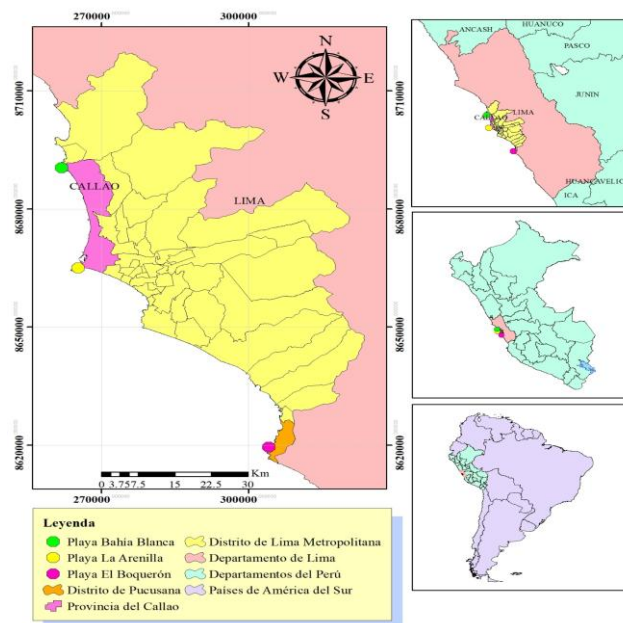


Figure 1. Location of the sampling sites at El Boquerón, La Arenilla, and Bahía Blanca beaches



Figure 2. Photographic record of the beaches: (A) El Boquerón, (B) La Arenilla, and (C) Bahía Blanca

At each beach, water samples were collected using a 1-L aluminum bucket previously washed with filtered distilled water. Samples were collected at depths ranging from 15 to 30 cm by submerging the container with its opening oriented opposite to the direction of the marine current. Temperature ( $^{\circ}\text{C}$ ), pH, and electrical conductivity ( $\text{mS}/\text{cm}$ ) were measured *in situ* using a portable multiparameter instrument (3630 IDS WTW, USA). In addition, the concentrations of diatoms ( $\mu\text{g}/\text{cm}^2$ ), green algae ( $\mu\text{g}/\text{cm}^2$ ),

and cyanobacteria ( $\mu\text{g}/\text{cm}^2$ ) were determined using a fluorometric method with a BenthosTorch Eijkelpkamp® instrument (The Netherlands) (Table 1).

**Table 1. Physicochemical characteristics of the water and algal parameters at three coastal beaches in Lima and Callao, Peru**

| Sampling site | T (°C) | pH  | Electrical conductivity (mS/cm) | Diatoms ( $\mu\text{g}/\text{cm}^2$ ) | Green algae ( $\mu\text{g}/\text{cm}^2$ ) | Cyanobacteria ( $\mu\text{g}/\text{cm}^2$ ) |
|---------------|--------|-----|---------------------------------|---------------------------------------|-------------------------------------------|---------------------------------------------|
| Pucusana      | 18.5   | 8.0 | 54.3                            | 6.17                                  | 0                                         | 6.66                                        |
| La Punta      | 24.0   | 7.9 | 52.6                            | 0.74                                  | 0.15                                      | 0.11                                        |
| Ventanilla    | 18.4   | 7.2 | 55.6                            | 0.79                                  | 0.13                                      | 1.03                                        |

#### Morphometric parameters of *Arbacia nigra*

Five morphometric variables were measured for each *A. nigra* individual: *i*) test diameter (TD, cm), measured with a Vernier caliper (precision  $\pm 0.01$  cm) as the maximum distance between two opposite points of the test, excluding the spines; *ii*) total weight (TW, g), recorded after gently blotting each individual with absorbent paper to remove excess water; *iii*) gonad weight (g), measured after the gonads were carefully removed using dissection forceps and residual connective tissue and adhering liquid were removed with absorbent paper; *iv*) intestine weight (g), measured after the intestine was completely separated from the esophagus to the rectum and the mesentery and other associated tissues were carefully removed; and *v*) coelomic fluid weight (g), obtained using a sterile Pasteur pipette and transferred to a previously tared container.

TW and the weights of the gonads, intestine, and coelomic fluid were determined using an analytical balance with a precision of  $\pm 0.001$  g. These morphometric variables allow the presence and load of MLPs to be interpreted, the data to be normalized, the physiological condition of the organisms to be assessed, and potential biological effects associated with exposure to these particles to be explored. TD is an indicator of body size and relative age in sea urchins, whereas TW represents organism biomass and body condition. The gonads constitute the primary organs for energy storage and reproduction in echinoids. Previous studies have indicated that prolonged exposure to MLPs may alter energy allocation, reduce the gonadal index, and affect gametogenesis. The intestine is the organ in which a large proportion of ingested MLPs accumulates before elimination. Likewise, determining coelomic fluid weight may allow the evaluation of potential physiological alterations associated with inflammatory processes, osmotic changes, or stress responses induced by MLPs (54-55). The sex of the collected *A. nigra* individuals was not determined (53). Species-level identification of *A. nigra* was performed using specialized taxonomic keys (56-57) (Figure. 3).



**Figure 3. Specimen of the black sea urchin *Arbacia nigra***

### **Quality control to prevent MLP contamination**

In the laboratory, airborne contamination was minimized by keeping doors and windows closed. The work area was disinfected with 70% ethanol previously filtered through nitrocellulose membranes with a pore size of 5  $\mu\text{m}$ . Blue nitrile gloves were worn throughout sea urchin handling, and all materials were disinfected before use. Materials used during sample processing, including Petri dishes, beakers, scissors, spatulas, and forceps, were made of metal or glass. Both distilled water and ethanol used to wash samples and laboratory materials were filtered through nitrocellulose membranes with a pore size of 0.45  $\mu\text{m}$ . When not in use, materials and reagents were covered with aluminum foil. To monitor contamination within the laminar-flow cabinet and laboratory, clean, moistened filter paper was placed in a Petri dish at the beginning of each working day. Before use, the filter paper was examined under a microscope to verify the absence of MLP contamination. At the end of the working day, the filters were examined again for the presence of particles. MLPs detected in these controls and attributed to laboratory contamination were subtracted from the number of MLPs recorded during the quantification of *A. nigra* samples <sup>(58)</sup>.

