

A First Comparison of Microplastics Abundance between Two Whale Shark Feeding Areas of the Gulf of California, Mexico.

Lara Roberta Cardelli

Instituto Politécnico Nacional Centro Interdisciplinario de Ciencias Marinas: Instituto Politecnico Nacional Centro Interdisciplinario de Ciencias Marinas

Francesca Pancaldi (✉ frampi@gmail.com)

Instituto Politécnico Nacional Centro Interdisciplinario de Ciencias Marinas: Instituto Politecnico Nacional Centro Interdisciplinario de Ciencias Marinas <https://orcid.org/0000-0003-4132-5402>

Felipe Galván Magaña

Instituto Politécnico Nacional Centro Interdisciplinario de Ciencias Marinas: Instituto Politecnico Nacional Centro Interdisciplinario de Ciencias Marinas

Darren Andrew Whitehead

pelagios kakunjá

Research Article

Keywords: endangered species, Gulf of California, contamination, microplastics, ingestion rate, whale shark

Posted Date: June 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-567472/v1>

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Abstract

Abundance and typology of microplastics (MPs) were measured in 12 water samples collected from two whale sharks feeding areas of the Gulf of California (Bahía de los Angeles (BLA) and Bahía de La Paz (LAP)). The concentration of MPs was 0.47 MPs/ m³ in BLA and 0.63 MPs/ m³ in LAP. Overall, films were the most abundant type of MPs (41%; n = 74) followed by line (37%; n = 68), fragments (20%; n = 36) and pellet (2%; n = 3), with significant differences between the types of microplastics. Theoretical ingestion rate per hour of MPs for the species was determined to be 288.58 and 386.82 in BLA and LAP, respectively. In faecal samples collected directly from animals of LAP (n = 3), 67% of the MPs were films, 25% were fragments, and 8% were lines. Results from this study indicate that MPs are common in the feeding areas of this endangered species and that whale sharks are likely ingesting MPs from filtering the surface water.

Main Text

Plastic pollution is a serious global threat in marine ecosystems (Villarrubia-Gómez et al. 2018). Anthropogenic activities such as poorly manage waste from urban site, aquaculture and fishing are increasingly responsible for the offload of plastics debris in the oceans (Rochman et al. 2013). It is estimated between 6 and 12 tons of plastics end up in the oceans every year (Jambeck et al. 2015). Once in the marine environment, these pollutants travel long distances and eventually (through mechanical, chemical, or biological processes) break down in microplastics (MPs), defined as plastics < 5 mm in diameter (Andrady 2011). Because of their small size, MPs are up taken by different organisms including microalgae (Zhang et al. 2017), zooplankton (Cole et al. 2013), teleost fish (Lusher et al. 2013) and megafauna (Fossi et al. 2017; Germanov et al. 2018). Consequences of these ingestions often include toxics effects (Zhang et al. 2017), gastrointestinal blockage, starvation, and death (Wright et al. 2013).

Filter feeder species are particularly vulnerable to MPs (Fossi et al. 2017; Germanov et al. 2019; Pennino et al. 2020). The whale shark, *Rhincodon typus* (Smith 1828) is the largest fish of the planet (Compagno 2001); it is a filter feeder with long life span and a circumglobal distribution (Stevens 2007; Rowat and Brooks 2012). In the Gulf of California, Mexico, feeding aggregations of whale shark are seasonally spotted in Bahía de los Angeles (Nelson and Eckert 2007) and Bahía de La Paz (Ketchum et al., 2013), where animals are seen feeding on dense patched of copepods, quetognats, and eufausids (Whitehead et al. 2019). Internationally, whale sharks are listed as “Endangered” by the International Union for the Conservation of Nature red list and in Mexico this species is protected under two national laws (DOF 2006, 2010). The presence of whale shark feeding aggregations in Bahía de los Angeles and Bahía de La Paz over the last two decades has risen to intense tourist activities and now represents an important economic source for the local community. In these areas, the increasing tourist pressure has forced local authorities to enforce management plans to guarantee the safety and conservation of the animals (SEMARNAT 2017).

