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First insight into plastics ingestion by fish in the Gulf of California, Mexico

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ABSTRACT

Plastic particle occurrence in the digestive tracts of fishes from a tropical estuarine system in the Gulf of California was investigated. A total of 1095 fish were analysed, representing 15 species. In total 1384 particles of plastic debris were recovered from the gastrointestinal tracts of 552 specimens belonging to 13 species, and all consisted of threads, the majority of which were small microplastics (0.23 to 1.89), followed by large microplastics (2.07 to 4.49), and few mesoplastics (5.4 to 19.86). Plastic particles were identified using ATR-FTIR spectroscopy. The mean frequency of occurrence of plastics in the gastrointestinal tracts of fishes from this system was 50.5%, which is higher than frequencies reported in similar systems in other areas. The polymers identified by ATR-FTIR were polyamide (51.2%), polyethylene (36.6%), polypropylene (7.3%), and polyacrylic (4.9%). These results show the first evidence of plastic contamination for estuarine biota in the Gulf of California.

1. Introduction

Ocean plastic pollution is of great concern because the most abundant type of marine debris is composed of plastic materials (Iñiguez et al., 2016), with recent annual production (2018) exceeding 350 million tonnes (Plastics Europe, 2020), implying a threat to aquatic wildlife and fisheries (Lusher et al., 2017; Ryan et al., 2009). Once in the aquatic environment marine debris tend to breakdown into meso (5–25 mm), micro (<5 mm), and nanoplastics (<1 μm) due to weathering processes which are caused by a suite of processes such as photodegradation, embrittlement, and hydrodynamic forces (GESAMP, 2019; Thompson et al., 2004).

Plastic debris (PD) are widely concentrated and available in the marine environment, meaning that they are able to interact with every trophic guild, as they are being directly ingested and subsequently transferred across trophic levels (Ferreira et al., 2018a), a reason for the ensuing high contamination rates which are found in top predator fishes (Ferreira et al., 2018a; Setälä et al., 2014). Top predators are one of the strongest links between humans and marine wildlife, because these include the most commercially valuable and consumed species by humans (Pauly et al., 1998), implying that PD may be indirectly affecting human populations due to fish consumption (Santillo et al., 2017; Wang et al., 2019). Top predators could be a pathway for the transport of harmful chemicals through the food web (United Nations Environment Programme, 2014). However, PD are not recognised as the primary vector for harmful chemicals (Koelmans et al., 2016), even though most chemicals used for producing plastic polymers are derived from non-renewable crude oil, many of which are hazardous, and that

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may be released during their disposal to the environment (Lithner et al., 2011).

Despite its potential threat to marine ecosystems, few experimental studies have documented the presence of plastics in marine and coastal environments in the Gulf of California and Mexican Pacific (Alvarez-Zeferino et al., 2020; Piñon-Colin et al., 2018; Ramírez-Álvarez et al., 2020; Retama et al., 2016), and no studies exist documenting the ingestion of PD by fish in the Gulf of California and Mexican Pacific, notwithstanding the importance of this region for the fisheries industry in Mexico, as this region is the principal area in Mexico where fish are landed (Díaz-Uribe et al., 2013; Ramírez-Rodríguez et al., 2014). Of the total catch, 50% to 70% comes from the small scale fisheries operating in estuarine systems (Díaz-Uribe et al., 2013; Spalding et al., 2007). The hydrodynamic complexity of these systems influences the inanimate material, including plastics debris, acting in their retention (Cole et al., 2011; Lima et al., 2014). Estuarine systems with mangrove forests are associated with low declivity sheltered areas and moderate tidal variations, increasing the deposition of PD (Cordeiro and Costa, 2010), with

