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*Research Article*

# Microplastic ingestion by copepods in a coastal environment of the Gulf of California, Mexico

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**ABSTRACT.** This study examined the ingestion of microplastics (MPs) by copepods in the Bay of La Paz, the largest and deepest coastal environment in the Gulf of California (GC), Mexico. The research was based on zooplankton samples collected during oceanographic cruises from 2003 to 2016 at a location near the urban area of La Paz city. In the laboratory, adult copepods were selected from the samples, and a dilution process was used to extract the MPs from their bodies. Were extracted 428 MPs, consisting of fibers, films, fragments, and pellets (microspheres). No evidence was found to suggest that the ingestion of MPs has increased over time. The year 2009 recorded the highest number of MPs, with 120 items, which may be attributed to the circulation pattern during that period, which was influenced by an old, decaying cyclonic eddy. In contrast, 2014 had the fewest MPs, with only 44 items. Throughout the study, transparent fibers were the most conspicuous material. Research on the ingestion of MPs by copepods in Mexico is still in its early stages. According to the available literature, this study represents the first documented report on this subject in coastal waterbodies within the GC.

**Keywords:** microplastics; copepods; Bay of La Paz; Gulf of California; Mexico

## INTRODUCTION

The Bay of La Paz (BLP) is situated in the southwestern part of the Gulf of California (GC), approximately 200 km from its connection with the open Pacific Ocean (Fig. 1). The bay, like the GC, is known for its high productivity and biodiversity levels, which are the result of a complex circulation pattern (Coria-Monter et al. 2024). One notable example is the presence of internal waves, which generate mixing and enrich the surface layers, benefiting phytoplankton (Coria-Monter et al. 2019). Additionally, hydraulic jumps caused by currents interact with the seafloor, leading to the resuspension of organic matter and nutrients that support plankton growth (Rocha-Díaz et al. 2021).

A key feature of the bay is the presence of a semi-permanent cyclonic eddy, which contributes to the upward movement of nutrients toward the surface through

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Ekman pumping. This process initially benefits phytoplankton (Coria-Monter et al. 2017) and results in a differential distribution of planktonic organisms (both phytoplankton and zooplankton) ranging from the center to the periphery of the eddy. The center primarily supports dinoflagellates, while the periphery is dominated by diatoms (Coria-Monter et al. 2014). Consequently, this differentiation alters the trophic composition of zooplankton groups, with herbivorous organisms prevailing in the eddy center and omnivorous organisms more common in its periphery (Durán-Campos et al. 2015).

These intricate bottom-up mechanisms support higher trophic levels, providing a habitat that offers refuge, growth, and feeding opportunities for several emblematic species, including endangered ones like whales, dolphins, and sea turtles (Durán-Campos et al. 2015). The bay also supports commercially important species, such as sardines, anchovies, and red snappers (Abitia-Cárdenas et al. 1994). Unfortunately, these species are facing numerous threats due to human activities. A recent study identified several anthropogenic pressures resulting from population growth in La Paz, a city with over 250,000 residents (Fig. 1). For instance, agricultural practices, port operations, and plastic waste generation pose significant environmental risks, endangering organisms across all trophic levels, including zooplankton (Coria-Monter et al. 2024).

Zooplankton is comprised of a highly diverse and heterogeneous group of organisms that fundamentally transfer energy and carbon throughout food webs (Steinberg & Landry 2017). The copepods are highlighted as an essential structural and functional component of the zooplankton. Copepods are small microcrustaceans, measuring between 0.5 and 3 mm, and are considered the most numerous organisms on Earth (Mauchline et al. 1998). In the marine ecosystem, they play a fundamental role due to the position they occupy in the food web because firstly, they consume a large amount of phytoplankton, and secondly the copepods are the food for numerous species (Turner 2004); therefore, this dynamic ensures an adequate flow of carbon and energy throughout water column.

Within the BLP, copepods are the dominant organisms within the zooplankton community (Coria-Monter et al. 2020). Their horizontal distribution is closely associated with a cyclonic eddy, which is semi-permanent but experiences significant seasonal variations in speed and diameter. For example, during winter, the presence of this eddy generates a "copepod belt" due to the mixing processes that occur at its edges, which ensures a continuous food supply for these species (Rocha-Díaz et al. 2021).

Copepods typically generate feeding currents using their mouthparts (Boltovskoy 1999). When potential prey enters this current, they capture or reject the particles suspended in the water column (Kiørboe 2011). As a result, copepods are highly susceptible to consuming a variety of materials, both natural and artificial, including microplastics (MPs). Recent research indicates that microcrustaceans prefer ingesting MPs of specific colors, particularly red, yellow, and transparent particles (Horie et al. 2024).