### **MLPs in *Arbacia nigra***

The procedure was based on a protocol for isolating microplastic particles from tissues of marine invertebrates <sup>(59)</sup>. For each *A. nigra* individual, the internal cavity was dissected, and the gonads, intestine, and coelomic fluid were separated. The tissues obtained as described in the morphometric parameters section were placed in 250- or 500-mL conical flasks.

Each sample was digested with a 10% potassium hydroxide (KOH) solution using a ratio of 5 mL of solution per gram of tissue (5:1, v/w). A standardized protocol was followed for the preparation of the 10% KOH solution <sup>(60)</sup>. Digestion was performed at 60 °C for 24 h under continuous agitation at 45 rpm to isolate MLPs from the organic tissue.

A 10% KOH solution was used instead of the Fenton reaction because KOH enables efficient digestion of biological tissues while preserving the physical integrity of most plastic polymers. In contrast, the Fenton reaction generates highly oxidizing hydroxyl radicals that may modify particle morphology or degrade certain polymer types, potentially introducing bias into the quantification and identification of MLPs <sup>(61-63)</sup>. MLPs were evaluated separately in the gonads, intestine, and coelomic fluid. After digestion, the particles and partially digested tissue were rinsed through 250- $\mu$ m stainless-steel sieves using previously filtered running water. The retained material was then transferred onto Whatman® Grade 540 hardened ashless filter paper, 90 mm in diameter and with an 11- $\mu$ m pore size (GE Healthcare Life Sciences, United Kingdom). The filters were dried at 60 °C, placed in glass Petri dishes, and sealed to prevent contamination before and during visual inspection. MLP counting was performed in a laminar-flow cabinet (Biobase, China).

### Physical characterization of MLPs

MLP samples stored in glass Petri dishes were examined using a NIKON SMZ745T stereomicroscope equipped with a camera and CMOS software (TOUPCAM E3ISPM12000KPB) <sup>(64-66)</sup>. Criteria used to classify a particle as an MLP included uniform coloration and the absence of organic or cellular structures. All particles with a maximum dimension <5 mm were photographed and classified according to their morphological characteristics, including shape, color, and size <sup>(67)</sup>.

MLPs were classified by shape as fragments, films, fibers, or foams <sup>(67-68)</sup>. Fragments were defined as particles with irregular shapes and edges, potentially originating from the fragmentation of larger particles. Films were also irregular in shape but were thinner than fragments and exhibited a flexible appearance. Fibers were characterized by one dimension, corresponding to length, being considerably greater than their second projected dimension, corresponding to width or diameter. In this study, microparticles with a length-to-width aspect ratio >4 were classified as fibers, whereas particles with a lower aspect ratio were classified as fragments or films. Foams were defined as solid, brittle, and porous particles <sup>(69-71)</sup>. Each MLP was photographed, and its size was measured <sup>(45)</sup>.

To characterize MLP morphology, five micrographs were obtained by scanning electron microscopy (SEM) using an FEI INSPECT S50 microscope operated under high-vacuum conditions at an accelerating voltage of 10 kV. Samples were previously critical-point dried, mounted on stubs using carbon tape, and coated with a layer of gold by sputter coating. They were subsequently observed and photographed at 150 $\times$  magnification <sup>(72)</sup>.

Although chemical identification of polymers using techniques such as  $\mu$ -FTIR or Raman spectroscopy is a reference approach for confirming the composition of microplastics, these analyses were not included because the primary objective of the study

was to evaluate the abundance and morphological characteristics of MLPs present in black sea urchins from the three evaluated beaches. These techniques require specialized instrumentation, trained personnel, prolonged analytical times, and high costs and are therefore not available in all laboratories. Nevertheless, the morphological characterization used in this study allowed a baseline to be established for the presence of MLPs and may provide a basis for future studies aimed at identifying polymer composition and potential sources of origin <sup>(73)</sup>.

### Data analysis for MLPs

Levene's test was used to assess homogeneity of variances, and the Shapiro–Wilk test was used to assess data normality. Morphometric measurements of *A. nigra* and MLP counts were compared among the three beaches using analysis of variance (ANOVA) when the corresponding assumptions were met or the nonparametric Kruskal–Wallis H test when they were not. Subsequent comparisons were performed using Tukey's test or the Mann–Whitney U test, respectively.

Pearson correlation analysis was performed between the number of MLPs in the different compartments of the black sea urchin and the morphometric measurements of *A. nigra* across the three beaches. The distributions of MLPs according to shape, color, and size in the different sea urchin compartments and beaches were analyzed using frequency tables and the chi-square test. In addition, Pearson correlation analysis was performed between the mean size of each total MLP size interval and its corresponding frequency for each beach. Statistical significance was set at  $p < 0.05$ . All descriptive and inferential statistical analyses were performed using IBM SPSS Statistics, version 28.0.

### Data availability

The dataset supporting the findings of this study is publicly available in Mendeley Data under the title: Iannacone J. *Partículas tipo microplástico en el erizo negro *Arbacia nigra* (Molina, 1782) (Echinoidea: Arbaciidae) en tres playas de la costa central del Perú – Dataset*. Mendeley Data, V1, 2026. <https://doi.org/10.17632/rhfg59byd2.1> <sup>(74)</sup>.

## RESULTS

### Morphometric parameters of *Arbacia nigra*

Significant differences among the three beaches in Lima and Callao, Peru, were detected for test diameter (TD), total weight (TW), and gonad weight (**Table 2**). *Arbacia nigra* individuals collected from La Arenilla (La Punta), Callao, showed the highest mean TD, TW, and gonad weight compared with specimens from El Boquerón (Pucusana), Lima, and Bahía Blanca (Ventanilla), Callao. No significant differences among beaches were observed for intestine weight or coelomic fluid weight (**Table 2**). A total of 905 microplastic-like particles (MLPs) were recovered from the 77 *A. nigra* individuals collected from the three beaches in Lima and Callao, corresponding to an overall mean of 11.75 MLPs per individual. The highest total abundance was recorded in Pucusana, with 334 MLPs (12.84 MLPs/individual), followed by La Punta, with 331 MLPs (13.24 MLPs/individual), and Ventanilla, with 240 MLPs (9.23 MLPs/individual) (**Table 3**).