Despite these measures, recent studies have showed that whale sharks from Bahía de La Paz are exposed to toxic substances such as heavy metals (Pancaldi et al. 2019 a,b), persistent organic pollutants and plastic pollution (Fossi et al. 2017) as a result of continuous feeding activity on the sea surface near costal urban areas. The aim of this study is to provide the first analysis of the abundance and type of MPs present in the surface waters in two whale sharks feeding areas of the Gulf of California (Bahía de los Angeles (BLA) and Bahía de La Paz (LAP)) during the period of maximum presence of the sharks in these areas. Analysis of MPs was also performed in faecal samples collected directly from animals in LAP.

Tows of water were performed in September 2016 in “La Mona” area, south of Bahía de los Angeles (BLA; 28.9519° N, 113.5624° W; Fig. 1) and from October to December 2016 in the “Mogote” area, south of Bahía de La Paz (LAP; 24.0832° N, 110.1839° W; Fig. 1). A manta net with a mesh of 333µm, 43.5 cm long and 14 cm wide was used to perform water tows. Tows were performed during the presence of foraging whale sharks, with wind speed between 2.5 and 7 knots. Whale shark presence was assessed visually and the estimation of total length (TL) of the individuals was established using an object (e.g., swimmer, boat) for comparison. Tows were trailed for 10 minutes at 2 knots, in a lineal path, parallel to the sharks’ movement. Once the net was recovered, the material was placed in a glass baking dish and subsequently stored in an additional glass storage jar. To avoid any possible contamination from plastic, the cap of the jar was covered with an aluminium foil before closing, then frozen at -20°C for ahead of microscope analysis.

Samples were unfrozen, moved into aliquots in a Petri dish and observed with a microscope Carl Zeiss model Stemi DV4. The particles, recognized morphologically as microplastics, were recovered with sterile tweezers, and placed in storage with cover glass objects. The microplastics were divided into 4 different classes of shape: fragment, line, pellet, and film (Rochman et al. 2013; Lorenzo- Navarro et al. 2018; Germanov et al. 2019). A “fragment” was considered as a piece of hard plastic with undetermined margin line (Fig. 2a); a “line” was considered a MP with elongated, cylindrical shape and high flexibility (Fig. 2b); a “pellet” was considered a compressed piece with a typical spheric shape (Fig. 2c); and a “film” was a thin and flexible piece with undetermined margin line (Fig. 2d). To avoid contamination, samples observation was performed in a sterilized room and above a fume hood (Choy and Drazen 2013; Avio et al. 2015).

The total volume of analysed water in each area was calculated by multiplying the length of the examined surface (distance between GPS start point and GPS end point of the trawling) and the area of the manta net mouth (which is calculated as the long side multiplied the short side) expressed in m³. To know the concentration of MPs per m³ found in each area, the total number of MPs was divided by the total volume of water analysed.

Faecal samples were collected in November 2016 in LAP (Mogote area; Fig. 1). Feces, identified as compact brownish to reddish material, were collected from a swimmer during opportunistic field observations after being defecated by the shark. The samples were collected in crystal jars and initially

stored on ice packs, then transferred to long-term storage at -20°C before analyses. Samples were contained less than a minute after being defecated by the shark, nevertheless, environmental contamination from microplastics may have occurred when they were collected from the water.

Whale shark theoretical MPs ingestion rates were calculated based on filtration volumes of captive whale shark individuals from the Georgia Aquarium and for free swimming animals based on the data published by Motta et al. (2010). As data followed a normal distribution, parametric tests were applied to the results.

