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First report of plastic contamination in batoids: Plastic ingestion by Haller's Round Ray (*Urobatis halleri*) in the Gulf of California

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ABSTRACT

The presence of microplastics has been reported in the marine environment and these pollutants have also been reported in food webs. Information about the presence of microplastics in the Haller's Round Ray (*Urobatis halleri*) and bottom sediments off the east coast of the Gulf of California is non-existent. The digestive tracts of individuals of this species and sediment samples were examined for plastic particles in this region. In total, 107 plastic particles were found in the sediment. All were fibers and 94.4% were microplastics, the rest were mesoplastics. The gastrointestinal tracts of 142 rays were analysed, and it was determined that this is a benthic feeder. A total of 386 plastic particles were recovered from 46 individuals (32.4%). On average 10.2 (±7.4) plastic particles were found per specimen, with plastic lengths ranging from 0.00821 mm to 0.953 mm. The FTIR-ATR analysis revealed the presence of six types of polymers: polyamide or nylon polyethylene, polypropylene, and polyacrylic were found in both sediments and gastrointestinal tracts of Haller's Round Ray. Polyethylene terephthalate and polyacrylamide were only found in the gastrointestinal tracts of the ray. These polymers are consistent with the human activities undertaken in this area, specifically intensive small-scale and industrial fisheries, as they are used for the elaboration of fishing nets, plastic bags, storage containers, clothing, and fishing boats maintenance. Our results show that benthic feeders are exposed to plastic debris, and its presence is another potential threat to batoids, which are already threatened by bycatch, overfishing, and other pollutants. However, studies on the ingestion of plastic debris in batoids and its presence in the sediment are still scarce or non-existent for this region. As such, these studies are necessary to help in the preservation of these species.

1. Introduction

Plastic litter in the marine environment has become an extremely serious threat, more than 80% of the waste found in the marine environment being plastics (Alvarez-Zeferino et al., 2020; Carbery et al., 2018). Plastics are persistent pollutants that have infested every ecological niche of the world, they can be transported over vast distances by ocean currents, winds, river outflow, and drift, making its presence heterogeneous and ubiquitous (Alimba and Faggio, 2019; Cole et al., 2011; Jambeck et al., 2015). Subsequently, gaining information on how these pollutants affect organisms, communities and ecosystems are extremely important (Cole et al., 2011). Nonetheless, the study of microplastics is still a challenge due to the difficulties in assessing its abundance, density, and distribution and due to limitations of environmental risk assessment methods (Cole et al., 2011; Piñon-Colin et al., 2018; Zhang et al., 2019). The fragmentation of larger plastics into microplastics as a consequence of physical, biological, and chemical processes, increase its availability to a wider range of organisms, including organisms at the lowest trophic level, which can result in a cascading effect in marine food webs, making microplastics more dangerous than macroplastics (Alvarez-Zeferino et al., 2020; Browne et al., 2008; Carbery et al., 2018). Specifically, it is known that

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microplastics can affect fish physically in different ways. Gastric obstruction was observed in larvae of *Dicentrarchus labrax* (Mazurais et al., 2015). Microplastics caused oxidative stress, inflammation, and disrupted energy metabolism in *Danio rerio* (Lu et al., 2016), reduced feeding and activity, behavioral and metabolic effects in *Carassius* (Mattsson et al., 2015).

Physiological effects of microplastic exposure have also been observed in different fish species. These include intestinal tract alterations and compromised intestinal function in *Dicentrarchus labrax* (Pedà et al., 2016); reduced predatory performance, abnormal swimming behavior, and lethargy in *Cynoscion acoupa* and *Pomatoschistus microps* (de Sá et al., 2015; Ferreira et al., 2016; Oliveira et al., 2013), and also it has been observed that microplastic exposure (concentration of 0.008 mg L-1 of polyethylene (PE)) induced liver toxicity, hepatic stress and changed endocrine function, as well as gene expression in *Oryzias laticeps* (Rochman et al., 2013, 2014). Due to their hydrophobicity and relatively large total surface area, they can efficiently absorb endocrine disruptive chemicals, pesticides, fertilizers, aqueous metals persistent organic pollutants (POPs), carcinogens, and mutagens, becoming vectors of contaminants by their ingestion (Betts, 2008; Carbery et al., 2018; Cole et al., 2011; Tosetto et al., 2017; Zhang et al., 2019). Their ability to absorb toxic compounds has been demonstrated in several studies, microplastic particles have been shown to hold concentrations of PCBs more than 1 million times higher than those in the surrounding water (Betts, 2008), polypropylene resin pellets contained PCBs, nonylphenol, and DDE at similar or higher concentrations than those found in sediments (Mato et al., 2001), and it is also known that a large suite of toxic compounds is absorbed by plastics in a kinetically slow process, however, once absorbed, these toxic compounds have great stability and do not degrade (Cole et al., 2011; Nerín et al., 1996).

However, studies assessing the susceptibility to plastic debris (PD) ingestion and its possible consequences in elasmobranchs are still scarce, and for the case of batoids (Elasmobranchii, Batoidea), these are nonexistent, even though these species are highly susceptible to accumulation of environmental pollution due to their intrinsic ecological and biological traits (i.e., low reproductive output, late maturation, slow growth) (Dulvy et al., 2017; Pierce and Bennett, 2010). Although habitat degradation and overexploitation are suggested as the most significant threats to elasmobranch populations (Dulvy et al., 2017), coastal and oceanic pollution represent a potential additional threat with unknown consequences to this taxonomic group, as plastic ingestion could be expected to cause physiological changes similar to those already described in bony fish species, representing inherent biological risks to already threatened species.