**Table 2. Variation in morphometric variables of *Arbacia nigra* collected from three beaches in Lima and Callao, Peru**

| Morphometric variable     | Pucusana Max–Min | Pucusana Mean $\pm$ SD         | La Punta Max–Min | La Punta Mean $\pm$ SD         | Ventanilla Max–Min | Ventanilla Mean $\pm$ SD      | F     | Sig. |
|---------------------------|------------------|--------------------------------|------------------|--------------------------------|--------------------|-------------------------------|-------|------|
| <b>TD (cm)</b>            | 5.70–3.40        | 4.58 $\pm$ 0.56 <sup>a</sup>   | 6.50–3.20        | 5.01 $\pm$ 0.69 <sup>b</sup>   | 5.40–3.80          | 4.47 $\pm$ 0.44 <sup>a</sup>  | 6.13  | *    |
| <b>TW (g)</b>             | 61.9–23.22       | 41.67 $\pm$ 10.14 <sup>a</sup> | 99.65–27.70      | 63.35 $\pm$ 20.30 <sup>b</sup> | 54.00–21.19        | 31.49 $\pm$ 7.26 <sup>a</sup> | 36.08 | *    |
| <b>Gonads (g)</b>         | 5.57–0.71        | 2.10 $\pm$ 1.07 <sup>a</sup>   | 16.39–0.74       | 6.85 $\pm$ 3.99 <sup>b</sup>   | 2.58–0.14          | 0.98 $\pm$ 0.70 <sup>a</sup>  | 35.9  | *    |
| <b>Intestine (g)</b>      | 4.41–0.01        | 0.70 $\pm$ 1.14 <sup>a</sup>   | 6.62–0.02        | 0.52 $\pm$ 1.31 <sup>a</sup>   | 0.34–0.01          | 0.11 $\pm$ 0.07 <sup>a</sup>  | 2.32  | 0.1  |
| <b>Coelomic fluid (g)</b> | 4.45–0.11        | 2.85 $\pm$ 0.91 <sup>a</sup>   | 6.5–1.52         | 3.40 $\pm$ 1.28 <sup>a</sup>   | 3.48–1.57          | 2.50 $\pm$ 0.50 <sup>a</sup>  | 3.83  | 0.2  |

**SD:** standard deviation; **TW:** total weight; **TD:** test diameter; Max–Min: maximum and minimum values. Identical lowercase letters within rows indicate no significant differences among means ( $p > 0.05$ ) according to Tukey's test. \* indicates statistical significance at  $p < 0.05$ .

### Physical characterization of MLPs

Fragments were the predominant particle shape (58.45%), followed by fibers (26.96%) (**Table 3**). Yellow was the most frequent color (44.98%), followed by blue (27.85%) (**Tables 4 and 5**). Regarding particle size, MLPs <500  $\mu$ m represented the most abundant size class (**Table 6**). Micrographs showing the different shapes and colors of MLPs recovered from *A. nigra* at the three study sites are presented in (**Figures 4–6**). Scanning electron microscopy images of fiber- and foam-like MLPs are shown in (**Figure. 7**).

**Table 3. Abundance of microplastic-like particles (MLPs) according to shape in the gonads, intestine, and coelomic fluid of *Arbacia nigra* collected from three beaches in Lima and Callao, Peru**

| Sampling site        | Compartment    | Fragments  | Fibers     | Foams     | Films      | Total      |
|----------------------|----------------|------------|------------|-----------|------------|------------|
| <b>Pucusana</b>      | Gonads         | 79         | 48         | 3         | 12         | 142        |
|                      | Intestine      | 47         | 28         | 3         | 21         | 99         |
|                      | Coelomic fluid | 48         | 25         | 1         | 19         | 93         |
|                      | <b>Total</b>   | <b>174</b> | <b>101</b> | <b>7</b>  | <b>52</b>  | <b>334</b> |
| <b>La Punta</b>      | Gonads         | 83         | 37         | 4         | 19         | 143        |
|                      | Intestine      | 66         | 28         | 1         | 11         | 106        |
|                      | Coelomic fluid | 51         | 20         | 0         | 11         | 82         |
|                      | <b>Total</b>   | <b>200</b> | <b>85</b>  | <b>5</b>  | <b>41</b>  | <b>331</b> |
| <b>Ventanilla</b>    | Gonads         | 61         | 28         | 1         | 14         | 104        |
|                      | Intestine      | 61         | 14         | 0         | 2          | 77         |
|                      | Coelomic fluid | 33         | 16         | 2         | 8          | 59         |
|                      | <b>Total</b>   | <b>155</b> | <b>58</b>  | <b>3</b>  | <b>24</b>  | <b>240</b> |
| <b>Overall total</b> |                | <b>529</b> | <b>244</b> | <b>15</b> | <b>117</b> | <b>905</b> |

**MLPg:** MLPs in gonads; **MLPi:** MLPs in intestine; **MLPc:** MLPs in coelomic fluid; **MLPp:** MLPs in individuals from Pucusana; **MLPip:** MLPs in individuals from La Punta; **MLPv:** MLPs in individuals from Ventanilla.

**Table 4. Abundance of microplastic-like particles (MLPs) according to color in the gonads, intestine, and coelomic fluid of *Arbacia nigra* collected from three beaches in Lima and Callao, Peru**

| Compartment           | Sampling site | Yellow     | Blue       | Black     | White     | Red        | Green     | Transparent | Total      |
|-----------------------|---------------|------------|------------|-----------|-----------|------------|-----------|-------------|------------|
| <b>Gonads</b>         | Pucusana      | 33         | 68         | 11        | 4         | 20         | 4         | 2           | 142        |
|                       | La Punta      | 69         | 30         | 10        | 5         | 21         | 6         | 2           | 143        |
|                       | Ventanilla    | 26         | 26         | 10        | 2         | 30         | 3         | 7           | 104        |
|                       | <b>Total</b>  | <b>128</b> | <b>124</b> | <b>31</b> | <b>11</b> | <b>71</b>  | <b>13</b> | <b>11</b>   | <b>389</b> |
| <b>Intestine</b>      | Pucusana      | 50         | 26         | 8         | 2         | 9          | 1         | 3           | 99         |
|                       | La Punta      | 61         | 26         | 5         | 2         | 8          | 1         | 3           | 106        |
|                       | Ventanilla    | 46         | 12         | 1         | 0         | 12         | 2         | 4           | 77         |
|                       | <b>Total</b>  | <b>157</b> | <b>64</b>  | <b>14</b> | <b>4</b>  | <b>29</b>  | <b>4</b>  | <b>10</b>   | <b>282</b> |
| <b>Coelomic fluid</b> | Pucusana      | 45         | 30         | 5         | 1         | 12         | 0         | 0           | 93         |
|                       | La Punta      | 47         | 20         | 5         | 0         | 8          | 0         | 2           | 82         |
|                       | Ventanilla    | 30         | 14         | 0         | 1         | 6          | 3         | 5           | 59         |
|                       | <b>Total</b>  | <b>122</b> | <b>64</b>  | <b>10</b> | <b>2</b>  | <b>26</b>  | <b>3</b>  | <b>7</b>    | <b>234</b> |
| <b>Overall total</b>  |               | <b>407</b> | <b>252</b> | <b>55</b> | <b>17</b> | <b>126</b> | <b>20</b> | <b>28</b>   | <b>905</b> |