A total of 12 water samples were collected: 5 in BLA and 7 in LAP. A total volume of 143.674 m³ was analysed in BLA and 190.01 m³ in LAP. In BLA a total of 27 whale sharks were observed during the sampling collections and 19 whale sharks were observed in LAP. Average total length (TL) of sharks was 6.2 m for BLA and 5.4 m for BLP. In BLA 61 MPs were encountered in the collected water (10 in the sample #1, 11 in the sample #2, 15 in the sample #3, 13 in the sample #4 and 12 in the sample #5; Table 1) and in LAP 120 MPs were reported (15 in the sample #1, 7 in the sample #2, 21 in the sample #3, 14 in the sample #4, 21 in the sample #5, 29 in the sample #6 and 13 in the sample #7; Table 1). The concentration of MPs was 0.47 MPs/ m³ in BLA and 0.63 MPs/ m³ in LAP. No significant differences were found between the number of MPs found in each site.

Table 1. Number of MPs found in water samples collected in BLA and LAP.

Site	Sample	Fragment	Line	Pellet	Film	Total
BLA	1	0	3	0	7	10
	2	3	4	0	4	11
	3	4	6	0	5	15
	4	3	8	0	2	13
	5	2	4	0	6	12
	Total	12	25	0	24	61
LAP	1	0	6	0	9	15
	2	0	5	1	1	7
	3	5	9	0	7	21
	4	5	3	2	4	14
	5	4	8	0	9	21
	6	3	11	0	15	29
	7	7	1	0	5	13
	Total	24	43	3	50	120
Total of two bays		36 ^{a,b,c}	68 ^{a,d}	5 ^{b,d,e}	74 ^{c,e}	181

Subscription letters indicate significative differences between type of MPs. Italic letters indicate p Student *t*-test < 0.05, bold letters indicate p Student *t*-test < 0.001. Statistic tests were performed using Statistica software.

Considering both study areas together (n tows = 12), 41% of MPs were films (n = 74), 37% line (n = 68), 20% fragments (n = 36) and 2% pellet (n = 3). Fig. 3. Student *t*-test indicated significative differences

between the number of fragments and pellet, line and pellet, film, and pellet ($P < 0.001$; Table 1), fragments and line and fragments and film ($P < 0.05$; Table 1). In BLA 44% of the total MPs found were films ($n = 24$), 38% line ($n = 25$), 18% fragments ($n = 12$) and no pellets were found (Fig. 3). In LAP 42% of the total MPs found were films ($n = 50$), 36% lines ($n = 43$), 20% fragments ($n = 24$) and 2% pellet ($n = 3$; Fig. 3). No significant differences were found between the MPs shape per site.

Three faecal samples were collected from three different specimens in Bahia de La Paz. Faecal sample # 1 showed a total of 3 MPs (all films), faecal sample #2 showed 5 films and 1 line, while faecal sample #3 showed 3 MPs (all fragments). In total, 67% of the MPs found in the faecal samples were films, 25% fragments and 8% lines.

Theoretical ingestion rate for whale sharks from BLA and LAP was calculated based on data published by Motta et al. (2010) which showed that a 622 cm whale shark from the Georgia Aquarium, Atlanta, can filter 614 m³ of water per hour. According to these data, whale sharks from this study can ingest 288.58 and 386.82 MPs / h in BLA and LAP, respectively. Considering that whale sharks from the Gulf of Mexico feeds during 7.5 hours per day (Motta et al. 2010) the theoretical ingestion rate per day in the sharks from BLA would be 2164.35 MPs / day and 2901.15 MPs / day in LAP.

Microplastics (MPs) were found in water samples and faecal samples indicating that these pollutants are present in the feeding areas of this endangered species and that whale sharks are likely ingesting MPs from filtering the surface water. In the water trawls, the number of MPs found in the total volume of water analysed (BLA = 0.47 MPs/ m³; LAP = 0.63 MPs/ m³) was higher than what was observed by Fossi et al. (from 0.00 items m³ to 0.14 items per m³; Fossi et al. 2017). The number of MPs per m³ from this study were in the same range of concentration of what was found by Germanov et al. 2019 (0.56 to 0.90 pieces m³) during trawl surveys performed in whale shark and the reef manta ray *Mobula alfredi* foraging area in Indonesia during the wet season. Indonesia contributes to about 14% of the global annual plastic pollution in the marine environment (Lebreton et al. 2017; Germanov et al. 2019) with highest input recorded during the wet season compared to the dry one (Germanov et al. 2019). Similarly, a significant quantity of plastics debris is also found in a similar foraging area Bahia de Banderas, Mexico, during the wet season due to river inputs (Pelamatti et al. 2019).