Batoids (superorder Batoidea) differ from sharks by their dorsoventrally flattened body morphology conferring them a widespread distribution in coastal and oceanic environments, most commonly found in benthic and demersal habitats (Stevens, 2005). Specifically, the family Urotrygonidae (American round stingrays) play crucial ecological functions in their food webs by directly (e.g., predation) or indirectly (e.g., bioturbation) structuring benthic communities (Bornatowski et al., 2014; García et al., 2008; Pierce et al., 2011). The life history of this family is directly linked to the bottom substrate where plastic debris often accumulates, increasing their exposure potential (Ling et al., 2017; Maes et al., 2017; Martin et al., 2017). However, studies regarding the feeding ecology of batoids, specifically the family Urotrygonidae are scarce (Valadez-González et al., 2001), and studies regarding plastic ingestion in this group as non-existent as previously stated. Therefore, adding information about such species will ensure its longevity and accurate information on the role of this particular species in trophic webs, considering the contamination threats they are facing.

In this work, we assessed the feeding ecology and the ingestion of plastic debris of the Haller's round ray (*Urobatis halleri*) through stomach content analysis (SCA) in the eastern Gulf of California. No previous studies have reported the presence of plastic debris in the area of this study and more specifically in the gastrointestinal tract of round rays, considering that this species is reported to feed on benthic organisms. Previous studies have, however, reported the presence of significant quantities of plastic debris in coastal sediments in other areas of the Gulf of California (Alvarez-Zeferino et al., 2020; Piñon-Colin et al., 2018). Considering this, our working hypothesis is that there will be an occurrence of PD in the gastrointestinal tracts of the Haller's round ray, with the main objectives of investigating the quantity and type of plastic ingestion in the studied area, determining differences in feeding habits, and plastic frequency and composition between fish body size, and sex.

2. Material and methods

2.1. Study area

Specimens of Haller's round ray were caught off the estuarine system of Santa Maria La Reforma, located in the southern part of the central Gulf of California. It is an arid climatic zone but with large agriculture fields thanks to highly technified agriculture activities, and shrimp farm ponds installed around this system with a recorded production in 2008 of about 130,000 t with a value of 486 million dollars (Berlanga-Robles et al., 2011; Ponce-Palafox et al., 2011). This region is also an important fishing ground for small-scale fisheries (Ramírez-Rodríguez et al., 2014). It is a wave-dominated coastal lagoon with a barrier island and permanent communication to the sea through two inlets, or a Type III 5 estuary (Amezcua et al., 2019). The area of this system is 53,140 m^2, populated mainly by mangrove forests (18,700 ha), which are the primary producers in terms of vegetal biomass (Flores-Verdugo et al., 1993).

2.2. Sediment sampling

At each sampling station, triplicates of sediment samples were collected with a Van Veen grab sampler. They were taken from the first 5 cm of the surface with a small wooden spoon. All samples were placed in hermetic bags (Ziploc) and transported to the laboratory in a cooler, which was maintained at approximately 4 °C.

2.3. Fish sampling

Samples were collected with a shrimp trawl net with a 24 m footrope and a 50 mm liner at the codend fitted to a 7.5 m boat with a single 115 hp outboard engine at a fishing ground located at 25.094346° N, −108.340280° W, at a depth of approximately 10 m. These were part of the bycatch of the shrimp trawl fishery during the shrimp fishing season running from September to December 2020 (Garcés-García et al., 2020; López-Martínez and Morales-Bojórquez, 2012). The individuals were placed on ice and transported to the laboratory, where they were kept in a freezer at −10 °C.

2.4. Sample processing

2.4.1. Sediments

A portion (25 g w/w) of each sediment sample was oven-dried at 50 °C, and used for both grain-size analysis and quantification of suspected PD. Each dried-sediment sample was weighed and placed in a 250-mL glass beaker and subsequently was treated with 50 mL of hydrogen peroxide (30%) to degrade the organic matter from the samples (Rodrigues et al., 2018). Sand, silt, and clay contents in the sediments were determined according to the sieving and pipetting method described by Folk (1974).

Suspected PD was extracted from the sand fraction of the sediments. A saturated sodium chloride (NaCl) solution was employed to separate suspected PD by density as suggested by Hanvey et al. (2017). All the sediment recovered from the sand fraction of the samples was placed in 250 mL glass beakers, mixed with 100 mL of purified MilliQ water (18.2 MΩ) and 50 g of NaCl (ρ = 1.2 g cm-3), and homogenized with a magnet and stirred until the salt was entirely dissolved. Afterwards the mixture was left to stand for 1 h to settle the sediment and the clean supernatant was decanted and vacuum filtered through filter paper (Whatman® GF/B glass microfiber pore size 1.0 μm). Filters with PD were placed in covered petri dishes, oven dried (60 °C for 30 min), and later observed under a binocular dissecting microscope.

