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Detection and quantification of microplastic pollution in the endangered Galapagos sea lion

Tesis

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ABSTRACT

Marine debris pollution poses a significant global threat to biodiversity, with plastics being the primary debris type found in oceans due to their low-cost production and high demand worldwide. Microplastics (MPs, <5 mm in size) are highly bioavailable to a wide range of marine taxa, including marine mammals, through direct and indirect ingestion routes (i.e., trophic transfer). Recently, MP pollution has been detected on the Galapagos Marine Reserve, so in this study we developed a baseline framework for MP pollution in the Galapagos sea lion (GSL, *Zalophus wollebaeki*) through scat-based analysis. We collected 180 GSL scat samples from the southeast region following strict quality assurance/quality control protocols to detect, quantify and characterize physical-chemical properties of MPs through visual observations and μFT-IR spectroscopy. We recovered 81 MPs of varying sizes and colors in 37% of samples (n=66), consisting mostly of fibers (69%, $\bar{x}$=0.31 ± 0.57 particles scat-1). The number of particles per gram of sample wet weight ranged from 0.02 to 0.22 ($\bar{x}$=0.04 ± 0.05 particles scat wet g-1). El Malecón and Punta Pitt rookeries at San Cristobal Island had the highest number of MPs ($\bar{x}$=0.67 ± 0.51 and 0.43 ± 0.41 particles scat-1, respectively), and blue-colored particles were the most common in all samples. We identified eleven polymers in 46 particles, consisting mostly of polypropylene-polyethylene copolymer, polypropylene, cellulose, polyethylene, and polyvinyl chloride. The textile, fishing, and packaging industries are likely significant sources of microfibers into this insular ecosystem. Our results suggest that the GSL is exposed to MPs due to anthropogenic contamination and bioaccumulation associated with trophic processes. These findings provide an important baseline framework and insights for future research on MP pollution in the region, as well as for management actions that will contribute to the long-term conservation of the GSL. **Keywords:** Galapagos Marine Reserve, microplastics, plastic pollution, scat-based analysis, trophic transfer, *Zalophus wollebaeki*

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INTRODUCTION

Marine debris pollution is a global threat to marine biodiversity (Alfaro-Núñez et al., 2021; Gall & Thompson, 2015; Pelamatti et al., 2021). Plastics (*i.e.*, synthetic polymers) (Geyer, 2020) are the dominant debris type in the ocean due to their extremely high demand, low production costs, high durability, and persistence; with global plastic production waste reaching 6.3 billion tons in recent years (Jepsen & de Bruyn, 2019; Du et al., 2022; Xiang et al., 2022). Demand for single-use plastic products increased significantly over the last few years to address the public health crisis caused by the SARS-CoV-2 virus outbreak, resulting in higher pollution rates (Peng et al., 2021). The widespread overuse of personal protective equipment (*e.g.*, facemasks, gloves, hospital supplies) and delivery packaging resulted in additional major sources of plastic pollution (Gibbons et al., 2022). If historical trends hold steady, annual global plastic production is likely to reach 1100 million metric tons by 2050, implying a predicted cumulative plastic production of 34 billion tons since 1950 (Geyer, 2020). Thus, the mass production of plastics and deficient waste management strategies have made these synthetic materials a persistent threat to the environment, especially for marine ecosystems (Jambeck et al., 2015; Peng et al., 2021).

Plastic litter degrades slowly but steadily in the ocean due to photolytic, mechanical, and biological action, resulting in meso- (5000 to 10000 µm), micro- (1 to 5000 µm), and nano- (0.001 to 1 µm) particles that are potential vectors of pathogens and chemical contaminants, with an estimated occurrence of over 170 trillion plastic particles within world’s oceans(da Costa, 2018; Zantis et al., 2021; Eriksen et al., 2023). There are primarily two different categories of microplastics (MPs). Primary MPs, which are particles directly available to the environment; and secondary MPs, which are the result of the fragmentation of larger debris (Laskar & Kumar, 2019). Similarly, the composition of these synthetic particles can either be made up from fossil-based or bio-based polymers. Fossil-based polymers (*e.g*., polyethylene, polypropylene, polystyrene, polyvinyl chloride) use fossil fuels as feedstock and encompass most plastic products (Walker & Rothman, 2020; Du et al., 2022). Whereas bio-based polymers (*e.g*., cellulose), rely on biological sources as feedstock (Walker & Rothman, 2020); however, some types of cellulose have been anthropogenically modified, thus altering the natural polymer's chemical composition to enhance its strength and durability (Sanchez-Vidal et al., 2018; Athey et al., 2020). Therefore, semi-synthetic cellulose is persistent in marine ecosystems and has a significant effect on microfiber abundance at a global scale (Athey et al., 2020; Adams et al., 2021).

Depending on various factors (*i.e*., size, density, abundance, color), MPs are bioavailable to a wide range of marine species at different trophic levels (Wright et al., 2013), either through direct (Gouin, 2020) or indirect ingestion of contaminated prey (Nelms et al., 2018). Thus, MPs can be transferred bottom-up, starting from the foundation of marine food webs (*i.e.*, planktonic organisms) and triggering a domino effect of bioaccumulation to higher trophic levels (Botterell et al., 2019; Costa et al., 2020; Miller et al., 2020). These characteristics make marine mammals, particularly cetaceans and pinnipeds, susceptible to direct and indirect MP ingestion routes depending on species-specific feeding strategies (Nelms et al., 2019a; Zantis et al., 2021). Raptorial feeding is the main trophic strategy in pinnipeds, meaning that they feed on prey using only their jaws and teeth, typically consuming them entirely (Hocking et al., 2017), thus suggesting trophic transfer as main route of MP ingestion for this group. Several methods have been developed to study the intake and abundance of MPs in marine mammals, being gut-content and scat-based analyses the most used (Zantis et al., 2021). Scat-based analysis has proven to be an effective non-invasive method for detecting and quantifying MPs in pinnipeds

(Eriksson & Burton, 2003; Nelms et al., 2018, 2019b; Perez-Venegas et al., 2018, 2020; Pérez-Guevara et al., 2021; McIvor et al., 2023).

MP pollution has been detected in the Galapagos Islands' southeast region (Alfaro-Núñez et al., 2021; Jones et al., 2021, 2022; Muñoz-Pérez et al., 2023), where the Galapagos sea lion (GSL, *Zalophus wollebaeki*) establishes its main reproductive rookeries (Páez-Rosas et al., 2021). This endemic pinniped is listed as endangered by the International Union for Conservation of Nature (IUCN) because of drastic population declines over the last four decades (Trillmich, 2015). Among its main threats are climate change, emerging diseases associated with invasive species, and impacts related to human activities near the archipelago (Páez-Rosas & Guevara, 2017; Riofrío-Lazo & Páez-Rosas, 2021). Plastic pollution has become one of the main anthropogenic threats to this species (Jones et al., 2021; Muñoz-Pérez et al., 2023), since the GSL is susceptible to biomagnification of persistent organic pollutants (POPs) (Alava et al., 2011; Alava & Ross, 2018), which are toxic contaminants that can be absorbed and transported by MPs. Nonetheless, there are no data on the abundance of MPs for this species, even though these particles are bioavailable in the food web and that the GSL has been catalogued as the mammal with the highest risk of ingesting plastic debris in the archipelago (Jones et al., 2021, 2022; Muñoz-Pérez et al., 2023).

The largest GSL rookeries are located on the southeast islands of the archipelago (*i.e.*, Española, Floreana, San Cristobal, and Santa Fe), with an estimated population of 2300 to 4100 individuals (Riofrío-Lazo et al., 2017; Páez-Rosas et al., 2021). The trophic efficiency of this region is significantly influenced by Humboldt current , which provides cold and highly productive waters (Riofrío-Lazo et al., 2021). However, this current is also an important driver of plastic debris pollution in the archipelago (Jones et al., 2021). Direct geographical exposure to this current has likely resulted in the prevalence of MPs in virtually all habitats of the southeast islands (Jones et al., 2021, 2022; Muñoz-Pérez et al., 2023). Therefore, the aim of this study is to establish a baseline framework for MP pollution in GSL rookeries from the southeast region of the Galapagos Marine Reserve. This information will be useful as reference values for further research, spatiotemporal monitoring of MPs in the Galapagos archipelago, and the planning of conservation strategies for the GSL.

METHODS

Sample collection

Fresh scats (n=180) of adult GSLs were collected along the coastline at five main rookeries of the southeast region of the Galapagos Marine Reserve in August 2021: El Malecón and Punta Pitt (n=60 and 30, respectively; San Cristobal Island), Punta Suárez (n=30; Española Island), Post Office (n=30; Floreana Island), and Bahía de Santa Fe (n=30; Santa Fe Island) (*Figure 1*). Some of the scat sampling was based on direct observations of depositions of adult individuals, while other scat samples were categorized within this age class based on their size and consistency. Scat samples were collected with metal forceps disinfected with 70% alcohol and placed inside labelled aluminum envelopes. Samples were then frozen at -20 °C until further laboratory processing.

Microplastic isolation

Scats were thawed at room temperature for 24 h, and standardized subsamples of approximately 15 g were separated to isolate MPs. Subsamples were separated from the core of the scat to avoid sand or debris that might have contained external MPs. Oxidative digestion with Fenton's Reagent (H_2O_2 + Fe catalyst) was employed by adding 150 ml of 30% H_2O_2 with 150 ml of $FeSO_4$ to the subsamples in Erlenmeyer flasks, to then incubate them for 72 h at 60 °C to maximize digestion efficiency. These reagents do not compromise the integrity of the polymers and present higher digestion rates compared to other commonly used oxidative reagents for organic matter digestion (Prata et al., 2019a, 2019b).

Digested samples were then transferred into a Sediment-Microplastic Isolation (SMI) unit along with 700 ml of a saline solution of 1.2 g/cm^3 density (337 g of NaCl dissolved in 1 L of ultra-filtered water) to isolate MPs from the digested solution using the principle of density floatation

(Masura et al., 2015; Coppock et al., 2017). The samples were left in the SMI unit for 24 hours, after which the valve was closed to separate the processed sample into two solutions. Only the supernatant was vacuum filtered using glass fiber filters (pore size 1.2 µm) and stored in Petri dishes sealed with Parafilm.