The small size of MPs (~5 mm) poses a significant threat to several populations. These tiny particles can be easily ingested by various organisms, including copepods (Malinowski et al. 2023). Traditionally, MPs have been classified primarily by size; however, the existing size categories are overly broad and lack biological or physical criteria for classification. In response to this issue, new categorization proposals have emerged in the literature, aiming to standardize the classification of MPs based on their interactions with biological organisms. For example, some recent proposals define MPs as particles measuring between 20 and 200 µm, a range corresponding to the sizes of several biological populations, both phytoplankton and zooplankton (Bermúdez & Swarzenski 2021).

Numerous studies have shown that MPs ingestion by copepods is high (Bai et al. 2021) and has been documented in numerous environments worldwide. These include the Atlantic Ocean (Cole et al. 2013), the English Channel (Botterell et al. 2020), the Mediterranean Sea (Costa et al. 2020), and coastal waters of the southwest Atlantic (Suwaki et al. 2020). In all these cases, negative impacts have been observed on crucial aspects of copepod life cycles, such as growth, reproduction, fecundity, and mobility.

In Mexico, current reports from coastal regions of the Gulf of Mexico indicate that copepods have a high ingestion of MPs, with blue and transparent fibers as the most commonly ingested, making up to 85% of all MPs found (Montoya-Melgoza et al. 2024). In the central Mexican Pacific, high levels of microplastic ingestion by copepods have been documented, with these organisms tending to consume a higher proportion of fragments, fibers, and spheres (Zavala-Alarcón et al. 2023). Although studies on the ingestion of MPs by crustaceans, particularly copepods, have gained attention, there is still a lack of research in coastal environments of Mexico, such as the BLP.

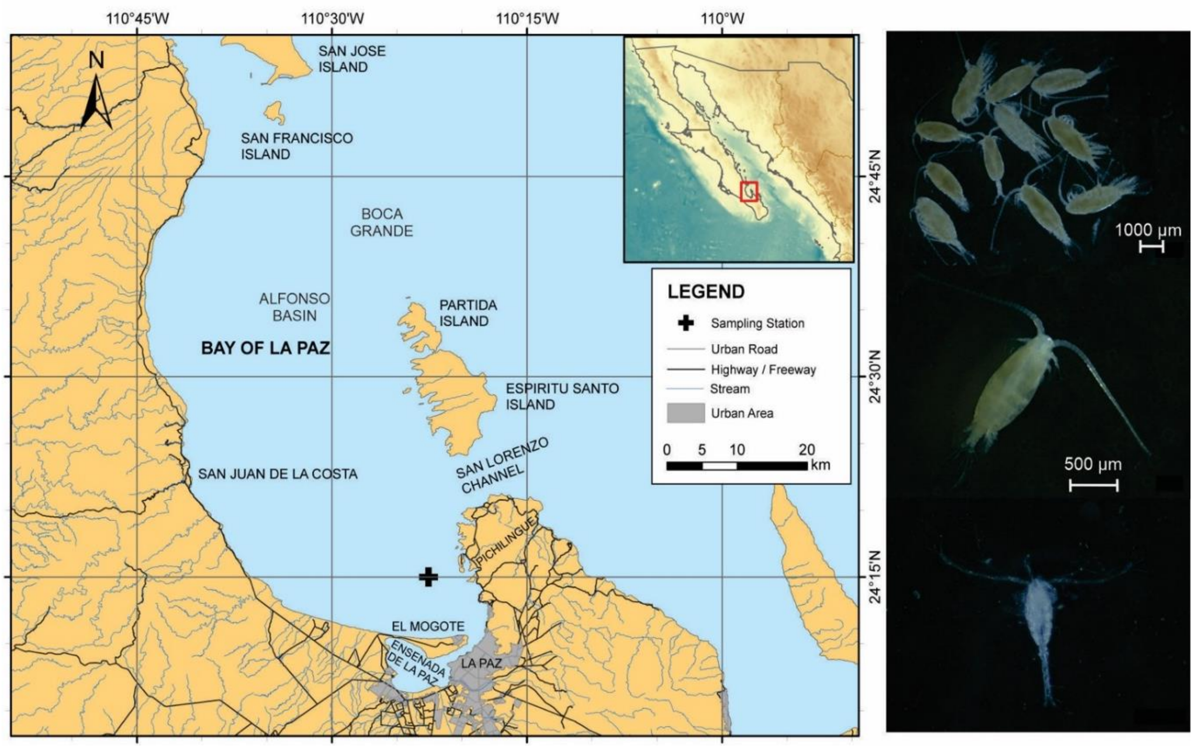


**Figure 1.** Left panel: the study area is the Bay of La Paz in the southwestern Gulf of California. The black plus sign (+) indicates the location where zooplankton samples were collected during various oceanographic cruises conducted from 2003 to 2016 (for more details, refer to Table 1). Right panel: examples of the copepods included in this study.