2.4.2. Ray individuals

In the laboratory, individuals were measured (total length in cm) and weighed (g). Sex was determined based on the presence (males) or absence (females) of claspers. Gastrointestinal tracts were removed from each ray from the top of the esophagus and cut away at the vent. Analysis of stomach contents was conducted looking for prey items and PD following the methodology proposed by Barletta et al. (2020). Precautions were taken to avoid contamination of samples with PD from other sources; the laboratory had restricted access and was previously cleaned to prevent contamination by PD from other sources, all personnel wore 100% cotton lab coats and disposable latex gloves during all the steps of the procedure, all laboratory instruments and work surfaces were washed with filtered distilled water, and all instruments were oven-dried, and checked for contamination before every use to prevent cross-contamination. Routine blank controls were done in all procedurals by placing four clean Petri dishes next to the work area which was analysed in parallel with the samples to determine any potential contamination from the laboratory atmosphere during the procedures that might have occurred despite all the care taken following the procedure described by Barboza et al. (2020). However, no contamination from external sources was found after checking the blank controls.

Gastrointestinal contents were identified under a stereoscopic microscope (Zeiss Stemi 508). Prey items were identified to the lowest taxonomic level due to partial digestion, and any ingested particle not resembling natural prey were separated from natural food items. Prey items were counted, weighed, and then grouped into ecological/taxonomic categories considering the taxonomy of the various prey items, in addition to their life-history traits. If prey items were not whole or nearly whole, numbers were based on countable parts, such as claws and legs for crustaceans, and beaks for cephalopods. Objects suspected of being PD were removed using tweezers and classified according to colour (Frias et al., 2010).

Upon identifying both prey and suspected PD in the organic tissues, these were submitted to chemical digestion using KOH 30% (potassium hydroxide) for 12 h at room temperature (Lusher et al., 2020). This method efficiently digested the whole gastrointestinal tract of the ray, and the PD were not discolored. After 12 h, the resulting liquid was submitted to vacuum filtration using a suction pump. For this filtration 1.5 μm filters with 4.7 cm diameter were used (Claessens et al., 2013). The filters were inspected under the microscope for the presence of microplastics that could not be detected in the previous step.

2.4.3. FTIR spectroscopy

All particles suspected to be PD from both sediments and gastrointestinal content of the Haller's round ray, were photographed under transmitted light using a binocular dissecting microscope (Zeiss Stemi 508; www.zeiss.com) equipped with a digital camera (Zeiss AxioCam ERc5s), and measured (length in mm) using the software Zen 2.3 Blue Edition, from Zeiss. The polymer composition was obtained under a laminar flow hood using Attenuated Total Reflectance - Fourier Transform Infrared (FTIR-ATR) spectroscopy (Thermo Scientific Nicolet iS5). The measurements were conducted in the range of 4000–400 cm^{-1}, with 30 scans at a resolution of 1.5 cm^{-1}. Identification was based on comparing each spectrum with different references of polymer spectra found in the National Institute of Advanced Industrial Science and Technology (https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi), FTIR Spectra of Polymers (http://www.ftir-polymers.com/soon.htm) and Pavia et al. (2016).

2.4.4. Data analysis

To identify different size groups of the Haller's round ray, and therefore determine ontogenetic changes in diet and trophic levels, length-frequency polygons were elaborated, and the cohorts were identified through a Kernel Density Estimate (KDE) (Silverman, 1986). The univariate kernel density estimator used is given by the equation:

$$\hat{f}(x) = \frac{1}{nh}\sum_{i=1}^{n} K\left(\frac{x - X_i}{h}\right)$$

Where *h* is the bandwidth and *K(x)* is the Gaussian kernel function. The Sheather-Jones bandwidth selection method for the kernel density estimation was used. The KDE procedure was performed on R. The mean and the standard deviation were obtained for every cohort found.

Randomized cumulative prey curves were constructed to determine whether the sample size was adequate to describe the diet of each size class of the Haller's round ray. To do this, the observed prey species were plotted by permuting the sample order 999 times, and the Michaelis-Menten model was used to estimate the richness (*S*) of the prey species (Magurran, 2004). When a cumulative prey curve tends towards an asymptote, the number of stomachs analysed were considered sufficient in describing the dietary habits of the predator studied, and the asymptote of the curve indicated the minimum sample size required to describe the diet adequately.

To quantitatively express the importance of various prey items in the diet of the corvine we used the index of relative importance (*IRI*), calculated with the formula: $IRI = (\%N + \%W) \times (\%F)$, where $\%N$ and $\%W$ represent the food items' quantities and wet weights, respectively. $\%F$ is the frequency of occurrence of each food item (presence-absence) in all stomachs that contained food, as described by (Pinkas and Oliphant, 1971).

Kendall's rank correlation was performed to assess the association between i) number of ingested microplastic particles and ray weight, ii) number of ingested microplastic particles and ray TL, and iii) size of ingested microplastic and ray TL. If there was a significant correlation a linear regression was performed to derive an equation predicting the relationship between the response variable and independent variable. For these statistical tests, only organisms with microplastic particles in the gastrointestinal tract were included and tests were completed using the statistical package R (R Core Team, 2021).

