frontiers | Frontiers in Marine Science

BRIEF RESEARCH REPORT
published: 26 May 2022
doi: 10.3389/fmars.2022.914391

Microplastics in Demersal Sharks From the Southeast Indian Coastal Region

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OPEN ACCESS

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Specialty section:
This article was submitted to Marine Pollution, a section of the journal Frontiers in Marine Science

Received: *06 April 2022*
Accepted: *20 April 2022*
Published: *26 May 2022*

Citation:
Janardhanam M, Sivakumar P, Srinivasan G, Sivakumar R, Marcus PN, Balasubramaniam S, Rajamanickam K, Raman T, Singaram G and Harikrishnan T (2022) Microplastics in Demersal Sharks From the Southeast Indian Coastal Region. Front. Mar. Sci. 9:914391. doi: 10.3389/fmars.2022.914391

Microplastic (MPs) contamination has emerged as a serious worldwide issue. Human activity, commercial enterprises, and fishing are concentrated around the seashore, causing high levels of MPs contamination in coastal and marine organisms. When it comes to their vulnerability to MPs ingestion, sharks are least studied organism. The objective of this study is to investigate MPs accumulation in sharks collected from the Southeast Indian coastal zone (Bay of Bengal). We present evidence of MPs ingestion in demersal sharks caught by the trawlers during trawling operations in marine waters beyond a depth of 80 m in the Southeast India coast. Shark samples were also checked for any gender or size differences in contaminant loading. Gill and gut (digestive tract) were examined in 40 sharks and 82.5% of samples contained at least one MP particle. The average number of MP particles was found to be 4.67 items per individual shark; the gastrointestinal tract showed more MPs than the gills. The majority of the MPs were blue and pale white followed by black and transparent particles with diameters ranging from 0.5 to 2 mm. The fibre fragments were prevalent in the intestines of the shark. Fourier Transform Infrared (FT-IR) spectroscopy revealed that the bulk of polymers were polypropylene (PP), polyacrylamides (PA), and polyethylene (PE). MPs contamination poses an unknown level of harm to shark species. The present study revealed the first scientific data of MPs and associated fibre ingestion in shark species in their habitat in the Bay of Bengal.

Keywords: shark, microplastics, marine pollution, fish, fisheries, aquaculture, bay of bengal

INTRODUCTION

The microplastics (MPs) pollution in oceans is a serious and ongoing global environmental issue. Annual worldwide plastics output has more than quadrupled over the previous half-century, with a 2016 estimate of > 335 Mt. (Galgani et al., 2015; Plastics Europe, 2018; Goswami et al., 2021). Plastics are widespread in both terrestrial and aquatic environments (including freshwater, estuaries, coastal, and marine), and they are increasingly becoming a major cause of marine pollution in addition to seawater quality (Jha et al., 2015), metal toxicity (Satheeswaran et al., 2019), and microbial contamination (Dheenan et al., 2016). The MPs pollution may threaten the marine life when they are ingested and have far-reaching effects on ecosystems (Nelms et al., 2018). Plastic manufacturing and usage have expanded considerably in recent years due to cost-effectiveness and economy, resulting in greater use of synthetic plastic polymers in terrestrial and aquatic environments (Thompson et al., 2009). Non-biodegradable compounds in the aquatic environment especially in the coastal regions, in particular abandoned fishing gear, carry bags, synthetic packaging materials, and plastic coverings, are harmful to marine life (Kaladharan et al., 2020). The MPs are small pieces of plastic with a diameter of 5 mm or less and are deposited in marine ecosystems (Sharma and Chatterjee, 2017). The MPs may be ingested by a wide range of marine species, including corals, plankton, marine invertebrates, fish, and whales, and are eventually transferred to the food chain (Thompson et al., 2009; Nelms et al., 2018; Kaladharan et al., 2020). These plastic polymers not only endanger marine life directly, but they also have an indirect impact on the environment by adsorbing other marine contaminants including organic and inorganic compounds. Further MPs quickly absorb hydrophobic pollutants from water systems due to their large surface area compare to that of any pollutants (Joo et al., 2021). As a result, MPs contamination is an issue primarily because of its negative impact on the health of the marine environment and biota.

