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Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary

Rocío S. Pazos[a,b,*], Tomás Maiztegui[a,b], Darío C. Colautti[a,b], Ariel H. Paracampo[a,b], Nora Gómez[a,b]

[a] Instituto de Limnología "Dr. Raúl A. Ringuelet", UNLP-CONICET (CCT La Plata), CC 712, 1900 La Plata, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

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ABSTRACT

The presence of microplastics (MPs) in gut contents of coastal freshwater fish of the Rio de la Plata estuary was studied. Samples were taken in six sites where 87 fish belonging to 11 species and four feeding habits were captured. Presence of MPs was verified in the 100% of fish. The fibres represented the 96% of MPs found. The number of MPs in gut contents was significantly higher close to sewage discharge. There was not found relationship between number of MPs and fish length, weight or feeding habit. The spatial differences in mean number of MPs in fish observed in this study, suggest that environmental availability of MPs could be of great importance to explain the differences found among sampling sites analysed. This work represents the first study about the interaction between MPs and aquatic organisms in this important estuarine ecosystem of South America.

1. Introduction

The annual production of plastic has increased significantly from 1.5 million t in the 1950s to an estimated 299 million t in 2013 (PlasticsEurope, 2015). As a direct consequence of the massive use of plastics in modern society, plastic waste is accumulating, especially in urbanized areas, where it often ends up in waterways and is ultimately transported into the ocean (Thompson et al., 2004; Browne et al., 2015). Through urban runoff, plastic debris carried by streams and rivers reach the coastal seas and oceans (Gregory, 2009; Andrady, 2011; Rech et al., 2014) and particularly in a greater extent in estuarine environments (Acha et al., 2003). However, plastic debris does not only come from land; fishing activities (e.g. gears lost or forsaken at sea, Tschernij and Larsson, 2003), marine traffic (e.g. accidental and deliberate dumping, Mato et al., 2001; Moore, 2008), and recreational coastal activities (e.g. irresponsible individual actions, Gregory, 1996; Bravo et al., 2009) are also important sources of coastal and marine litter. Because plastic polymers show minimal biological degradation, they remain in the environment for hundreds to thousands of years, where they break down into smaller pieces owing to ultraviolet radiation, physical forces and hydrolysis (Moore, 2008). Different studies have shown the detrimental effects of plastics on the biota, such as physical entanglement, decreased nutrition from intestinal blockage, and suffocation or decreased mobility (Derraik, 2002, reviewed in Gregory, 2009).

A particular fraction of plastic debris are the microplastics (MPs), size < 5 mm (Arthur et al., 2009), which have recently drawn attention because they not only make their way into the marine environment but are also more easily ingested by marine organisms; they may thus act as vectors for the chemical transfer of pollutants within the food chain (Teuten et al., 2009). The MPs comprise either manufactured plastics of microscopic size, such as scrubbers (Gregory, 1996; Fendall and Sewell, 2009) and industrial pellets that serve as precursors for plastic industry (primary sources), or fragments or fibres of plastics derived from the breakdown of larger plastic products (secondary sources)(Cole et al., 2010; Browne et al., 2011; Arthur et al., 2009).

Records referring to environmental problems caused by MPs were reported mainly in marine environments, however the information about other environments is less frequent (Li et al., 2016) and particularly in estuaries (Thornton and Jackson, 1998; Browne et al., 2010; Costa et al., 2011). On the other hand, there have been few studies specifically examining the occurrence of MPs in natural populations (Lusher et al., 2013). In relation to fish populations, Carpenter et al. (1972) identified the ingestion of plastic by fish including pieces < 16 mm in Atlantic silversides, *Menidia menidia* (Linnaeus, 1766). In addition, Hoss and Settle (1990) reviewed previous papers finding pieces < 50 mm in the European flounder, *Platichthys flesus* (Linnaeus, 1758). Boerger et al. (2010) found that MPs (< 2.79 mm) were

* Corresponding author at: Instituto de Limnología "Dr. Raúl A. Ringuelet", UNLP-CONICET (CCT La Plata), CC 712, 1900 La Plata, Argentina.
E-mail address: rpazos@ilpla.edu.ar (R.S. Pazos).