Multivariate analyses (PRIMER 6) were used to compare the diet and ingestion of PD particles in the rays captured. A matrix containing every analysed ray as columns and prey item's IRI as rows (including micro and meso plastics of each colour), was created and Bray-Curtis similarity matrices were generated from this. The assigned factors were size, obtained from the KDE analysis, and sex (male-female). To test both H_0 that the diet and ingestion of PD of the analysed species did not differ according to these factors PERMANOVA was employed.

Principal Coordinates Analysis (PCO) was performed to visualize how the different fish species were grouped where the PERMANOVA revealed statistically different results and also to determine which factors best-explained group separation.

3. Results

3.1. Plastic debris in sediments

Sediments were mainly sand (79.8%), with similar silt and clay contents. A total of 107 PD items were found, and all of them consisted of filaments, with a total concentration of PD of 3.58 items kg^{-1}. Most PD belonged to microplastics (104), both large and small, and no correlation was observed between the size of the sediment and its plastic content. Only 3 of the plastics found belonged to the mesoplastic class. Transparent (39.3%) and blue (38.3%) microplastic filaments dominate over the red and black microplastic filaments (11.2% for both cases). Twenty-six representative samples of PD were selected based on their colour to be analysed using ATR-FTIR.

3.2. Analysed individuals of Haller's round ray

A total of 142 individuals of Haller's round ray were captured and analysed at the laboratory. The mean TL was 17.8 cm (SD = 4.6), the mean disc length was 10.0 cm (SD = 2.6), and the mean disc width was 10.3 cm (SD = 2.6). Mean weight was 66.3g (SD = 91.5). Regarding sex, 57 were females and 41 were males. The KDE function analyzing the TL of the rays, identified three cohort or size groups: Small = 22< cm, Medium = 22.1–30 cm, Large= >30.1 cm (Supplementary material Fig. 1).

3.3. Prey and plastic ingestion

Four prey categories were found: crustaceans (78.3%), teleost fish (12.3%), annelids (9.2%), and molluscs (1.2%). Only 6 rays had an empty digestive system. In the case of plastic debris, a total of 386 plastic particles were extracted of which 381 were fibers (98.7%), two had an irregular shape (0.5%) and one had a round shape (0.2%). A total of 46 individuals of Haller's round ray had PD on their gastrointestinal tract (32.4%). In the individuals which contained PD on their tracts, on average 10.2 (±7.4) plastic particles were found, being the dominant colour blue (61.5%) followed by black (17.6%), yellow (7.2%), red (5.7%), white (3.1%), pink (2.3%), transparent (1.0%), green (0.7%), brown and purple (0.2% each) (Fig. 1) (Table 1), with lengths ranging from 0.00821 mm to 0.953 mm (Table 2).

The Michaelis-Menten model indicated that the number of analysed stomachs was representative for a meaningful statistical analysis of the diet (Supplementary material Fig. 2). Haller's round ray ingested the majority of prey types present in the sampling location.

Kendall's rank correlations indicated no significant correlations ($p > 0.05$) between the quantity of ingested plastic particles and TL, between the quantity of ingested plastic particles and weight, neither between the size of ingested plastic particles and TL.

The PERMANOVA results revealed that no significant differences existed in the feeding habits and plastic ingestion of this species in relation to sex ($\text{Pseudo-F}_{1,71} = 1.0112$, $p > 0.05$), size ($\text{Pseudo-F}_{2,71} = 1.6597$, $p > 0.05$), or the interaction sex-size ($\text{Pseudo-F}_{1,71} = 0.57461$, $p > 0.05$). These results were corroborated with the PCO, as no clear-cut groups were formed according to any of the factors analysed. Blue vectors indicate the importance of the different prey items and the ingested PD of the analysed individuals. In terms of prey items, the most important were crustaceans, followed by demersal fish, molluscs, and annelids, as already revealed by the IRI, although there is a group of rays that preyed only on fish. These types of prey indicate that the rays were feeding at the bottom. In the case of the ingestion of PD, the PCO showed the vectors of the different PD particles close to the centroid, indicating that the null hypothesis, which is that there are no differences in the PD contents in gastrointestinal tracts of fish according to the proposed factors, is true. (Fig. 2).

3.4. FTIR analysis

The FTIR-ATR analysis revealed the presence of four types of polymers in the sediment, and six types of polymers in the gastrointestinal tracts of the Haller's round ray. In the sediment the identified polymers were nylon polyamide (49.3%), polyethylene (38.5%), polypropylene (8.3%), and polyacrylic (3.9%). In the ray, the identified polymers were nylon polyamide (45.3%), polyethylene terephthalate (polyester, 30.2%), polyethylene (12.8%), polyacrylamide (5.8%), polypropylene (3.5%) and polyacrylic (2.3%).

The most common polymer in both sediments and gastrointestinal tracts was nylon polyamide with spectra (cm^{-1}) at 3385 (N–H stretching) (reference 3500–3000), 2937 and 2870 (C–H stretching) (reference 3000–2850), 1469 (C–H stretching) (reference 1470–1465), 1633 (C=O amide) (reference 1690–1630), and 1062 (C–O amide) (reference 1200–1000) (Fig. 3A).