MPs have been demonstrated to be ingested directly by fish and pelagic invertebrates due to their physical resemblance to their food (Phillips and Bonner, 2015). According to global studies on marine MPs, they are more prevalent near the shorelines, and a definite association between the size of terrestrial pollution and the degree of marine pollution has been documented (Harris, 2020). Plastic debris that finds its way to and is dumped in the water is broken by physical, chemical, and biological processes, resulting in the production of MPs in coastal regions (Thompson et al., 2009). Because of the broad dispersion of MPs in aquatic habitats and along the shorelines, MPs pollution has gained relevance as an attractive scientific issue over the last decade (Galgani et al., 2015).

MPs have been discovered in the stomachs of a variety of marine organisms, including invertebrates and vertebrates. We have a better knowledge of the implications of MPs consumption in the preceding group, with evidence revealing dose-dependent detrimental effects on feeding behavior, development, reproduction, and lifespan (Issac and Kandasubramanian, 2021). Despite the fact that elasmobranchs are largely understudied in terms of plastic pollution risks, their susceptibility to MPs ingestion has been documented (Parton et al., 2020). Because of their feeding habits or habitat utilization, some elasmobranch species are thought to be particularly vulnerable to MPs ingestion. Filter-feeding species (such as whale sharks and basking sharks) are considered to be more sensitive to MPs ingestion in habitats that overlap with regions with high amounts of plastic pollution. Many shark species, on the other hand, are non-filter feeders, preying on larger organisms including fish, crabs, marine turtles, and marine mammals, all of which have been proven to ingest MPs (Parton et al., 2020).

The Bay of Bengal is a habitat for a variety of shark and ray species, including tiny to medium-sized demersal sharks. These species may be found at depths ranging from 5 to 900 m and favor benthic settings (Tyabji et al., 2020). They feed on a range of small teleost fishes, crabs, and cephalopods. Because of their habitat preference, they are usually taken as by catch in demersal fisheries; nevertheless, specialist fisheries for these species exist. MPs exposure to demersal shark species is currently understudied, with just a few examples of plastic ingestion, mainly in and around the Mediterranean Sea (Cózar et al., 2015). However, there have been multiple studies of plastic ingestion in bony fish, with ingestion rates varying from 0 to 100% have been reported (Thiele et al., 2021). To the best of our knowledge, there are no studies on MPs accumulation in sharks caught as commercial fish in the southern coastal region of the Bay of Bengal. The findings of the first thorough investigation on MPs ingestion in sharks in the Bay of Bengal are discussed in detail in the present study.

MATERIALS AND METHODS

The samples for this study were collected from the southern coastal region of the Bay of Bengal, India. The shark fish [*Rhizoprionodon acutus* (Rüppell) 1837] was taken as a by catch in a demersal fishery trawling in and around the Bay of Bengal at a depth of 80 m and landed in the fish harbour of Royapuram, Chennai. The samples were collected during the period of November 2020, June-2021 and November, 2021. The number of shark collected during different sampling period is given in **Table 1**. Forty sharks were investigated, comprising 16 males and 24 females. All of the sharks used were subjected to standard morphometric measurements.

The standard dissection method was followed to remove the complete digestive tract from the individual shark samples (Boerger et al., 2010; Davison and Asch, 2011) which were then placed in a watch glass and weighed, the intestines after

TABLE 1 | Microplastics (MPs) in shark collected from Royapuram Fish landing centre, Chennai.