Microplastic identification

Glass fiber filters were examined with a Nikon SMZ1000 stereomicroscope in search of potential polymer-based particles, classifying them as either fibers, fragments, or films. Recovered potential MPs were photographed and characterized (*i.e.*, coloration and length, which was the measurement of the farthest points of each particle). Chemical composition was also characterized by micro-Fourier-Transform Infrared (µFT-IR) spectroscopy on a PerkinElmer Frontier µFT-IR spectrometer to confirm anthropogenic origin of the particles. The µFT-IR spectrometer records were compared with a spectral library at the Scientific and Technological Centres of Universitat de Barcelona (CCiTUB).

Quality Assurance/Quality Control (QA/QC) protocol

A strict QA/QC protocol was followed for each step involving interaction with samples (field and laboratory phases). In the field, for sample collection, environmental controls (*i.e.*, exposed glass fiber filters) were placed in an open area of each study site to recover potential airborne fiber contamination. Sand controls were also collected, although subsequently discarded as we worked with core subsamples, which were not in contact with the substrate.

In the laboratory, sample processing, including reagent preparation and filtration processes, was always carried out inside a laminar flow hood with positive pressure. Procedural blanks (*i.e.*, exposed glass fiber filters) were placed at all workspaces to recover potential airborne fiber contamination. Nitrile gloves and cotton laboratory coats were always required. All materials used (*e.g.*, glass containers, forceps, SMI units, etc.) were carefully rinsed with ultra-filtered water before use and between samples. Hydrogen peroxide and iron catalyst solutions were vacuum filtered with glass fiber filters (pore size 1.2 μm) before oxidative digestion of scats. Reagent controls were also used to recover potential airborne fiber contamination in the digestion process. Finally, saline solution controls were implemented to measure potential external MP contamination in this reagent.

Control correction

Fiber contamination confirmed by μFT-IR was shown in procedural blanks ($\bar{x}$=0.21 fibers per control), reagent ($\bar{x}$=0.23 fibers per control), and saline solution controls ($\bar{x}$=1.07 fibers per control). Four polymers were identified: cellophane, polyethylene, cellulose, and polystyrene. To correct for this external contamination, the mean values for each polymer were determined in each individual control, and then added up; as shown in the following equation: $\bar{x}_{polymer} = \bar{x}_{PB} + \bar{x}_{RC} + \bar{x}_{SC}$, where $\bar{x}_{polymer}$ refers to the overall mean particles for each polymer in all controls, $\bar{x}_{PB}$ to the mean of the specific polymer in procedural blanks, $\bar{x}_{RC}$ to its mean in reagent controls, and $\bar{x}_{SC}$ to its mean in saline solution controls.

Approximately one cellophane particle of contamination per control sample was determined. Therefore, all particles corresponding to this polymer type were discarded (n=9), except for one cellophane-mesoplastic film which was found directly in the sample. Means of the remaining polymer types were <0.4, so they were not discarded in the dataset. Two cellulose particles (n=2) were also visually discarded due to similarities with natural fibers. With these adjustments, 32% of the 143 potential particles analyzed by μFT-IR had a confirmed anthropogenic origin determined by its chemical composition. However, 253 potential particles were originally recovered from all samples. To extrapolate the control data to the full dataset, the proportion of particles discarded by µFT-IR (68%) was removed proportionally for each sample (n=172 discarded particles).

Statistical analyses

Statistical analyses (significance level of α=0.05) were performed with STATISTICA 7 software. Shapiro-Wilk tests were performed for normality check. Since data did not satisfy normality, non-parametric Kruskal-Wallis tests were performed to analyze potential statistical differences in total recovered MPs, MPs scat g^{-1}, and MPs sizes among all studied rookeries.

RESULTS

Microplastic quantification and characterization

A total of 81 MP particles were retrieved in 37% of the collected GSL scats ($\bar{x}$=0.45 ± 0.65 per scat subsample, hereafter referred to simply as scat) (*Figure 2*). Fibers were the most frequent MP shape found (69%, $\bar{x}$=0.31 ± 0.57 particles scat^{-1}), followed by fragments (26%, $\bar{x}$=0.12 ± 0.35), and films (5%, $\bar{x}$=0.02 ± 0.15). El Malecón and Punta Pitt rookeries at San Cristobal Island had the highest number of MPs ($\bar{x}$=0.67 ± 0.51 and 0.43 ± 0.41 particles scat^{-1}, respectively), followed by Punta Suárez ($\bar{x}$=0.33 ± 0.32), Post Office ($\bar{x}$=0.33 ± 0.38), and Bahía de Santa Fe ($\bar{x}$=0.27 ± 0.29) (see *Table 1* and *Figure 3*). The number of particles per gram of sample wet weight ranged from 0.02 to 0.22 ($\bar{x}$=0.04 ± 0.05 particles scat wet g^{-1}). Kruskal-Wallis tests showed statistical differences in total MPs recovered ($H_{(4)}$=10.37, p=0.04) and MPs scat g^{-1} ($H_{(4)}$=10.22, p=0.04) among rookeries, specifically, between El Malecón and Bahía de Santa Fe (multiple comparisons of median ranks, p<0.05).

MP particles sizes ranged from 20 to 4340 µm ($\bar{x}$=864.09 ± 833.82 µm). Particles registered in each rookery were grouped into five size-classes: Class A (20 to 1000 µm), Class B (1001 to 2000 µm), Class C (2001 to 3000 µm), Class D (3001 to 4000 µm), and Class E (4001 to 5000 µm). Class A was dominant in all rookeries (*Figure 4*), despite significant differences observed in MP size-composition among rookeries ($H_{(4)}$=28.83, p<0.01), mainly among San Cristobal Island rookeries (*i.e.*, El Malecón and Punta Pitt) and Punta Suárez and Post Office rookeries (multiple comparisons of median ranks, p<0.05) (*Table 2*).

Blue (45%) and black (32%) were the predominant MP colorations in all rookeries (*Figure 5*). MP prevalence in scats varied along sampled rookeries; being San Cristobal rookeries those that showed the highest values: El Malecón and Punta Pitt (52% and 33%, respectively) (*Table 3*).

Chemical characterization by µFT-IR spectrometry

Anthropogenic origin of 46 particles was confirmed through chemical characterization by µFT-IR spectrometry. A spectrum of eleven different polymers was identified, being the most common polypropylene-polyethylene copolymer (PP-PE); polypropylene (PP); cellulose; polyethylene (PE); and polyvinyl chloride (PVC): with 22%, 18%, 17%, 11%, and 11%, respectively. These were followed by polyacrylonitrile (PAN); polyester tere-&iso-phthalate (PETP); poly(ethylacrylate:st:acrylamide) copolymer (PEAA:ST:AA); polystyrene-polyacrylonitrile copolymer (PS-PAN); polystyrene (PS); and cellophane.

Chemical composition at each rookery varied in diversity and dominance of polymers. El Malecón rookery had the greatest diversity of polymers (eight different types), being PP dominant. Punta Suárez rookery was composed of six different polymers, with cellulose being dominant. Punta Pitt and Post Office rookeries was composed of seven and three different polymers (all equally represented). Finally, Bahía de Santa Fe rookery was composed of two different polymers, being PP-PE copolymer the dominant (*Figure 6*).

DISCUSSION

Quantitative and physical properties of recovered microplastics

Fibers were the predominant shape of MPs retrieved from scats of GSL rookeries at the southeast of the Galapagos Marine Reserve, consistent with a trend of predominance of these anthropogenic particles in marine environments (Browne et al., 2011; Lusher et al., 2017; Athey et al., 2020) and its subsequent bioaccumulation in marine mammals (Zantis et al., 2021). The use of synthetic fibers has displaced natural fibers (*e.g.*, cotton, wool) due to its low production costs and high demand (Lusher et al., 2017); being textiles, fisheries (*e.g.*, lines, nets, ropes) and packaging, the most representative industries that contribute with microfiber fluxes into the marine environment (Lusher et al., 2017; Gago et al., 2018; Jensen et al., 2019). Microfibers recorded in previous studies in the Galapagos Marine Reserve were primarily composed of semisynthetic celluloses, PP, polyester (PES), and nylon (Jones et al., 2021), which is consistent with the polymers observed in our study. Browne et al. (2011) suggested that sewage affluents are a major source of microfiber discharges into the environment through washing clothes, where a single garment can release up to 1900 fibers per wash. Considering that the urban centers of the Galapagos have poor wastewater management strategies (Ragazzi et al., 2016; Mateus et al., 2020), this could be a potential source of contamination. Therefore, it is crucial to address this environmental risk when proposing mitigation strategies aimed at countering MP pollution in coastal environments (Liu et al., 2022).

Small sized MPs are the most bioavailable around the Galapagos Marine Reserve, due to the constant fragmentation processes that synthetic particles undergo (Alfaro-Núñez et al., 2021). This agrees with our results, where MPs grouped into Class A (20 to 1000 µm) were the most represented in all rookeries. MP-size-class composition showed significant differences among San Cristobal Island rookeries and other rookeries/islands, which were associated with a greater abundance of MPs between 20 and 1000 μm in El Malecón and Punta Pitt rookeries. A possible explanation could be the abundance of MP pollution sources, which can be land- (75-90%) or sea-based (10-25%) (Perumal & Muthuramalingam, 2022). San Cristobal Island maintains urbanized areas with a main fishing and tourist port of the archipelago (Páez-Rosas & Guevara, 2017; Walsh et al., 2019). These conditions increase the exposition to both terrestrial and marine sources of contamination for this island's rookeries; unlike the other rookeries that are located on geographically remote islands with no urbanization, and consequently less exposed to pollution sources. In urbanized areas, industrial activities, runoff, and sewage may provide additional inputs of micro debris into the ocean, causing nearby sites to contain higher particulate discharges and thus more diverse physical characteristics (Dris et al., 2015; Perumal & Muthuramalingam, 2022).

Blue was the main color in our samples, followed by black. This agrees with several studies of MPs in marine mammals (Zantis et al., 2021). Ingestion of marine debris of anthropogenic origin is driven by its detectability in marine ecosystems and the foraging strategy of different animal groups, based on Thayer's law (*i.e.*, the detectability of a prey item is reduced by counter-shadowing, in visual predators) applied to debris (Santos et al., 2016). Blue colored MPs are the most common in aquatic taxa, probably due to their visual similarities to animal prey (Santos et al., 2021; Du et al., 2022). Therefore, reporting this information is important as it provides comparability between studies (Zantis et al., 2021).

