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Research Article

Microplastics in gastro-intestinal tract of estuarine fish from the mangrove ecosystem of Indian Sundarbans

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Abstract

Mangrove ecosystems all around the globe are affected by microplastic (MPs) pollution. The Sundarban Biosphere reserve, the world's largest mangrove forest is not an exception. The study aims to identify the occurrence of MPs in the gastro-intestinal tract (GI tract) of estuarine fish from Indian Sundarbans and the relationship between the presence of MPs with the morphology and feeding niche of fish. Total of 13 fish species were collected from the Saptamukhi River near Lothian Island, India and MPs were isolated from GI tract contents. Morphological parameters like body size, mouth aperture length, eyeball diameter and GI tract length of fish were measured. Niche breadths and niche overlapping of these 13 species were analyzed using Levin niche breadth and Pianka niche-overlap indices. The fiber-shaped MPs were ubiquitously detected in all fish species followed by films and fragments. The size of MPs was ranged from 100µm to <5mm. A significant variation (p<0.0001) in respect of the trophic morphology of fish and length of MPs (p<0.05) were found. The length of MPs had a positive correlation with body size and mouth aperture but in the case of GI tract length and eyeball diameter, the correlation was negative. It was noticed that the presences of MPs in GI tract of those 13 estuarine fish species did not bother their niche breadth and niche overlap.

Keywords: Microplastic, Fish, GI tract, Sundarban, Mangrove, Morphology, Niche

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Introduction

Every year around 1.15 – 2.41 million tonnes of plastics enter the oceans from various riverine sources (Lebreton *et al.*, 2017). In the meantime, Asia contributes nearly the world's 67% ocean going plastic materials as garbage (Agarwal, 2018). The generation rate of plastic waste in India is exponentially booming with present production at around 25,940 tons per day (TPD), of which 6.92% originated as plastic material from municipal solid wastes (CPCB, 2015). Around 15,600 TPD or 60% of total plastics are recycled but on the other hand, more than 9,400 tons ultimately end up in the riverine waterways, bays, seas, and oceans or in the terrestrial soil layer (Shrivastav, 2019). In the case of the Indian state wise plastic consumption, per capita consumption of plastics in West Bengal is at around 5 kg per year on average and still growing up to 15% nationally in each succeeding year (PTI, 2017).

Large plastic products are generally degraded by solar UV radiation, heat and/or microbial-chemical actions (Amorim *et al.*, 2020) and finally disintegrate into very tiny micro-sized plastics (length <5 mm) often called Microplastics (MPs). MPs were globally reported from marine environments (Yang *et al.*, 2021) and various mangrove regions (Deng *et al.*, 2021). It is observed that the coastal ecosystems (such as mangrove forests, estuarine rivers, and coastal beaches) show a high degree of MPs pollution as these are usually in the junction of receiving end of any ecotone structures, where, exponential anthropogenic pressure, unsustainable tourism practices, intensive fish farming, etc. play major roles (Llorca, 2020).These plastic pieces are taken up by various aquatic organisms and in turn accumulate inside their gastro-intestinal tract, and/or gills of fish and ultimately lead to their unnatural death or destabilizing malfunctions (Reddy, 2018). MPs are generally get ingested and enter inside the fish body during their foraging period which in turn deposited inside gastro-intestinal (GI) tract (Gove *et al*., 2019). It is found that MPs were also accumulated in tissues of other organs like the brain, gills, and muscle of fish (Yin *et al.*, 2022). Alike MPs, Nano-plastic (1 to 1000 nm) is also get bio-accumulated within fish tissues that might disrupt several physiological and behavioral functions (Guerrera *et al.*, 2021). The morphological characteristics like body size (Gad and Midway, 2022), oral, and nasal cavity (Zhang *et al.*, 2022) had been found to be related with availability of MPs in some fish. But, other studies also found that the MPs had no significant relationship with feeding guild (Parker *et al.*, 2022), functional trophic groups and feeding habitats (Koraltan *et al.*, 2022) of various fish. MPs were found to affect embryonic development in the case of sea urchin, *Lytechinus variegaatus* (Nobre *et al.*, 2015) experimentally. MPs also adhere to the external chitinous carapace and appendages of exposed zooplankton and it was found that 7.3μm sized MPs can significantly reduce their filter feeding rate and opportunities (Cole *et al.*, 2013). Polystyrene MPs are said to be related to micro-biome dysbiosis and create inflammation in the GI tract of male Zebra fish, *Danio rerio* (Jin *et al.*, 2018). A study showed that the presence of MPs inside the GI tract, further induces heavy metal accumulation and increases oxidative stress related systemic lethality in aquatic organisms (Barboza *et al.*, 2018). Exposure to MPs (polyethylene) in low concentration can increase the feeding rate of larvae of California Grunion fish, *Leuresthes tenuis* and also shows the trophic transfer of MPs under experimental conditions (Uy and Johnson, 2022).

Indian Sundarbans is a detritus-based mangrove ecosystem. An huge amount of solid waste materials is regularly carried out from the upstream to the downstream of rivers through the large estuaries and mixed thoroughly with tides coming from the Bay of Bengal. Like other mangrove forests, the Sundarban estuary is a natural nursery ground for a wide variety of fish as it provides shelter and food (Paillon *et al.*, 2014) to them. Around 172 species of fish recorded from Indian Sundarbans (Jhingran, 1977) are broadly categorized as omnivore, planktivore, detritivore, carnivore, etc. based on their primary choice of food and food materials found in their gastro-intestinal tract (GI tract) (Chaudhuri *et al.*, 2014). The detection of MPs from sediment and water from the Sundarban Biosphere reserve increases concern and demand for immediate and collective monitoring approaches from all arenas (Kumar *et al.*, 2022). Yet very few studies have been carried out in the context of Indian Sundarbans to know whether the MPs are present inside the GI tract of estuarine fish of Sundarbans or not. Thus, the objectives of the present study were to focus on the presence of MPs in the gastro-intestinal (GI) tracts of fish, sampled from Indian Sundarbans and the relation of the presence of MPs with morphological characters and niches of fish.