At the beginning of the 2000s, the National Autonomous University of Mexico (UNAM, by its Spanish acronym) initiated a multidisciplinary monitoring program in the BLP. This program revealed that the region is highly dynamic, characterized by numerous hydrodynamic processes that enhance biological productivity. As part of this monitoring effort, numerous zooplankton samples were collected over the years and analyzed for multiple purposes. However, one aspect that has not yet been explored is the ingestion of MPs by zooplankton and copepods in particular.

This study aims to evaluate the ingestion of MPs by copepods, based on zooplankton samples collected during six research cruises from 2003 to 2016. We hypothesize that ingesting MPs by copepods in this region will be evident across all the studied years, with an increasing trend over time. This study highlights the threats that MPs pose to zooplankton. Additionally, we seek to advance knowledge further regarding global issues related to MPs.

## MATERIALS AND METHODS

### Study area

The BLP is the GC's largest and deepest coastal environment, covering approximately 2,400 km$^2$ (Fig. 1). The bay is separated from the gulf by two main islands: Partida and Espíritu Santo. It shares its waters with the gulf through two main connections: Boca Grande to the north and the San Lorenzo Channel to the south (Monreal-Gómez et al. 2001). The bay's maximum depth, reaching 420 m, is found in the central region, specifically in the Alfonso Basin. Depths gradually decrease towards the south, where they reach just 20 m.

In climatic terms, the bay is influenced by a changing wind pattern; during winter, strong (>12 m s$^{-1}$) and persistent winds present a northwesterly component, while during summer, they present a southeastern component. The riverine discharge into the bay is null, and evaporation exceeds precipitation (Monreal-Gómez et al. 2001).

### Fieldwork

This study used historical zooplankton samples collected in six oceanographic research cruises from 2003 to 2016 (see details in Table 1) carried out on board the R/V El Puma operated by UNAM. For the objective of this study, we selected a station (indicated by the black plus symbol in Fig. 1), located in the southern region of the bay with the coordinates 24°14'59.28"N, 110°22'35.04"W, near the area of influence of La Paz city. The depth at the sampled station was 45 m.

In each oceanographic research cruise, organisms were collected using oblique zooplankton hauls using bongo nets with a 60 cm diameter mouth and a mesh size of 333 µm. Each haul was performed from near the bottom (approximately 5 m) to the surface at a speed of 2 kn for 15 min. Once on board, the samples were immediately fixed with 4% formalin buffered with sodium borate. After 24 h, the samples were transferred to glass jars containing a 70% ethanol solution for final preservation.

To ensure the integrity of the organisms, strict precautions were taken during storage. For instance, the lids of the jars were changed periodically (usually every two months), and the ethanol was replaced regularly (again, typically every two months) to prevent degradation of the samples. This preservation protocol has been successfully implemented in the zooplankton laboratory at the Institute of Marine Sciences and Limnology of UNAM for the past 20 years.

### Laboratory analyses

In the laboratory, adult copepods were selected from the samples using a Carl Zeiss stemi 508 stereomicroscope, which offers magnification from 0.5 to 4.0x. This setup is equipped with a Zeiss Axiocam 105 color camera. This study focused on magnifications of 3.0 and 4.0x to identify adult copepods from Calanoida and Cyclopoida, as these orders are the most prevalent in the marine environment (Harris 2001). For our research objectives, we did not identify the organisms at species level; instead, we classified them at the order level based on their primary feeding habits. These copepods trap materials from seawater using fine hairs on their mouthparts (Harris 2001), making them particularly susceptible to ingesting MPs. Our selection criteria for adult organisms (Fig. 1, right panel) were based on two factors: first, at this stage, all their body structures are fully developed, and second, adult organisms tend to be preferential grazers (Harris 2001).

Following the protocols outlined in Montoya-Melgoza et al. (2024), we selected 1,000 organisms for each research cruise and placed them into 10 mL glass vials. A deep visual inspection of the organisms was conducted under the stereomicroscope to remove any MPs adhered to their bodies. Throughout this process, we took precautions to prevent contamination from foreign MPs by using only glass and stainless steel materials. Additionally, we always wore cotton coats, clothing, caps, and masks. To further minimize the risk of contamination from external fibers, we restricted the movement of scientific personnel in the laboratory.

