In the jaws of pollution: quantifying micro-plastic and heavy metal burden in commercial sharks in the Andaman Islands

A Thesis

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by

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Certificate

This is to certify that this dissertation entitled **In the jaws of pollution: quantifying micro-plastic and heavy metal burden in commercial sharks in the Andaman Islands** towards the partial fulfilment of the BS-MS dual degree program at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Sumit SurenderKumar Yadav** at **Indian Institute of Science, Bangalore** under the supervision of **Dr. Kartik Shanker, Professor, Center for Ecological Sciences** during the academic year 2023-2024.

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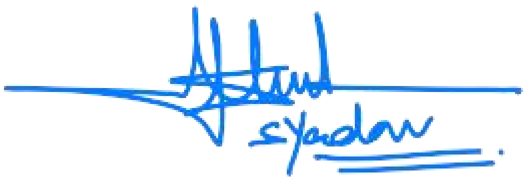
This thesis is dedicated to my parents and their unwavering faith.

Declaration

I hereby declare that the matter embodied in the report entitled "**In the jaws of pollution: quantifying micro-plastic and heavy metal burden in commercial sharks in the Andaman Islands**" are the results of the work carried out by me at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore, under the supervision of Professor Kartik Shanker for the partial fulfilment of the BS-MS dual degree program at Indian Institute of Science Education and Research, Pune and the same has not been submitted elsewhere for any other degree. Wherever others contribute, every effort is made to indicate this clearly, with due reference to the literature and acknowledgement of collaborative research and discussions.



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Abstract

The world is witnessing an unprecedented level of pollutants in the oceans today. With this rise in pollution, there is an increase in concentrations of contaminants such as heavy metals and micro-plastics, which pose a threat to marine ecosystem health and function as well as human health. This study focussed on the quantification of these heavy metal and micro-plastic contaminants in the most commonly landed shark species in the Andaman and Nicobar Islands, India. Tissue samples were collected and analyzed for contamination levels to identify habitat-specific vulnerabilities in contaminant accumulation. Results showed a wide range of micro-plastic types, with fibres and fragments being the most common while white/transparent and blue colours were observed to occur more frequently. However, no significant differences were found in contamination levels across age, sex, or tissue types of the sampled sharks. Similarly, no significant difference was observed in contamination level between habitats, but significant differences were observed between certain types of micro-plastics across two different size categories in reef-dwelling species.

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Contributions

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-	Software
-	Validation
Shawn Dsouza, Garima Bora	Formal analysis
-	Investigation
Princia Dsouza	Resources
-	Data Curation
-	Writing - original draft preparation
Garima Bora, Shawn Dsouza, Prof. Kartik Shanker	Writing - review and editing
Shawn Dsouza, Garima Bora	Visualization
Prof. Kartik Shanker	Supervision
Garima Bora, Chandana Pusapati	Project administration
Chandana Pusapati	Funding acquisition

This contributor syntax is based on the Journal of Cell Science CRediT Taxonomy[1].

[1] https://journals.biologists.com/jcs/pages/author-contributions

1. Introduction

The world's oceans today face an unprecedented pollution crisis with an incessant increase in the concentration of contaminants such as heavy metals and micro-plastics due to unregulated spillage and waste mismanagement. This surge in pollutant levels threatens the delicate balance of marine ecosystems and jeopardises the health and survival of countless species. Furthermore, contamination can also lead to cascading effects, such as threatening species distributions, biodiversity, and ecosystem functioning. Hence quantifying their presence and understanding their impact is of utmost importance not only for the conservation of the affected marine life but also for public health as a vast number of humans depend on the oceans for their livelihood and consumption.

1.1 What are micro-plastics?

Micro-plastics (MPs here on) are ‘any synthetic solid particle or polymeric matrix with size ranging between 1 µm to 5 millimetres regardless of its shape and is of either primary or secondary manufacturing origin while being insoluble in water’ (Frias and Nash 2019). MPs can be classified into two categories based on their origin. Primary MPs are plastic particles or fragments that are already small enough to be categorized as MPs before they enter the ocean, often intentionally manufactured at the size class mentioned above for industrial use cases such as in the case of MP pellets utilized in the cosmetic industry (Bhuyan 2022). Secondary MPs result from the breakdown of much larger pieces due to various forms of weathering through their exposure to wave action, wind abrasion, UV radiation, and sometimes even the biological agents such as through organisms nibbling and chewing them whilst their attempts to consume them (Dawson et al. 2018; Conkle, Báez Del Valle, and Turner 2018). MPs can be categorized based on their colour and shape. Some more commonly encountered shapes include micro-fibres, pellets, fragments, and films. Fibres tend to be uniform across their breadth, pellets or beads tend to be rounded off of their edges, and fragments tend to be highly irregular in shape.