The second most common polymer in the gastrointestinal tract was polyester (polyethylene terephthalate) with spectra (cm-1) at 3030 ($–CH_2–$ asymmetrical and symmetrical) (reference 3000–2850), 1719 (C=O ester) (reference 1750–1705), 1604 and 1457 (C=C aromatic ring) (reference 1604–1475), 1407 and 1345 (C–H) (reference 1471–1409 and 1340), 1248 (C–C(O)–O amide) (reference 1260–1250), 1095 (-O-C-) (reference 1130–1120) and 2455 (CO_2) (reference 2380) (Fig. 3B).

The third polymer most found was in the gastrointestinal tract, and second in the sediments polyethylene with spectra (cm^{-1}) at 2926 (CH stretching) (reference 2965–2915), 2855 (CH stretching) (reference 2865–2840), 1421 (CH_2 in plane) (reference 1470–1465), and 712 (C–H bending formation) (reference 720–710) (Fig. 3C).

The fourth polymer most found in the gastrointestinal tract was polyacrylamide with spectra (cm^{-1}) at 3873 (-O-H) (reference 3750–3300), 3260 (N–H stretching) (reference 3500–3000), 1522 (N–H

Fig. 1. Representative images of the most abundant plastic material identified in the gastrointestinal tract of Haller's round ray. The text under each photograph indicates the colour and the percentage of PD from that colour found in the analysed individuals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Number and colour of PD particles found in the stomach and intestine of female and male Haller's round ray. I: Intestine. S: Stomach.

	Blue	Black	Yellow	Red	Trans-parent	Pink	White	Green	Brown	Purple	Total
Female											
I	79	25	10	7	5	4		2		1	133
S	75	21	11	7	4	4	2	1	1		126
Male											
I	37	9	1	2	1	1	2				53
S	51	10	6	6	1						74
Total	242	65	28	22	11	9	4	3	1	1	386

Table 2
Mean length (mm) of plastic debris of each colour found in stomachs and intestines of females and males of Haller's round ray (SD = standard deviation). I: Intestine. S: Stomach.

	Blue	Black	Yellow	Red	Trans-parent	Pink	White	Green	Brown	Purple
Female	0.148 (SD ± 0.12)	0.127 (SD ± 0.09)	0.27 (SD ± 0.18)	0.182 (SD ± 0.13)	0.561 (SD ± 0.53)	0.154 (SD ± 0.1)	0.077 (SD ± 0)	0.041 (SD ± 0.01)	0.352 (SD ± 0)	0.023 (SD ± 0)
I	0.155 (SD ± 0.13)	0.149 (SD ± 0.09)	0.302 (SD ± 0.22)	0.201 (SD ± 0.13)	0.67 (SD ± 0.68)	0.164 (SD ± 0.12)		0.04 (SD ± 0.02)		0.023 (SD ± 0)
S	0.142 (SD ± 0.10)	0.101 (SD ± 0.08)	0.242 (SD ± 0.13)	0.162 (SD ± 0.15)	0.424 (SD ± 0.28)	0.144 (SD ± 0.09)	0.077 (SD ± 0)	0.042 (SD ± 0)	0.352 (SD ± 0)	0 (SD ± 0)
Male	0.153 (SD ± 0.12)	0.155 (SD ± 0.15)	0.298 (SD ± 0.14)	0.175 (SD ± 0.10)	0.106 (SD ± 0.12)	0.693 (SD ± 0)	0.324 (SD ± 0.01)			
I	0.171 (SD ± 0.11)	0.144(SD ± 0.14)	0.337 (SD ± 0)	0.173 (SD ± 0.16)	0.191 (SD ± 0)	0.693 (SD ± 0)	0.324 (SD ± 0.01)			
S	0.14 (SD ± 0.13)	0.165 (SD ± 0.16)	0.292 (SD ± 0.15)	0.175 (SD ± 0.1)	0.022(SD ± 0)					

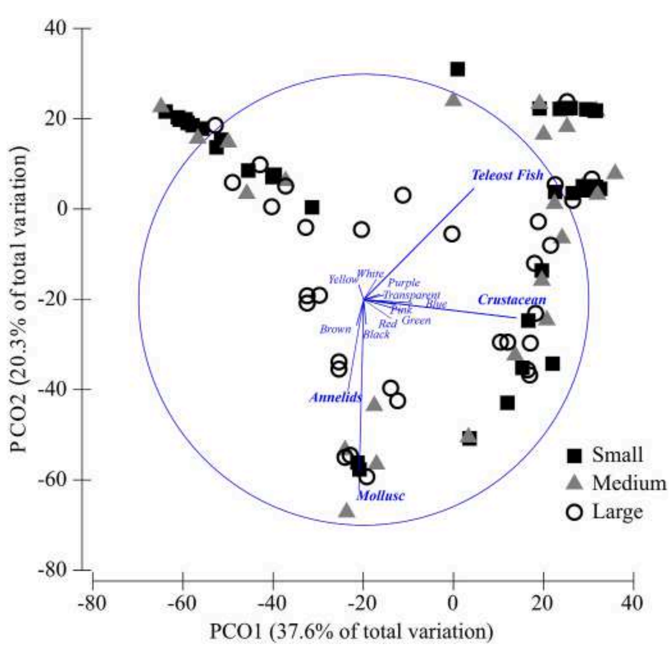

Fig. 2. Principal Coordinates Analysis (PCO) describing the feeding patterns and plastic ingestion of the different sizes of Haller's round ray. Blue vectors indicate the importance of each prey item and colour of PD found in the gastrointestinal tract of this species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

flexed) (reference 1640–1550), 691 (N–H flexed out plane) (reference 600–700), 2958 and 2870 (CH stretching) (reference 3000–3850), 1457 (CH_2 in plane) (reference 1470–1465), 1737 (C=O stretched amide) (reference 1690–1630), 1024 (C–N stretching) (reference 1200–1000), and 2313 (CO_2) (reference 2380) (Fig. 4A).