To extract the MPs from the interior of the copepods, we followed the method proposed by Desforges et al. (2015). This technique completely dissolves the organisms using nitric acid ($HNO_3$), which releases all the plastic particles. Specifically, we added 5 mL of $HNO_3$ at a concentration of 68% to each vial and placed them in a water bath (Cole-Parmer Polystat) for 25 min at a constant temperature of 80ºC. After this period, we filtered the acid from each vial through glass fiber membranes (Whatman GF/F) to capture all the MPs released from the copepods. Each membrane was then placed in glass Petri dishes for observation under the stereomicroscope, allowing us to count, classify, and photograph the MPs. Finally, the recovered MPs were mounted for observation under a Scanning Electron Microscope (JEOL JSM6380 LV) following standard protocols. This step was performed to analyze the recovered items' shape, texture, and fragmentation areas more precisely.

Bivariate correlation analyses were performed to assess the statistical significance of the dataset generated. This process involves examining two variables to determine the nature of their relationship, identifying whether a statistical association exists, assessing the strength of that association if it does, and evaluating whether one variable can be predicted based on the other (Michalos et al. 2005). The analysis was conducted using standard routines in Statistica software.

## RESULTS

### Oceanographic context

This study presents historical data collected from six research cruises between 2003 and 2016. This information has been utilized for various purposes and reported in several scientific articles (e.g. Coria-Monter et al. 2014, Durán-Campos et al. 2020, Sánchez-Mejía et al. 2020, Ramos-de-la-Cruz et al. 2021, Rocha-Díaz et al. 2021). Therefore, we will provide a summary of the oceanographic context.

**Table 1.** Microplastics (number of items) ingested by copepods in the Bay of La Paz in different years.

| Cruise | Mont/Year | Fibers | Films | Fragments | Pellets | MPs for each cruise |
|---|---|---|---|---|---|---|
| PALEO-XI | August 2003 | 35 | 0 | 7 | 3 | 45 |
| DIPAL-I | February 2006 | 74 | 2 | 13 | 2 | 91 |
| DIPAL-III | August 2009 | 106 | 1 | 10 | 3 | 120 |
| DIPAL-V | December 2012 | 56 | 0 | 4 | 2 | 62 |
| PALEOMAR-I | November 2014 | 34 | 5 | 3 | 2 | 44 |
| PALEOMAR-II | June 2016 | 55 | 2 | 6 | 3 | 66 |
| | | | | | | Total 428 |

A strong seasonal variability was observed throughout the cruises. During winter, low surface temperatures (<19ºC) are recorded, which lead to elevated chlorophyll-*a* levels (>5 mg $m^{-3}$). Conversely, as summer approaches, surface temperatures rise above 30ºC, resulting in significant water column stratification and a consequent drop in chlorophyll-*a* levels, with values falling below 1 mg $m^{-3}$.

In all cases, the circulation pattern was dominated by a cyclonic eddy located in the central portion of the bay, in Alfonso Basin. This eddy uplifts the isotherms and transports nutrients from subsurface waters to the surface, benefiting phytoplankton communities.

In the southern part of the study area, at the sampling station considered in this research, east-west currents are observed (Coria-Monter et al. 2014, in their Fig. 1e; Sánchez-Mejía et al. 2020, in their Fig. 3a), flowing from the city of La Paz towards the interior of the bay.

This study examined research cruises conducted during different climatic seasons: summer, autumn, and winter (see details in Table 1). The average surface temperatures and salinity levels recorded in the sampling site considered in this study were as follows: summer (28.1ºC and 35.26), autumn (26.6ºC and 34.90), and winter (20.1ºC and 35.57).

### Microplastics

The results showed a wide variety of MPs items that were recovered from the interior of the copepods: 428 MPs were counted, including miscellaneous material such as fibers, films, fragments, and pellets with different colors such as blue, yellow, gray, red, multicolor, transparent, and green. The most abundant items were fibers that reached 360 particles, followed by fragments (43 items), pellets (15 items), and films (10 items) (Table 1, Fig. 2). The year 2009 presented the highest number of MPs with a total of 120 particles, of which 106 were fibers, 10 fragments, 1 film, and 3 pellets. On the other hand, 2014 recorded the lowest number of MPs, with 44 MPs, including 34 fibers, 3 fragments, 5 films, and 2 pellets (Table 1, Fig. 2).

The percentages of each material recovered from the interior of the copepods are shown in Table 2. Fibers were the most prominent material, making up over 77% of all cases.

Our statistical analyses of the dataset reveal statistical significance in all cases, with a *P*-value of less than 0.05 (Table 3).

Figure 3 illustrates the material recovered from the interiors of copepods collected in the BLP. The material displayed a high diversity in shapes, including round, curvilinear, triangular, and rhomboid forms. It varied in size, ranging from 20 µm to over 1,000 µm, and included a range of colors. Notable examples are transparent fibers (Fig. 3a), blue fibers (Fig. 3b-c), red fragments (Fig. 3d), transparent films (Fig. 3e), and gray pellets (Fig. 3f).