Spatial variability in microplastic contamination patterns

San Cristobal is one of the islands with the highest levels of MP pollution in surface waters ($\bar{x}$=0.89 particles m^{-3}), as well as in benthic sediments, mostly composed of fibers (Jones et al., 2021). This explains our results, since El Malecón rookery, located within an urban center of San Cristobal Island, had predominance of fibers in scats. This condition was maintained in

Punta Pitt rookery, despite being more distant from the urban center of San Cristobal Island (*i.e.*, it is located at the north-east end of the island). However, their exposure to MPs is high because it is geographically located at a highly exposed area to the accumulation of plastic debris due to the influence of marine currents and windward effects, with high particle concentrations previously recorded at the coastline ($\bar{x}$=381 ± 68 particles m^{-2}) (Jones et al., 2021, 2022; Muñoz-Pérez et al., 2023).

Punta Suárez (Española Island), Post Office (Floreana Island), and Bahía de Santa Fe (Santa Fe Island) rookeries reported lower concentrations of MPs and similar abundances among them. Since these three sites are not urbanized, MP pollution would be mostly influenced by sea-based anthropogenic sources (*e.g.*, fishing) (Perumal & Muthuramalingam, 2022). The areas designated for artisanal fishing at these rookeries are in proximity (or even overlapping) to the foraging grounds of GSLs (Jeglinski et al., 2012; Páez-Rosas & Aurioles-Gamboa, 2014; Páez-Rosas et al., 2017). Abandoned, lost, or discarded fishing gear (*i.e.*, "ghost fishing") are weathered under environmental conditions, causing materials such as nets or ropes to degrade over time and to fragment into smaller particles (*e.g.*, microfibers) (Montarsolo et al., 2018), which is consistent with the types of polymers found at these rookeries (*e.g.*, PP-PE, PP, PE). Muñoz-Pérez et al., (2023) found that artisanal and industrial fishing gear (*e.g.*, monofilament lines, nets, ropes, strings, etc.) were a major source of macroplastic pollution throughout the Galapagos Marine Reserve. Therefore, it is suggested that the fishing industry has a significant effect on the prevalence of MPs in our samples.

Chemical identification and diversity of polymers

We identified eleven distinct polymers in GSL scats, being polypropylene-polyethylene copolymer (PP-PE) the most common. PP-PE is created by copolymerizing PP and PE, which allows it to exhibit the most important features of both polymers, enhancing its thermal and mechanical properties when compared to either polymer in isolation (Teh et al., 1994). PP copolymers are a significant source of marine debris due to their widespread use in the production of synthetic materials, such as ropes (Jang et al., 2014), pipelines, or packaging materials (Zhang et al., 2020). Our samples also contained a significant amount of PP and PE, which are among the most abundant polymers in the ocean, accounting for 46% of global plastic production, especially for packaging and fishing materials (Andrady, 2017; Geyer, 2020). Cellulose fibers were also highly represented in our samples, which was expected considering that cellulosic fibers are highly abundant in the oceans due to their importance in textiles (Suaria et al., 2020). Although some authors do not classify cellulosic particles strictly as microplastics, their increased persistence when chemically modified makes them a risk for marine organisms (Athey et al., 2020; Adams et al., 2021).

The trophic flexibility of the GSL could account for the high diversity of polymers found in our samples. Previous studies have shown that there is food resource partitioning in GSL rookeries, employing at least three trophic strategies: epipelagic, mesopelagic, and benthic (Villegas-Amtmann et al., 2008; Páez-Rosas & Aurioles-Gamboa, 2010; Schwarz et al., 2021). These conditions increase the ecological niches occupied by this species and their prey catalog, consequently increasing the potential for trophic transfer and accumulation of MPs of different densities. Polymer density directly affects MPs buoyancy (van Sebille et al., 2015; Ajith et al., 2020; Wang et al., 2020). Low-density polymers (*e.g.*, PP-PE, PP, PE) are positively buoyant, and therefore concentrate in surface waters (epipelagic zone); whereas high-density polymers (*e.g.*, PVC, PAN), are negatively buoyant and tend to accumulate in the sediment (benthic zone) (Ajith et al., 2020). This spatial variation, based on polymer density, allows MPs to be widely available to different taxonomic groups (Wang et al., 2020). Therefore, the trophic breadth of GSLs would explain the high polymer diversity observed at rookeries with high prey species richness, such as El Malecón and Punta Suárez (Páez-Rosas & Aurioles-Gamboa, 2014), as well as the low diversity of polymers observed in Bahía de Santa Fe rookery, where GSLs maintain a reduced trophic spectrum (Urquía et al., unpublished data).

Nonetheless, although the buoyancy of MPs is relevant to predict its spatial distribution, it can be altered by external factors, such as biofouling or aggregations with other debris (Corcoran et al., 2015; Kooi et al., 2017). Likewise, the spatial dynamics of MPs are driven by a multifactorial set of interactions, including the physical-chemical characteristics of the particles, proximity to sources, ocean current patterns, wind strength and direction, climatic events (*e.g.*, El Niño Southern Oscillation), etc. (van Sebille et al., 2012, 2015; Jensen et al., 2019; Wang et al., 2020; Muñoz-Pérez et al., 2023).

Comparison with previous otariid studies

The empirical evidence on the incidence of MPs in otariids is increasing, including at a regional scale (Perez-Venegas et al., 2018, 2020; Ortega-Borchardt et al., 2023). Therefore, comparability between studies through standardized methodological and experimental approaches is essential to objectively understand the dynamics of MP pollution at different scales (Pérez-Guevara et al., 2021). McIvor et al. (2023) emphasized the importance of selecting appropriate relative measures for MP abundance to achieve comparability between studies, since inherent variations in experimental designs can lead to biased interpretations (*e.g.*, water content, total mass of the sampled scat, etc.). For this reason, we used different relative measures, such as particle number per scat/gram, prevalence, etc., to enable objective comparability with other studies (*Table 4*).

Our findings indicate a relatively low prevalence of MPs in scat samples of the GSL, when compared to other studies of otariids in northern hemisphere (Donohue et al., 2019; Ayala et al., 2021). Regionally, Perez-Venegas et al. (2018, 2020) reported abundances of 2.7-13.35 and 2-15 particles g^{-1} for three otariid species along the Peruvian and Chilean coasts, which differs widely from our results (0.02-0.22 particles wet g^{-1}). This could be explained by the low MP concentrations previously reported in southeast Galapagos archipelago, relative to other regions of the planet (Alfaro-Núñez et al., 2021; Jones et al., 2021). At a basin scale, it is suggested that MPs reach the Galapagos Marine Reserve from oceanic basins of South America, with Peru being a major source (van Sebille et al., 2019; Muñoz-Pérez et al., 2023). Thus, the observed difference between regional-scale concentrations of MPs in otariids would likely be due to geographic proximity to large-scale sources of MPs. However, the high relative abundance of fibers reported in other otariid species suggests that this type of micro debris bioaccumulates in their prey, moving through different trophic boundaries until they reach top predators (Andrady, 2017).

Ortega-Borchardt et al., (2023) found a high variation of polymer types in scat samples of California sea lions (*Zalophus californianus*), which was associated with the high diversity of marine debris types within their environment. Polyethylene terephthalate (PET), PP, and PE were the predominant polymers found in that study, which partially agrees with our results, where PP and PE, as well as the copolymer of both (PP-PE), were the most representative. Likewise, Donohue et al., (2019) reported PE as the only polymer in samples from Northern fur seals (*Callorhinus ursinus*), and although the number of samples chemically analyzed was very low, it demonstrates the ubiquity of this polymer in marine ecosystems.

Most studies on otariids reported blue as the predominant color of MPs found in scats (Perez-Venegas et al., 2018, 2020; Ayala et al., 2021; Ortega-Borchardt et al., 2023). While it has been suggested that this might be explained due to physical resemblance between synthetic particles and food items, there are more complex evolutionary approaches. Santos et al. (2021) suggested that there are three traits that determine the likelihood of plastic ingestion in an animal: (1) the level of resemblance of plastics to prey, both visually and chemically, (2) food selectivity (generalist to specialist), and (3) nutritional status of the animal. Páez-Rosas et al. (2017) suggested that GSLs have a certain level of specialization in their diet (high frequency consumption of a limited number of prey items), although they may also be flexible in their trophic spectrum and incorporate different prey to reduce intraspecific competition, or in response to fluctuation in resource abundance. Since this species exhibits a certain level of prey selectivity, it is unlikely that it intentionally ingests plastics; therefore, MP ingestion would not depend on vision but on trophic transfer, as has been reported in other marine predators (Nelms et al., 2018). In this context, Santos et al. (2021) suggested that to achieve a reduction in the probability of encounter between plastic items and animals, the only approach is to reduce plastic production.

CONCLUSION

We have quantified MP pollution in a top predator of the Galapagos Marine Reserve, establishing a baseline to monitor its impacts on Galapagos sea lion populations. The increasing marine debris pollution derived from plastics could affect the survival of coastal marine species, so it is important to detect spatiotemporal changes in the abundance of this pollutants through time. We emphasize the importance of (1) further research into the ingestion of MPs by potential prey, especially those at higher trophic levels, to evaluate potential bioaccumulation routes, (2) assessments in different regions of the Galapagos Marine Reserve, where, although oceanographic conditions and anthropogenic impacts differ, plastic pollution persists, and (3) evaluation of the effects of MP ingestion in Galapagos sea lions, specifically with regard to the biomagnification of chemical pollutants. We encourage the use of our results in the planning of conservation and plastic pollution mitigation strategies for this species.

Limitations and recommendations

The standardization of methods for quantifying MPs through scat-based analysis is essential, yet very complicated to achieve. Accurately quantifying MPs in scats is dependent on several factors, such as experimental design, sample weight, reagent efficiency to digest samples, quality controls, correction factors, etc. In this study, some limitations were identified and carefully addressed to guarantee the reliability of our data. The principle of density floatation was applied with sodium chloride to maximize the efficiency of MP retrieval, even though this salt is not ideal because it theoretically only recovers MPs formed by polymers with densities lower than 1.2 g/cm^3. However, some high-density polymers were recovered in our samples (*e.g.*, PVC, PAN), probably because they aggregated with organic residues in the supernatant solution of the SMI units.