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consumed by fish feeding in the water column in the North Pacific Gyre, which is known to have substantial accumulation of debris (Moore, 2008). In addition, Davison and Asch (2011) found mesopelagic fish to have ingested plastic fibres; filaments and films (mean length 2.2 mm). Plastic ingestion by fish from estuarine waters in Northeast Brazil, Goiana Estuary (7.5° S 34.5°W), was found in different ontogenic phases in *Cathorops spixii* (Agassiz, 1829), *Cathorops agassizii* (Eigenmann and Eigenmann, 1888), *Sciades herzbergii* (Bloch, 1794) (Possatto et al., 2011), *Eugerres brasilianus* (Cuvier, 1830), *Eucinostomus melanopterus* (Bleeker, 1863), *Diapterus rhombeus* (Cuvier, 1829) (Ramos et al., 2012) and in Scianidae (Dantas et al., 2012; Ferreira et al., 2016). Also in this estuary, was analysed the relationship between the concentration of MPs and ictioplankton, highlighting the impact of this emergent pollutant (Lima et al., 2014, 2015, 2016).

The Río de la Plata (RLP) is one of the largest estuarine systems of South America and is the fifth largest in the world and drains the second largest basin in South America (Baigun et al., 2016). The Argentinean margin of the freshwater sector is located in the most important industrial and urban centre of the country accounting for about 15 million inhabitants, being Buenos Aires City the main urban conglomerate. Despite the coast of this area having developed recreational activities and commercial fisheries, the sewage, urban and industrial discharges are conducted to the estuary, and are considered to be poorly treated (Gómez et al., 2012; Gómez and Cochero, 2013) Studies performed in the RLP turbidity front (brackish estuary sector) have documented plastic pieces (Acha et al., 2003) and MPs in the sediment of the Uruguayean coastal line in the mouth of the estuary (Lozoya et al., 2016). However, the presence of MPs in the biota of this wide environment is still unknown.

The aim of this study is to analyse the presence, occurrence, type and abundance of MPs in gut contents of fish of several feeding habits in the freshwater sector of the RLP estuary. This work represents the first study about the interaction between MPs and aquatic organisms in this important estuarine ecosystem of South America.

2. Material and methods

2.1. Study area

This study was carried out on the Argentinean coastline of the RLP estuary (Southern Coastal Fringe) near La Plata City, between 34°46′49″S 58°0′57″W and 34°55′45″S 57°42′56″W, in the freshwater zone (salinity < 0.5 PSU) (Fig. 1).

La Plata City and its surroundings is the southernmost urban and agropecuary conglomerate that discharge into the RLP estuary. The water drains by mean of streams, channels or pipelines from areas with different land uses. Six sampling sites were placed along 35 km of coastline, each one close to water discharges from natural, semi-natural or artificial drainage systems of the region (Table 1).

Table 1
Sampling sites, geographical position, drainage system and land uses.

Sites	Locality	GPS coordinates	Drainage systems	Land uses
PL 1	Punta Lara	34°46′49″S 58° 0′57″W	Semi-natural (channeled streams)	Urban, rural and forest
PL 2	Punta Lara	34°47′29″S 57°59′46″W	Semi-natural (channeled streams)	Urban, rural, forest and plantations
PL 3	Punta Lara	34°48′32″S 57°58′44″W	Semi-natural (channeled streams)	Urban, rural and plantations
BE 4	Berisso	34°52′22″S 57°48′39″W	Artificial (pipeline)	Urban
BE 5	Berisso	34°54′31″S 57°45′35″W	Natural	Rural, wetlands and forest
BE 6	Berisso	34°55′44″S 57°42′56″W	Natural	Rural and wetlands