From scanning electron microscopy photographs, Figures 4a-c show fibers with different textures, sizes, and twists, some smooth and flattened and others with striations along their length. Figure 4d is a fragment that presents irregular angles, faces, and parallel lines, showing detachment. Besides, smooth triangular-shaped pieces were recovered, with very well-defined sharp angles (Fig. 4e), and small smooth microspheres were recovered (Fig. 4f).

## DISCUSSION

The techniques used in our study enabled us to identify and characterize the MPs ingested by copepods in the BLP. Since 2003, we have observed that these organisms consume MPs without evidence suggesting this trend has increased. In our study, 2009 marked the highest number of MPs ingested. This peak may be attributed to the circulation patterns influenced by an old decaying cyclonic eddy (Coria-Monter et al. 2014,

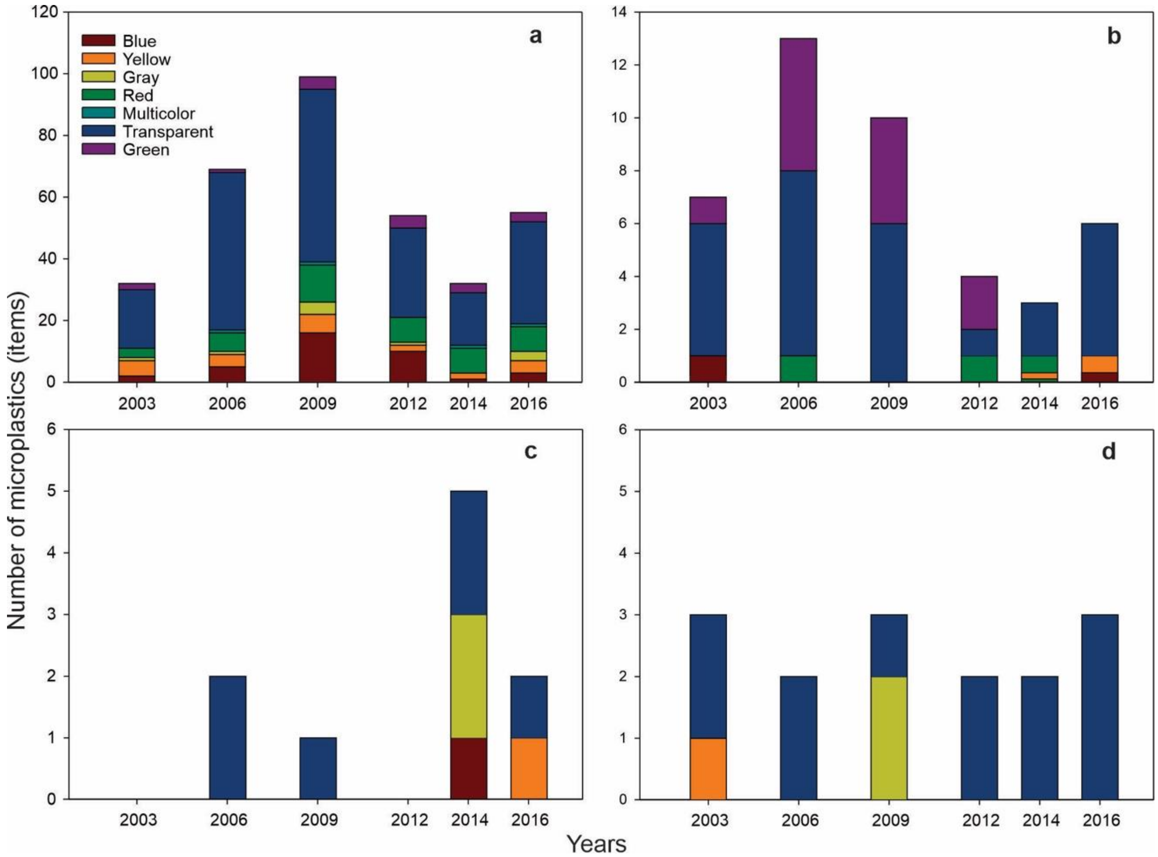


**Figure 2.** Number of microplastics ingested by copepods in the Bay of La Paz by year. a) Fibers, b) fragments, c) film, and d) pellets. Note the change in scale on the Y axis.