We recommend that future studies seek to maximize the efficiency of oxidative digestion of scats to avoid the use of additional reagents (thus, additional potential contamination sources), while increasing the total mass of the scat sample to be digested. Thereby, saving both time and budget in the study, and granting more reliability when quantifying synthetic particles through scat-based analysis. We also encourage researchers on MPs in wildlife to be transparent regarding communicating the limitations of their studies, so that they can be considered in the interpretation of the results. This information is expected to be useful to the scientific community and contribute to the development of new, more accurate and efficient quantification methods.

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REFERENCES

Adams, J. K., Dean, B. Y., Athey, S. N., Jantunen, L. M., Bernstein, S., Stern, G., Diamond, M. L., & Finkelstein, S. A. (2021). Anthropogenic particles (including microfibers and microplastics) in marine sediments of the Canadian Arctic. *Science of The Total Environment*, *784*, 147155. https://doi.org/10.1016/j.scitotenv.2021.147155

Ajith, N., Arumugam, S., Parthasarathy, S., Manupoori, S., & Janakiraman, S. (2020). Global distribution of microplastics and its impact on marine environment—A review. *Environmental Science and Pollution Research*, *27*(21), 25970–25986. https://doi.org/10.1007/s11356-020-09015-5

Alava, J. J., & Ross, P. S. (2018). Chapter 8 - Pollutants in Tropical Marine Mammals of the Galápagos Islands, Ecuador: An Ecotoxicological Quest to the Last Eden. In M. C. Fossi & C. Panti (Eds.), *Marine Mammal Ecotoxicology* (pp. 213–234). Academic Press. https://doi.org/10.1016/B978-0-12-812144-3.00008-5

Alava, J. J., Salazar, S., Cruz, M., Jiménez-Uzcátegui, G., Villegas-Amtmann, S., Paéz-Rosas, D., Costa, D. P., Ross, P. S., Ikonomou, M. G., & Gobas, F. A. P. C. (2011). DDT Strikes Back: Galapagos Sea Lions Face Increasing Health Risks. *AMBIO*, *40*(4), 425–430. https://doi.org/10.1007/s13280-011-0136-6

Alfaro-Núñez, A., Astorga, D., Cáceres-Farías, L., Bastidas, L., Soto Villegas, C., Macay, K., & Christensen, J. H. (2021). Microplastic pollution in seawater and marine organisms across the Tropical Eastern Pacific and Galápagos. *Scientific Reports*, *11*(1), 6424. https://doi.org/10.1038/s41598-021-85939-3

Andrady, A. L. (2017). The plastic in microplastics: A review. *Marine Pollution Bulletin*, *119*(1), 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082

Athey, S. N., Adams, J. K., Erdle, L. M., Jantunen, L. M., Helm, P. A., Finkelstein, S. A., & Diamond, M. L. (2020). The Widespread Environmental Footprint of Indigo Denim Microfibers from Blue Jeans. *Environmental Science & Technology Letters*, *7*(11), 840–847. https://doi.org/10.1021/acs.estlett.0c00498

Ayala, F., Cardeña, M., & Cárdenas-Alayza, S. (2021). Registro preliminar de microplásticos en fecas del león marino sudamericano (*Otaria byronia* [de Blainville 1820]) recolectadas en Punta San Juan, Perú. *Revista Internacional de Contaminación Ambiental*, *37*, 273–279. https://doi.org/10.20937/RICA.53745

Botterell, Z. L. R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R. C., & Lindeque, P. K. (2019). Bioavailability and effects of microplastics on marine zooplankton: A review. *Environmental Pollution*, *245*, 98–110. https://doi.org/10.1016/j.envpol.2018.10.065

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T., & Thompson, R. (2011). Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environmental Science & Technology*, *45*(21), 9175–9179. https://doi.org/10.1021/es201811s

Coppock, R. L., Cole, M., Lindeque, P. K., Queirós, A. M., & Galloway, T. S. (2017). A small-scale, portable method for extracting microplastics from marine sediments. *Environmental Pollution*, *230*, 829–837. https://doi.org/10.1016/j.envpol.2017.07.017

Corcoran, P. L., Norris, T., Ceccanese, T., Walzak, M. J., Helm, P. A., & Marvin, C. H. (2015). Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. *Environmental Pollution*, *204*, 17–25. https://doi.org/10.1016/j.envpol.2015.04.009

Costa, E., Piazza, V., Lavorano, S., Faimali, M., Garaventa, F., & Gambardella, C. (2020). Trophic Transfer of Microplastics From Copepods to Jellyfish in the Marine

Environment. *Frontiers in Environmental Science*, *8*. https://doi.org/10.3389/fenvs.2020.571732

da Costa. (2018). Micro- and nanoplastics in the environment: Research and policymaking. *Current Opinion in Environmental Science & Health*, *1*, 12–16. https://doi.org/10.1016/j.coesh.2017.11.002

Donohue, M. J., Masura, J., Gelatt, T., Ream, R., Baker, J. D., Faulhaber, K., & Lerner, D. T. (2019). Evaluating exposure of northern fur seals, *Callorhinus ursinus*, to microplastic pollution through fecal analysis. *Marine Pollution Bulletin*, *138*, 213–221. https://doi.org/10.1016/j.marpolbul.2018.11.036

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., & Tassin, B. (2015). Microplastic contamination in an urban area: A case study in Greater Paris. *Environmental Chemistry*, *12*(5), 592. https://doi.org/10.1071/EN14167

Du, Y., Liu, X., Dong, X., & Yin, Z. (2022). A review on marine plastisphere: Biodiversity, formation, and role in degradation. *Computational and Structural Biotechnology Journal*, *20*, 975–988. https://doi.org/10.1016/j.csbj.2022.02.008

Eriksen, M., Cowger, W., Erdle, L. M., Coffin, S., Villarrubia-Gómez, P., Moore, C. J., Carpenter, E. J., Day, R. H., Thiel, M., & Wilcox, C. (2023). A growing plastic smog, now estimated to be over 170 trillion plastic particles afloat in the world's oceans—Urgent solutions required. *PLOS ONE*, *18*(3), e0281596. https://doi.org/10.1371/journal.pone.0281596

Eriksson, C., & Burton, H. (2003). Origins and Biological Accumulation of Small Plastic Particles in Fur Seals from Macquarie Island. *AMBIO: A Journal of the Human Environment*, *32*(6), 380–384. https://doi.org/10.1579/0044-7447-32.6.380

Gago, J., Carretero, O., Filgueiras, A. V., & Viñas, L. (2018). Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. *Marine Pollution Bulletin*, *127*, 365–376. https://doi.org/10.1016/j.marpolbul.2017.11.070

Gall, S. C., & Thompson, R. C. (2015). The impact of debris on marine life. *Marine Pollution Bulletin*, *92*(1), 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041

Geyer, R. (2020). Production, use, and fate of synthetic polymers. In *Plastic Waste and Recycling* (pp. 13–32). Elsevier. https://doi.org/10.1016/B978-0-12-817880-5.00002-5

Gibbons, D. W., Sandbrook, C., Sutherland, W. J., Akter, R., Bradbury, R., Broad, S., Clements, A., Crick, H. Q. P., Elliott, J., Gyeltshen, N., Heath, M., Hughes, J., Jenkins, R. K. B., Jones, A. H., Lopez de la Lama, R., Macfarlane, N. B. W., Maunder, M., Prasad, R., Romero-Muñoz, A., … Ockendon, N. (2022). The relative importance of COVID-19 pandemic impacts on biodiversity conservation globally. *Conservation Biology*, *36*(1). https://doi.org/10.1111/cobi.13781

Gouin, T. (2020). Toward an Improved Understanding of the Ingestion and Trophic Transfer of Microplastic Particles: Critical Review and Implications for Future Research. *Environmental Toxicology and Chemistry*, *39*(6), 1119–1137. https://doi.org/10.1002/etc.4718

Hocking, D. P., Marx, F. G., Park, T., Fitzgerald, E. M. G., & Evans, A. R. (2017). A behavioural framework for the evolution of feeding in predatory aquatic mammals. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1850), 20162750. https://doi.org/10.1098/rspb.2016.2750

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, *347*(6223), 768–771. https://doi.org/10.1126/science.1260352

Jang, Y. C., Lee, J., Hong, S., Lee, J. S., Shim, W. J., & Song, Y. K. (2014). Sources of plastic marine debris on beaches of Korea: More from the ocean than the land. *Ocean Science Journal*, *49*(2), 151–162. https://doi.org/10.1007/s12601-014-0015-8

Jeglinski, J. W. E., Werner, C., Robinson, P. W., Costa, D. P., & Trillmich, F. (2012). Age, body mass and environmental variation shape the foraging ontogeny of Galapagos sea lions. *Marine Ecology Progress Series*, *453*, 279–296. https://doi.org/10.3354/meps09649

Jensen, L. H., Motti, C. A., Garm, A. L., Tonin, H., & Kroon, F. J. (2019). Sources, distribution and fate of microfibres on the Great Barrier Reef, Australia. *Scientific Reports*, *9*(1), 9021. https://doi.org/10.1038/s41598-019-45340-7

Jepsen, E. M., & de Bruyn, P. J. N. (2019). Pinniped entanglement in oceanic plastic pollution: A global review. *Marine Pollution Bulletin*, *145*, 295–305. https://doi.org/10.1016/j.marpolbul.2019.05.042

Jones, J. S., Guézou, A., Medor, S., Nickson, C., Savage, G., Alarcón-Ruales, D., Galloway, T. S., Muñoz-Pérez, J. P., Nelms, S. E., Porter, A., Thiel, M., & Lewis, C. (2022). Microplastic distribution and composition on two Galápagos island beaches, Ecuador: Verifying the use of citizen science derived data in long-term monitoring. *Environmental Pollution*, *311*, 120011. https://doi.org/10.1016/j.envpol.2022.120011

Jones, J. S., Porter, A., Muñoz-Pérez, J. P., Alarcón-Ruales, D., Galloway, T. S., Godley, B. J., Santillo, D., Vagg, J., & Lewis, C. (2021). Plastic contamination of a Galapagos Island (Ecuador) and the relative risks to native marine species. *Science of The Total Environment*, *789*, 147704. https://doi.org/10.1016/j.scitotenv.2021.147704