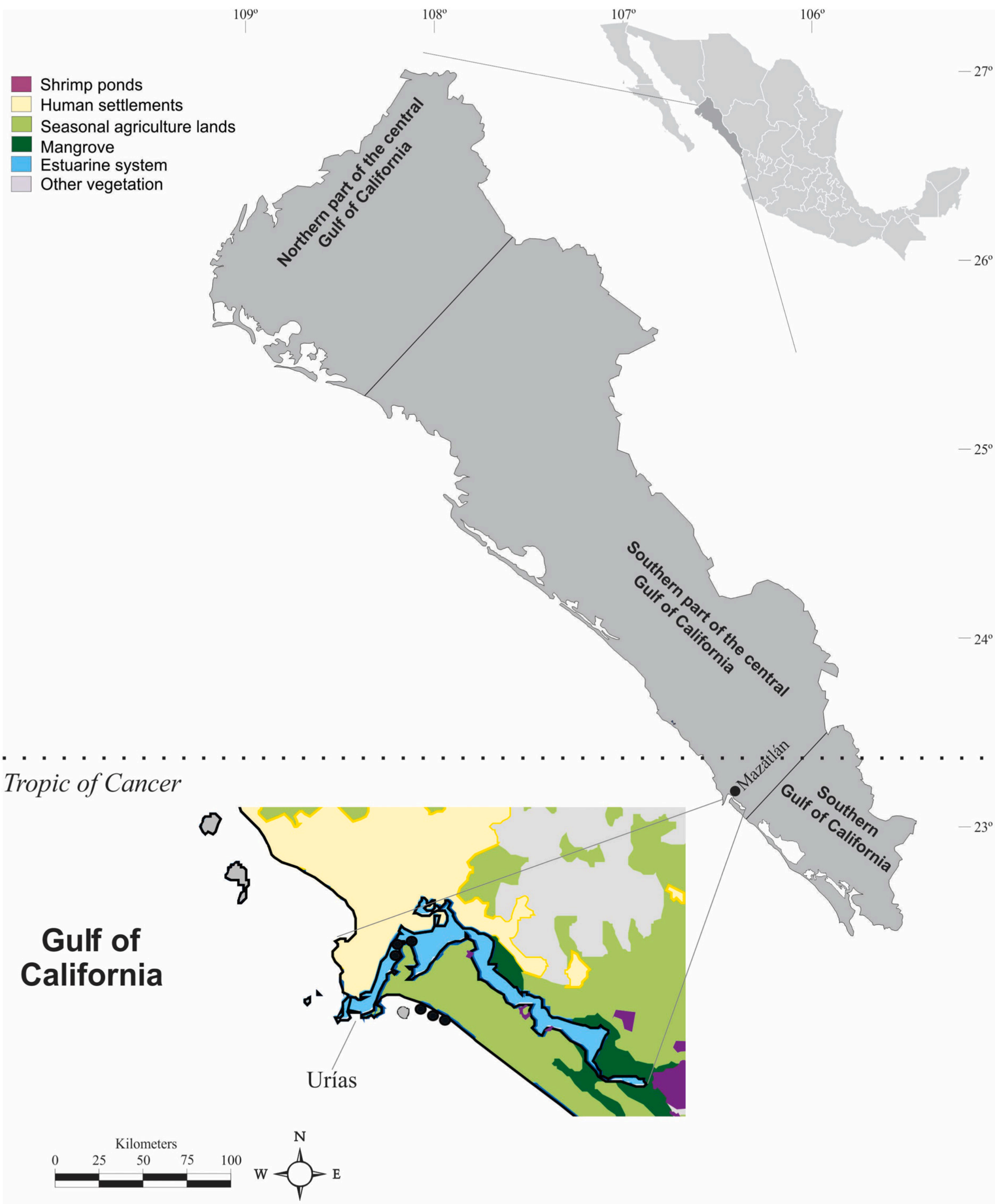

Fig. 1. Studied area in the south eastern Gulf of California, Mexico. Black dots show the sampling sites at the beach adjacent to the system designated as Isla de la Piedra Beach (IPB), and the beach inside the system was designated as Urias Estuary Beach (UEB).

the consequence that at present the estuarine systems concentrate a large quantity of these objects which eventually will break down into microplastics (Collignon et al., 2012; Cózar et al., 2014; Frias et al., 2014; Lima et al., 2014), because of the high amount of PD that is introduced in and around these systems (Le Roux, 2005; Nordstrom et al., 2006).

The present work was undertaken at Urias estuarine system and an adjacent beach, in the southeast Gulf of California. This is an urbanised system (with circa 380,000 inhabitants) that hosts a variety of installations and activities such as shipyards, seafood processing industries, shrimp-aquaculture facilities, a thermoelectric power plant, and fishing, petroleum, and merchant fleets (Cardoso-Mohedano et al., 2016). All these activities have contributed to the pollution of the system through discharges from shrimp farms, industry, urban settlements, and the thermoelectric power plant (Frías-Espericueta et al., 2005), in addition to being an important fishing ground for small scale fisheries targeting fin fish (Ramírez-Rodríguez et al., 2014). In addition, the urban sewage of the city of Mazatlan is discharged outside of this estuary (JUMAPAM, 2021). Based on these anthropogenic activities, we hypothesise that there will be an occurrence of PD in the gastrointestinal tracts of fish, with the main objective of investigating the quantity and type of plastic ingestion by fishes of two locations in the Urias estuarine system. Other aims were to determine differences in the microplastic frequency and composition between pelagic and demersal fish, and between fish collected inside the estuary and fish collected in the beach outside of the estuary next to the inlet. A further aim was to assess whether the quantity, type, and size of PD ingested by fish varied between sites, between pelagic and demersal species, and fish body size. Additionally, a final goal was to compare the results obtained in this work with those reported in similar systems from other tropic estuarine systems.

2. Materials and methods

2.1. Study area

Urias estuary (23.19°N–106.36°W, Fig. 1) is located in the southeast Gulf of California, Mexican Pacific, next to the city of Mazatlan. It as a tide-dominated estuary, characterised by a mixed tide with an average range of 1.0 m (Amezcua et al., 2019). Within the navigation channel there is a strong tidal current (up to 0.60 m/s) which characterises the flow of water into the lagoon during the flood tide, with a reversal in direction of the flow during the ebb. The inner part of the lagoon experiences the occurrence of low velocity tidal currents (<0.30 m/s) as the lagoon becomes very shallow (1–4 m water depth). It is inhabited by mangroves along the borders.

The samples of fish were collected at two localities: on an adjacent beach outside the estuary, known as Isla de la Piedra, and designated as Isla de la Piedra Beach (IPB), and at a beach inside the system designated as Urias Estuary Beach (UEB). IPB was selected for being a touristic site with several restaurants and hotels, which are potential sources of plastics, and is exposed to the open sea. UEB was selected for sampling purposes because it is on the opposite side of the mainland to the harbour, where most of the anthropogenic activities are carried out, and also as it is located at the intersection between the main tidal channel and the intermediate lagoon (Cardoso-Mohedano et al., 2016), and it is not urbanised; it is a beach with mangroves that, because of its location at the end of the high energy of the estuary's main tidal channel, receives a high quantity of PD (Fig. 1).