**MLPg:** MLPs in gonads; **MLPi:** MLPs in intestine; **MLPc:** MLPs in coelomic fluid.

**Table 5. Abundance of microplastic-like particles (MLPs) according to color and shape in *Arbacia nigra* collected from three beaches in Lima and Callao, Peru**

| Sampling site     | Shape        | Yellow     | Blue       | Black     | White    | Red       | Green    | Transparent | Total      |
|-------------------|--------------|------------|------------|-----------|----------|-----------|----------|-------------|------------|
| <b>Pucusana</b>   | Fragments    | 99         | 42         | 4         | 0        | 23        | 5        | 1           | 174        |
|                   | Fibers       | 11         | 56         | 16        | 1        | 14        | 0        | 3           | 101        |
|                   | Foams        | 1          | 0          | 0         | 6        | 0         | 0        | 0           | 7          |
|                   | Films        | 17         | 26         | 4         | 0        | 4         | 0        | 1           | 52         |
|                   | <b>Total</b> | <b>128</b> | <b>124</b> | <b>24</b> | <b>7</b> | <b>41</b> | <b>5</b> | <b>5</b>    | <b>334</b> |
| <b>La Punta</b>   | Fragments    | 153        | 15         | 2         | 2        | 17        | 6        | 5           | 200        |
|                   | Fibers       | 17         | 39         | 15        | 0        | 12        | 0        | 2           | 85         |
|                   | Foams        | 0          | 0          | 0         | 5        | 0         | 0        | 0           | 5          |
|                   | Films        | 7          | 22         | 3         | 0        | 8         | 1        | 0           | 41         |
|                   | <b>Total</b> | <b>177</b> | <b>76</b>  | <b>20</b> | <b>7</b> | <b>37</b> | <b>7</b> | <b>7</b>    | <b>331</b> |
| <b>Ventanilla</b> | Fragments    | 80         | 16         | 1         | 0        | 42        | 6        | 10          | 155        |
|                   | Fibers       | 18         | 23         | 8         | 1        | 4         | 1        | 3           | 58         |
|                   | Foams        | 0          | 0          | 0         | 2        | 0         | 1        | 0           | 3          |
|                   | Films        | 4          | 13         | 2         | 0        | 2         | 0        | 3           | 24         |
|                   | <b>Total</b> | <b>102</b> | <b>52</b>  | <b>11</b> | <b>3</b> | <b>48</b> | <b>8</b> | <b>16</b>   | <b>240</b> |

**TMLP:** total microplastic-like particles.

**Table 6. Abundance of microplastic-like particles (MLPs) according to size range in the gonads, intestine, and coelomic fluid of *Arbacia nigra* collected from three beaches in Lima and Callao, Peru**

| Size range (µm) | Pucusana g | Pucusana i | Pucusana c | La Punta g | La Punta i | La Punta c | Ventanilla g | Ventanilla i | Ventanilla c | Total MLPs |
|-----------------|------------|------------|------------|------------|------------|------------|--------------|--------------|--------------|------------|
| <500            | 88         | 63         | 55         | 89         | 72         | 58         | 68           | 65           | 38           | 596        |
| 501-1000        | 28         | 12         | 21         | 23         | 16         | 12         | 17           | 5            | 5            | 139        |
| 1001-1500       | 12         | 11         | 6          | 9          | 7          | 6          | 4            | 3            | 8            | 66         |
| 1501-2000       | 4          | 4          | 2          | 4          | 3          | 1          | 5            | 1            | 3            | 27         |
| 2001-2500       | 6          | 1          | 3          | 6          | 3          | 1          | 2            | 1            | 4            | 27         |
| 2501-3000       | 1          | 1          | 3          | 5          | 2          | 2          | 4            | 0            | 1            | 19         |
| 3001-3500       | 1          | 4          | 1          | 5          | 2          | 0          | 2            | 0            | 0            | 15         |
| 3501-4000       | 2          | 0          | 0          | 1          | 1          | 1          | 0            | 1            | 0            | 6          |
| 4001-4500       | 0          | 0          | 1          | 1          | 0          | 1          | 1            | 1            | 0            | 5          |
| >4500           | 0          | 3          | 1          | 0          | 0          | 0          | 1            | 0            | 0            | 5          |

**g:** MLPs in gonads; **i:** MLPs in intestine; **c:** MLPs in coelomic fluid; **Total MLPs:** total microplastic-like particles.