Kooi, M., Nes, E. H. van, Scheffer, M., & Koelmans, A. A. (2017). Ups and Downs in the Ocean: Effects of Biofouling on Vertical Transport of Microplastics. *Environmental Science & Technology*, *51*(14), 7963–7971. https://doi.org/10.1021/acs.est.6b04702

Laskar, N., & Kumar, U. (2019). Plastics and microplastics: A threat to environment. *Environmental Technology & Innovation*, *14*, 100352. https://doi.org/10.1016/j.eti.2019.100352

Liu, D., Guo, Z.-F., Xu, Y.-Y., Ka Shun Chan, F., Xu, Y.-Y., Johnson, M., & Zhu, Y.-G. (2022). Widespread occurrence of microplastics in marine bays with diverse drivers and environmental risk. *Environment International*, *168*, 107483. https://doi.org/10.1016/j.envint.2022.107483

Lusher, A., Hollman, P. C. H., & Mendoza-Hill, J. (2017). *Microplastics in fisheries and aquaculture: Status of knowledge on their occurrence and implications for aquatic organisms and food safety*. Food and Agriculture Organization of the United Nations. https://www.fao.org/publications/card/en/c/59bfa1fc-0875-4216-bd33-55b6003cfad8/

Masura, J., Baker, J., Foster, G., Arthur, C., & Herring, C. E. (2015). *Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for Quantifying Synthetic Particles in Waters and Sediments*. https://repository.library.noaa.gov/view/noaa/10296

Mateus, C., Valencia, M., DiFrancesco, K., Ochoa-Herrera, V., Gartner, T., & Quiroga, D. (2020). Governance Mechanisms and Barriers for Achieving Water Quality Improvements in Galapagos. *Sustainability*, *12*(21), Article 21. https://doi.org/10.3390/su12218851

McIvor, A. J., Pires, R., Lopes, C., Raimundo, J., Campos, P. F., Pais, M. P., Canning-Clode, J., & Dinis, A. (2023). Assessing microplastic exposure of the Critically Endangered Mediterranean monk seal (*Monachus monachus*) on a remote oceanic island. *Science of The Total Environment*, *856*, 159077. https://doi.org/10.1016/j.scitotenv.2022.159077

Miller, M. E., Hamann, M., & Kroon, F. J. (2020). Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. *PLOS ONE*, *15*(10), e0240792. https://doi.org/10.1371/journal.pone.0240792

Montarsolo, A., Mossotti, R., Patrucco, A., Caringella, R., Zoccola, M., Pozzo, P. D., & Tonin, C. (2018). Study on the microplastics release from fishing nets. *The European Physical Journal Plus*, *133*(11), Article 11. https://doi.org/10.1140/epjp/i2018-12415-1

Muñoz-Pérez, J. P., Lewbart, G. A., Alarcón-Ruales, D., Skehel, A., Cobos, E., Rivera, R., Jaramillo, A., Vivanco, H., Zurita-Arthos, L., Wallace, B., Valle, C. A., & Townsend, K. A. (2023). Galápagos and the plastic problem. *Frontiers in Sustainability*, *4*. https://doi.org/10.3389/frsus.2023.1091516

Nelms, S. E., Barnett, J., Brownlow, A., Davison, N. J., Deaville, R., Galloway, T. S., Lindeque, P. K., Santillo, D., & Godley, B. J. (2019a). Microplastics in marine mammals stranded around the British coast: Ubiquitous but transitory? *Scientific Reports*, *9*(1), 1–8. https://doi.org/10.1038/s41598-018-37428-3

Nelms, S. E., Galloway, T. S., Godley, B. J., Jarvis, D. S., & Lindeque, P. K. (2018). Investigating microplastic trophic transfer in marine top predators. *Environmental Pollution*, *238*, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016

Nelms, S. E., Parry, H. E., Bennett, K. A., Galloway, T. S., Godley, B. J., Santillo, D., & Lindeque, P. K. (2019b). What goes in, must come out: Combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator. *Methods in Ecology and Evolution*, *10*(10), 1712–1722. https://doi.org/10.1111/2041-210X.13271

Ortega-Borchardt, J. Á., Ramírez-Álvarez, N., Rios Mendoza, L. M., Gallo-Reynoso, J. P., Barba-Acuña, I. D., García-Hernández, J., Égido-Villarreal, J., & Kubenik, T. (2023).

Detection of microplastic particles in scats from different colonies of California sea lions (*Zalophus californianus*) in the Gulf of California, Mexico: A preliminary study. *Marine Pollution Bulletin*, *186*, 114433. https://doi.org/10.1016/j.marpolbul.2022.114433

Páez-Rosas, D., & Aurioles-Gamboa, D. (2010). Alimentary niche partitioning in the Galapagos sea lion, *Zalophus wollebaeki*. *Marine Biology*, *157*(12), 2769–2781. https://doi.org/10.1007/s00227-010-1535-0

Páez-Rosas, D., & Aurioles-Gamboa, D. (2014). Spatial variation in the foraging behaviour of the Galapagos sea lions (*Zalophus wollebaeki*) assessed using scat collections and stable isotope analysis. *Journal of the Marine Biological Association of the United Kingdom*, *94*(6), 1099–1107. https://doi.org/10.1017/S002531541300163X

Páez-Rosas, D., & Guevara, N. (2017). Management Strategies and Conservation Status of Galapagos Sea Lion Populations at San Cristobal Island, Galapagos, Ecuador. In J. J. Alava (Ed.), *Tropical pinnipeds: Bio-ecology, threats, and conservation* (pp. 159–175). CRC Press, Taylor & Francis Group.

Páez-Rosas, D., Torres, J., Espinoza, E., Marchetti, A., Seim, H., & Riofrío-Lazo, M. (2021). Declines and recovery in endangered Galapagos pinnipeds during the El Niño event. *Scientific Reports*, *11*(1), 8785. https://doi.org/10.1038/s41598-021-88350-0

Páez-Rosas, D., Villegas-Amtmann, S., & Costa, D. (2017). Intraspecific variation in feeding strategies of Galapagos sea lions: A case of trophic specialization. *PLOS ONE*, *12*(10), e0185165. https://doi.org/10.1371/journal.pone.0185165

Pelamatti, T., Rios-Mendoza, L. M., Hoyos-Padilla, E. M., Galván-Magaña, F., De Camillis, R., Marmolejo-Rodríguez, A. J., & González-Armas, R. (2021). Contamination knows no borders: Toxic organic compounds pollute plastics in the biodiversity hotspot of

Revillagigedo Archipelago National Park, Mexico. *Marine Pollution Bulletin*, *170*, 112623. https://doi.org/10.1016/j.marpolbul.2021.112623

Peng, Y., Wu, P., Schartup, A. T., & Zhang, Y. (2021). Plastic waste release caused by COVID-19 and its fate in the global ocean. *Proceedings of the National Academy of Sciences*, *118*(47), e2111530118. https://doi.org/10.1073/pnas.2111530118

Pérez-Guevara, F., Kutralam-Muniasamy, G., & Shruti, V. C. (2021). Critical review on microplastics in fecal matter: Research progress, analytical methods and future outlook. *Science of The Total Environment*, *778*, 146395. https://doi.org/10.1016/j.scitotenv.2021.146395

Perez-Venegas, D. J., Seguel, M., Pavés, H., Pulgar, J., Urbina, M., Ahrendt, C., & Galbán-Malagón, C. (2018). First detection of plastic microfibers in a wild population of South American fur seals (*Arctocephalus australis*) in the Chilean Northern Patagonia. *Marine Pollution Bulletin*, *136*, 50–54. https://doi.org/10.1016/j.marpolbul.2018.08.065

Perez-Venegas, D. J., Toro-Valdivieso, C., Ayala, F., Brito, B., Iturra, L., Arriagada, M., Seguel, M., Barrios, C., Sepúlveda, M., & Oliva, D. (2020). Monitoring the occurrence of microplastic ingestion in Otariids along the Peruvian and Chilean coasts. *Marine Pollution Bulletin*, *153*, 110966. https://doi.org/10.1016/j.marpolbul.2020.110966

Perumal, K., & Muthuramalingam, S. (2022). Global sources, abundance, size, and distribution of microplastics in marine sediments—A critical review. *Estuarine, Coastal and Shelf Science*, *264*, 107702. https://doi.org/10.1016/j.ecss.2021.107702

Prata, J. C., da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2019a). Methods for sampling and detection of microplastics in water and sediment: A critical review. *TrAC Trends in Analytical Chemistry*, *110*, 150–159. https://doi.org/10.1016/j.trac.2018.10.029

Prata, J. C., da Costa, J. P., Girão, A. V., Lopes, I., Duarte, A. C., & Rocha-Santos, T. (2019b). Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. *Science of The Total Environment*, *686*, 131–139. https://doi.org/10.1016/j.scitotenv.2019.05.456

Ragazzi, M., Catellani, R., Rada, E. C., Torretta, V., & Salazar-Valenzuela, X. (2016). Management of Urban Wastewater on One of the Galapagos Islands. *Sustainability*, *8*(3), Article 3. https://doi.org/10.3390/su8030208

Riofrío-Lazo, M., Arreguín-Sánchez, F., & Páez-Rosas, D. (2017). Population Abundance of the Endangered Galapagos Sea Lion *Zalophus wollebaeki* in the Southeastern Galapagos Archipelago. *PLOS ONE*, *12*(1), e0168829. https://doi.org/10.1371/journal.pone.0168829

Riofrío-Lazo, M., & Páez-Rosas, D. (2021). Galapagos Sea Lions and Fur Seals, Adapted to a Variable World. In C. Campagna & R. Harcourt (Eds.), *Ethology and Behavioral Ecology of Otariids and the Odobenid* (pp. 643–661). Springer International Publishing. https://doi.org/10.1007/978-3-030-59184-7_30

Riofrío-Lazo, M., Reck, G., Páez-Rosas, D., Zetina-Rejón, M. J., Del Monte-Luna, P., Reyes, H., Murillo-Posada, J. C., Hernández-Padilla, J. C., & Arreguín-Sánchez, F. (2021). Food web modeling of the southeastern Galapagos shelf ecosystem. *Ecological Indicators*, *132*, 108270. https://doi.org/10.1016/j.ecolind.2021.108270