2.2. Fish sampling and laboratory analysis

Experimental fishing was performed in April and September of 2016. At each sampling site three fyke nets (Colautti, 1998) were used during one night. Fish were identified following: Azpelicueta and Braga (1991), Lopez and Miquelarena (1991), Braga (1994), Casciotta et al. (2005), Miquelarena and Menni (2005), Almirón et al. (2008). Captured species in each sampling site were sorted according to their feeding habits, considering the studies on their trophic ecology (Ringuelet et al., 1967; Oliva et al., 1981; Colautti, 1997; Gealh and Hahn, 1998; Menni, 2004; Casciotta et al., 2005; Novakowski et al., 2007; Corrêa and Piedras, 2009; González Sagrario and Ferrero, 2013; Gottlieb Almeida et al., 2013; Llamazares Vegh et al., 2014). Five specimens of each trophic group were euthanized in ice, transported in coolers and preserved in freezer in the laboratory. The individuals were measured (TL, cm), weighed (W, g) (Ohaus precision balance, 1 g) and their guts extracted and digested with a solution of Hydrogen peroxide (H_2O_2) 30% to 60 °C until its total digestion, following Avio et al. (2015). Previously, to prevent sample contamination, glass beakers and petri dishes used for observation of the sample were cleaned with distilled water. Later the sample was observed under a stereomicroscope 5.6 × (Olympus SZX7), recovering the MPs. The total number of MPs

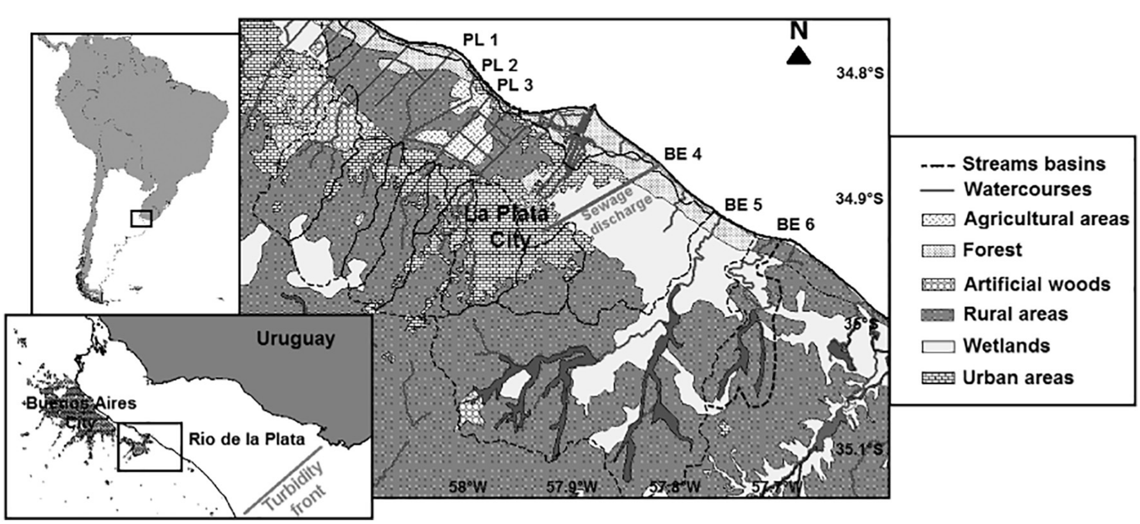

Fig. 1. Location of Río de la Plata estuary indicating the six sampling sites, their drainage systems and the land uses in each streams basins.

found in each specimen was registered and classified by type, colour and size (maximum length).

During gut extraction, storing, digestion and until visual identification, the samples were covered with foil paper. Control samples were processed as the same time of gut samples following the described protocol.

2.3. Statistical analysis

The mean number of MPs per individual at each site, were analysed by a non-parametric test (Kruskal-Wallis) and differences were analysed a posteriori by the Dunn method. Data on the mean number of MPs per feeding habit were analysed using one-way ANOVA and the relationship between the number of MPs, TL and W, was assessed using the Pearson correlation coefficient.

3. Results

During the sampling period, 87 fish were captured, belonging to 11 species and four feeding habits: detritivore, planktivore, omnivore and ichthyophagous. TL of the fish ranged between 6 and 51.6 cm, and the W between 2 and 3160 g (Table 2). The number of specimens selected for analysis per site fluctuated from 10 to 21 and the percentage distribution per feeding habit in each site is shown in Fig. 2.

The gut analysis contents allowed the identification of fibres and 'other types of MPs' hereafter referred to as 'others' (hard pieces of regular and irregular shapes). In both cases, the colours detected were diverse (red, green, yellow, white, black and blue, the latter was dominant) (Fig. 3). In the control samples a maximum of 2 fibres were found. It is important to highlight that in all individuals analysed, some type of the MPs was found. Fibres were present in 100% of the samples, meanwhile 'others' were found in 30% of the fish analysed.