1.2 Effects of MPs as pollutants

The presence of MPs can have a huge effect on the environment. They can degrade the benthic habitat through smothering, where plastics form a thin layer on the top of the benthos (Critchell and Hoogenboom 2018). Moreover, they can get trapped between coral reefs which leads to an increase in their persistence in the region, thereby also increasing the probability of uptake by organisms mistaking them for diatoms (Ding et al. 2019). They can also smoothen phytoplankton in pelagic waters, thereby compromising the foundation of the food chain (Mbugani, Machiwa, Shilla, Joseph, et al. 2022).

Properties such as the density of the plastic also play a huge role in the kind of organisms that are more likely to come across them. The majority of the MPs are less dense than water, however flocculation can lead to the creation of much denser chunks of MPs which can then sink to the bottom, making them available for all possible habitats one can encounter in a marine ecosystem (Mbugani, Machiwa, Shilla, Joseph, et al. 2022) For example, demersal fish would come across a greater number of dense MPs while pelagic species would be prone to low-density plastics. Filter and deposit feeder fish, due to their non-selective feeding strategies tend to be more vulnerable to MP ingestion when compared to predatory fish (Bhuyan 2022; Critchell and Hoogenboom 2018). Ingestion of MPs can be active, where the organism confuses the plastics for potential prey and thereby actively consumes it, or passive where MPs get absorbed through the gut wall due to their ingestion during particle filtration or through a consumed organism previously carrying the plastic particles (Foley et al. 2018).

Once ingested, MPs can have a wide range of effects. Ingestion can lead to blockages in the digestive tract, thereby affecting nutrient absorption, creating a false sense of fullness, or choking the organism, which can lead to death (Critchell and Hoogenboom 2018; Foley et al. 2018). MPs can get embedded into the lining of the GI tract from where they can get taken up by the organism, they can rupture the walls of the intestines, thereby changing the morphology of the wall and affecting the presence of the microbiota present in the tract (Mbugani, Machiwa, Shilla, Joseph, et al. 2022; Mbugani, Machiwa, Shilla, Kimaro, et al. 2022). The extent of MP assimilated by the organism also tends to be a function of particle size. Small-sized particles tend to get assimilated into the GI tract within a week of exposure. The presence of MPs can lead to inflammation stress, potentially weakening the immune system of the organism and making it prone to other life-threatening diseases (Bhuyan 2022). If sufficiently small then the particles can be taken up by the organism and assimilated into other organs via the circulatory and lymphatic systems. MPs also work as vectors, transporting germs and toxins deep inside organisms, and protecting them from immune responses.

Ingestion of MPs also tends to have complex behavioural and hormonal effects on organisms. These effects can vary from changes in levels of restlessness to an increase in territorial behaviour. Fish displayed a greater number of aggressive encounters, with the aggressor travelling much larger distances as observed (Critchell and Hoogenboom 2018)). Such behavioural changes can lead to impaired growth and maturation, reproductive ability, or survival.

1.3 What are Heavy Metals?

Heavy metal (HM here on) refers to a particular group of elements, such as mercury(Hg), and lead(Pb), and their compounds. The term itself is however a conflicted one as it can imply very different means of categorization in different contexts. In general biological terms, it is a categorization based on the density of the metals, which only happens to be a relevant property for a few studies (Duffus 2001). A more accurate definition in biological terms would refer to them as metallic chemical elements with relatively high density when compared to more abundant metals present in living organisms, and which also possess the ability to be toxic and/or poisonous to the organism due to them being non-degradable and their tendency to accumulate in biological systems, through which they can lead to the detriment of organisms natural functioning with potential to affect broader ecosystem functions due to small variations in their concentration (generally in ppm or ppb) (Nkwunonwo, Odika, and Onyia 2020).