Sanchez-Vidal, A., Thompson, R. C., Canals, M., & de Haan, W. P. (2018). The imprint of microfibres in southern European deep seas. *PLOS ONE*, *13*(11), e0207033. https://doi.org/10.1371/journal.pone.0207033

Santos, R. G., Andrades, R., Fardim, L. M., & Martins, A. S. (2016). Marine debris ingestion and Thayer's law – The importance of plastic color. *Environmental Pollution*, *214*, 585–588. https://doi.org/10.1016/j.envpol.2016.04.024

Santos, R. G., Machovsky-Capuska, G. E., & Andrades, R. (2021). Plastic ingestion as an evolutionary trap: Toward a holistic understanding. *Science*, *373*(6550), 56–60. https://doi.org/10.1126/science.abh0945

Schwarz, J. F. L., Mews, S., DeRango, E. J., Langrock, R., Piedrahita, P., Páez-Rosas, D., & Krüger, O. (2021). Individuality counts: A new comprehensive approach to foraging strategies of a tropical marine predator. *Oecologia*, *195*(2), 313–325. https://doi.org/10.1007/s00442-021-04850-w

Suaria, G., Achtypi, A., Perold, V., Lee, J. R., Pierucci, A., Bornman, T. G., Aliani, S., & Ryan, P. G. (2020). Microfibers in oceanic surface waters: A global characterization. *Science Advances*, *6*(23), eaay8493. https://doi.org/10.1126/sciadv.aay8493

Teh, J. W., Rudin, A., & Keung, J. C. (1994). A review of polyethylene–polypropylene blends and their compatibilization. *Advances in Polymer Technology*, *13*(1), 1–23. https://doi.org/10.1002/adv.1994.060130101

Trillmich. (2015). *Zalophus wollebaeki. The IUCN Red List of Threatened Species 2015:* E.T41668A45230540. https://doi.org/10.2305/IUCN.UK.2015-2.RLTS.T41668A45230540.en

Urquía, D. O., Vences, M., Anslan, S., Asadobay, P., Moreira-Mendieta, A., Chaves, J., & Páez-Rosas, D. (unpublished data). *DNA-metabarcoding confirms trophic flexibility and reveals new prey species in southeastern Galapagos sea lion rookeries* [Manuscript in preparation].

van Sebille, E., Delandmeter, P., Schofield, J., Hardesty, B. D., Jones, J., & Donnelly, A. (2019). Basin-scale sources and pathways of microplastic that ends up in the Galápagos Archipelago. *Ocean Science*, *15*(5), 1341–1349. https://doi.org/10.5194/os-15-1341-2019 van Sebille, E., England, M. H., & Froyland, G. (2012). Origin, dynamics and evolution of ocean garbage patches from observed surface drifters. *Environmental Research Letters*, *7*(4), 044040. https://doi.org/10.1088/1748-9326/7/4/044040

van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B. D., van Franeker, J. A., Eriksen, M., Siegel, D., Galgani, F., & Law, K. L. (2015). A global inventory of small floating plastic debris. *Environmental Research Letters*, *10*(12), 124006. https://doi.org/10.1088/1748-9326/10/12/124006

Villegas-Amtmann, S., Costa, D. P., Tremblay, Y., Salazar, S., & Aurioles-Gamboa, D. (2008). Multiple foraging strategies in a marine apex predator, the Galapagos sea lion *Zalophus wollebaeki*. *Marine Ecology Progress Series*, *363*, 299–309. https://doi.org/10.3354/meps07457

Walker, S., & Rothman, R. (2020). Life cycle assessment of bio-based and fossil-based plastic: A review. *Journal of Cleaner Production*, *261*, 121158. https://doi.org/10.1016/j.jclepro.2020.121158

Walsh, S. J., Engie, K., Page, P. H., & Frizzelle, B. G. (2019). Demographics of Change: Modeling the Transition of Fishers to Tourism in the Galapagos Islands. In T. Kvan & J. Karakiewicz (Eds.), *Urban Galapagos: Transition to Sustainability in Complex Adaptive Systems* (pp. 61–83). Springer International Publishing. https://doi.org/10.1007/978-3-319-99534-2_5

Wang, W., Ge, J., & Yu, X. (2020). Bioavailability and toxicity of microplastics to fish species: A review. *Ecotoxicology and Environmental Safety*, *189*, 109913. https://doi.org/10.1016/j.ecoenv.2019.109913

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, *178*, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Xiang, Y., Jiang, L., Zhou, Y., Luo, Z., Zhi, D., Yang, J., & Lam, S. S. (2022). Microplastics and environmental pollutants: Key interaction and toxicology in aquatic and soil environments. *Journal of Hazardous Materials*, *422*, 126843. https://doi.org/10.1016/j.jhazmat.2021.126843

Zantis, L. J., Carroll, E. L., Nelms, S. E., & Bosker, T. (2021). Marine mammals and microplastics: A systematic review and call for standardisation. *Environmental Pollution*, *269*, 116142. https://doi.org/10.1016/j.envpol.2020.116142

Zhang, D., Liu, X., Huang, W., Li, J., Wang, C., Zhang, D., & Zhang, C. (2020). Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. *Environmental Pollution*, *259*, 113948. https://doi.org/10.1016/j.envpol.2020.113948

TABLES

Table 1. MP abundance in scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago. Number of scats analyzed (n), number of the different shapes of MPs found per rookery, and total mean with standard deviation (x̄ ± SD) of MPs per scat rookery and rookery are reported.

Rookery	Island	n	Fibers	Fragments	Films	Total	x̄ ± SD
El Malecón	San Cristobal	60	28	11	1	40	0.67 ± 0.51
Punta Pitt	San Cristobal	30	6	6	1	13	0.43 ± 0.41
Punta Suárez	Española	30	8	1	1	10	0.33 ± 0.32
Post Office	Floreana	30	8	2	0	10	0.33 ± 0.38
Bahía de Santa Fe	Santa Fe	30	6	1	1	8	0.27 ± 0.29
Total		180	56	21	4	81	0.45 ± 0.65

Table 2. Multiple comparisons of median ranks of MPs particle sizes found on scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago. Highlighted values (*) show significant statistical differences.

Rookery	El Malecón	Punta Pitt	Punta Suárez	Post Office	Bahía de Santa Fe
El Malecón	—	—	—	—	—
Punta Pitt	1.0000	—	—	—	—
Punta Suárez	**0.0009***	**0.0143***	—	—	—
Post Office	**0.0029***	**0.0357***	1.0000	—	**—**
Bahía de Santa Fe	0.0666	0.2468	1.0000	1.0000	—

Table 3. MP prevalence in scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago.

Rookery	**Island**	**n**	**Samples w/ MPs**	**Samples w/ no MPs**	**Prevalence (%)**
El Malecón	San Cristobal	60	31	29	52
Punta Pitt	San Cristobal	30	10	20	33
Punta Suárez	Española	30	7	23	23
Post Office	Floreana	30	10	20	33
Bahía de Santa Fe	Santa Fe	30	8	22	27
Total		180	66	114	37

Table 4. Characterization of MPs found on different otariid species though scat-based analyses*.

Common name	**Species**	**Country**	**Positive samples**	**Prevalence (%)**	**Abundance**	**Chemically confirmed MPs**	**Polymer ID**	**Colors (>10%)**	**Shape**	**Reference**
Galapagos Sea Lion	*Zalophus wollebaeki*	Ecuador	66 (180)	37	81 particles; 0.45 ± 0.65 particles scat^{-1}; 0.02–0.22 particles wet g^{-1}; 0.04 ± 0.05 particles wet g^{-1}	46 particles	PP-PE, PP, PE, PVC, PAN, PEAA:ST:AA, PETP, PS, PS-PAN, cellulose, cellophane	Blue (45%), black (32%), non-colored (11%)	Fibers: 69%; Fragments: 26% Films: 5%	*This study*
California Sea Lion	*Zalophus californianus*	Mexico	–	–	294 potential particles**; 0.14 ± 0.32 particles g^{-1}	77 particles	PET, PP, PE, ABS, PAN, PVA, PV, PEA, PEU, rayon, cellulose	Blue (54%), black (24%)	Fibers: 92%; Fragments: 8%	(Ortega-Borchardt et al., 2023)
South American fur seal	*Arctocephalus australis*	Chile/Peru	34 (51)	67	2.7–13.35 particles wet g^{-1}	–	–	Blue (45%), white (24%), black (16%), and red (15%)	Fibers: 100%	(Perez-Venegas et al., 2018)
South American fur seal	*Arctocephalus australis*	Chile/Peru	44(205)	21	2–15 particles g^{-1}	6 scats***	PET, cotton, nylon	Blue, white, and red	Mostly fibers	(Perez-Venegas et al., 2020)
Juan Fernández fur seal	*Arctocephalus philippii*	Chile/Peru								
South American sea lion	*Otaria flavescens*	Chile/Peru								
South American sea lion	*Otaria flavescens*	Chile/Peru	8 (10)	80	47 potential particles**	–	–	Mostly blue	Fragments: 91%; Fibers: 9%	(Ayala et al., 2021)
Northern fur seal	*Callorhinus ursinus*	United States of America	44 (44)	100	584 potential particles**	2 particles	LDPE	Fragments: White (99%); Fibers: Black, white, purple, blue,	Fragments: 55%; Fibers: 41%	(Donohue et al., 2019)

								red, yellow, transparent (no %)		
Antarctic fur seal	*Arctocephalus gazella*	Western Antarctica	0 (42)	0	–	–	–	–	–	(Garcia-Garin et al., 2020)
Antarctic fur seal	*Arctocephalus gazella*	Tasmania	145	–	164 particles; 1–4 particles scat^{-1}	164 particles	PE, PP, poly(1-Cl-1-butenylene) polychloroprene, melamine-urea (phenol) (formaldehyde) resin	White (33%), brown (19%), blue (15%), green (15%), yellow (15%)	Particles and fibers	(Eriksson & Burton, 2003)
Subantarctic fur seal	*Arctocephalus tropicalis*	Tasmania	–							

* Modified from McIvor et al., 2023, and Ortega-Borchardt et al., 2023.

** "Potential particles" refers to the fact that this study did not undergo through control correction, or chemically confirmation of the anthropogenic origin of the particles before reporting the total abundance of MPs.