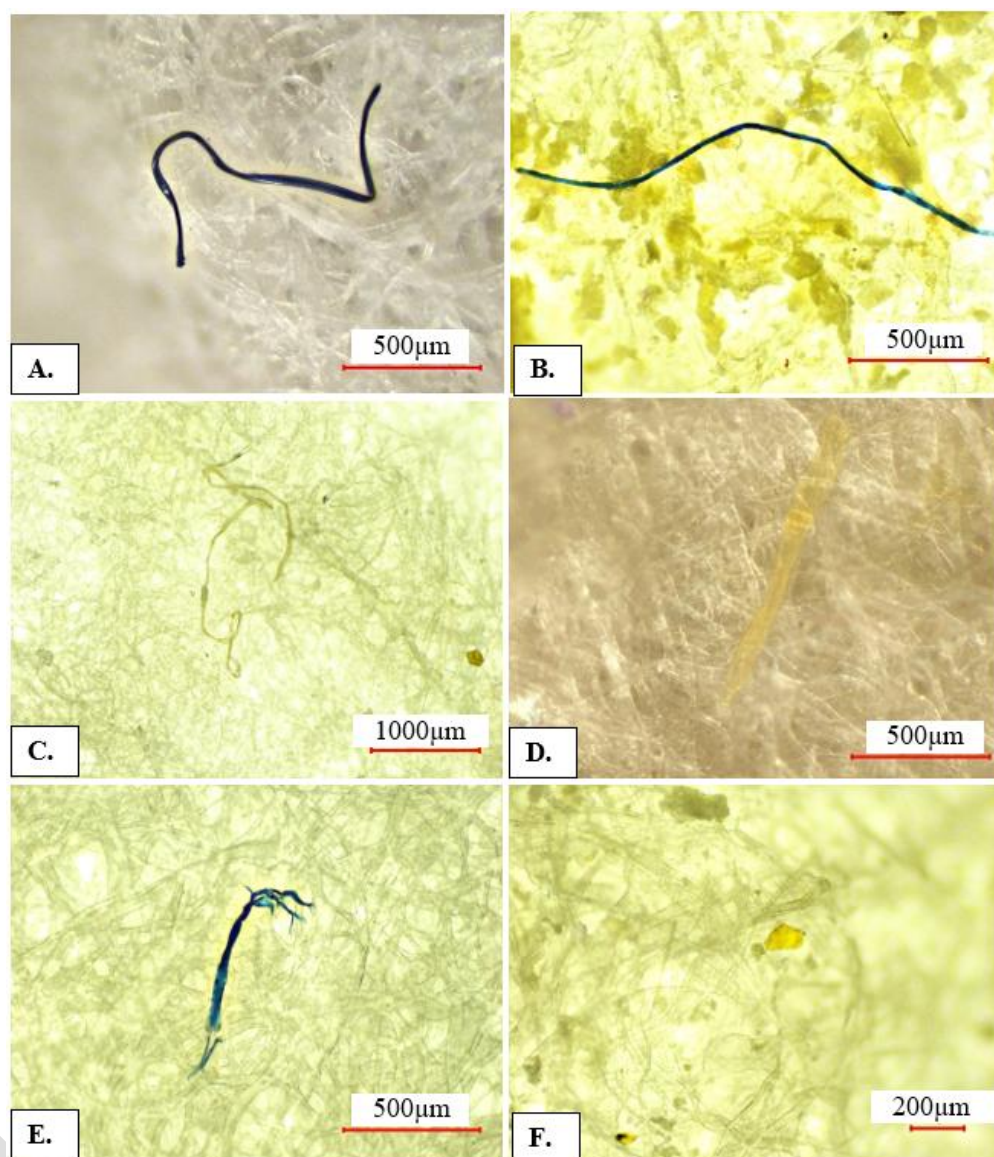


**Figure 4.** Micrographs showing different shapes and colors of microplastic-like particles found in *Arbacia nigra* from Pucusana, Lima, Peru. (A) Blue fiber; (B) blue fiber; (C) transparent fiber; (D) yellow film; (E) blue fiber; (F) yellow fragment.

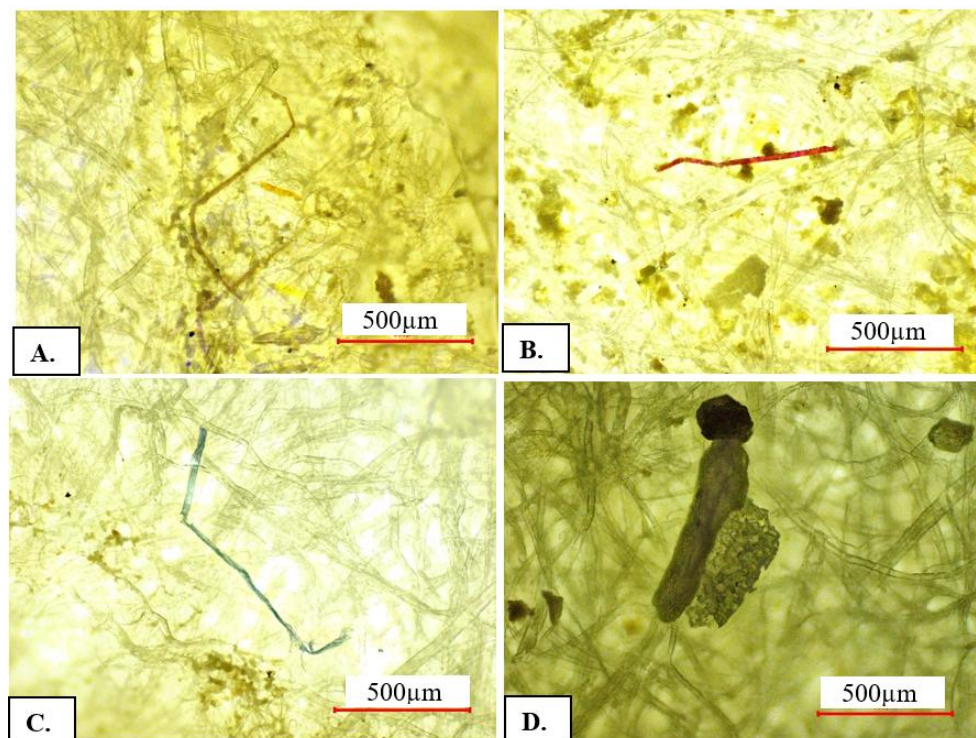


**Figure 5.** Micrographs showing different shapes and colors of microplastic-like particles found in *Arbacia nigra* from La Punta, Callao, Peru. (A) Red fiber and yellow fragment; (B) red fiber; (C) blue film; (D) white foam.

#### Associations between morphometric parameters of *Arbacia nigra* and MLP abundance

In Pucusana, no significant correlations were found between TD or TW of *A. nigra* and MLP abundance in the gonads, intestine, coelomic fluid, or whole individual ( $r = -0.11$  to  $0.32$ ,  $p > 0.05$ ). Similarly, in La Punta, no significant associations were observed between TD and MLP abundance in the whole individual, gonads, intestine, or coelomic fluid ( $r = 0.17$  to  $0.33$ ,  $p > 0.05$ ). However, significant positive associations were observed between TW and MLP abundance in the intestine and whole individual at La Punta ( $r = 0.52$ – $0.56$ ,  $p < 0.05$ ). In Ventanilla, no significant correlations were found between TD or TW and MLP abundance in the gonads, intestine, coelomic fluid, or whole individual ( $r = -0.33$  to  $0.07$ ,  $p > 0.05$ ).

Individuals from Ventanilla showed a lower mean MLP abundance per individual ( $9.23 \pm 5.53$ ) than those from Pucusana ( $12.84 \pm 6.48$ ) and La Punta ( $13.24 \pm 5.88$ ), with a significant overall difference among beaches ( $F = 4.24$ ,  $p = 0.01$ ). When each tissue compartment was compared separately among the three beaches, no significant differences in MLP abundance were detected ( $F = 0.15$ – $2.12$ ,  $p = 0.12$ – $0.83$ ).