Period of Collection	Species	Total Length(cm)	Wet Weight (g)	Total No. of Shark	No. of MPs	Average Item/per Shark
November- 2020	*Rhizoprionodon acutus*	40.90 ± 5.60	280.9 ± 29.60	16	72	4.50 ± 1.59
June-2021	*Rhizoprionodon acutus*	38.20 ± 4.08	260.8 ± 32.60	11	63	5.72 ± 1.61
November-2021	*Rhizoprionodon acutus*	32.78 ± 1.05	242.8 ± 20.95	13	52	4.00 ± 1.82

weighing were digested with a solution of hydrogen peroxide (30% H_2O_2) at 60°C in a glass beaker (Rochman et al., 2015). MPs were detected by observing the sample under a NIKON stereoscopic microscope fitted with a digital camera. The total number of MPs found in each sample was recorded and classified by type, color, and size (maximum length) (Wessel et al., 2016; Jung et al., 2018). Samples were covered with foil prior to recovery, storage, intestinal digestion, and visual identification.

Non-plastic components of various size classes were manually sorted under a stereomicroscope and removed from each sample using stainless steel forceps prior to segregation (Karthik et al., 2018). Using the method described by Free et al. (2014), MPs and their form were recognized. Granules (spherical, cylindrical particles), film (thin, soft, transparent particles), fragments (small angular, irregular shaped particles), fibre/line (elongated, thin, straight particles), and foam (lightweight particles with spongy texture) were the five categories (Robin et al., 2020). The plastic particles were then divided into seven colour categories: blue, transparent, green, pale white, black, red, and yellow (Young and Elliott, 2016).

SEM AND FTIR ANALYSIS

In most cases, MPs are identified visually before a polymer type is identified. Although smaller particles can be seen with the naked eye, microparticles can be seen clearly with a scanning electron microscope (SEM), and the images were captured using these instruments (Tudor and Williams, 2004). Fourier Transform Infrared Spectroscopy (FT-IR) is a technique used to determine the type of plastic substances present in the observed samples. These methods rely on the energy transmittance of polymer particles' characteristic functional groups. A Bruker ALPHA FT-IR spectrometer with a single reflection diamond Attenuated Total Reflectance (ATR) accessory was used to collect the infrared spectra. A fixed load was applied to a sample of approximately 1 g placed directly on the internal reflection element (IRE) to ensure full contact with the diamond ATR. For each sample, twenty-four scans were averaged at a resolution of 4 cm^{-1} within the wave number range of 400 to 4000 cm^{-1} (default Bruker OPUS 6.5 software settings), and the resulting averaged spectrum was recorded. To confirm the significance of differences in MPs particle numbers between sampling periods, a statistical analysis was performed using the SPSS software (Version 20.0). The T-test was used to differentiate between gender and size groups. A one-way analysis of variance (ANOVA) test was used to determine whether there was a significant difference between the sampling periods. Results from Pearson's correlation co-efficient two-tailed test revealed no correlation between MPs and fish length/weight.

RESULTS

In total, 40 sharks were studied for the presence of MPs, of which 40% were males and 60% were females. The entire sample caught was a mixture of adults and juveniles. Fibres, granules, fragments, films and foams are the different types of MPs identified in sharks (**Figure 1C**). Among 40 sharks analysed in this study, 82.5% of the samples (33/40) contained at least one MP particle. Among the MPs, the number of fibres was about 42%, followed by fragments and granules with 26 and 20%, respectively. Film (5%) and foam (7%) also constituted the total MPs in sharks (**Figures 1A–C**). No significant variations in number of MPs were found between different sampling periods ($p < 0.058$). Similarly, t-test analysis revealed no significant differences in MPs accumulation between male and female sharks over the sample periods ($p < 0.2735$ for November 2020; $p < 0.611$ for June 2021and $p < 0.572$ for November 2021). Additionally, we also analysed microplastic size variations and accumulation and found no significant difference ($p < 0.245$).