Despite that significative differences were not found (probably because of the low sample size), water collected from LAP showed higher concentration of MPs per m³ compared to BLA. The whale shark feeding area of Bahia de La Paz (Mogote area; Fig. 1) is located in close proximity to the city of La Paz, the second most inhabitant city of Baja California Sur (272.211 habitants recorded in 2015; INEGI; <http://cuentame.inegi.org.mx/monografias/informacion/bcs/poblacion/default.aspx?tema=me&e=03>). In addition, the whale shark feeding aggregation season in Bahía de La Paz coincides with the period of strong north wind that could potentially accelerates the influx of plastic debris from the urban area to the adjacent foraging area of the sharks.

Considering both study areas, film was the MPs with the highest incidence followed by line, fragments, and pellet. Films were also the main MPs shape found in the whale shark feces collected in LAP, which confirms the high presence of these pollutants and the risks associated to them. Pellet was the MPs with a significantly lower incidence than the other types ($P < 0.001$). Pellets are primary MPs (Barnes et al. 2009) used mainly in cosmetic, pharmaceutical and textile industry (Gregory 1996; Fendall and Sewell 2009).

Considering that neither La Paz nor Bahia de los Angeles are industrial cities, it is probable why pellets showed such low incidence compared to the other MPs shapes. On the contrary, film, fragment and line are secondary MPs (Barnes et al. 2009) derived from the breakup of daily use products (cloths, plastic bags, bottles, fishing gears) and, in our study areas they are likely to ended up on local beaches and/or washed by currents into the whale shark feeding areas. Daily theoretical ingestion rate for whale sharks from this study was higher (2164.35 MPs / day in BLA, and 2901.15 MPs / day in LAP) compared to a previous study performed in Bahía de La Paz (171 MPs / daily; Fossi et al. 2017) and to another study performed in Indonesia (326 MPs / h; Germanov et al. 2019). Risks associated with ingestion of plastic debris by megafauna include possible toxic effects provoked by the presence of plastic associated pollutants such as organochlorine compounds and heavy metals (Fossi et al. 2017; Pancaldi et al. 2019a,b). The latter ones have been previously detected in skin biopsies of feeding whale sharks from Bahía de La Paz (Pancaldi et al. 2019a) and organs of stranded whale sharks from Bahía de La Paz and Punta Bufeo (Pancaldi et al. 2019b), even if this contamination has not been linked to exposure through plastic ingestion. On the contrary, organochlorine compounds (PCBs, DDTs), and polybrominated diphenyl ethers (PBDEs) plastic additives associated to plastic materials has been detected in skin biopsies of whale sharks from La Paz Bay (Fossi et al. 2017).

In conclusion, the presence of microplastics in whale shark feeding areas represents a threat to whale sharks which uptake these items by continuously filter feeding on the water surface. Local policies that regulate the use of plastic items should be enforced especially in areas adjacent to feeding grounds of protected megafauna. Further research should be carried out to evaluate the risks related to the ingestion of microplastics in this endangered species.

Declarations

- Ethics approval and consent to participate

This study was performed on a protected species under the Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) permit number: SGPA/DGVS 05605/17. No animal was killed during this study

- Consent for publication

Not applicable

- Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

- Competing interests

The authors declare that they have no competing interests.

- Funding

Comisión de Operación y Fomento de Actividades Académicas (COFAA) fellowship: collection and analysis of data

Education Department Costa Edutainment Spa: collection and analysis of data

Centro Interdisciplinario de Ciencias Marinas (CICIMAR) Project “Tiburones y Rayas” and “Cambios estacionales de las presas zooplanctónicas clave del tiburón ballena *Rhincodon typus* en Bahía de La Paz, B.C.S.”. Grant number SIP: 20170585 and to the SIP-IPN Project “Ecología trófica de los tiburones de la costa occidental del Golfo de California”. Grant number SIP: 20181417: collection and analysis of data

- Author’s contributions

Lara R. Cardelli: Formal analysis; Writing - review & editing; Methodology; Field work. Francesca Pancaldi: Conceptualization; Data curation; Formal analysis; Software; Writing - Original draft; Writing - review & editing; Visualization. Felipe Galván Magaña: Funding acquisition; Project administration; Resources; Supervision; Writing - review & editing; Validation. Darren A. Whitehead: Investigation; Writing- review.