Materials and methods

Fish sampling

The study was conducted near Lothian Island Wildlife Sanctuary, West Bengal, India, in the southern part of Saptamukhi River of Indian Sundarbans (Fig. 1) during post-monsoon period from November 2018 to February 2019. Fish samples were collected fortnightly during both low tide and high tide (high tide- 21°42’0”N, 88°20’9”E and low tide- 21°42’3”N, 88°20’9”E) in the respective months of the sampling period. All samples were collected with the help of local fisher folks by using Benti/ Badhar Jal (purse seine or conical-shaped net with two extensions) having mesh size of 10±3mm. The collected samples were immediately preserved in 2% formaldehyde solution on the spot. Identification of fish species was done by using web databases and standard taxonomic literature (www.fishbase.org; Day, 1889; Talwar and Kacker, 1984). For further analysis, only 13 fish species (total catch size= 303 individuals) among the total captured fish taxa were selected based on their regular presence in both high and low tide. The selected 13 fin fish species were *Harpadon nehereus* (n=15), *Lepturacanthus savala* (n=8), *Bregmaceros mcclellandi* (n=18), *Otolithoides pama* (n=38), *Arius arius* (n=8), *Stolephorus indicus* (n=51), *Cynoglossus lingua* (n=8), *Coilia ramcarati* (n=50), *Pellona ditchella* (n=9), *Tetraodon cutcutia* (n=7), *Pisodonophis boro* (n=6), *Panna microdon* (n=12) and *Coilia dussumeri* (n=73) (Fig. S1).

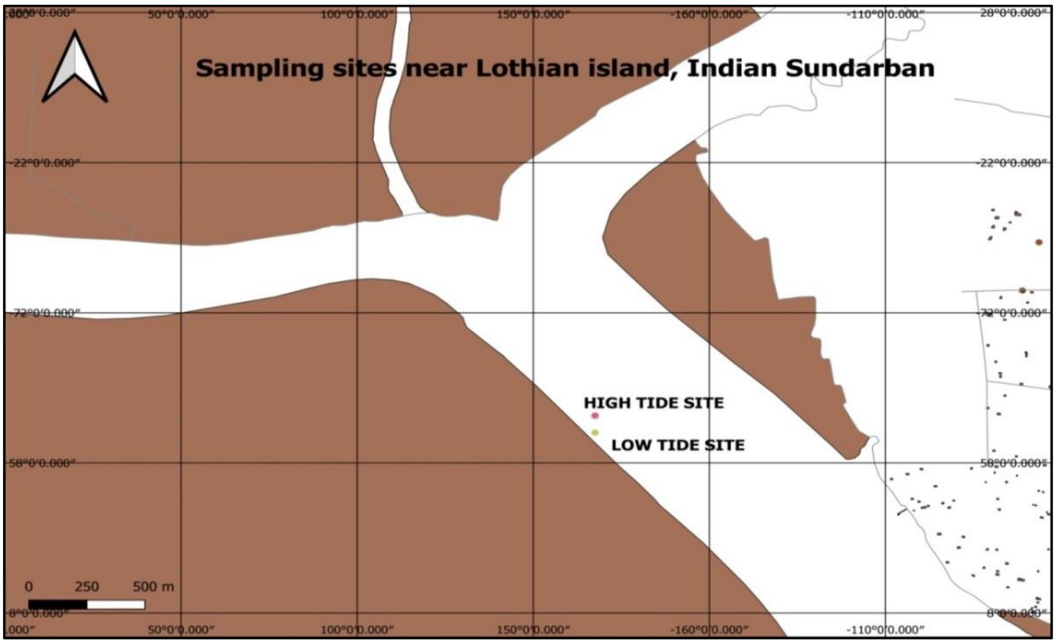

Figure 1: Sampling sites (high and low tide site) on Saptamukhi River near Lothian Island Wildlife Sanctuary of Sundasrban Biosphere Reserve, West Bengal, India.

Measurement of trophic morphological characters

Morphometric measurements of an organism which have a direct correlation with the feeding habitats, specifically feeding ecology, are termed as trophic morphological parameters. Consumption of MPs might be depending on trophic morphology because it could influence both the feeding performance and diet of fish (Hulsey *et al.*, 2005) and it is also varied among different taxa of fish (Fugi *et al.*, 2001). Trophic morphological features such as body size (total body length, TBL), mouth aperture and eye ball diameter and gastro-intestinal (GI) tract length were minutely measured by using slide calipers. Trophic morphological features in turn were related to GI tract content and simultaneous MPs ingestion (Kamboj and Kamboj, 2019; Anumudu and Mojekwu, 2015; Khan *et al.*, 2013).

GI tract content, Niche breadth and overlap analysis

The fish were dissected ventrally by following an incision near the anus and the entire GI tract was taken out carefully. Water flow was passed (after measurement of GI tract length) from one open end of GI tract and the flushed out GI tract contents were then collected in a 100mL glass beaker for further analysis. Fish samples having empty GI tract (Total no.=205) were recorded and discarded from the analysis. 1mL of GI tract content samples (with 5 replicates for each species) were taken in Sedgewick rafter and observed under compound microscopes (Olympus CH20i microscope, 10x magnifications). The frequency of occurrence method was used to assess the GI tract contents as per Podder *et al.* (2021) and Chaudhuri *et al.* (2014) with some modifications. The GI tract content compositions were categorized into eight groups by visual identification- phytoplankton, zooplankton, prawn, crab, aquatic insects, plant parts, soil-sand particles, and unidentified debris. Further measurements of species-specific feeding niche differentiation (niche breadth and niche overlap analysis) were performed. Niche breadth was measured according to Levins (Levins, 1968):

$B= 1/\sum p^2$

Where, B= Levins measure of niche breadth and *pi* proportion of individual found using the resource (*i*). Niche overlaps were measured for different species according to Pianka, 1986. The measure ranges from 0 (no resources are common) to 1 (complete overlap):

$O_{jk}=\sum p_{ij}p_{ik}/\sum pij^2 p_{ik}^2$,

Where, O_{jk}= Pianka's measure of Niche overlap between species (*i*) and (*k*), (p_{ij}) is the proportion of that resource (*i*) that is used by species (*j*), (p_{ik}) is the proportion of that resource (*i*) which is exploited by another species (*k*).