**Table 2.** Percentages of microplastics (MPs) ingested by copepods in the Bay of La Paz in different years.

| Research cruise | Month/Year | Total of MPs | Fibers (%) | Films (%) | Fragments (%) | Pellets (%) |
|---|---|---|---|---|---|---|
| PALEO-XI | August 2003 | 45 | 77.7 | 0.0 | 15.6 | 6.7 |
| DIPAL-I | February 2006 | 91 | 81.3 | 2.2 | 14.3 | 2.2 |
| DIPAL-III | August 2009 | 120 | 88.3 | 0.9 | 8.3 | 2.5 |
| DIPAL-V | December 2012 | 62 | 90.3 | 0.0 | 6.5 | 3.2 |
| PALEOMAR-I | November 2014 | 44 | 77.3 | 11.4 | 6.8 | 4.5 |
| PALEOMAR-II | June 2016 | 66 | 83.3 | 3.0 | 9.2 | 4.5 |

see their Fig. 5) and currents that flow from the city of La Paz into the interior of the bay (Coria-Monter et al. 2014, see their Fig. 1e).

Research on the ingestion of MPs by marine life has become increasingly important due to the potential risks to their health. The ingestion of MPs by copepods has been documented in several environments, including the Atlantic Ocean (Cole et al. 2013), the Mediterranean Sea (Costa et al. 2020), the English Channel (Botterell et al. 2020), the Yellow Sea (Zheng et al. 2021), and even the Arctic (Rodríguez-Torres et al. 2020). In all these cases, it was observed that copepods ingest MPs of various shapes and colors, leading to effects on several aspects of their life cycle, such as reproduction, feeding behavior, and vertical migration patterns, among others. In Mexican waters, a few reports document MPs' consumption by copepods, particularly in the Gulf of Mexico (Montoya-Melgoza et al. 2024) and the Pacific Ocean (Zavala-Alarcón et al. 2023). In both areas, high levels of MPs consumption

**Table 3.** *P*-values derived from bivariate correlation analyses.

| | PALEO-XI | DIPAL-I | DIPAL-III | DIPAL-V | PALEOMAR-I | PALEOMAR-II |
|---|---|---|---|---|---|---|
| PALEO-XI | * | 0.0030 | 0.0057 | 0.0071 | 0.0278 | 0.0066 |
| DIPAL-I | 0.0030 | * | 0.0030 | 0.0053 | 0.0169 | 0.0039 |
| DIPAL-III | 0.0057 | 0.0030 | * | 0.0003 | 0.0086 | 0.00005 |
| DIPAL-V | 0.0071 | 0.0053 | 0.0003 | * | 0.0083 | 0.0002 |
| PALEOMAR-I | 0.0278 | 0.0169 | 0.0086 | 0.0083 | * | 0.0077 |
| PALEOMAR-II | 0.0066 | 0.0039 | 0.00005 | 0.0002 | 0.0077 | * |

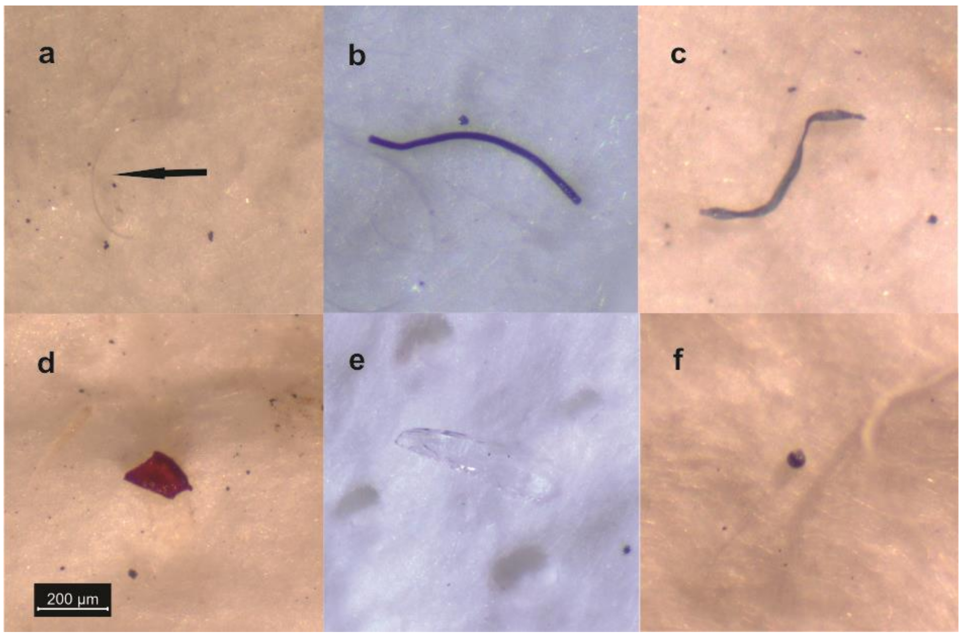


**Figure 3.** Microplastics (MPs) recovered from the copepods' interior in the Bay of La Paz. a) Transparent fiber, b) blue fiber, c) blue fiber, d) red fragment, e) transparent film, and f) gray pellet. The black arrow points to the MPs.

have been recorded, with observations indicating that copepods tend to favor blue, red, and transparent particles. However, there is a noticeable lack of studies in other environments, such as in the GC.