- Acknowledgements

We would like to thank Dr. Stefano Furlati from the Education Department Costa Edutainment Spa for the donation of the field material. Thanks to the Instituto Politécnico Nacional for the support received through the -Contracting Excellence Program- and Fellowship EDI and COFAA. Thanks to CICIMAR Project “Tiburones y Rayas” and “Cambios estacionales de las presas zooplanctónicas clave del tiburón ballena *Rhincodon typus* en Bahía de La Paz, B.C.S.”. Grant number SIP: 20170585.

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Figures

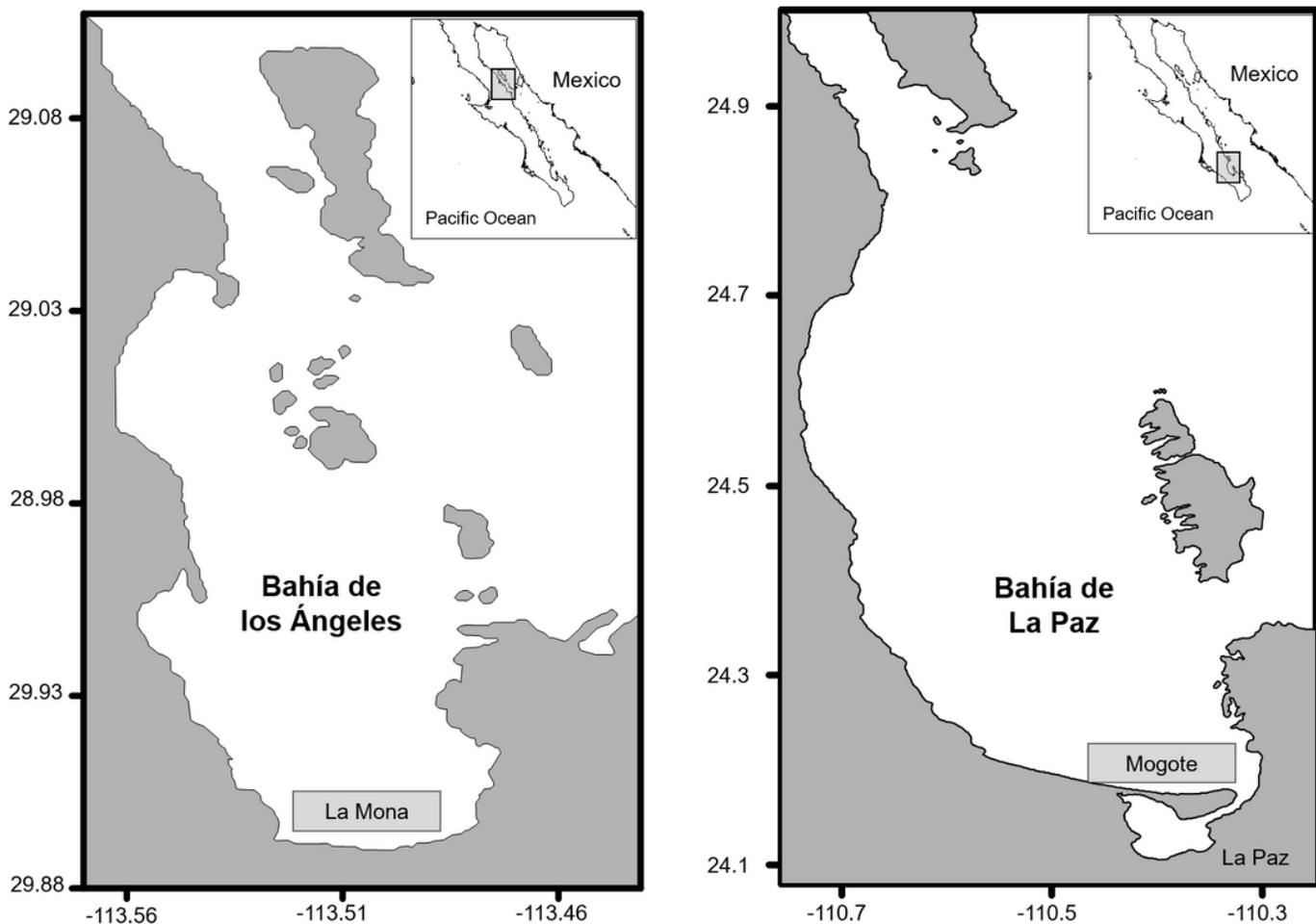


Figure 1

Map of the study area: Bahía de los Angeles (BLA) and Bahía de La Paz (LAP), Mexico. Grey boxes indicate sampling areas. Map designed with Surfer Plot program. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