2.2. Fish sampling

Fish were collected diurnally during two consecutive days (July 23–24, 2019) using a silk seine net (3 m long, codend of 4.5 m). Three diurnal replicates were made on consecutive days, first on IPB, and next at UEB. The same area was towed in all sampling events.

2.3. Sample processing

2.3.1. Fish dissections and stomach content analysis

Fish were identified, counted, weighed (total weight, W in g), and measured (total length, TL, cm). Gastrointestinal tracts were removed from each fish from the top of the oesophagus 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 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 were analysed in parallel with the samples in order to determine any potential contamination from 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 visually sorted using a stereomicroscope (Zeiss Stemi 508). Any ingested particle not resembling natural prey were separated from natural food items. The natural food items were counted, weighed, and identified to family or the lowest taxonomic level, and then grouped into ecological/taxonomic categories considering the taxonomy of the various prey items, in addition to their life history traits.

2.3.2. Identification of plastic particles

Objects suspected of being PD were removed using tweezers and their colour was recorded. These were photographed and measured (length in mm) under transmitted light using a binocular dissecting microscope (Zeiss Stemi 508; www.zeiss.com) equipped with a digital camera (Zeiss AxioCam ERc5s) and software (Zen 2.3 Blue Edition; Zeiss).

The polymer composition for each suspected PD particle was analysed using Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) 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 comparing each spectrum with different references of polymer spectra (Compa et al., 2018).

2.4. Data analysis

The ingested prey items were quantified according to the criteria and the Index of Relative Importance (IRI) as proposed by Barletta et al., 2020. PD ingestion was quantified using the following criteria: the number of fish individuals in which debris was found, or the frequency of occurrence estimated as the proportion of all fish individuals examined with or without microplastic debris in their stomachs; the number of elements of debris in each fish's stomach contents; and the weight (mass) of the debris in each animal's stomach contents. The population average was estimated in all cases. The ingested PD were grouped into: small microplastics (0.2–2 mm), large microplastics (2–5 mm), and mesoplastics (5–25 mm) (Romeo et al., 2016).

Length frequency histograms of the TL of the captured fish, and the length of PD were plotted for each zone. Cohorts were identified using a Kernel Density Estimate (KDE) (Silverman, 1986). The following equation shows the univariate kernel density estimator used:

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

where *K(x)* is the Gaussian kernel function and *h* is the bandwidth. After this analysis an ANOVA (STATISTICA 13) was used to determine if the lengths of fish and microplastics differed between sites; homogeneity of variances were tested with Cochran's C test.

Kendall's rank correlation was performed to assess the association between i) number of ingested microplastic particles and fish weight, ii) number of ingested microplastic particles and fish TL, and iii) size of ingested microplastic and fish 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, 2020).

Multivariate analyses (PRIMER 6) were used to compare the diet and ingestion of PD particles in the fish species captured in both analysed zones. A matrix containing every analysed fish specimen 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 site (IPB, UEB), fish species, habitat (demersal or benthopelagic), and size of fish obtained from the TL frequency histograms (XS = <5 cm>, S = 5–9 cm, M = 9–14 cm, L ≥ 14). To test both H_0 that the diet and ingestion of microplastic of the analysed species did not differ according to these factors PERMANOVA was employed.

Principal Coordinates Analysis (PCO) was performed to visualise 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. Fish species analysed

A total of 1095 organisms were analysed, representing 15 species from 9 families (Table 1). The KDE function (Fig. 2) analysing fish TL, indicated that fish caught at IPB were larger and more abundant, with the majority measuring between 5 and 9.5 cm, whilst fish caught at EUB were less abundant and most measured between 2 and 8 cm TL. These results were corroborated by ANOVA, as significant differences in TL were found between the two sites ($F_{1,1121} = 82.03$, $p < 0.01$); at IPB the mean TL averaged 7.25 cm (±0.32), whilst at EUB the mean TL averaged 5.97 cm (±0.24).

3.2. Prey ingestion

Six prey categories were found: infaunal benthic crustaceans, chaetognaths, bivalves, polychaetes, teleostei, and algae. PERMANOVA revealed no statistically significant differences in the diet between fish of different habitats (demersal & benthopelagic, pseudo-$F_{1,76} = 0.47$, $p > 0.9$). However significant discrimination was found among the diets of fish from different sizes (pseudo-$F_{4,76} = 6.79$, $p < 0.01$), species (pseudo-$F_{11,76} = 11.95$, $p < 0.01$), and zones (estuarine and coastal zonepseudo-$F_{1,76} = 9.88$, $p < 0.01$). Vectors from the PCO analysis (Fig. 4) in blue indicate the importance of the different prey items and vectors in black indicate the importance of the different microplastics to the diet of the analysed fish species.

The diets of the organisms found at UEB (upper-centre part) were bivalves and polychaetes for small fish, whilst larger fish ate mainly algae and infaunal benthic crustaceans. At IPB (left and lower central left part) the diet of pelagic fish included polychaeta, chaetognaths, and infaunal benthic crustaceans, whilst larger individuals also ate fish. Demersal fish ate almost exclusively infaunal benthic crustaceans regardless of size.

3.3. Plastic ingestion

In total 1384 plastic particles were extracted from the gastrointestinal tracts of 552 specimens belonging to 13 species (Table 1). All PD found were threads of three colours: the most commonly ingested threads (45.2%) were blue, followed by transparent threads (26.9%), and red threads were the least ingested (8.6%), (Fig. 3). Of the total number of PD, 971 (70.1%) were taken from the stomachs of 434 specimens representing 8 species, and 413 particles (29.9%) were taken from the intestines of 413 specimens representing 9 species (Table 1). On average per fish 1.26 (±1.8) particles were found. The TL and weight of fish individuals containing ingested plastics varied from 4.4 to 25.6 cm, and between 1.0 and 305.0 g.