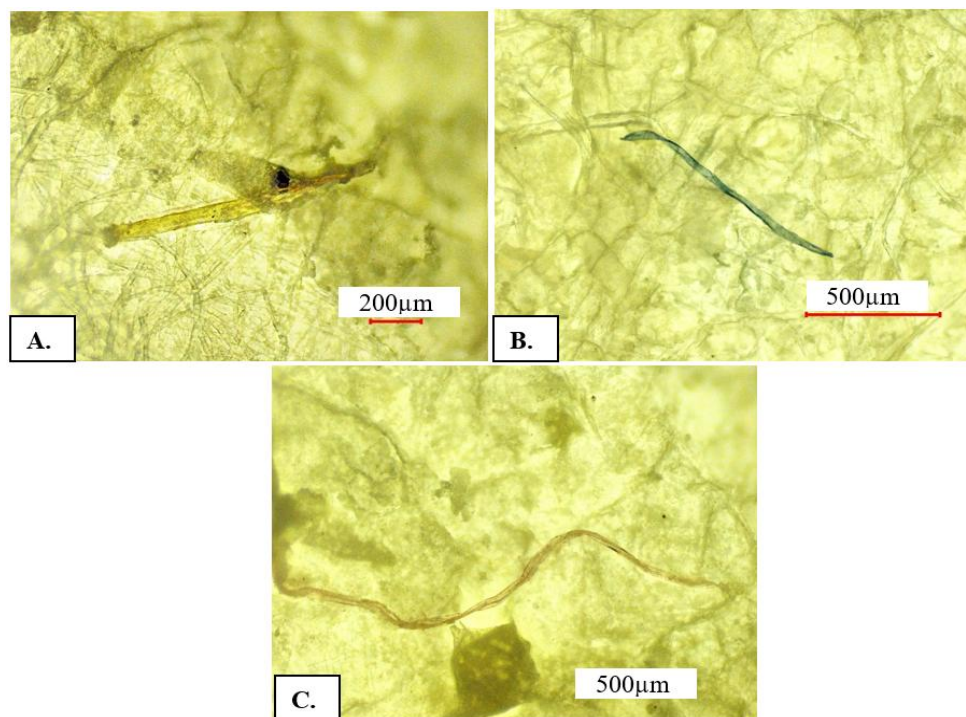


**Figure 6.** Micrographs showing different shapes and colors of microplastic-like particles found in *Arbacia nigra* from Ventanilla, Callao, Peru. (A) Yellow fiber; (B) blue fiber; (C) yellow fiber.

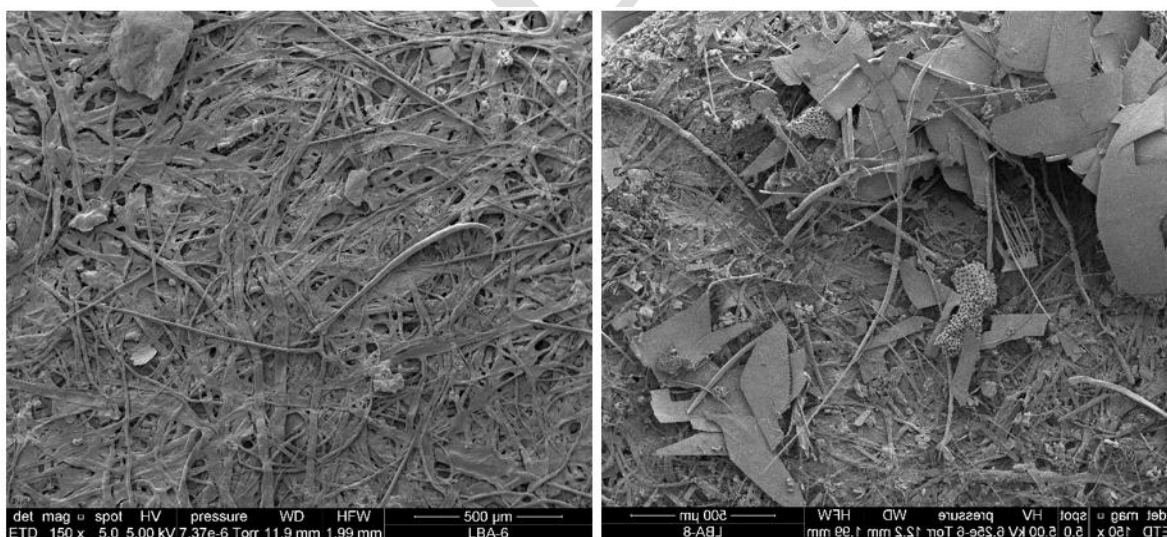


**Figure 7.** Scanning electron micrographs of microplastic-like particles. (A) Fiber; (B) foam.

Within Pucusana, mean MLP abundance did not differ significantly among the gonads, intestine, and coelomic fluid ( $F = 2.61$ ,  $p = 0.08$ ; Levene's test for homogeneity of variances,  $p = 0.71$ ). In contrast, significant differences were observed at La Punta between the gonads ( $5.72 \pm 3.62$  MLPs/individual) and coelomic fluid ( $3.28 \pm 2.15$  MLPs/individual), whereas the intestine ( $4.24 \pm 3.56$  MLPs/individual) did not differ significantly from either compartment ( $F = 3.72$ ,  $p = 0.02$ ; Levene's test,  $p = 0.18$ ). Similarly, at Ventanilla, mean MLP abundance differed significantly between the gonads ( $4.00 \pm 2.21$  MLPs/individual) and coelomic fluid ( $2.27 \pm 2.16$  MLPs/individual), whereas the intestine ( $2.96 \pm 1.48$  MLPs/individual) did not differ significantly from either compartment ( $F = 5.03$ ,  $p = 0.008$ ; Levene's test,  $p = 0.30$ ).

### Microplastic-like particles according to shape

Fragments and fibers were the most abundant MLP shapes in the gonads, intestine, and coelomic fluid of *A. nigra* from the three beaches in Lima and Callao (Table 3). No significant association was found between MLP shape and tissue compartment in individuals from Pucusana and La Punta ( $\chi^2 = 3.85$ – $10.75$ ,  $p = 0.09$ – $0.69$ ). In contrast, a significant association between MLP shape and tissue compartment was observed in *A. nigra* from Ventanilla ( $\chi^2 = 14.89$ ,  $p = 0.02$ ).