*** This study did not report the number of chemically confirmed particles, but only the number of scats subjected to μFT-IR spectrometry analysis

FIGURES

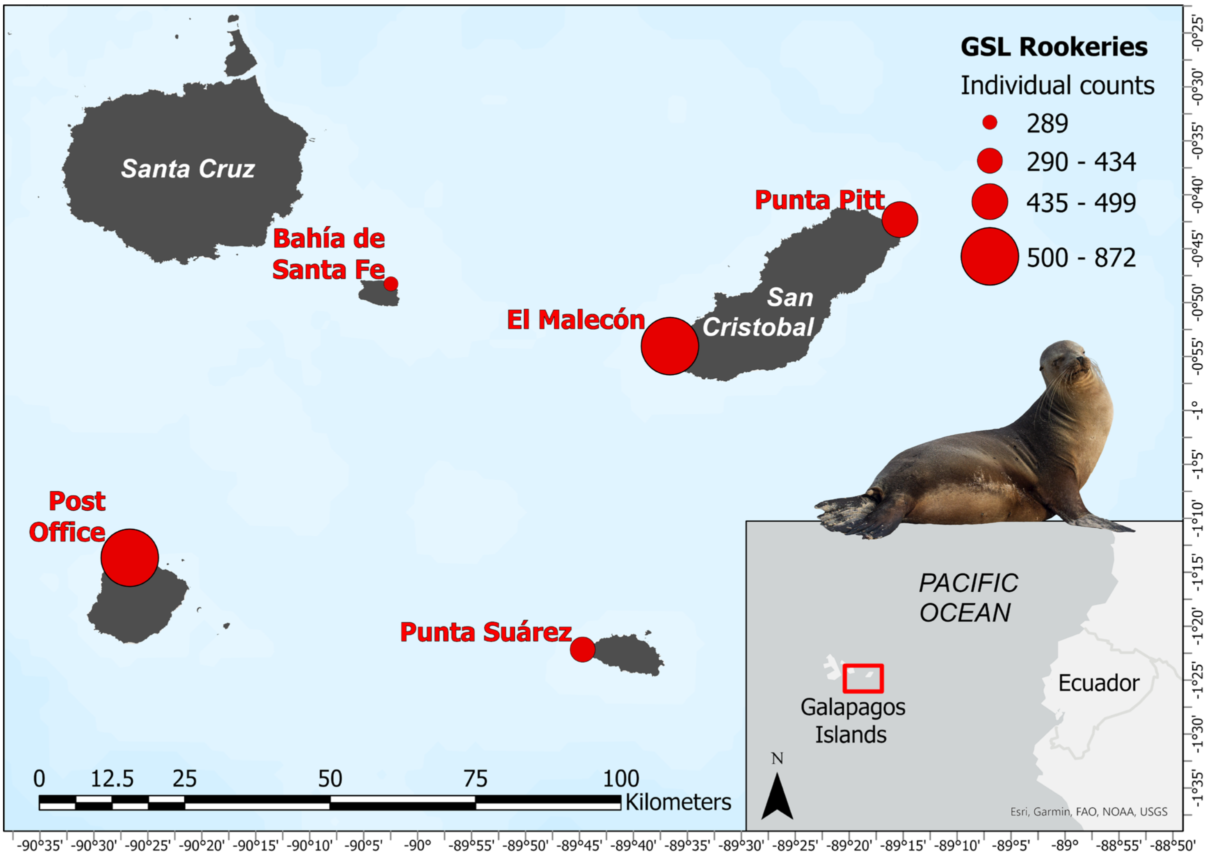

Figure 1. Study areas showing the locations of Galapagos sea lion rookeries from the southeast Galapagos archipelago. Circle sizes are based on animals counted on each rookery during field work.

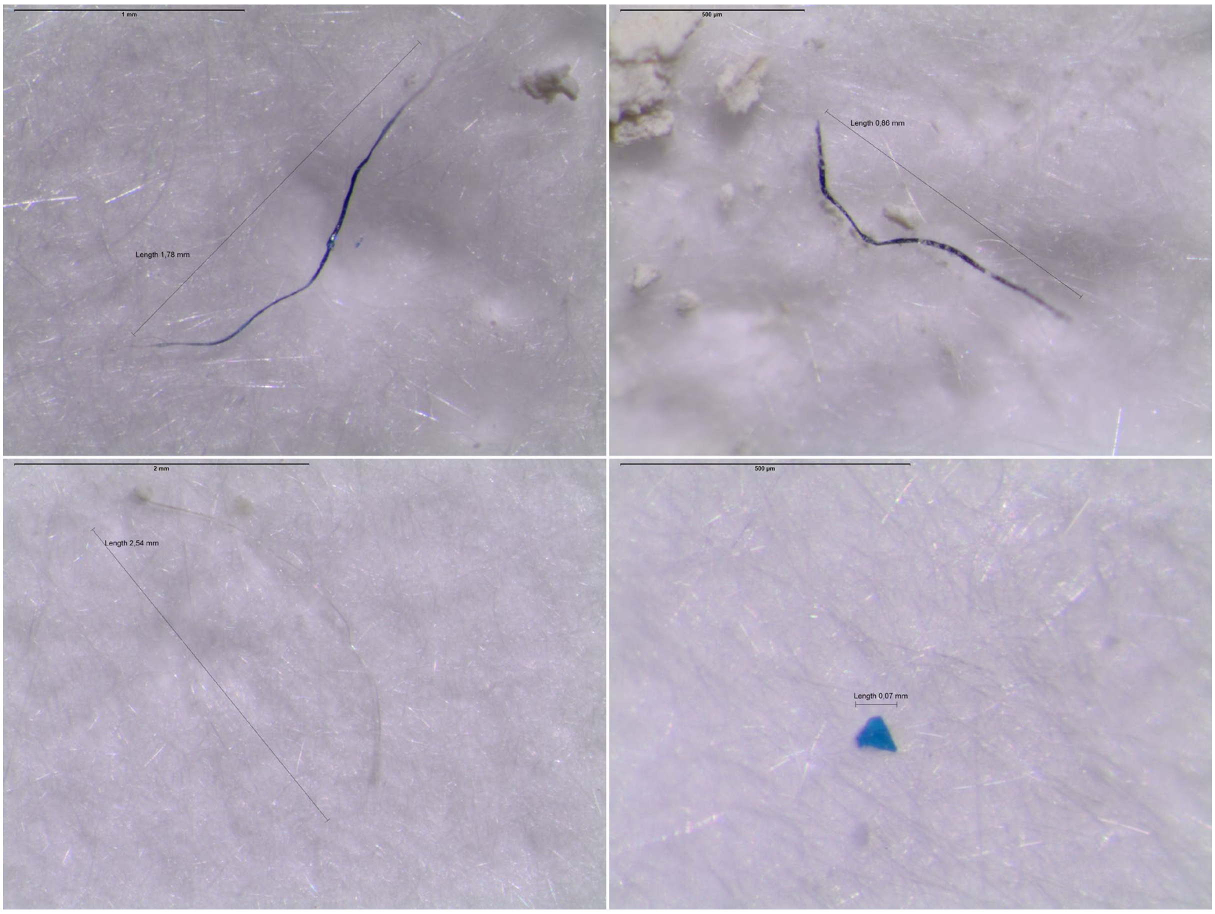

Figure 2. MPs retrieved in scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago.

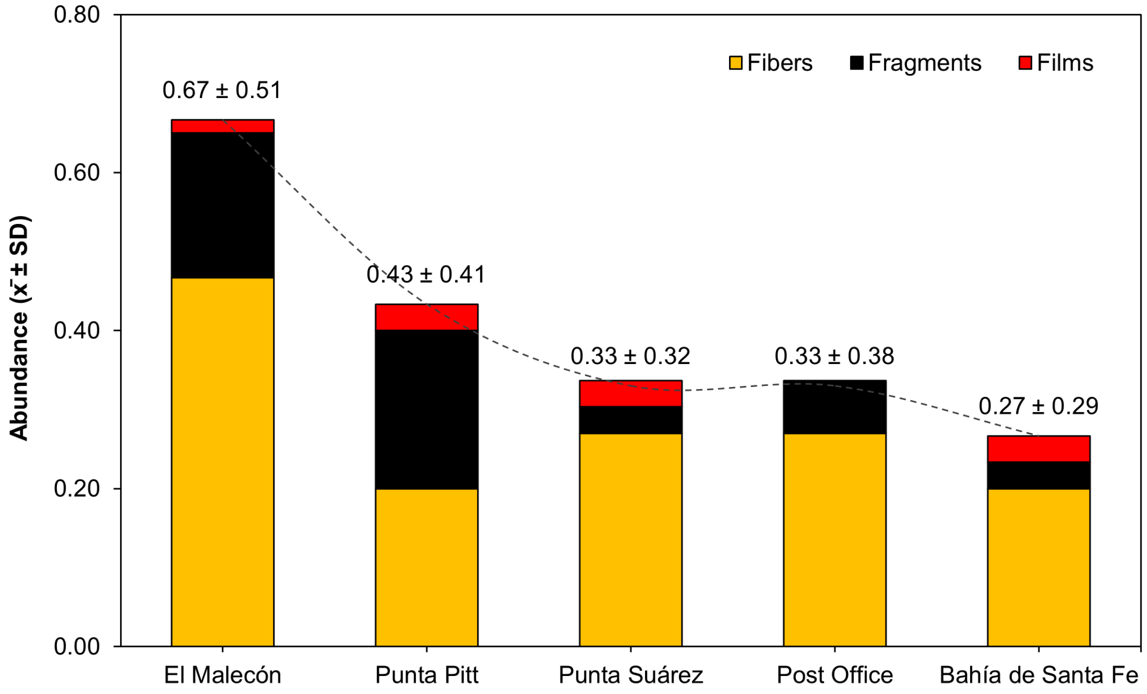

Figure 3. MP abundance in scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago. Mean and standard deviation (x̄ ± SD) of particles per scat and rookery are reported above the plotted bars.