A number of 1679 pieces of MPs were counted, corresponding to 96% fibres and 4% to 'others'. Their sizes ranged between 0.06 and 4.7 mm. The average number of MPs per fish was 18.5 ($\pm$ 18.9) fibres and 0.7 ($\pm$ 1.7) for 'others'. Regarding abundance, for fibres the minimum number of pieces found per individual was 1 (in BE 6) and the maximum was 89 (in BE 4). While for 'others' the minimum was 1 (PL 1, 2, 3, BE 5 and 6) and the maximum was 8 (PL 3) (Table 3).

The average number of MPs per individual was significantly higher in BE 4 ($p < 0.05$), corresponding exclusively to fibres (Fig. 4).

Correlations between the abundance of MPs with TL (fibres: $p = 0.2$ and 'others': $p = 0.3$) and W (fibres: $p = 0.1$ and 'others': $p = 0.1$) of the specimens were not significant. The ANOVA performed between number of MPs ingested and feeding habits was not significant for either, fibres or 'others' (Fig. 5).

4. Discussion

According to the results of this study 100% of fish gut contents that were analysed exhibited MPs indicating that this kind of pollutants are

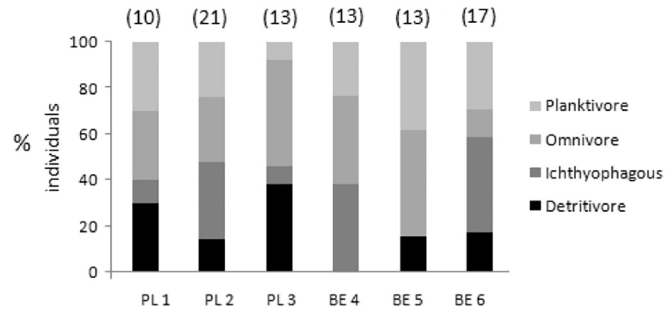

Fig. 2. Percentage per feeding habit in each sampling site and number of individuals analysed, between brackets.

interacting with fish community in the RLP estuary and that the ingestion could be related to the extent of exposition. This is in concordance with Browne et al. (2010), who reported that MPs are more abundant than larger plastics debris and this increases the risk of ingestion and other consequences related to plastics pollution at sea. As the fragmentation of larger plastics debris at sea is a common phenomenon (Barnes et al., 2009) this can also generate the progressive increment of such pollutants.

Another remarkable observation was that the main type of MPs found were fibres (96%), being the highest percentage documented in the bibliography. This finding shows the high chance that fish consume fibres. Nevertheless, similar percentages were also reported by Boerger et al. (2010) (94%) and Lusher et al. (2013) (68.3%). The origin of the fibres in aquatic ecosystems is diverse, according to Pruter (1987) who found that the main polymers were polyamide and polyester which are commonly used in the fishing industry. Lusher et al. (2013) also found that rayon made up over half of the polymers identified (57.8%) having their possible sources in clothing, furnishing, female hygiene products and nappies and hence high levels could be a result of indirect input through sewage. Items made of rayon can disintegrate rapidly (Park et al., 2004) which could explain their abundance. This is in agreement with the findings of this study, because in BE 4 (sewage discharge) the highest abundance of MPs in gut content of analysed fish was documented. Browne et al. (2011) conducted studies in marine environments and identified that fibres enter the ecosystem mainly via wastewater discharge (a single synthetic clothing garment can release > 1900 MPs fibres per wash) and can be higher in the more in densely populated areas. They also concluded that water in close proximity to sewage discharge sites can contain proportions of fibres resembling the proportions used in synthetic clothing. This is in agreement with the results of this study, not only by the maximum levels of MPs found in BE 4 but also with the decreasing levels of MPs found as function to the distance from this sampling site to the others. Therefore, the diverse activities developed in the basin of each other discharge appear not to be relevant source of MPs contamination.

The number of MPs found was not related to fish TL or W, neither

Table 2
Species captured in the study, number of specimens analysed, their feeding habits and total length (TL) and weight (W) fish ranges.