The largest number of ingested plastics per specimen (6 particles) was recorded for white mullet (*Mugil curema*); however, the species responsible for the majority of all recovered plastic particles was the Pacific crevalle jack (*Caranx caninus*, with 766 plastic particles, and a population average number of plastic particles per stomach of 1.36). This species was the most abundant (n = 562). The frequency of occurrence (FO%) of plastic per species among those with four or more examined specimens varied between 16.67% for the white mullet, to 100% for the paloma pompano (*Trachinotus paitensis*).

Mean plastic length was 2.71 mm (±2.95), both the smallest and largest plastic threads were found in gastrointestinal tracts of fish caught at IPB. The smallest measured 0.23 mm and the largest measured 19.86 mm. Mean plastic length at UEB was 2.43 mm (±2.13), and at IPB was 2.98 mm (±3.57). Both KDE and ANOVA indicated that the lengths of the ingested plastics were not statistically different ($F_{1,97} = 0.88$, $p > 0.1$) in both sites, with the majority of the plastics ingested corresponding to the category of small microplastics, followed by large microplastics, with few mesoplastics.

No significant correlations were found ($p > 0.05$) between size of ingested plastic particles and fish TL, the quantity of ingested plastic particles and fish TL, or between the quantity of ingested plastic particles and fish weight.

PERMANOVA revealed no statistically significant differences in the ingestion of micro and mesoplastics between fish of different habitats (demersal & benthopelagic, pseudo-$F_{1,36} = 0.52$, $p > 0.05$), fish from different sizes (pseudo-$F_{4,36} = 1.49$, $p > 0.1$), species (pseudo-$F_{9,36} = 1.85$, $p > 0.05$), or zones (estuarine and coastal zone pseudo-$F_{1,36} = 0.83$, $p > 0.1$). In the PCO it can be observed that the vectors of the micro and mesoplastics are close to the centroid, indicating that the null hypothesis, which is that there are no differences in the microplastic contents in gastrointestinal tracts of fish according to the proposed factors, is true.

3.4. FT-IR analysis

Plastic polymers were identified using ATR-FTIR and comparison with reference spectra. All threads suspected to be PD were effectively classified as such. Four types of particles were found. The most common was polyamide (nylon) found in 51.2% of the samples, and these were classified as blue, black, and red threads with spectra (cm^{-1}) at 3300 (N—H stretching) (reference 3500–3000), 2917 (C—H stretching) (reference 3000–2850), 1690 (C═O amide) (reference 1690–1630), 1570 (N—H bending) (reference 1640–1550), 1400 (C—H stretching) (reference 1470–1465), and 1030 (C—O amide) (reference 1200–1000) (Fig. 5a). The next particle was polyethylene found in 36.6% of the samples, and classified as blue and black threads with spectra (cm^{-1}) at 2926 (C—H stretching) (reference 2965–2915), 2853 (C—H stretching) (reference 2865–2840), 1542 (CH_2 in plane, symmetric) (reference 1470–1465) and 700 (C—H bending deformation) (reference 720–710) (Fig. 5b). Polypropylene was of third greatest importance (7.3%), and it was classified as transparent threads with spectra (cm^{-1}) at 2912 (C—H stretching) (reference 2900), 1461 (C—C tensing) (reference 1350–1450), and 999 (CH_3 bending) (reference 1200–1000) (Fig. 5c).

Table 1
Summary of analysed fish species and their microplastic contents. UEB = Urias estuary beach, IPB = Isla de la Piedra beach.

Family	Species	Common name	No of individuals	Min-max & mean length (cm)	Habitat	Location	No of individuals with microplastics	Frequency (%)	N° of microplastic particles in gastrointestinal tract	Mean N° of microplastic particles/ specimen	N° of fish with microplastic in stomach	N° of microplastic particles in stomach	N° of fish with microplastic in intestine	N° of microplastic particles in intestine
Pristigasteridae	*Opisthopterus dovii*	Dove's longfin herring	204	4.3–10.0 & 6.5	Pelagic, neritic	IPB	93	45.45	204	2.2	74	130	56	74
Clupeidae	*Lile stolifera*	Striped herring	6	6.0–6.3 & 6.2	Pelagic, neritic	UE	4	66.67	8	2	3	4	2	4
	Opisthonema libertate	Pacific thread herring	2	7.2–7.9 & 7.5	Pelagic, neritic	IPB	1	50	0	0	0	0	0	0
Mugilidae	*Mugil curema*	White mullet	6	20.0–22.8 & 20.7	Pelagic, neritic	IPB & UE	1	16.67	6	6	1	4	1	2
Hemiramphidae	*Hyporhamphus unifasciatus*	Common halfbeak	2	13.7–25.0 & 19.3	Pelagic, neritic	UE	0	0	0	0	0	0	0	0
Carangidae	*Caranx caninus*	Pacific crevalle jack	562	6.0–13.2 & 7.4	Pelagic	IPB & UE	281	50	766	2.7	230	664	102	102
	Trachinotus kennedyi	Blackblotch pompano	4	1.8–9.8 & 5.2	Demersal, neritic	IPB	2	50	0	0	0	0	0	0
	Trachinotus paitensis	Paloma pompano	4	3.1–5.7 & 4.4	Demersal, neritic	IPB	4	100	12	3	4	8	4	4
	Lutjanus argentiventris	Yellow snapper	1	2.9	Demersal, neritic	UE	1	100	0	0	0	0	0	0
Lutjanidae	*Lutjanus colorado*	Colorado snapper	2	25.5–25.6 & 25.55	Demersal, neritic	IPB	2	100	2	1	0	0	2	2
	Eucinostomus entomelas	Dark-spot mojarra	254	2.0–11.4 & 5.8	Demersal, neritic	UE	156	61.54	371	2.4	117	156	98	215
Gerreidae	*Gerres cinereus*	Yellow fin mojarra	2	19.3–20.7 & 20.0	Demersal, neritic	UE	2	100	9	4.5	1	1	2	8
	Polydactylus approximans	Blue bobo	34	3.8–10.7 & 6.1	Demersal, neritic	IPB	3	10	3	1	3	3	0	0
Polynemidae	*Menticirrhus elongatus*	Pacific kingcroaker	4	4.3–10.3 & 7.4	Demersal, neritic	IPB	2	50	2	1	0	0	2	2
Sciaenidae	*Umbrina xanti*	Polla drum	8	6.5–9.5 & 8.2	Demersal, neritic	IPB	0	0	0	0	0	0	0	0
Total			1095				552	50.45	1384	1.3	434	971	269	413