Sample preparation for MPs isolation

The digestion of GI tract-derived samples was carried out as per Karami *et al.* (2017) and Su *et al.* (2019) with few required modifications. 100 mL of 30% hydrogen peroxide (H_2O_2, Merck) was mixed in a glass beaker with the desired samples and kept at 65°C in 80 rpm (revolutions per minute) for 24 hours in a B.O.D incubator. After that, 100 mL saturated NaCl (1.2g/mL) solution was added to the sample for density separation and kept at room temperature. The entire sample was then divided into two equal portions, one part for the stereomicroscopic identification (LAS EZ) and the other part for fluorescence based identification of MPs. Both samples were then carefully passed through a glass filtration apparatus with Whatman Grade 1 (47mm, 11µm pore size, UK) filter paper (Cheung *et al.*, 2018) and kept the filter paper covered under glass Petri dish and dried within incubator at 40-45°C for another 24 hours.

Staining of MPs for fluorescence study was done by following the standard protocol using Nile red stain (Shim *et al.*, 2016; Erni-Cassola *et al.*, 2017).The stained samples were observed under a fluorescence microscope (Olympus IX 71 inverted microscope) with excitation wavelength between a range of 430-490nm and emission wavelength between 510-560nm at 40x magnification.

Visualization, categorization and length measurement of MPs

The categorization of MPs were performed under a stereo-microscope (Leica EZ4) according to their apparent shape. Large irregular pieces of MPs were named as fragments, whereas, the thin strip-like shaped MPs were categorized as films and thread-like MPs were classified as fiber (Vendel *et al.*, 2017). The presence of all types of MPs was recorded for each species. The length of MPs was measured. The fluorescence photograph of MPs for each species was also captured accordingly.

Statistical analysis

Depending on data size, Kruskal-Wallis test was selected to find out the variation of trophic morphology and length of MPs between species. The Wilcoxon signed rank test was performed to understand the statistically significant variation of niche breadth among 13 fish species. The Spearman rank correlation test was used to understand the relationship between trophic morphology and the length of MPs. All the statistical analysis, map and graphs were developed by using SPSS 17.0, QGIS 3.22 and GraphPad Prism 8 software, respectively.

Quality control and Quality assurance (QC/QA)

The entire work was performed using plastic-free components under the bio-safety cabinet. Gloves and borosilicate glass products were used in all required steps. The glass products and stainless steel-made dissection tools were pre-cleaned with isopropyl alcohol followed by acid-water wash (a few drops of Nitric acid dissolved in double distilled water). For filtration and chemical preparation, only double distilled water was used after filtering through a glass filtration unit. Prior to microplastic trapping, the blank filter papers were first examined under stereomicroscope to minimize any external contamination.

Results

Variation in trophic morphology of fish

The value (Mean±SE) of all measured trophic morphological characters – Body size (Total body length), mouth aperture, eye-ball aperture, and GI tract lengths were shown in Table 1. These trophic morphologies were found to be significantly different (p<0.0001) among 13 fish species (Table S1) after the Kruskal-Wallis test was done.

GI tract contents composition

Among eight different GI tract content prawn body parts was the most common type (~28%) of food items found in most samples. The second and third most observed food items were zooplankton (~21%) and phytoplankton (~15%), respectively.

Table 1: Measurements of trophic morphology of 13 estuarine fish species selected in the present study.

Fish species	Total No.	Full GI tract	Empty GI tract	Total body length (cm)		Mouth aperture (cm)		Eyeball diameter (cm)		GI tract length (cm)	
				Mean	SE	Mean	SE	Mean	SE	Mean	SE
Harpadon nehereus	15	8	7	10.753	1.357	1.24	0.185	0.349	0.044	1.178	0.104
Lepturacanthus savala	8	5	3	16.224	0.658	0.48	0.052	0.524	0.037	4.468	0.242
Bregmaceros mcclellandi	18	8	10	6.205	0.492	0.405	0.065	0.185	0.019	2.38	0.143
Otolithoide pama	38	8	30	7.159	0.505	0.635	0.090	0.365	0.040	2.813	0.139
Arius arius	8	6	2	6.997	0.438	0.59	0.074	0.237	0.044	5.837	0.793
Stolephorus indicus	51	7	44	6.234	0.345	0.76	0.047	0.274	0.028	2.623	0.276
Cynoglossus lingua	8	8	0	8.765	0.546	0.3175	0.012	0.15	0.017	3.753	0.544
Coilia ramcarati	50	10	40	11.12	0.795	0.925	0.046	0.574	0.028	3.58	0.174
Pellona ditchella	9	8	1	9.48	0.383	0.655	0.044	0.458	0.020	3.44	0.087
Tetraodon cutcutia	7	6	1	5.553	0.411	0.24	0.017	0.647	0.047	2.553	0.298
Pisodonophis boro	6	6	0	5.203	1.206	0.243	0.028	0.13	0.013	2.143	0.111
Panna microdon	12	8	4	1.753	0.119	0.678	0.067	0.485	0.028	2.765	0.142
Coilia dussumeri	73	10	63	12.864	0.332	0.92	0.035	0.351	0.023	3.706	0.252

The percentage of the rest of the GI tract contents in all samples were parts of crab (5%), aquatic insect (~5%), plant part (3%), soil (2%), and unidentified debris (16%). The species-specific GI tract content percentage for each species was shown in Figure 2. The highest variation of food items (six different food types) were obtained from the GI tract content of *A. arius*, *P. boro* and *P. microdon*. Phytoplankton was found within the GI tract of every species except *H. nehereus*, *O. pama*, *C. lingua*, and *T. cutcutia*. Except *L. savala*, different zooplanktons were common within GI tracts of other fish and except *T. cutcutia* prawns were observed in the GI tracts of remaining 12 species. Only *L. savala* and *P. boro* had shown plant debris within their GI tract. Aquatic insect's body parts were available within the GI tracts of *H. nehereus*, *C. lingua* and *P. microdon* and body parts of small crabs were found among the GI tracts of *H. nehereus*, *O. pama*, *A. arius*, *P. boro*, and *P. microdon*. Except, *H. nehereus, L. savala*, *O. pama*, *P. ditchella, S. indicus* and *P. microdon* soil was detected within the GI tracts of the remaining species.