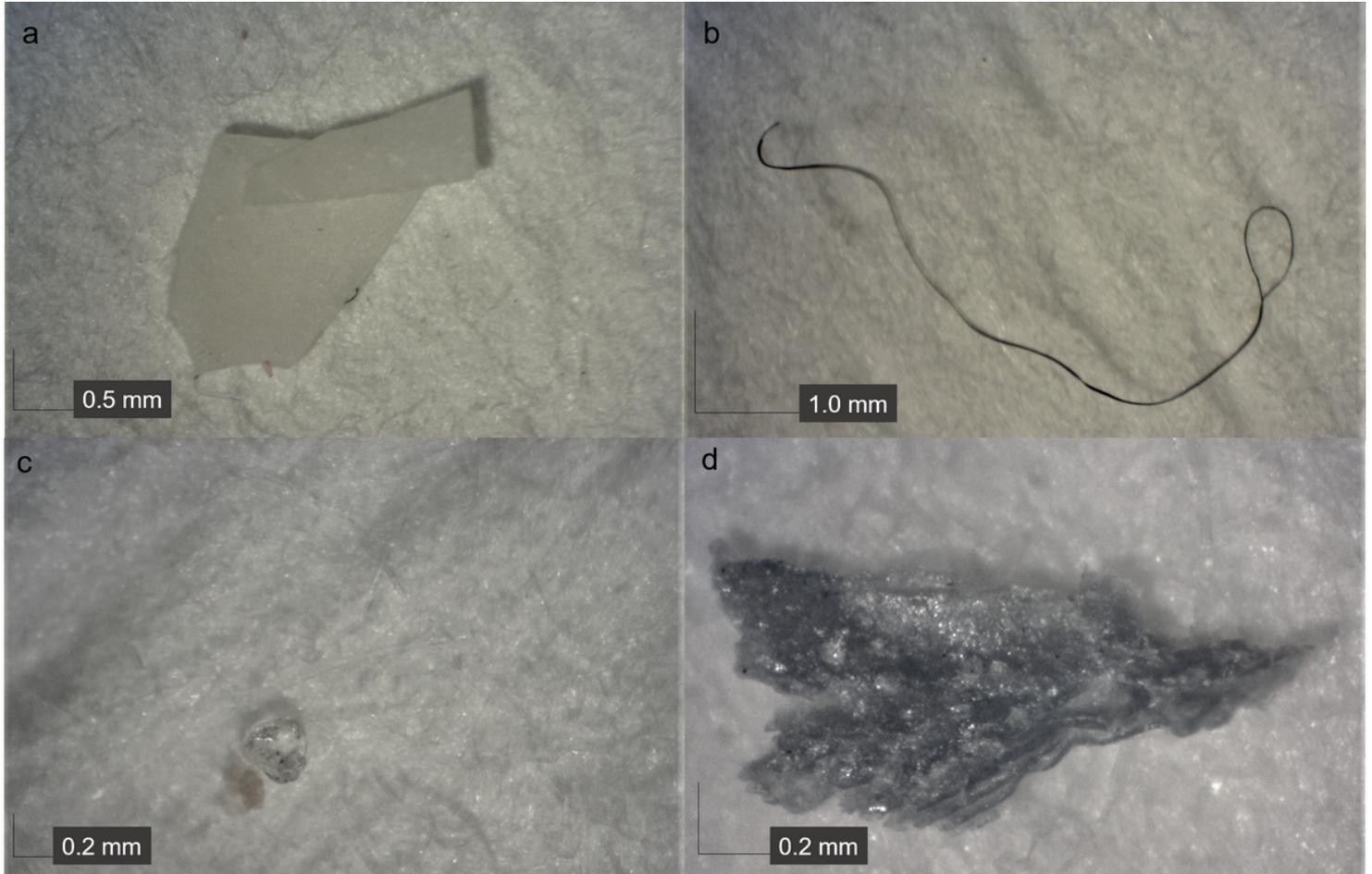


Figure 2

Images of microplastics types: fragment (a), line (b), pellet (c) and film (d) found in water samples from the whale shark feeding area in La Paz bay.