The second least polymer found in the gastrointestinal tract, and the third in the sediments was polypropylene with spectra (cm^{-1}) at 2964, 2993, 2855 (C–H stretching) (reference 2900), 1466 (C–C tension) (reference 1350–1450), and 1100, 883 (CO_2) (reference 1200–1000) (Fig. 4B).

Lastly, polyacrylic was the least polymer in both sediments and gastrointestinal tracts with spectra (cm^{-1}) at 2926 (C–H stretching) (reference 2965–2915), 2896 (C–H stretching) (reference 2865–2840), 2896 (CH_2 in plane) (reference 1470–1465), 700 (C–H deformed out plane) (reference 720–710), 1716 (C=O ester) (reference 1750–1730) and 1100, 1092 (C–O) (reference 1300–1000) (Fig. 4C).

4. Discussion

Although there are few studies on the feeding habits of this species in the Mexican Pacific (Flores-Ortega et al., 2011, 2015; Valadez-González et al., 2001), this study has generated data for the first time about the contamination of plastic debris in the digestive tract of Haller's round ray, and for that matter, the first report of PD presence in batoids. Also, it contributes to the documentation of PD presence in sediments from the Gulf of California. Considering the high occurrence of PD in estuarine and marine environments, because of their extensive use in all human activities, and improper disposal practices over the last century (Costa and Barletta, 2015), such studies are important because the reality of the presence of these particles in batoids and marine sediments from this zone was unknown until now.

In general, studies assessing environmental contamination in this group of elasmobranchs are few (Bezerra et al., 2019; Consales and Marsili, 2021), and studies on the presence of plastic debris in gastrointestinal tracts of batoids are nonexistent, despite their importance and key role in aquatic ecosystems. Also, for the area of study, works on plastic debris in the marine environment and biota are scarce (Fossi et al., 2016, 2017). These studies are essential as food webs are vulnerable to a suite of toxins and contaminants, potentially leading to bioaccumulation, so it is critical to study the consequences and the fate of PD and their effects on marine food webs (Carbery et al., 2018).

4.1. Feeding habits and plastic ingestion

Considering that the Haller's round ray reaches maturity in both

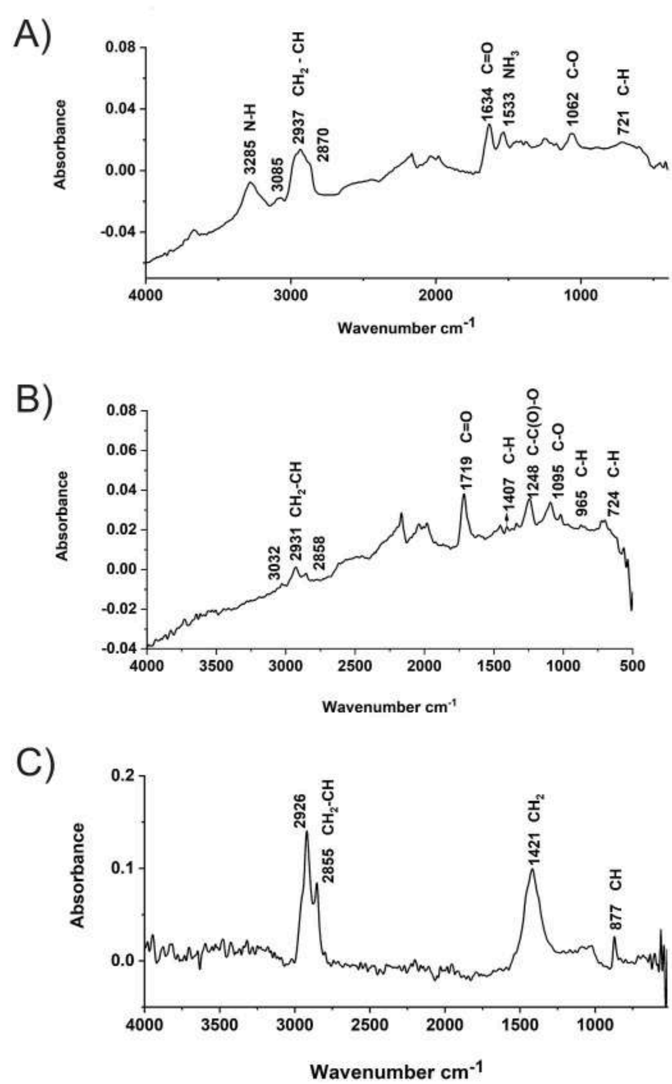

Fig. 3. ATR-FTIR spectra of plastic particles found in the gastric content of Haller's round ray: A) polyamide, B) polyethylene terephthalate, C) polyethylene.