Fibres ranged in length from 0.3 to 10.0 mm and had an average length of 3.2 ± 2.2mm. About 75% of the MPs were less than 2 mm in size (**Figures 2A–H**). The vast majority of fibres were blue and pale white (54.0%) in colour, with the remaining colours including transparent, black, red, yellow and other each making up to 13% of maximum [**Supplementary Figure 1A**, (SF 1A)]. Among the organ the gut contained 92% of MPs and gill 8% (**Figure SF1B**). Fibres larger than 5 mm (n = 4) were considered here as macroplastics and were excluded from the analysis. The SEM investigations of MPs of different types are given in **Figures 2I–L**. The surface morphology of fragments, fibre and granules were visible through the SEM investigations. The presence of polyethylene (PE), polyamide (PA) and polypropylene (PP) in the FTIR spectrum was determined by their characteristic wave numbers. **Figure 2M** show representative ATR-FTIR spectra for the three distinct kinds of particles. PE, PP, and PA were identified as the principal categories of MPs based on sample analysis. The existence of PE was determined by the presence of typical wave numbers ranging from 1462 to 1465 cm^{-1}. Similarly, PP was found using characteristic wave numbers in areas of 1737cm^{-1} and 1745 cm^{-1} that showed CH_2 stretching (Veerasingam et al. (2016).

DISCUSSION

MPs pose critical threat to marine species due to the fact that these animals tend to swallow these compounds present in water leading to widespread physiological effects (Barnes, 2002; Dharani et al., 2003). In order to understand the effect of anthropogenic activities on marine ecosystem, more research needs to be focussed on the prevalence, characteristics and residence time of MPs in aquatic food webs. Due to their small size, they are less likely to damage a fish's digestive tract. However, studies of negative physical and biochemical effects of MPs on fish physiology, especially in coastal waters of India, are limited.

Our research is the first to show the presence of MPs in sharks caught in the Bay of Bengal. Despite the fact that there were no significant variations in MPs uptake across the sharks sampled (seasonal, gender and size dependent), the study provides an