Niche breadth and overlap

It was observed that the niche breadths of these 13 fish species were significantly different (sum of signed rank, W=91.00, p=0.002***: statistically significant variation).

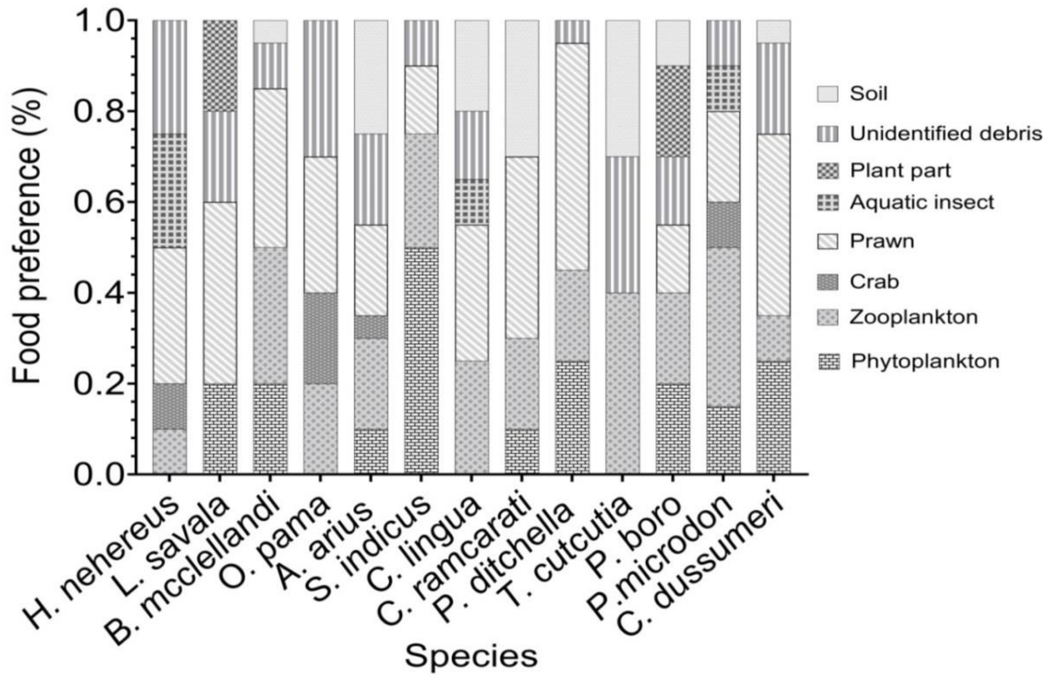

Figure 2: Percentage of food preferences in 13 estuarine fish species sampled from the Saptamukhi river of Indian Sundarbans, West Bengal, India.

Most of the species used around 4-5 types of resources for feeding among selected 8 types reflected by their niche breadth index (range from 3.54–5.40). Among fish, *A. arius* showed the highest value in Levin's niche breadth index indicating generalized feeding habits within its span of resources (Fig. 3). After performing the Pianka niche overlap index, it was found that many fish species showed higher niche overlapping with others (higher than 0.7 niche overlap index value), whereas few moderately overlapped with others (index value within 0.4-0.7) and in very few cases lower niche overlapping were found (index value less than 0.4) (Table 2). Most of the fish species showed higher Pianka niche overlap index values indicating their coexistence with great degrees of overlapping within the mangrove ecosystem of Indian Sundarbans.

MPs in fish and their relation with morphology

MPs illuminate green fluorescence after Nile red staining under a fluorescence microscope (Fig. 4). On the other hand, total of three different shapes of MPs were detected under stereomicroscope, among which MP-fibers were predominantly observed in all samples (Fig. 5). Film-shaped MPs were frequently found after the fiber type where the fragments were found to be not as much that numerous as the fibers.

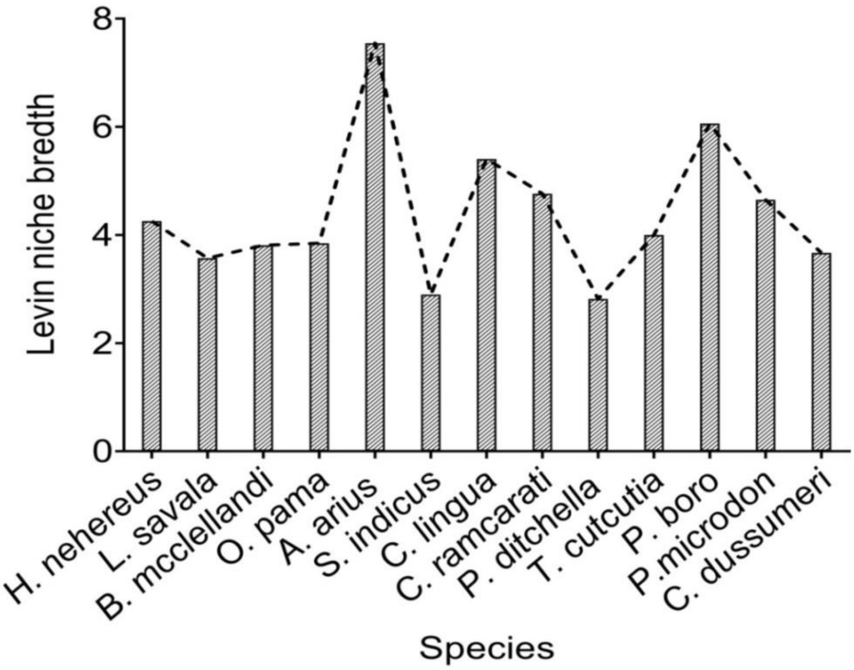

Figure 3: Levin niche breadth value of 13 estuarine fish species sampled from the Saptamukhi river of Indian Sundarbans, West Bengal, India.