Species	Number of fish	Feeding habit	TL (cm)	W (g)
Luciopimelodus pati (Valenciennes, 1836)	9	Ichthyophagous	21.2–30	44.6–178.4
Pseudoplatystoma corruscans (Spix & Agassiz, 1829)	2	Ichthyophagous	30	155–200
Oligosarcus oligolepis (Steindachner, 1867)	5	Ichthyophagous	15.7–18.7	36–73
Parapimelodus valenciennis (Lütken, 1874)	21	Planktivore	9.7–24.5	6–126
Odontesthes bonariensis (Valenciennes, 1835)	1	Planktivore	28.9	159
Astyanax rutilus (Jenyns, 1842)	12	Omnivore	6–9.5	2–9.2
Cyprinus carpio Linnaeus, 1758	2	Omnivore	19.2–51.6	112.4–3160
Pimelodus maculatus Lacepéde, 1803	14	Omnivore	10.5–19.2	10.9–65.2
Prochilodus lineatus (Valenciennes, 1836)	5	Detritivore	9.3–17.2	10.7–72
Hypostomus commersoni Valenciennes, 1836	2	Detritivore	12.7–16.7	19–44.5
Cyphocharax voga (Hensel, 1836)	14	Detritivore	11.5–18.4	19.5–120

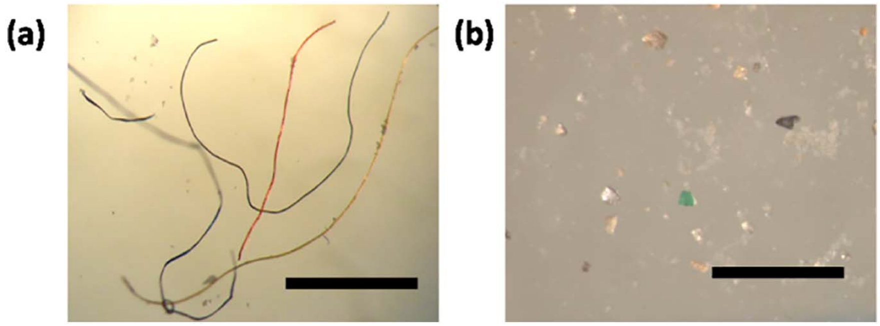

Fig. 3. (a) Example of fibres and (b) 'others'. Scale bar represents 1 mm.

Table 3
Number and ranges of fibres and 'others types of MPs' found in fish analysed per site.

Sites	Number of fibres	Number of 'others types of MPs'	Number of fibres min–max	Number of 'others types of MPs' min–max
PL 1	65	3	2–14	1–2
PL 2	237	13	2–23	1–4
PL 3	227	31	4–55	1–8
BE 4	691	0	30–89	0
BE 5	296	9	14–39	1–2
BE 6	99	8	1–11	1–4
Total	1615	64		

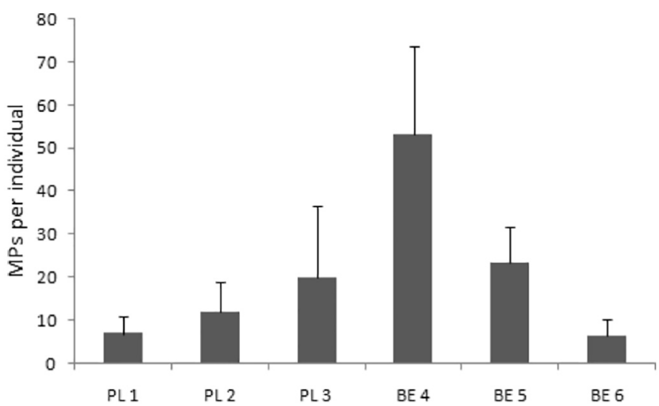

Fig. 4. Average number of MPs in fish analysed in each sampling site.