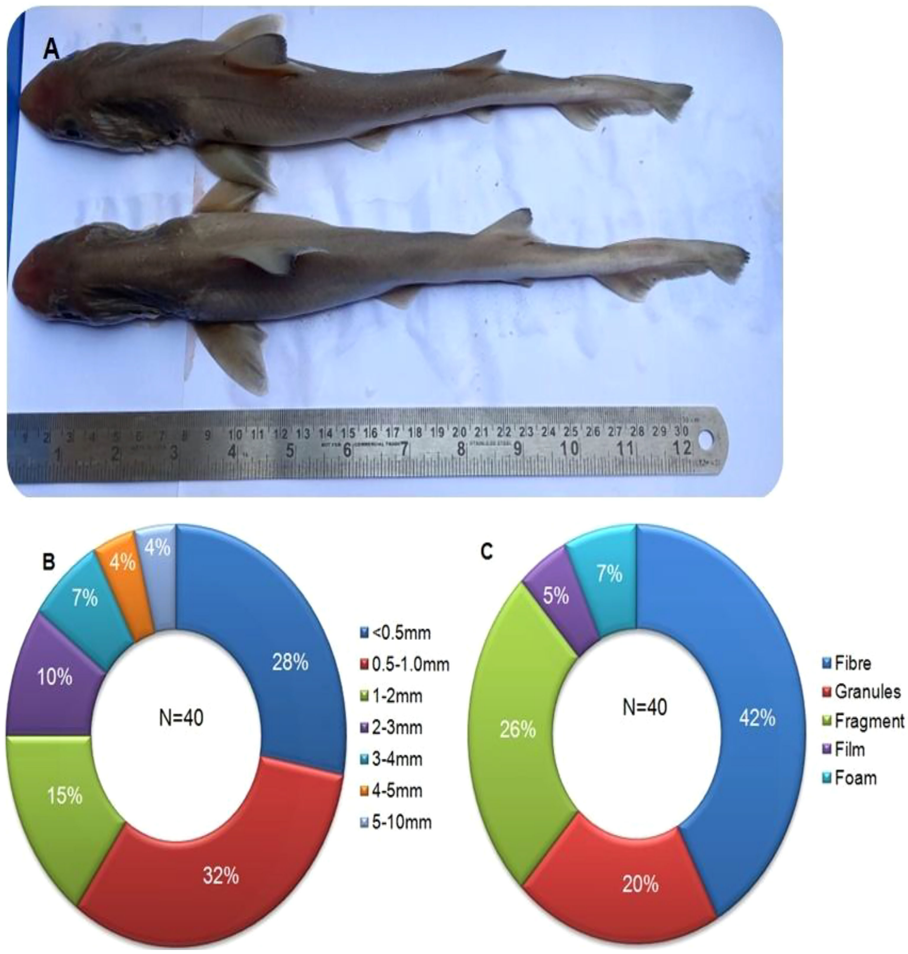

FIGURE 1 | Shark fish samples collected and analyzed for microplastics **(A)** Juvenile male shark **(B)** Pie diagram showing percentage of different size of microplastics **(C)** Pie diagram information showing percentage of different type of microplastics.

essential empirical baseline for future research into contaminant levels in shark fish habitat in Indian coastal and marine regions. From these we could predict that fish habitat in shore regions of South India's coast with poor water quality may be exposed to high amounts of plastic pollution (Veerasingam et al., 2016; Karthik et al., 2018; Robin et al., 2020). Although we haven't investigated its impact on health of sharks, the existence of these particle pollutants suggests their pervasiveness in the marine ecosystem, especially closer to the shores, which can probably cause long term health effects on fishes including sharks number of such marine contaminants is projected to rise as global plastic manufacturing and its ubiquity in everyday items increase (Villarrubia-Gómez et al., 2018).

Very few studies across the world have demonstrated the existence of MPs in shark (Valente et al., 2019; Parton et al., 2020). Patron et al. (2020) reported the presence of MPs in numerous species of shark in the UK's North-East Atlantic coastline area. In our study, over 92% of all sharks examined had at least one MP particle in their digestive system, and 8% showed the presence of MPs in the gills. We also found no gender differences in MPs accumulation during the sampling period, nor any notable changes in MPs accumulation based on shark size. These findings clearly show that MPs accumulation was found in the majority of the samples regardless of size or gender or sampling period. The percentage of sample with MPs was still very high and alarming. The quantity of MP fibres was found to be more in the case of larger sharks compared to smaller ones, indicating the ability of larger sharks to ingest more food and thus indirectly more MP accumulation. For this study, sample collection was done deep in the waters of Bay of Bengal by fishermen and hence we were not able to accurately pinpoint the location or habitat from which these sharks were caught and hence this aspect needs to be looked further. Our results also lead us to hypothesize that nutrition could be an important influencing factor on MPs accumulation in sharks.

The type of MPs in the marine ecosystem could help one to understand the possible sources, and fate of these MPs. The use of FT-IR spectroscopy to analyse environmental materials is a