Table 2: Pianka niche overlap value of 13 estuarine fish species showing different degrees of niche overlapping (>0.7=High niche overlap, 0.4-0.7= moderate niche overlap, <0.4= Low niche overlap).

	H. nehereus	***L. savala***	***B. mcclellandi***	***O. pama***	***A. arius***	***S. indicus***	***C. lingua***	***C. ramcarati***	***P. ditchella***	***T. cutcutia***	***P. boro***	***P. microdon***	***C. dussumeri***
H. nehereus	-	0.6627	0.4382	0.8293*	0.5586	0.3669	0.7719*	0.5273	0.7839*	0.4068	0.4331	0.5923	0.6986
L. savala		-	0.4403	0.6671	0.5583	0.4757	0.5976	0.6211	0.8521*	0.1945	0.483	0.4591	0.8889*
B. mcclellandi			-	0.5375	0.9784*	0.9223*	0.8811*	0.9132*	0.5605	0.7991*	0.6339	0.6734	0.6897
O. pama				-	0.6759	0.4722	0.7648*	0.5729	0.859*	0.5718	0.3415	0.6497	0.7379*
A. arius					-	0.9159*	0.9082*	0.9214*	0.6819	0.7811*	0.7641*	0.7068*	0.7874*
S. indicus						-	0.744*	0.7992*	0.5992	0.7413*	0.646	0.7251*	0.7309*
C. lingua							-	0.8853*	0.7469*	0.7412*	0.5709	0.7334*	0.195
C. ramcarati								-	0.6233	0.5323	0.6444	0.6653	0.8072*
P. ditchella									-	0.5081	0.4234	0.5904	0.9271*
T. cutcutia										-	0.4905	0.5302	0.4194
P. boro											-	0.4435	0.5496
P. microdon												-	0.6452
C. dussumeri													-

*= High niche overlap value

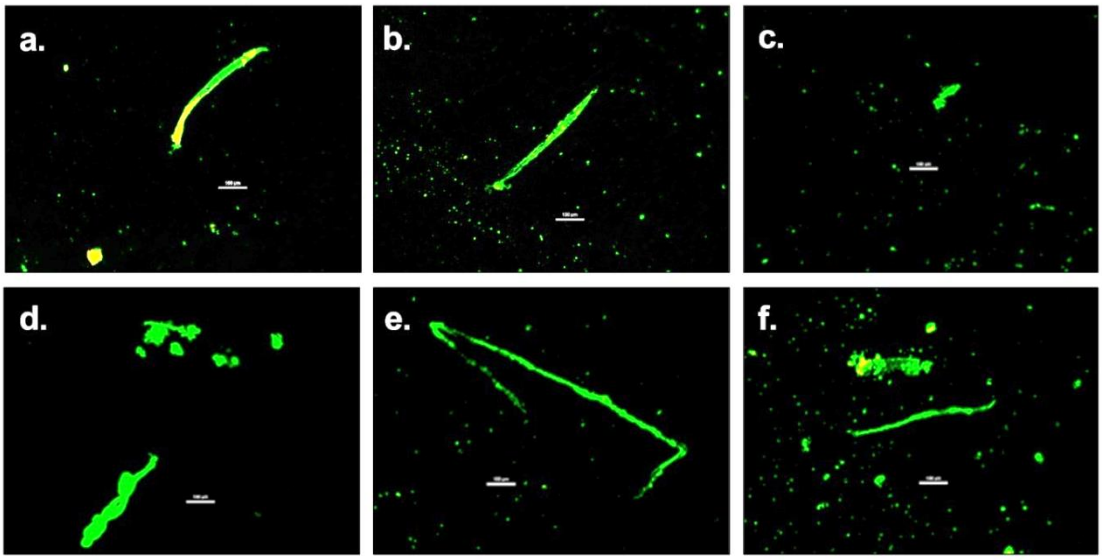

Figure 4: Representative fluorescence microscopic images of microplastics detected after stained with Nile red at excitation wavelength 430-490 nm and emission wavelength 510-560 nm.