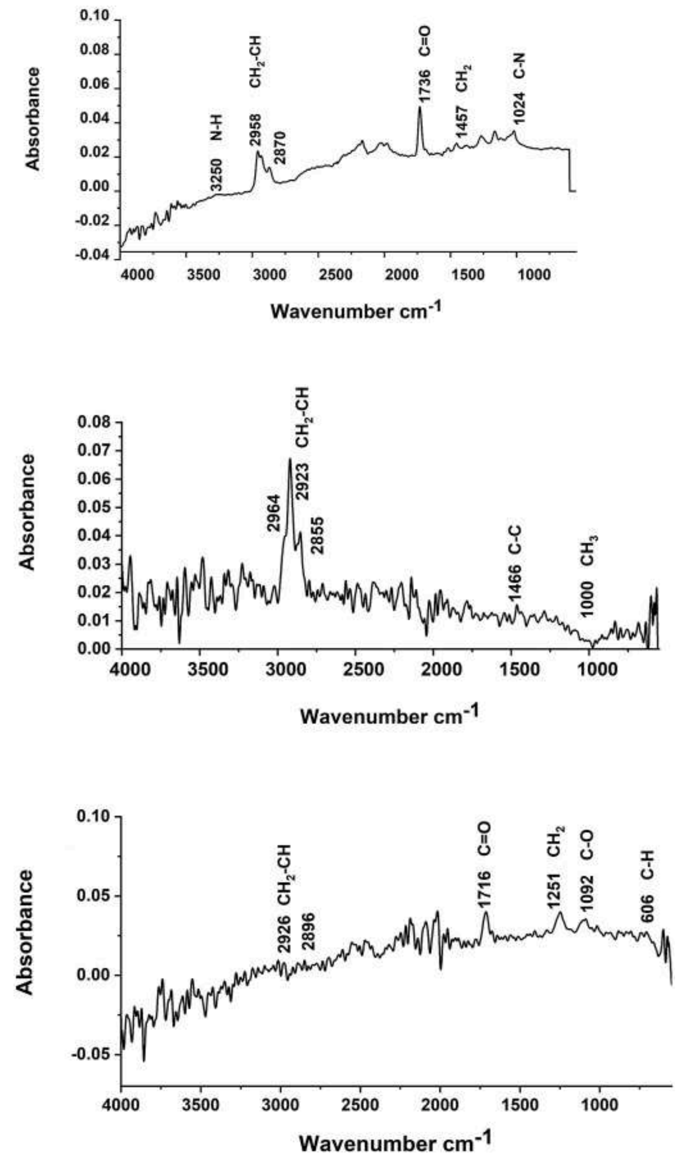

Fig. 4. ATR-FTIR spectra of the plastic particles found in the gastric content of Haller's round ray: A) polyacrylamide, B) polypropylene, C) polyacrylic.

sexes at approximately 26 cm (Hale and Lowe, 2008), this study included both juvenile and adult specimens, therefore, we can state that the results are representative of the entire population in the studied area.

As previously stated, the feeding habits of the Haller's round ray found in this study agree with previous results for the species also in the Mexican Pacific (Flores-Ortega et al., 2011, 2015; Valadez-González et al., 2001), as the main prey items were benthic crustaceans, and demersal fish, and to a lesser extent, benthic molluscs and annelids, which are the same prey groups reported in the other studies. No differences were found in the preying behavior about size or sex. Therefore, it is possible to say that this species is a benthic feeder through all its life span, burrowing in the substrate, and as such, the ingested plastics were likely those present in the sediments. Although the amount of PD in the sediments analysed can be considered as low when compared with similar studies, and similar regions and environments, such as beaches in Baja peninsula (Alvarez-Zeferino et al., 2020; Piñon-Colin et al., 2018), the frequency and number of plastics found in the Haller's round ray can be considered high when compared to data from other fish species in similar environments (Dantas et al., 2012; Lusher et al., 2017). A reason for this could be that in those studies, no attempt was made to use any digestion-based method, which is known to be more efficient in recovering PD from the gastrointestinal tracts of fish (Lakshmi Kavya et al., 2020). In our study, we used the method proposed by Claessens et al. (2013), which yielded good results. However, as this is a relatively new area, at the moment the methodological options to extract PD from fish are too variable, and for the case of rays, this was the first attempt to do such analyses. Further studies are needed to establish the best method to extract PD from rays and establish a standard protocol, although the method used in this study yielded favorable results.

All PD found in the sediments as well as those ingested by the Haller's round ray consisted of fibers, which has been previously reported by other works assessing PD in sediments (Piñon-Colin et al., 2018), and for other demersal fish in tropical environments (Barletta et al., 2020; Lusher et al., 2013; Pegado et al., 2018; Possatto et al., 2011; Salazar-Pérez et al., 2021). This intake might occur as a result of the rapid sinking of certain common polymers, such as polyamide, acrylic, and PET (GESAMP, 2019), which makes it readily available for accidental ingestion during benthic foraging (Ramos et al., 2012; Vendel et al., 2017). In addition, fibers may resemble natural food items (amphipods, copepods, and polychaetes) (Thompson, 2004), with the result of them being preyed upon accidently (Ferreira et al., 2018).