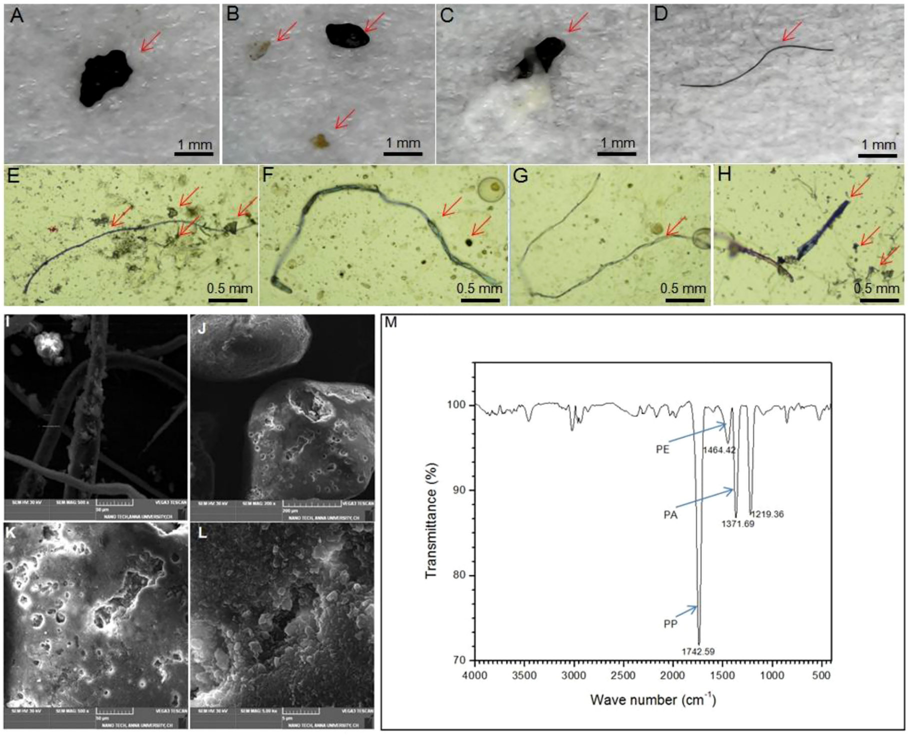

FIGURE 2 | Presence of different types of microplastics in Shark **(A–C)** Fragments and film **(D–H)** Fibre and granules **(I–L)**. SEM images of microplastics **(I)** Fibre **(J, K)** Fragment **(L)** Granules **(M)**. FTIR spectra representing PE, PA and PP type.

reliable approach for detecting polymer make-up and should be central to any future research. The polymers we discovered are similar to the polymer variety of MPs found across the world, with polypropylene being one of the most prevalent polymers found. The presence of PE and PP in the fish gut was verified using FT-IR analysis and all three types were found in the gut of the sharks, suggesting that microplastics of all kinds are an environmental problem. Veerasingam et al. (2016) reported similar spectra for microbeads while researching MPs on the Chennai coast. Together these results clearly show the presence of microplastics in the waters of Bay of Bengal which were revealed by their significant presence in the gut of demersal sharks that were collected during routine fishing. However, MPs presence in these sharks was not influenced by animal size, gender or season and hence this particular kind of pollutant could pose a year round problem for marine animals. In addition, presence of MPs of PE, PP and PA types suggests that these pollutants are widespread and their persistence could pose enormous challenges to all marine ecosystems.

CONCLUSION

Our study shows the presence and accumulation of microplastics in the sharks from Bay of Bengal. The size, gender of the animal as well as the season of collection did not have any apparent influence on MPs accumulation in sharks, indicating that MPs could be persistent pollutants in marine ecosystem similar to persistent organic pollutants. Furthermore finding of all the three types of MPs in the sharks indicate that MPs of all kinds are serious marine pollutants. Since MPs in marine waters is a result of anthropogenic activities, this study can be used as a reference for future research on MPs pollution in commercial fish in coastal and estuarine region, as well as for governmental organisations developing management strategies and policies against microplastic pollution.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because it's a commercial fish.

AUTHOR CONTRIBUTIONS

MJ, PS, GSrin, RS, PM, sample collection, laboratory analysis, methodology validation, data processing, and writing-original draft. SB and KR, conceptualization, sampling design, data validation, and manuscript review. TR, manuscript corrections and critical suggestions. GSing and TH, reviewing the manuscript, suggestion, and project administration. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors thank Head, Research department of Zoology and also the Principal Pachaiyappa's College for Men, Chennai- 600 030, and Head, Research department of Zoology and also the Principal Government Arts College for men (Autonomous), Chennai-600 035 India for extending their support through the experiment.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.914391/full#supplementary-material

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