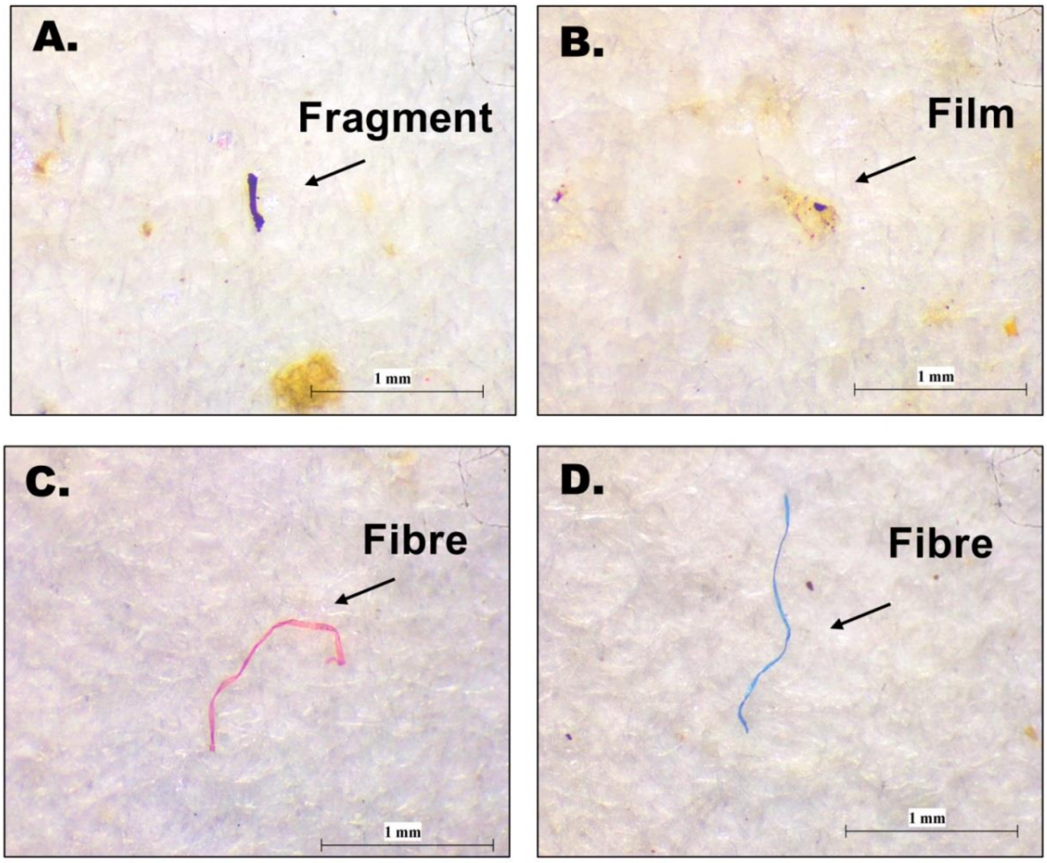

Figure 5: Various shaped stereo-microscopic pictures of microplastics observed in gastro-intestinal tract of 13 estuarine fish species. A=Fragment, B=Film and C, D=Fiber.

The fragment-shaped MPs were only observed within the GI tracts of *P. ditchella*, *T. cutcutia*, and *A. arius*. The presences of film-type MPs were almost ubiquitous in all fish species after fibers (Table 3).

Table 3: Different microplastic types found in 13 estuarine fish species during analysis. OB- Observed types of microplastics in the maximum amount.

Sl. No.	Species name	Microplastic types		
1	*H. nehereus*	-	-	OB
2	*L. savala*	-	OB	OB
3	*B. mcclellandi*	-	-	OB
4	*O. pama*	-	OB	OB
5	*A.. arius*	OB	-	OB
6	*S. indicus*	-	OB	OB
7	*C. lingua*	-	OB	OB
8	*C. ramcarati*	-	-	OB
9	*P. ditchella*	OB	OB	OB
10	*T. cutcutia*	OB	OB	OB
11	*P. boro*	-	OB	OB
12	*P. microdon*	-	-	OB
13	*C. dussumeri*	-	OB	OB

Approximately 130 MPs from all fish species were taken into account for the length measurement. The observed length of MPs found in 13 fish species is shown in Figure 6. The length of MPs in all samples ranged from 100µm to <5mm. A statistically significant variation (p<0.05) was observed in the length of MPs among 13 fish species (see Table S1). But, the length of MPs was found to have a positive correlation with body size and mouth aperture of fish but had a negative correlation with eyeball diameter and GI tract length. The result was obtained after performing Spearman rank correlation analysis (Fig. 7).

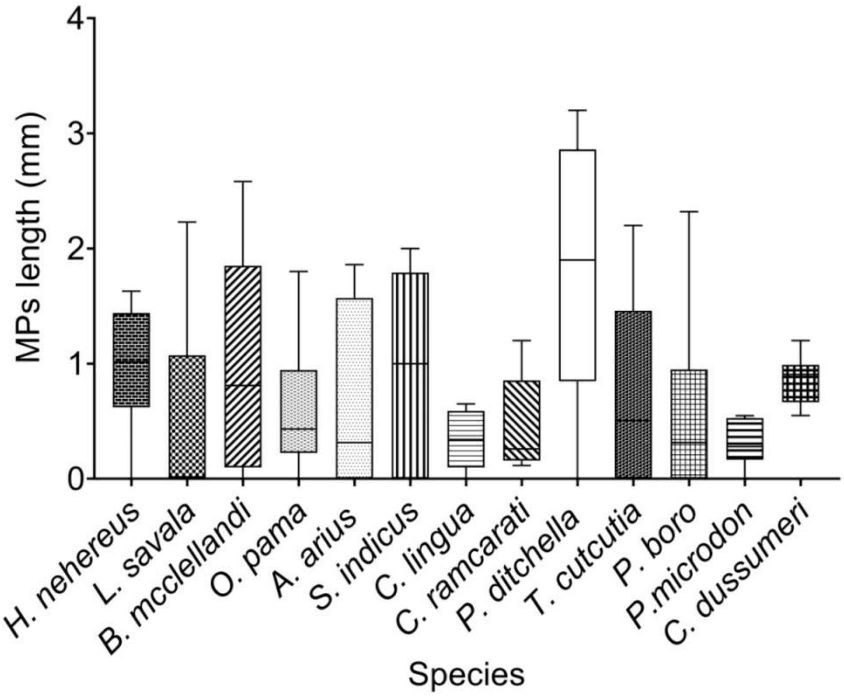

Figure 6: Box plot shows microplastic lengths (Mean ± SE) obtained from gastro-intestinal tract of 13 different fish species.

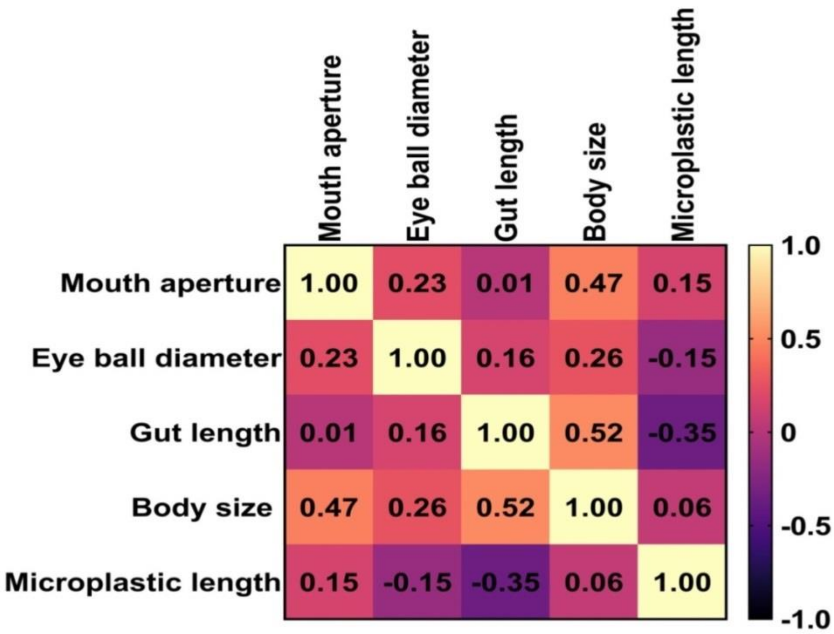

Figure 7: Spearman rank correlation analysis shows microplastic length is positively correlated with mouth aperture, body size but negative relation had found with eyeball diameter and gastro-intestinal tract length.