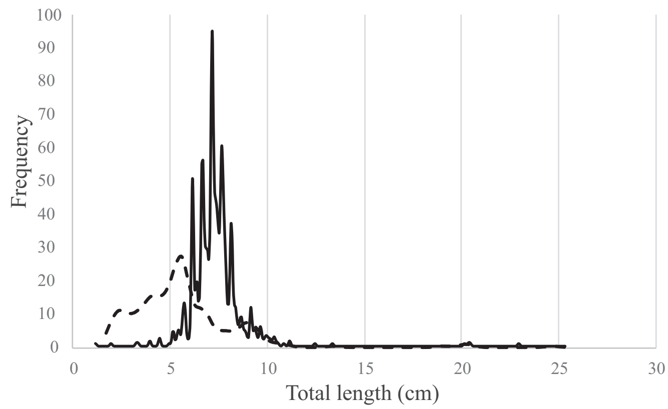

Fig. 2. KDE function analysing fish lengths in both study sites. Dotted line indicates fish at UEB. Continuous line indicates fish from IPB.

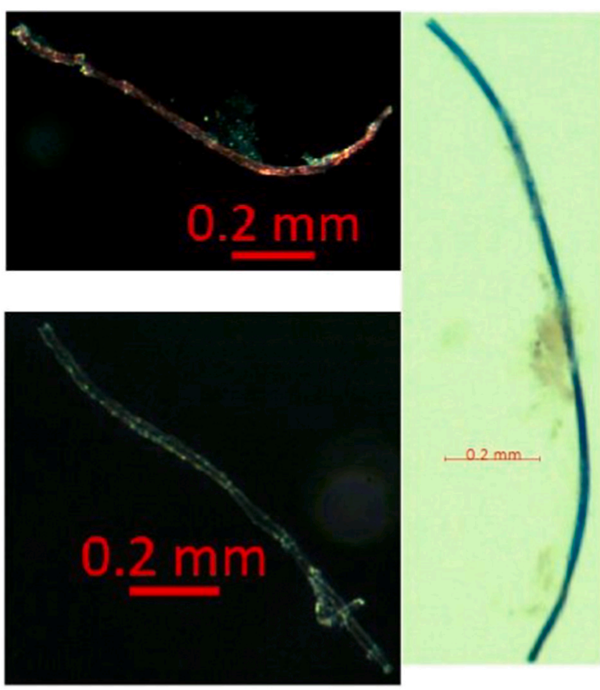

Fig. 3. Examples of the three different colours of threads found in the gastric contents of the fish analysed in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Finally, polyacrylic (4.9%) was classified as light blue threads with spectra (cm^{-1}) at 2974 (C—H stretching) (reference 2965–2915), 2885 (C—H stretching) (reference 2865–2840), 1714 (C═O ester) (reference 1750–1730), 1407 (CH_2 in plane, symmetric) (reference 1470–1465), 1096 (C—O) (reference 1300–1000), and 727 (C—H bending deformation) (reference 720–710) (Fig. 5d). All references were obtained from Pavia et al. (2016).

4. Discussion

This study offers the first evidence of PD ingestion by fishes from the Gulf of California and Mexican Pacific. The frequency of occurrence of PD in fish in our study was high when compared to the studies of Possatto et al. (2011) and Pegado et al. (2018); as they report frequencies of occurrence from 13% to 23% respectively in similar environments from Brazil. Our study found a detection frequency of 50.5%, and in the three most abundant species the frequencies varied from 45.5% (Dove's longfin herring) to 61.5% (Mojarra).

4.1. *Feeding habits of the analysed fish species*

All fish found were demersal or benthopelagic organisms preying near the bottom for algae, polychaetes, and infaunal crustaceans, and in the water column for zooplanktivorous fish and chaetognaths. These results indicate the complexity of the trophic relations in such systems, but also indicate that microplastics are widely available for all fish predators and different trophic levels. Considering the differential use of the available resources and that the different species are likely preying on different areas of the water column, the fact that no differences were found on the type, size, or quantity of microplastics indicates the availability of these throughout the water column.