### Microplastic-like particles according to color

Among the seven MLP colors recorded in the gonads, intestine, and coelomic fluid of *A. nigra* from the three study beaches, yellow and blue were the most abundant, followed by red and black. Significant associations were found between MLP color and beach of origin for particles recovered from the gonads and coelomic fluid ( $\chi^2 = 24.86$ – $52.47$ ,  $p = 0.001$ – $0.01$ ). In contrast, no significant association between MLP color and beach of origin was observed for particles recovered from the intestine ( $\chi^2 = 13.61$ ,  $p = 0.32$ ) (Table 4).

### Microplastic-like particles according to color and shape

Fragments and fibers were the most abundant MLP shapes and were predominantly yellow, blue, and red. A significant association between MLP color and shape was observed at the three study beaches ( $\chi^2 = 82.11$ – $375.75$ ,  $p < 0.001$ ) (Table 5).

### Microplastic-like particles according to size

The MLPs recovered from *A. nigra* were distributed across 10 size classes, ranging from  $<500 \mu\text{m}$  to  $>4500 \mu\text{m}$ . Particles  $<500 \mu\text{m}$  were the most abundant, followed by those in the  $501$ – $1000 \mu\text{m}$  size class. Larger particles were progressively less represented, showing an overall decrease in MLP abundance with increasing particle size (Table 6). This pattern was reflected by significant negative correlations between MLP size and total MLP abundance in Pucusana ( $r = -0.69$ ,  $p = 0.02$ ), La Punta ( $r = -0.66$ ,  $p = 0.03$ ), and Ventanilla ( $r = -0.63$ ,  $p = 0.04$ ).

## DISCUSSION

### Morphometric parameters of *Arbacia nigra*

Although differences in body size and gonad weight of *Arbacia nigra* were observed among the evaluated beaches, these results do not allow such variations to be directly attributed to the presence of MLPs. Growth and reproductive investment in sea urchins are determined by multiple environmental factors, including food availability, hydrodynamics, temperature, primary productivity, and the reproductive condition of the populations. Consequently, the observed morphometric differences probably reflect the interaction of several ecological factors <sup>(54)</sup>. Sea urchins such as *A. nigra* are common invertebrates of rocky marine benthic habitats, and they have recently been suggested to act as potential sinks for MPs and as a biological pathway through which these particles may enter marine-coastal food webs <sup>(36,39,40,45)</sup>.

### Physical characterization of MLPs

A total of **905** MLPs were recovered from the 77 *A. nigra* individuals collected from the three beaches in Lima and Callao, Peru, corresponding to a mean of 11.75 MLPs per individual. In the sea urchin *Diadema africanum* collected in Tenerife, Canary Islands, Spain, a mean concentration of  $9.7 \pm 3.9$  particles/individual was reported, which is comparable to that observed in the present study <sup>(40)</sup>. For *Paracentrotus lividus*, a mean abundance of  $1.0 \pm 0.30$  MPs per individual has been reported in specimens collected from Sardinia, Italy <sup>(39)</sup>. In *Triploneustes gratilla*, values of up to 233 particles/individual have been reported <sup>(36)</sup>. Differences in MLP abundance among studies may reflect site-specific exposure levels associated with local pollution sources, habitat characteristics, or feeding behavior specific to each sea urchin species <sup>(45)</sup>. Likewise, when comparing concentrations of anthropogenic particles among studies, differences in methodological protocols, quality control and quality assurance procedures, organism size, and the tissues analyzed should be considered because these factors may considerably influence the results obtained <sup>(45)</sup>. MLPs detected in adult *A. nigra* individuals could enter through at least two pathways described in other sea urchin species: ingestion of food and the entry of water through the madreporite plate <sup>(44)</sup>.

### Associations between morphometric parameters of *Arbacia nigra* and the physical characteristics of MLPs

In general, no significant correlations were observed between test diameter (TD), total weight (TW), and MLP abundance in the gonads, intestine, coelomic fluid, or whole individual. However, at La Punta, significant positive associations were observed between TW and MLP abundance in the intestine and in the whole individual. Results reported in the literature suggest an inverse relationship between sea urchin size and the number of ingested MLPs <sup>(44)</sup>. In smaller individuals of *Strongylocentrotus intermedius*, *Temnopleurus hardwickii*, *Temnopleurus reevesii*, and *Hemicentrotus pulcherrimus*, evaluated using test diameter and body weight, a higher abundance of MPs has been reported. Likewise, a higher particle load has been associated with increased

digestive tract weight and with a reduction in gonadal index or gonadal quality, which could indicate effects on reproductive condition <sup>(43)</sup>. MLP abundance in the gonads of *Diadema setosum* has also been reported to decrease as gonad weight increases <sup>(75)</sup>. Although most studies on plastic contamination in sea urchins have focused on the intestine, some investigations have also included the gonads and other compartments, such as the coelomic fluid <sup>(45)</sup>. In the present study with *A. nigra*, MLPs were evaluated separately in the intestine, gonads, and coelomic fluid.

*A. nigra* individuals from Ventanilla showed a lower mean MLP abundance per individual than those from Pucusana and La Punta. These differences among beaches could reflect site-specific exposure levels related to local pollution sources. During the study, solid waste, including food wrappers, bottles, and plastic bags, was observed in the rocky intertidal zone and near the piers at the three beaches. Street vendors and artisanal fishers were also observed at La Arenilla and Bahía Blanca. Other factors that could influence the results include the presence of bathers, proximity to urbanized areas, and proximity to industrial sources. At El Boquerón, the presence of a nearby sewage system could also have an influence.

### Microplastic-like particles according to shape

The predominant MLP shapes in *A. nigra* were fragments (58.45%) and fibers (26.96%). In *D. africanum* collected from the Canary Islands, Spain, fibers and fragments also predominated, although in different proportions: stereomicroscopic analysis showed 97.5% microfibers and 1.9% fragments <sup>(40)</sup>. For *P. lividus*, proportions closer to those observed in the present study have been reported, with 22% fibers, 56% fragments, 13% foams, and 9.0% films <sup>(39)</sup>. In *Parenchinus angulosus*, filaments or fibers and fragments represented 93% and 6%, respectively <sup>(76)</sup>. These differences among studies may reflect regional variation in particle sources, environmental conditions, and the methodological procedures used <sup>(45)</sup>. The predominance of fibers and fragments could be related to anthropogenic sources, including wastewater and fragmentation of plastic materials. Fibers, in particular, may originate from the fragmentation of synthetic textiles or fishing gear <sup>(40,45)</sup>.