Discussion

The results revealed the presence of MPs in the gastrointestinal tract of 13 different estuarine fish species. The body length of fish was found to be positively correlated with MPs lengths which shows basic similarity with the study of MPs in fish from Han River, South Korea by Park *et al.* (2022). A recent study from Ulhas River estuary, India showed that mouth structure can selectively filter the MP particles and the different shape of MPs except fibers was trapped in the pharyngeal apparatus of mud-dwelling amphibious mudskipper, *Boleophthalmus dussumieri* (Kumkar *et al.*, 2021). It was also found from the above-mentioned study that the stomach fullness index (SFI), and the hepato-somatic index (HSI) had a negative correlation with MPs. This is quite similar to the present study as it reflects that the MPs length has a relationship with the mouth aperture of fish. The work of Vries *et al.* (2020) concluded that fullness of the GI tract was not at all related to plastic consumption which is also close to our observation as the present study also found that GI tract length has a negative correlation with MPs lengths. Therefore, we can hypothesize that the oral structures can selectively regulate the entry of different shapes and sizes of MPs. Nevertheless, the morphological parameters can also alter the abundance of MPs in the GI tract of fish but may not be able to selectively exclude them to enter the body of fish as the existence of MPs in the coastal environment are largely ubiquitous. The ingestion of MPs may not be voluntarily and deliberately done by the fish species. The study of Collard *et al.* (2017) showed that clupeiformes mistakenly intake the MPs which were similar to their natural prey. Even the structure of zooplanktons often showed apparent closeness with the shapes of MPs and that in turn compelled the fish to intake it erroneously, instead of its original food items (Ory *et al.*, 2017).

Even though the variation in MPs accumulation in the fish body can be altered with habitats, feeding habits (Koongolla *et al.*, 2022)and feeding behaviors (Kumkar *et al.*, 2021) of different fish species, the present study detects the presence of MPs within estuarine fish irrespective of their niche breadths and overlaps. All of the 13-fish species described in this study have different taxonomic classification, IUCN status, migration pattern, feeding habitat and commercial values, as stated in, Table S2 with varied percentages of GI tract content (that indicates their different feeding preferences), but MPs were detected in all samples. A previous study on freshwater fish from Beijiang and Pearl River Delta regions in South China observed that omnivorous fish possess twice MPs load as carnivorous ones (Wang *et al.*, 2020). The concentration of MPs had varied between demersal and pelagic fish of Campeche Bay, Mexico, which was related to plastic density in that particular habitat (Borges-Ramírez *et al.*, 2020). These above-mentioned studies were strictly done based on the variation of MPs abundance among different feeding habits and habitats, while our present study was focused on the presence of MPs in estuarine fish. Thus, the concentration of MPs in fish may be varied but, the presence of MPs inside their GI tract is irrespective of their dispersal strata and feeding habits which is obviously an alarming issue. As the present work also detected MPs within 13 fish species with different niche breadth and niche overlaps, it can be stated that the presence of MPs did not bother the feeding assemblage pattern or the feeding guild structure of those fish species. Though, the feeding composition was greatly varied with guild structure the MPs often crossed the niche barrier and entered within the fish body which may create great concern about bio-accumulation and tropic transfer of these pollutants. From Bangladesh part of Sundarban, Sarker *et al.* (2022) conceded the trophic transfer of MPs from zooplankton, fish to higher predators (*e.g.* Turtle). Another study by Sultan *et al.* (2023) also from Bangladesh part of Sundarban detected the presence of MPs from the muscle and gastrointestinal tract of twenty estuarine fish and shellfish species in higher concentrations and also comments on the exposure of MPs to humans. Various aquatic and terrestrial organisms (including birds) consume fish as their food source, therefore it is said that the MPs might be transferred into their body by following successive food chains.

The Sundarban Biosphere reserve, the world’s largest mangrove delta shared by India and Bangladesh, and both of the countries have been placed under twelve mismanaged plastics waste nations (Jambeck *et al.*, 2015). Thus, becoming a cesspit of plastic pollution

(Adyel and Macreadie, 2022) with wastes coming from upstream rivers and adjoined coastal bays. Our study found the ubiquitous presence of microfiber-type MPs in all fish specimens. Micro-fiber type of MPs is predominantly (67.3%) observed in marine biota (Ugwu *et al.*, 2021) globally, which can validate our work. The studies of MPs from mangrove estuaries of different countries of Indian subcontinent also concluded the predominance of fiber in the body of fish (Table S3). In Indian or global context, fishing gears, tourism or recreational activities (Vaid *et al.*, 2021), and textile sources including washing and dyeing (Periyasamy and Tehrani-Bagha, 2022) are some of the biggest contributors of MPs in aquatic environments. MPs reported from ballast waters of vessels often serve as 'Microplastic hotspots' and help in spreading (Naik *et al.*, 2019). On a different note, various metals, antibiotics, toxic chemicals, pathogenic bacteria (*Vibrio cholerae*), and harmful algal bloom (HAB)-forming dinoflagellates (Naik *et al.*, 2019) across the continents hitchhikes on MPs particles to cover large distances, often transcontinental distances (Andrade *et al.*, 2021). Huge domestic and industrial wastewaters are dumped in the estuarine environment of Indian Sundarbans due to huge anthropogenic pressure and industrial runoff, which may be the primary potential sources of MPs (Sadia *et al.*, 2022). Due to the large specific surface area and hydrophobic surface of MPs, the persistent organic pollutants, metals, and pathogens are easily adsorbed on the surface of MPs (Zhang and Chen, 2020).