4.2. *Plastic ingestion by fish*

One of the main goals of this work was to compare our results with others from similar tropical systems in order to have an idea of the magnitude of PD contamination. Being a new topic, results from other similar estuarine systems in tropical regions in the American continent are scarce, being Brazil the only region where such studies have already been undertaken (Barletta et al., 2020; Ferreira et al., 2018b; Pegado et al., 2018; Possatto et al., 2011). In order to make our results comparable to these works, the exact same methodology was employed, in which the gastrointestinal tract of fish is dissected and visually inspected for both preys and plastic debris. Although such method can underestimate the quantity of PD ingested by fish (Lakshmi Kavya et al., 2020), this method was used as we intended to couple the analysis of the diet with that of PD, as was done in the previously mentioned studies, and so any digestion-based method was excluded even if more efficient in PD recovery. Also, as Lakshmi Kavya et al. (2020) suggest, determining suitable extraction methods becomes crucial for further studies, and at the moment the methodological options to extract PD from organisms are too variable, precluding comparisons as there are no established standard protocols. Further studies are needed in order to establish the best method to extract microplastics from estuarine fish, and establish a standard protocol.

All PD ingested by the fish analysed in this study consisted of threads, which has been previously described for other demersal fish in similar tropical environments (Dantas et al., 2012; Lusher et al., 2017). This high intake might occur as a result in one part 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), and in other part because, as pointed out by Lima et al. (2015) the current amount of PD in the water column of coastal zones is similar to or even higher than the abundance of zooplanktonic organisms, because several other polymers tend to float, such as polystyrene, polypropylene, and polyethylene (GESAMP, 2019), thus increasing the chances of interactions between fauna and PD through ingestion of these particles during pelagic feeding. In addition, threads may resemble natural food items (amphipods, copepods, and polychaetes) (Thompson et al., 2004), with the result of them being preyed upon accidentally, mainly by juveniles and sub-adult fish (Ferreira et al., 2018a).Therefore, it is likely that the ingested microplastic particles were consumed both in the benthic environment and the water column. However further studies are needed comparing the amount of PD in both sediment and water to understand the origin of the PD found in the biota.

Most of the threads found in the gastrointestinal tracts of fish were small microplastics, which are the most commonly ingested type of microplastic particle (Pegado et al., 2018), this also indicates that these might have a coastal origin (Ferreira et al., 2018a). Strong hydrodynamic forces are associated with the coastal environment, and PD is exposed to high wind, wave, and tidal action, resulting in the breakdown