There are very few reports on the ingestion of MPs by the biota of the GC; however, at the time of writing, it is known that populations of sea lions, fish, and shrimp are threatened. Indeed, it has recently been reported that different sea lion *Zalophus californicus* colonies ingest MPs, particularly blue-colored fibers (Ortega-Borchardt et al. 2023). Furthermore, the presence of plastic debris in the gastric contents of teleost fishes has been noticed (Salazar-Pérez et al. 2021), and the presence of fibers in the gastrointestinal tract, gills, and exoskeleton of commercial shrimps of the gulf has recently been reported as a serious threat (Valencia-Castañeda et al. 2024).

In our study, transparent fibers were the most conspicuous material, agreeing with previous reports on ingesting MPs by the macrofauna inside the GC (e.g. Valencia-Castañeda et al. 2024). The presence of transparent fibers in coastal environments has been related to the deposition of wraps, bags, and containers (Flores-Cortés & Armstrong-Altrin 2022) and considering the proximity of La Paz city to the sampling site of our study (see plus black symbol in Fig. 1), it could explain the high proportion of this material that is available for consumption by the marine fauna inside the bay, in this case, the copepods. Furthermore, transparent fibers have been related to the residence time in the environment, being more aged (Frias et al. 2018), and in this sense, some works have shown evidence that copepods tend to consume aged MPs (Vroom et al. 2017).

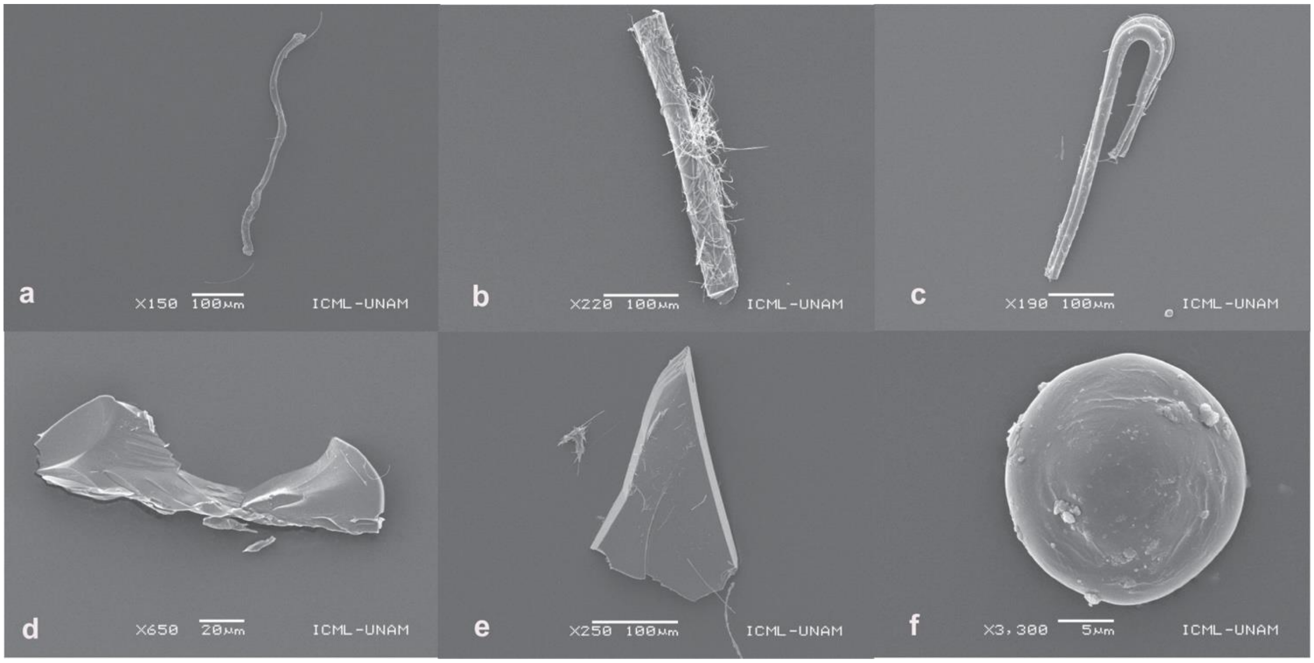


**Figure 4.** Scanning electron microscopy photographs of the microplastics recovered from the interior of the copepods in the Bay of La Paz, Gulf of California, Mexico, showing details on their texture, shape, and fragmentation: a-c) fibers, d-e) fragments, and f) microsphere.