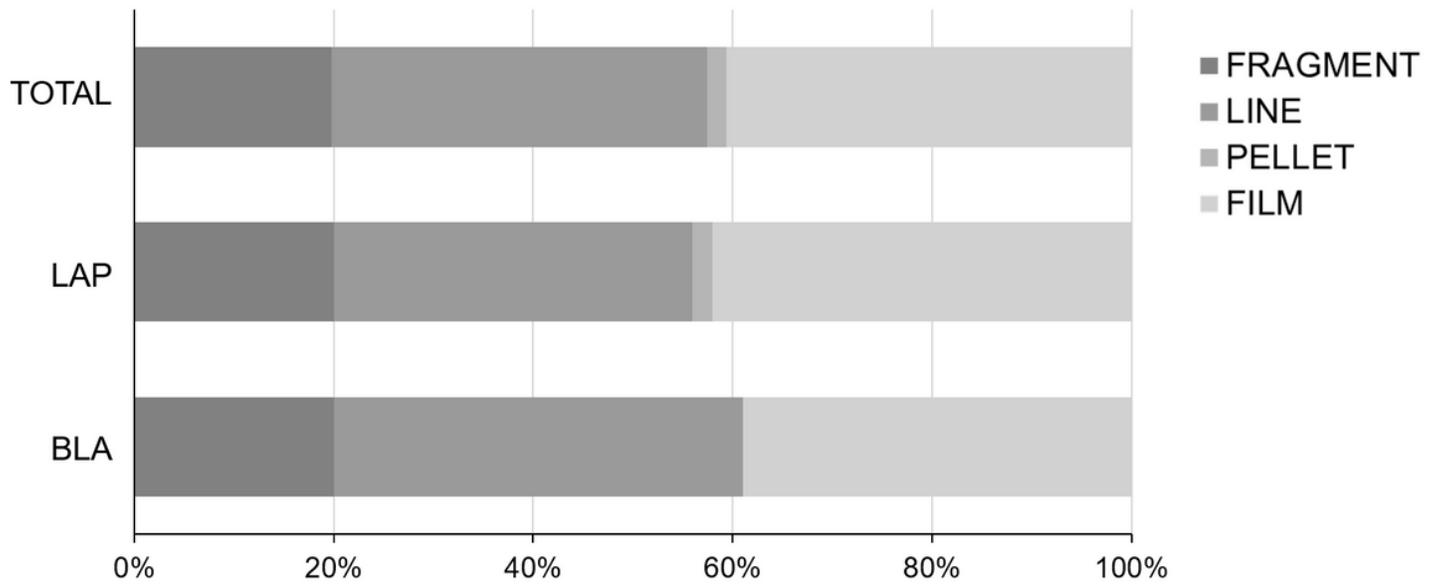


Figure 3

Percentage of fragment, line, pellet, and film found in water samples collected in Bahía de los Angeles, Bahía de La Paz and in total.