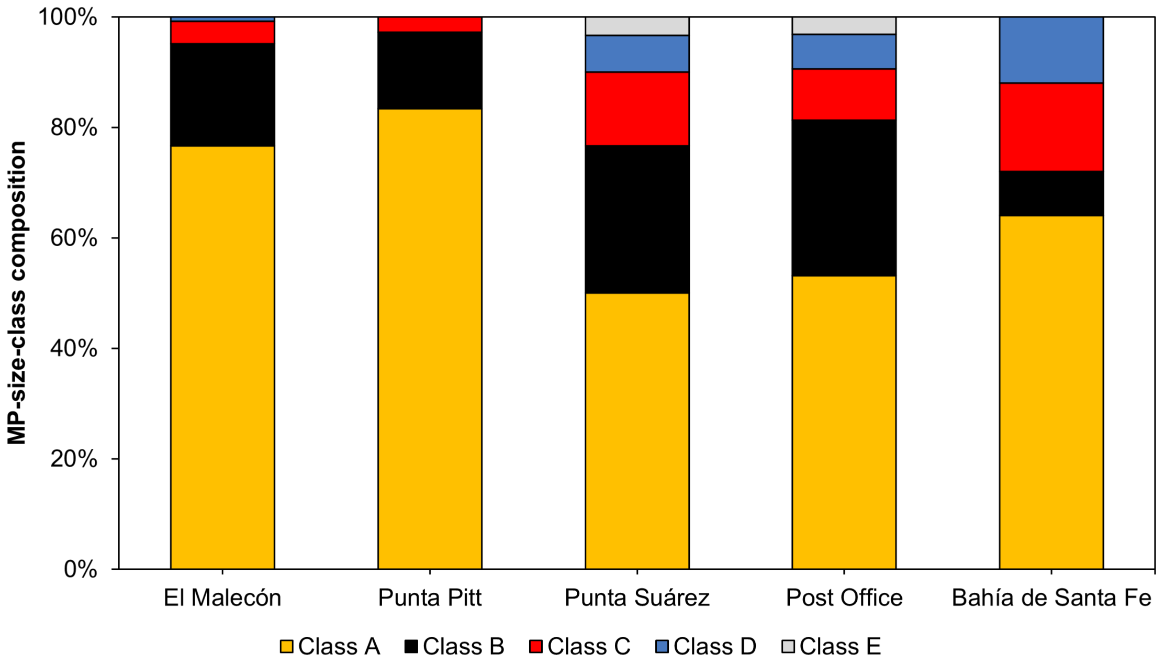

Figure 4. MP-size-class composition found on scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago. Class A: 20 to 1000 µm, Class B: 1001 to 2000 µm, Class C: 2001 to 3000 µm Class D: 3001 to 4000 µm, Class E: 4001 to 5000 µm.

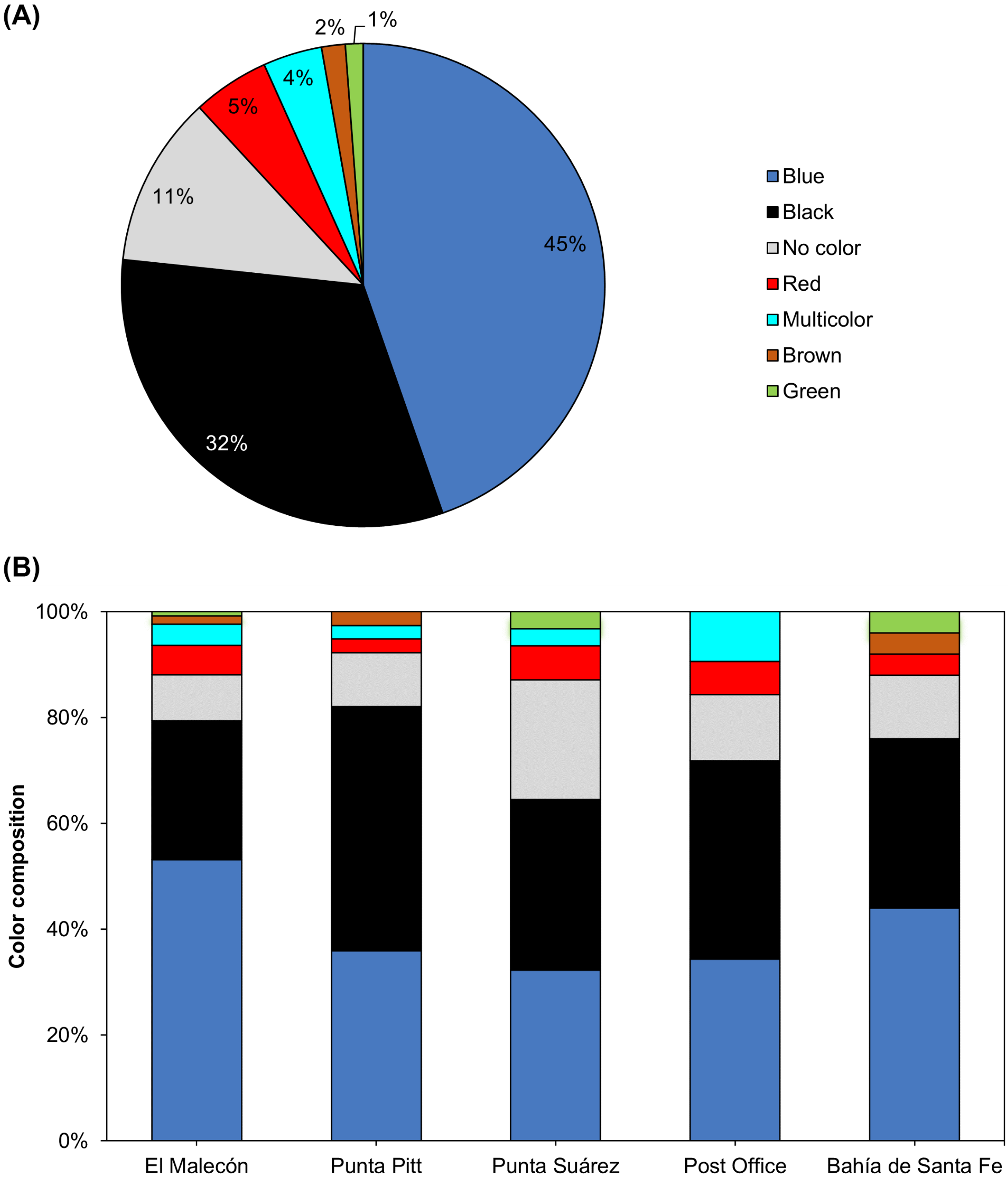

Figure 5. Percentage of MP colorations found on scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago. (A) Total average. (B) In each of the different rookeries.

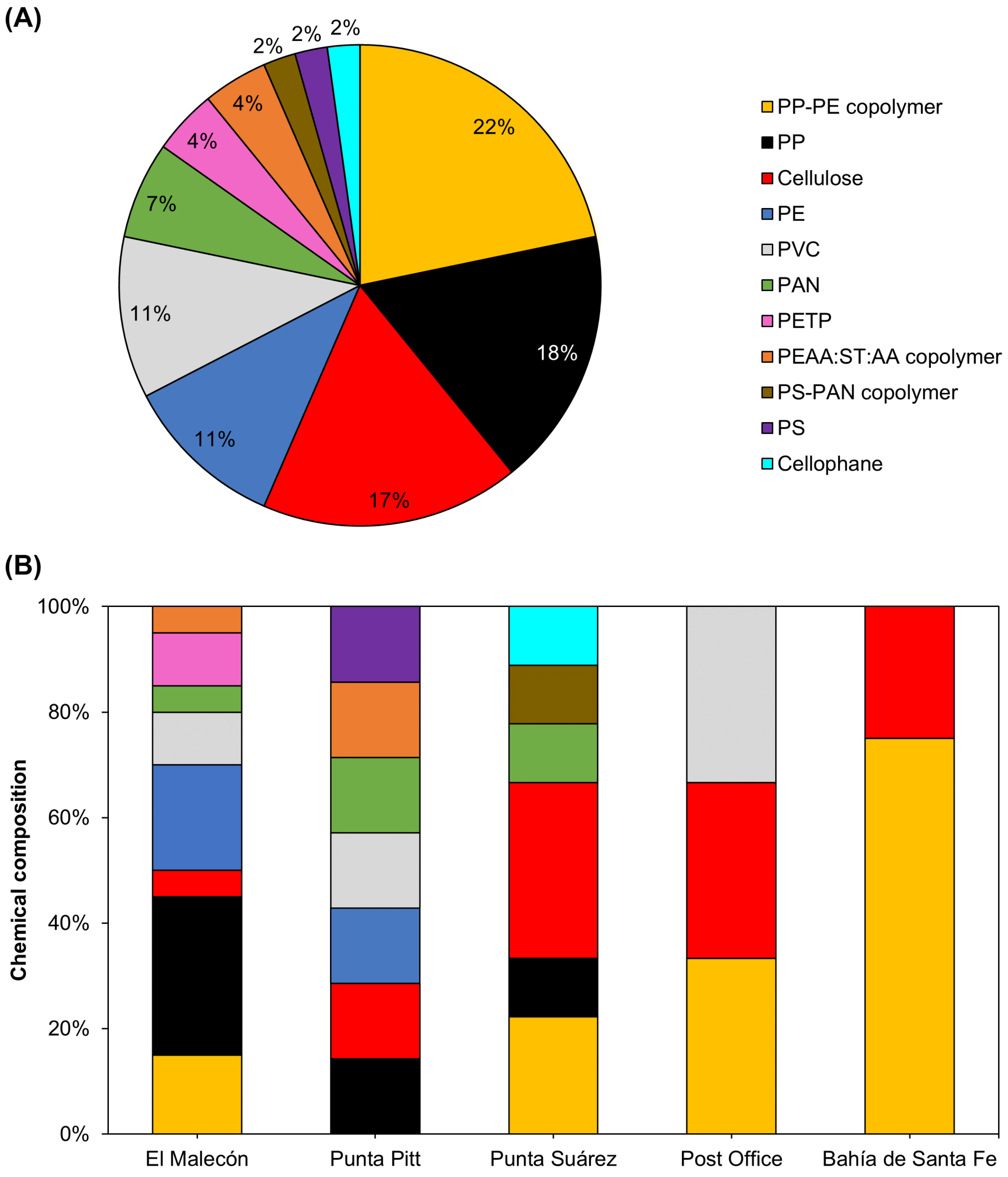

Figure 6. Percentage of µFT-IR confirmed polymers found on scat samples of Galapagos sea lion rookeries from the southeast Galapagos archipelago. (A) Total average. (B) In each of the different rookeries.