In Mexico, research on ingesting MPs by zooplankton, particularly copepods, is still in its early stages. In contrast, numerous studies conducted globally have documented significant negative effects on the life cycles of copepods due to MPs. One of the earliest reports was by Cole et al. (2013), who found that copepods in the Atlantic Ocean not only ingest MPs but also adhere easily to their bodies. This adhesion impairs their mobility and affects their hunting behavior. Similar findings were presented by Suwaki et al. (2020), who demonstrated that ingesting MPs by copepods in the Flamengo Inlet, Brazil, can reduce their swimming speed by up to 40%. In the Mediterranean Sea, ingesting MPs by copepods has become a serious concern, as it leads to transferring these particles to higher trophic levels, posing risks to numerous species, including humans (Costa et al. 2020). More recently, research on the calanoid copepod *Centropages furcatus* in the Gulf of Thailand highlighted the issue of MPs ingestion, as this organism preferentially consumes MPs fragments that can be transferred through the pelagic food web (Alfonso et al. 2024). These problems may also be affecting copepod populations in the BLP, making multidisciplinary research essential.

In this study, we unfortunately do not have measurements of the chemical composition of the MPs recovered from the interior of the copepods. However, previous research has identified several types of polymers in other marine organisms. For instance, polymers such as polyethylene, nylon, polyethylene terephthalate, polystyrene, polypropylene, and polyamide have been found in the interior of *Penaeus vannamei* shrimp from the GC (Valencia-Castañeda et al. 2024). Similarly, polyamide, polyethylene, polypropylene, and polyacrylic have been retrieved from the interiors of teleost fish in the Gulf (Salazar-Pérez et al. 2021). These polymers may constitute significant components of the MPs detected in our study. Nonetheless, based on the SEM photographs presented in this study and supported by previous literature utilizing a similar approach (e.g. Flores-Cortés & Armstrong-Altrin 2022, Montoya-Melgoza et al. 2024), it is likely that the primary sources of the MPs found in our study are plastic bags, wrappers, and clothing.

## CONCLUSIONS

The results presented here indicate that the ingestion of MPs by the copepods from the BLP has been detectable since 2003. While the quantity of MPs found inside the copepods varied from year to year without showing a clear upward trend, it is evident that MPs remain a persistent issue.

Two thousand nine saw the highest MP levels ingested by copepods in the bay. The explanation for this phenomenon may be found in the oceanographic conditions of the area. As previously mentioned, the BLP is a highly dynamic environment, with significant differences between its northern and southern regions. The southern region, where the sampling location for this study is situated, is characterized by shallow waters (less than 50 m deep) and currents that flow from east to west, specifically from La Paz toward the bay's interior. This current pattern, observed in the summer of 2009 by Coria-Monter et al. (2014) (their Fig. 1e), may explain the increased availability of microplastics for copepod consumption, as these currents transport the MPs into the bay.

Our findings highlight the threats facing the biota within BLP. In this context, copepods occupy the base of the food chain. As they are preyed upon by various species, some of which are consumed by humans, there is a potential for MPs to accumulate along the food chain, ultimately impacting human health. Further research is necessary to investigate the chemical components found in MPs, as they may be toxic and pose risks to ecosystem health and the human population. According to the available literature, the results presented here represent the first report on MPs ingestion by copepods in the BLP. In the region, this problem has begun to gain scientific relevance; however, these studies have been limited only to the evaluation of some populations. In this sense, it is necessary to increase monitoring efforts to identify the threats to which the organisms living inside the BLP are exposed, and thus be able to propose conservation actions for marine fauna to policymakers.

### Credit author contribution

D. López-Mendoza: conceptualization, validation, methodology, formal analysis, writing-original draft; E. Coria-Monter: conceptualization, validation, methodology, formal analysis, writing-original draft, review, and editing; M.A. Monreal-Gómez: conceptualization, validation, methodology, formal analysis, funding acquisition, writing-original draft, review, and editing; E. Durán-Campos: conceptualization, validation, methodology, formal analysis, funding acquisition, writing-original draft, review, and editing; D.A. Salas-de-León: conceptualization, validation, methodology, formal analysis, funding acquisition, writing-original draft, review, and editing; A. Montoya-Melgoza: conceptualization, validation, methodology, formal analysis; F.A. Rocha-Díaz: conceptualization, validation, methodology, formal analysis. All authors have read and accepted the published version of the manuscript.

### Conflict of interest

The authors declare no conflict of interest.

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