During the field visit, the observer group noticed that earth (soil) and sand-filled woven plastic fiber bags (Polypropylene polymer) are used instead of biodegradable gunny bags (Jute derived) for the sake of maintenance and conservation of river embankments in various rural parts of Indian Sundarbans (Fig. 8). In accompanying woven bags, polypropylene sheets are also being used for embankment restoration from such belief that it can increase slope stability by protecting the eroding soil in Sundanban area (Bera, 2012). But due to the course of regular tidal action, poly-fibers of woven bags and plastic sheets are directly entered into this fragile ecosystem abundantly. The size analysis of the degraded plastic fibers of those bags showed all of the fibers are less than 5 mm in breadth. Similar polypropylene and polyethylene made woven bags were also used in mollusk aquaculture farms and become a major source of the MPs in Qinzhou Bay, China (Li *et al.*, 2018).Therefore, it may be possible that degraded small-sized plastic fibers released from earth and sand filled plastic bags may often contribute a portion of MPs in Indian Sundarbans.

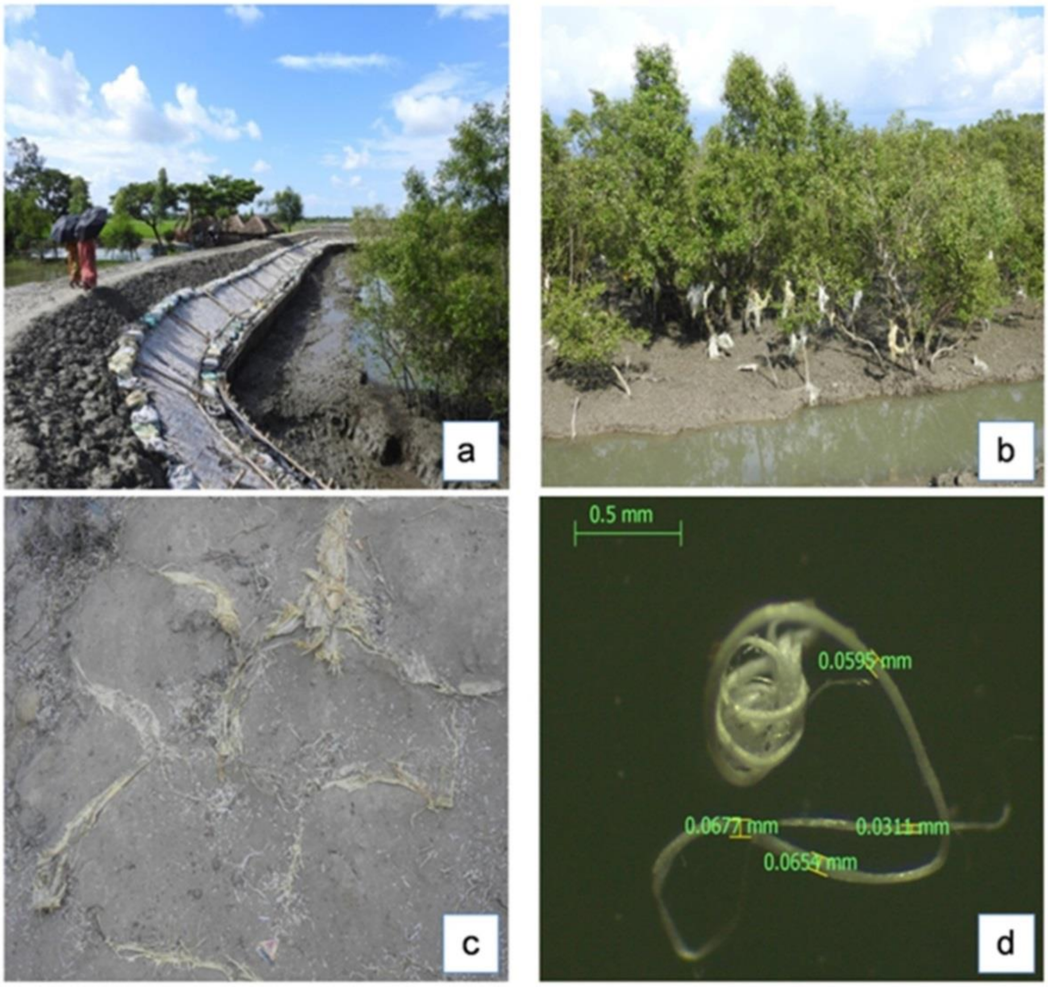

Figure 8: (a) Earth and sand-filled woven polymer bags ("Bosta" in the local Bengali language) are used very commonly for the conservation of the embankment of rivers in Sundarban. b) Earth and sand material gradually washed out and the bags enter into the ambient media, spreading throughout the ecosystem by tidal action. (c) By the process of weathering, large ploy-fiber bags get fragmented and become a continuous source of microplastics. (d) Fibers of the degraded bags were observed under stereomicroscope and breadths were measured.

But, to prove this speculation, future research on quantification and polymer characterization of MPs in various mangrove-associated fauna and their ambient environment are highly recommended.

In conclusion, the study reports the presence of MPs particles in the GI tract of 13 estuarine fish species from Indian Sundarbans. The fiber-shaped MPs were mostly observed in all samples followed by films and fragments. The length of MPs had a positive correlation with the body length and mouth aperture of fish whereas eyeball diameter and GI tract length were not at all related to the length of MPs. The presence of MPs were found in fish irrespective of their niche breadth and niche overlapping. Along with other marine and land-based sources, field observation summarized that rampant use of earth and sand-filled plastic-made woven bags for restoration of river embankment might be a potential local source of MPs in Indian Sundarbans.

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