The majority of the fibers found both in the sediment and gastrointestinal tracts of Haller's round ray were microplastics which are the most commonly ingested type of microplastic particle (Pegado et al., 2018), and all the polymers identified in the sediments were also found in the gastrointestinal tract of the ray. This indicates that these might have a coastal origin considering the region in which these specimens were caught, i.e., the coastal region close to the shore (Ferreira et al., 2018). Strong hydrodynamic forces are associated with the coastal environment, and PD is exposed to high wind, wave, and tidal action, resulting in the breakdown into smaller particles because of the stronger weathering processes (Browne et al., 2007). However, our results also show that it is necessary to increase the sampling effort of PD in the sediments, as there are surely other polymers available for the biota, since the PD ingested by the studied species included more polymers than those found in the analysed samples, which were taken in the same stations where the rays were collected.

4.2. FTIR analysis

The types of microplastics identified in this study, namely, polyamide (nylon), polyethylene terephthalate (polyester), polyethylene, polyacrylamide, polypropylene, and polyacrylic, are consistent with the human activities being undertaken in the area of our study. Polyamide and polypropylene are used in fishing gear, which provides durability and strength to fishing nets (GESAMP, 2019).

Polyethylene terephthalate and polyethylene are both commonly produced polymers used to make plastic bags and storage containers (GESAMP, 2019). It is known that globally these are the most abundant polymers found in the environment (Hidalgo-Ruz et al., 2012).

Polyacrylamide is used in the textile industry, which provides anti-wrinkle and anti-mole properties to materials, and it can also prevent fabric static and is flame retardant; it adds higher adhesion to the product and can also be used as a stabilizer for bleaching non-silicon polymers (Piñon-Colin et al., 2018). Finally, polyacrylic can be used in anti-fouling paints for boats (Hu et al., 2020).

4.3. Plastic sources

The studied area is a very important fishing ground in the Gulf of California; it is the largest estuarine system in northwest Mexico and is highly productive in terms of fishing and the economic activities derived from it. At present, approximately 2000 skiffs are fishing in the area, and the presence of the industrial fleet catching shrimp and sardines is common (Breceda-Martos, 2020). There are five human settlements located around the system, being the fishing village of La Reforma the most important with a human population of approximately 6900 inhabitants (INEGI), and approximately 2200 registered fishers (Lyle Fritch, 2003). The other settlements have a population together of about 1500 inhabitants. As such, the human activities in and around the system are high, and with this, constant pollution from plastic debris into the system. The fishing industry has been estimated to be responsible for up to 20% of all plastic debris found in the oceans (Chenillat et al., 2021). The small-scale fisheries operating in the area predominantly use gillnets and cast nets (Ramírez-Rodríguez et al., 2014). However, these nets can be either accidently lost or purposedly discarded at sea, resulting in a sort of persistent marine pollution called ghost nets (Andrady, 2017), and also they can suffer several degradation processes over time, decomposing into small particles (Godoy et al., 2019). Besides that, fishers usually discard their litter at sea, which includes high plastic bags and food containers. And finally, as all these human settlements are undeveloped (Breceda-Martos, 2020), the sewage is poured directly into the system, without any previous treatment. It is known that the textiles release a high amount of fiber dirt (Zhi Shang Chemical, 2021), so considering the presence of human settlements interacting directly with this system, it is not surprising that polymers found in textiles are abundant in the area.

As previously stated, it has been already demonstrated that microplastics are widely available in the marine environment (Alvarez-Zeferino et al., 2020; Browne et al., 2008; Carbery et al., 2018), and, because of their characteristics, they can efficiently absorb endocrine disruptive chemicals, pesticides, fertilizers, aqueous metals persistent organic pollutants (POPs), carcinogens and mutagens, becoming vectors of contaminants by their ingestion (Betts, 2008; Carbery et al., 2018; Cole et al., 2011; Tosetto et al., 2017; Zhang et al., 2019). It has already been demonstrated that microplastic ingestion affects different fish species both physically and physiological in different ways that include gastric obstruction and compromised intestinal function, oxidative stress, inflammation, disrupted energy metabolism, behavioral and metabolic effects, reduced feeding and predatory performance, abnormal swimming behavior and lethargy, as well as liver toxicity, hepatic stress and changed endocrine function, as well as gene expression (de Sá et al., 2015; Ferreira et al., 2016; Lu et al., 2016; Mattsson et al., 2015; Mazurais et al., 2015; Oliveira et al., 2013; Pedà et al., 2016; Rochman et al., 2013, 2014).

Although such affectations have not yet been reported in batoids, PD ingestion represents an actual threat to these species, considering the amount that these organisms are ingesting, and that it seems that these materials are widely available in the studied area. It is already known that marine pollutants are a substantial threat to sea turtles (Casini et al., 2018), cetaceans (Fossi et al., 2016, 2017), and sea birds (Costantini et al., 2017; Dietz et al., 2019), so it is likely that these pollutants are also affecting batoids. Therefore it is necessary to continue with this kind of study for other similar species, to help in the preservation of these species.

5. Conclusion

Haller's round ray is a benthic feeder exposed to PD found in the sediment. Results show a high ingestion of PD both in terms of quantities and frequency of occurrence. Microplastic ingestion, therefore, can be added to the threats batoids face, besides bycatch and overfishing, as well as by other pollutants such as persistent organic pollutants, PAHs, and heavy metals (Consales and Marsili, 2021).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.113077.

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