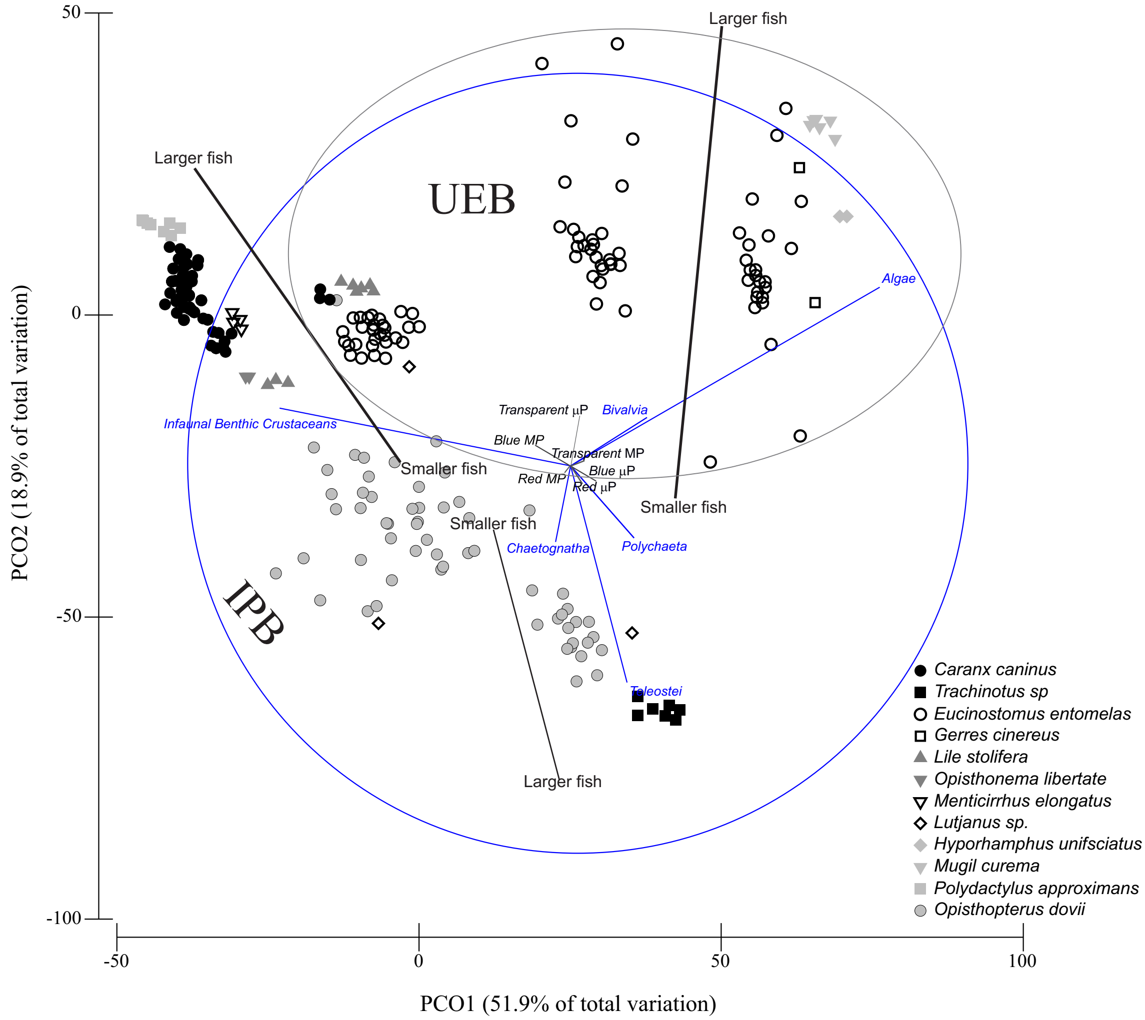

Fig. 4. Principal Coordinates analysis (PCO) describing the feeding patterns and plastic ingestion of the ichthyofauna in both analysed zones, UEB: Urias Estuary Beach, IPB: Isla de la Piedra Beach. Symbols inside the grey ellipse belong to fish analysed from UEB. The rest belong to fish analysed from IPB. Blue vector indicates the importance of each prey item, black vector indicates the importance of each colour of plastic found. μP = microplastics; MP = mesoplastics. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

into smaller particles because of the stronger weathering processes (Browne et al., 2007).

This particular estuarine system has a high influence from the sea, as it serves as a harbour for many human activities, and there is a strong tide, inputting water from the open sea. The hydrodynamic complexity of estuaries influences the inanimate material, including plastic debris, acting in their retention (Cole et al., 2011; Lima et al., 2014). Specifically, in relation to every sampling site, at IPB there are intense touristic activities, whilst UEB on the opposite side of the mainland, receives plastic debris from fishers and people inhabiting the surrounding area. UEB also has the presence of mangroves, increasing the deposition of plastic debris (Cordeiro and Costa, 2010) which eventually will break-down into microplastics and be ingested by the fauna. In addition, a large part of the city's sewage is discharged into the sea after being treated in a location approximately two kilometres west of the inlet of this estuarine system. Wastewater treatment plants play an important role in releasing microplastics to the environment (Sun et al., 2019), therefore it is likely that the water column and sediments close to this plant have a higher content of PD because of this proximity.

4.3. FTIR analysis

The types of microplastics identified in this study are consistent with the anthropic activities previously described; from the ATR-FTIR analysis polyamide (Nylon) represented 51.2% of all PD ingested by fishes in our sample. Most fishing gear is manufactured from nylon (GESAMP, 2019; Timmers et al., 2005), and the fishing industry has been estimated to contribute approximately 18% of all plastic debris found in the oceans (Andrady, 2011). In this region there is intense small scale fishing activity, which predominantly uses gillnets and cast nets, gear manufactured with this polymer (Ramírez-Rodríguez et al., 2014). Also, the textile industry uses nylon (Piñon-Colin et al., 2018), and considering the presence of the wastewater treatment plant in the vicinity, it is not surprising that this nylon-type polyamide appears as the most abundant in the area. In fact threads of this polymer were also reported to be an important component of microplastic debris of beaches in the Baja Peninsula, west of the location of the present study (Piñon-Colin et al., 2018).

The other important polymer was polyethylene (PE), a commonly produced polymer used to make plastic bags and storage containers