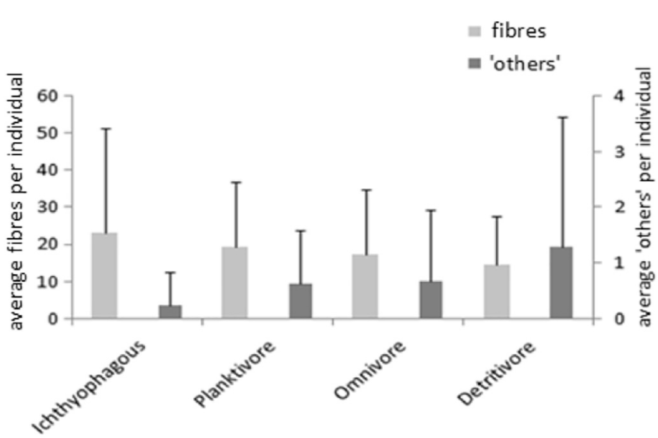

Fig. 5. The average number of MPs per fish per feeding habit, discriminating between fibres and 'others'.

with their feeding habits, which indicates that the ingestion of MPs is dependent of other variables. This is in concordance with the observations of Possatto et al. (2011) in the Goiana estuary, who also reported that the ingestion of plastic debris probably happened during the fish's normal feeding activity. In the same estuary was documented the differential ingestion of MPs according to ontogenetic phases (Dantas et al., 2012; Ferreira et al., 2016; Ramos et al., 2012). Although in the present study this issue was not analysed, MPs were found in all fish regardless their lengths. The lack of relationship among number of MPs ingested and feeding habit are indicative that other factors are involved in fish quantitative MPs intake. In fact, Neves et al. (2015) could not find significant differences among species or feeding habits regarding MPs ingestion despite huge variability in the feeding habits, and of density and size distribution of MPs in the habitat where the study was carried out. The spatial differences in mean number of MPs in fish observed in this study, suggest that environmental availability of MPs could be of greater importance to explain the differences found among the sampling sites analysed. So it seems to be important analyse the availability and distribution of MPs in the freshwater coastal sector of the RLP estuary, to clarify the strength of this relationship. Labropoulou and Eleftheriou (1997) and Murua (2010) related MPs ingestion with the fish feeding habits, or with an incorrect prey identification. Also Masó et al. (2003) and Cyrus and Blaber (1983) suggest that certain organisms (e.g. diatoms) can aggregate on the surfaces of plastic debris and construct a biofilm that could be attractive to fish. Despite these, the referred phenomena were not sustained by our results.

This study also found that the number of MPs found in the gut contents of fish were related to the site of sampling despite the fish size or feeding habit, suggesting that the dynamics of these pollutants in gut is not accumulative and likely follow the digestive transit circulation. This hypothesis was reinforced by the fact that the study was performed in an open system and fish are mobile organisms. As consequence of this thinking, the number of MPs in gut contents could be considered as an indirect instantaneous measure of MPs in the environment.

On the other hand, potentially toxic effects to fish from contamination of MPs with persistent, bioaccumulative and toxic pollutants have been assessed in laboratory experiments, using the Japanese medaka (*Danio rerio* (Hamilton, 1822)). Rochman et al. (2013) demonstrated liver toxicity under exposure to MPs with contaminants adsorbed from the environment in food. It also reported altered endocrine system function when the fish were exposed to environmentally relevant concentrations of MPs (Rochman et al., 2014). However when considering wild fish, the contribution of MPs to the body burden of contaminants remains to be demonstrated, as fish are also exposed to the complex mixture of contaminants present in the water. Nevertheless, it is known that MPs have the potential to sorb persistent organic pollutants (Ogata et al., 2009; Bakir et al., 2012) and that desorption under gut conditions could be up to 30 times greaterthan in seawater alone (Bakir et al., 2014). Taking into account that there are reports of high concentrations of aliphatic hydrocarbons and polychlorinated biphenyls (PCBs) in the muscle tissue of *Prochilodus lineatus* (Valenciennes, 1836), *Cyprinus carpio* (Linnaeus, 1758) and *Mugil platanus* (Günther, 1880) from the coastal area of the RLP estuary (Colombo et al., 2000, 2007), it is likely that the proven consumption of MPs by the fish in this area, would contribute to potentiate their contamination.

This study represents the southernmost latitudinal record in South America regarding MPs ingestion by estuarine fish, constituting a warning sign for fish inhabiting the freshwater coastal sector of RLP estuary. In addition, the widespread occurrence of MPs in fish indicates that future research across a wider range of species and habitats, together with environmental availability of MPs should be considered in order to fully establish the potential effects of MPs in the area and clarify about the mechanistic dynamics involved in the interaction between fish and MPs.

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