### Microplastic-like particles according to color

The predominant colors of MLPs found in *A. nigra* were yellow, blue, and red. In *D. africanum* from Tenerife, other colors predominated, mainly blue (45.15%) and translucent white (36%), with black occurring at a lower proportion <sup>(40)</sup>. A different pattern has also been described in *P. lividus*, with gray (65%), brown (22%), black (9.0%), and blue (4.0%) particles <sup>(39)</sup>. In *Arbacia lixula*, the predominant colors were colorless/translucent (63.6%) and blue (21.9%), followed by pink (6.6%) and black (3.3%) <sup>(45)</sup>. Blue particles have predominated in several studies conducted on marine species, unlike the present study, in which yellow was the most frequent color, and blue ranked second <sup>(44)</sup>. A higher occurrence of dark-colored MLPs, particularly black and blue, has also been described in other sea urchin species <sup>(76)</sup>. Some studies suggest that selection of particular particles may be related to the similarity of their color to natural food sources <sup>(44)</sup>.

Variations in MLP color may be related to the original color of the plastic source and to modifications occurring during their residence in the environment. Likewise, prolonged exposure to ultraviolet radiation may promote oxidative processes and changes in particle coloration <sup>(45)</sup>. The high occurrence of yellow particles observed in this study could be related to their visual similarity to algal fragments or other organic particles, which could favor their ingestion. They could also originate from materials such as fishing nets, packaging, marine paints, or other yellow plastic objects <sup>(36)</sup>. Environmental degradation processes can also produce changes in the coloration of plastic materials <sup>(45,77-78)</sup>.

### Microplastic-like particles according to size

MLPs <500 µm were the most abundant, followed by those ranging from 501 to 1000 µm. In *D. africanum*, most of the recorded microfibers ranged from 250 to 2250 µm in size <sup>(40)</sup>, whereas MLPs >1000 µm have also been identified in the digestive tract of other sea urchin species <sup>(79)</sup>.

### Microplastic-like particles according to beach

At La Punta and Ventanilla, significant differences in mean MLP abundance per individual were observed between the gonads and coelomic fluid, whereas the abundance recorded in the intestine did not differ significantly from that observed in either of these compartments. In *Diadema setosum*, higher MLP levels have been recorded in the digestive tract than in the gonads, with significant differences between the two tissues <sup>(37)</sup>.

The presence of MLPs in the gonads of *A. nigra* is relevant because interactions between these particles and gonadal components have been suggested to affect processes related to reproductive function <sup>(36)</sup>. In addition, the gonads of certain sea urchin species are used for human consumption, raising the possibility of exposure to particles present in these tissues <sup>(80)</sup>. More detailed studies are required to evaluate the potential risks associated with the consumption of hydrobiological products containing MLPs. Marine organisms such as small fish, bivalves, gastropods, and echinoderms may represent a potential pathway of human exposure to MLPs because of the presence of these particles in tissues and organs such as the gastrointestinal tract and gills <sup>(44)</sup>. In addition, policies and regulations aimed at reducing plastic use and plastic-associated pollution, particularly from single-use plastic products, have been implemented in different countries. However, the effectiveness of these measures depends on their appropriate implementation and enforcement <sup>(81)</sup>.

### Scope and limitations

One limitation of the present study was the absence of chemical characterization of polymers using spectroscopic techniques such as µ-FTIR or Raman spectroscopy. Consequently, particle identification was based on morphological and physical criteria, which allowed the presence and characteristics of MLPs to be quantified and their distribution among the three evaluated beaches

to be compared, but did not allow confirmation of their polymer composition or precise identification of their specific sources. Nevertheless, the morphological characterization performed provides information on the abundance and physical characteristics of MLPs present in *A. nigra* and constitutes a baseline for future studies incorporating chemical polymer identification techniques.

## CONCLUSIONS

When the morphometric measurements of *Arbacia nigra*, including test diameter (TD), total weight (TW), gonad weight, intestine weight, and coelomic fluid weight, were compared among the three beaches in Lima and Callao, Peru, individuals from La Punta, Callao, showed the highest values for TD, TW, and gonad weight compared with those from Pucusana and Ventanilla. In general, no significant correlations were found between TD or TW and MLP abundance in the gonads, intestine, coelomic fluid, or whole individual; however, at La Punta, significant positive associations were observed between TW and MLP abundance in the intestine and whole individual. Black sea urchins from Ventanilla showed a lower MLP abundance per individual than those from Pucusana and La Punta. The most abundant MLPs were fragments and fibers, predominantly yellow, blue, and red. Overall, MLP abundance decreased as particle size increased. These findings highlight the need to implement strategies aimed at managing and reducing plastic waste in marine-coastal areas and underscore the importance of additional studies involving organisms from different trophic levels to better understand the associated ecological risks.

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## ETHICAL CONSIDERATIONS

The authors state that all applicable national and international institutional regulations were followed during sample collection, biosafety procedures, and the final management of chemical and biological waste generated during the study. MLPs are currently considered non-hazardous waste and were therefore disposed of in accordance with Supreme Decree No. 014-2017-MINAM (82), which establishes that non-hazardous solid waste generated by environmental testing laboratories should be managed through the municipal public cleaning service. The project was approved by the Research, Innovation, and Entrepreneurship Unit and the Ethics Committee of the Faculty of Natural Sciences and Mathematics, Universidad Nacional Federico Villarreal, through Evaluation Report No. 01-2024-CEI-FCCNM-UNFV.

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## DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest

## AUTHOR CONTRIBUTIONS

**Iannacone J:** study conception and design, data collection, data analysis and interpretation, writing of the original draft, and critical revision of the manuscript. **Obregón-Ruiz VA:** study conception and design, data collection, data analysis and interpretation, and writing of the original draft. **Núñez-Bolaños FA** and **Rojas-Casamayor N:** study conception and design, data collection, and writing of the original draft. **Canchari-Madueño FI, Alvaríño-Flores L, and Castañeda-Pérez L:** study conception and design, data analysis and interpretation, writing of the original draft, and critical revision of the manuscript. **Sernaque-Auccahuasi FA** and **Carvalho-Viana D:** study conception and design, data analysis and interpretation, and writing of the original draft.

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