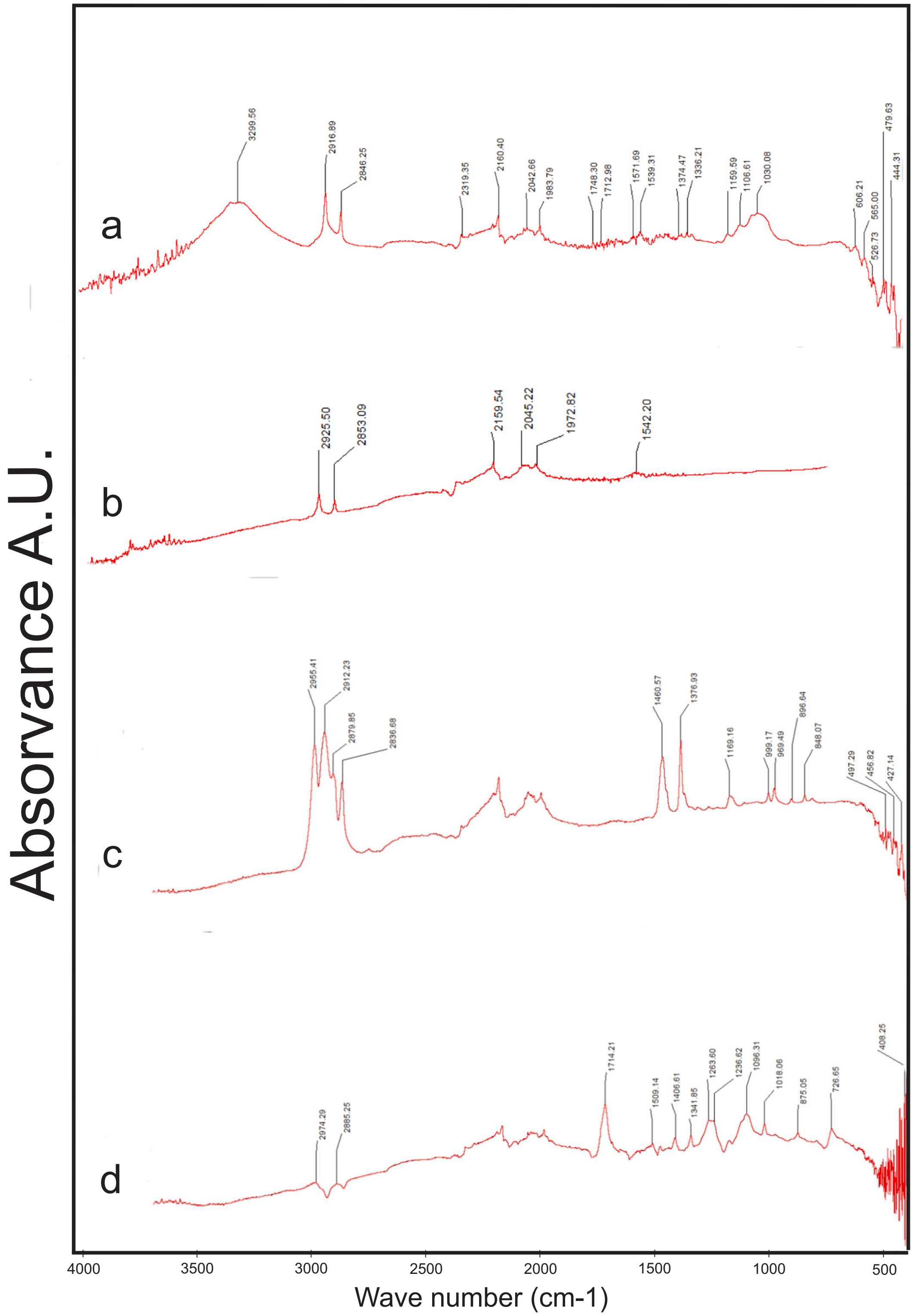

Fig. 5. ATR-FTIR spectra of the plastic particles found in the gastric content of the analysed fish: a) polyamide, b) polyethylene, c) polypropylene, and d) polyacrylic.

(GESAMP, 2019), which represented 36.6% of the ingested PD. In both IPB and EU there was a high amount of PD in the form of plastic bags and discarded containers. It is known that globally PE is the most abundant polymer found in the environment (Hidalgo-Ruz et al., 2012).

Of much less importance were polypropylene and polyacrylonitrile (acrylic); the former is used to manufacture rope, bottle caps, gear and strapping, which is also consistent with the PD observed in the area in the form of used fishing ropes and bottle caps from plastic bottles. Acrylic is used in textile manufacture, indicating that there is also an acrylic influence on the sewage discharge in the system.

4.4. Concluding remarks

The principal objective of this study was to determine whether fish species from an impacted area in the Gulf of California are ingesting PD, and the results show not only that they are consuming PD, but also that the quantities and frequency of occurrence are high when compared with other studies from similar environments. All fish species analysed are important for the local small-scale fisheries (Ramírez-Rodríguez et al., 2014). The implications are that these fish are widely eaten by the local human population, and their consumption can cause harm, as microplastics may contain toxic additives which can also be a vector for organic pollutants (Rochman et al., 2013; Santillo et al., 2017) due to their ability to adsorb and release pollutants. Once they come into contact with the intestinal dermis of a contaminated fish microplastics can potentially cause bioaccumulation and biomagnification of organic contaminants (Rochman et al., 2013). This should therefore raise food safety concerns for human populations.

Another source of concern is that the fish analysed in the present work were small jueveniles, that are likely prey items of larger fish from this system and the adjacent sea. There is a current gap in knowledge of the trophic transfer of PD within aquatic ecosystems, therefore uncertainty exists on the full impact it has on the ecology of coastal environments. Considering that the Gulf of California is where most fishing activity occurs in Mexico it is urgent to continue such studies in order to improve our knowledge on the dynamics of PD, and the ecology of PD ingestion by fish, as well as their ecological impact on marine ecosystems as well as human health implications. Detailed studies are needed in order to link year-round microplastic contamination levels through the foodweb and across ontogenetic stages.

This is only an initial insight into microplastic occurrence in fish, and the results are worrisome. The frequency of PD found in the gastric system of fish from the study site is much higher than those reported for similar tropical areas. Therefore further studies need to be urgently undertaken to determine the quantity and type of plastic debris in the water column, sediment, on all ontogenetic phases of fish, and also in different estuarine systems, coastal zones, and the open sea in this region, as well as on the relationship between PD content in fish and the presence of pollutants on their tissues.

CRediT authorship contribution statement

C. Salazar-Pérez: Formal analysis, Investigation, Methodology, Writing – original draft. **F. Amezcua:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Supervision, Writing – original draft, Writing – review & editing. **A. Rosales-Valencia:** Formal analysis, Investigation, Methodology, Writing – original draft. **L. Green:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **J.E. Pollorena-Melendrez:** Formal analysis, Investigation, Methodology, Writing – original draft. **M.A. Sarmiento-Martínez:** Formal analysis, Investigation, Methodology, Writing – original draft. **I. Tomita Ramírez:** Formal analysis, Investigation, Methodology, Writing – original draft. **B.D. Gil-Manrique:** Formal analysis, Investigation, Methodology. **M.Y. Hernandez-Lozano:** Formal analysis, Investigation, Methodology. **V.M. Muro-Torres:** Formal analysis, Investigation, Methodology, Writing - original draft. **C. Green-Ruiz:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **T.D.J. Piñon-Colin:** Formal analysis, Methodology, Writing – original draft. **F.T. Wakida:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **M. Barletta:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing.

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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