In the context of the present study, the term refers to metallic elements that fall under the definition mentioned above with the addition that they tend to get pumped into the natural environment through anthropogenic activities in much larger concentrations than what they naturally exist in. These metals tend to play essential roles in ecological processes but tend to become hazardous pollutants at elevated concentrations.

1.4 Effects of Heavy Metals as Pollutants

Broadly these metals tend to induce mutagenesis and possess carcinogenic properties along with their ability to accumulate in the upper rungs of the food chain. however, "Heavy metals" tends to be a broad term, which can often mask the complexity of the effects as well as the specific risks associated with individual metals. Different metals tend to have different properties and behave differently in ecological systems and hence it becomes important to consider the individual and collective effects they can have.

Arsenic for example can disrupt cellular respiration and mitosis, along with causing severe degeneration of the kidney and the liver. Excess copper can cause an oxidative stress response, which can lead to cell death while causing poor immune response and fertility problems. Copper can also be neurotoxic and lead to olfactory disruption. Lead can cause impotency and growth retardation in organisms. Prolonged exposure in fish leads to distortion of the liver and gill cells. Mercury can cause abnormal structural and biochemical changes to the nervous system even at sublethal concentration levels. Nickel tends to induce behavioral changes in fish and can induce a change in blood parameters such as decreased levels of hemoglobin and white blood cells. Zinc toxicity in organisms can depend on many environmental factors such as temperature and dissolved oxygen species (in the case of fish). It can induce mortality in fish by causing hypocalcemia (decrease in calcium concentration in the blood). However, nickel and Zinc are essential metals at low concentrations. Certain metals, such as mercury, also tend to affect the distribution and retention rates of other metals and their compounds (Garai, Banerjee, and Mondal 2021; Javed and Usmani 2019; K. Al Taee, H., and Kh. Ismail 2020; Negahdari et al. 2021). While these pollutants have enough devastating effects on their own, their compounds can also get absorbed by MPs, which then act as carriers for these toxins and deliver them deep inside the organism unfortunate enough to ingest them.

1.5 Elasmobranchs and the status of sharks

Elasmobranchs are a diverse subclass of cartilaginous fish of which sharks and rays are a part. There are over 125 species of sharks, rays, and chimaeras, many of which have persisted on the Earth for over 400 million years (Dulvy et al. 2017). They happen to be one of the largest predatory fishes and most often occur at the top of the food chain. For an organism to be considered a pollution bioindicator, it needs to fulfil the following general criteria - it should be relevant both socially and ecologically, it should have an appropriate distribution while being easily accessible, should preferably be large in size, should be long lived in order to be able of accumulating detectable amounts of contaminants without dying (Alves et al. 2022). Due to their large geographical distribution, easy access through commercial and recreational fishing, and their size and position in the food chain, Elasmobranchs tend to fit the criteria perfectly.

Among the elasmobranchs, sharks are the most evolutionarily and functionally diverse vertebrate radiations and are the most threatened lineage after amphibians (Pacoureau et al. 2021). Shark landing peaked around 2003 after which the biomass captured in fisheries has decreased up to 89 percent since the beginning of commercial shark fishing. Sharks have been overfished due to the lack of any regulation on their fishing limits. Sharks also happened to be a cheap source of meat in developing countries due to their high urea content and shark fin soups are particularly sought after in certain south asian cultures. This demand for shark fins also leads to the practice of " finning" sharks. India, a developing nation located on the southern side of Asia, plays a huge role as one of the largest exporters of shark meat. Thus due to the constant decline in shark populations in the world (Pacoureau et al. 2021) and their consumption in humans, it becomes important that one investigate the extent of contamination in these beautiful organisms.

1.6 Objectives

The objective of the study was to quantify the kind of MP and heavy metal contamination present in the sharks being landed in the Andamans with the overarching agenda of identifying habitat-specific vulnerabilities among the commonly landed sharks.

The objectives of the study were the following:

1. To quantify the extent of micro-plastic contamination in commonly landed shark species
2. To quantify the extent of heavy metal contamination in commonly landed shark species
3. To examine the extent of contamination assimilation across tissues
4. To explore variation in contamination across habitats

2. Materials and Methods

2.1 Field site

The Andaman and Nicobar Islands, located in the Bay of Bengal, happen to be an archipelago of unmatched ecological importance. They boast of a rich diversity of terrestrial and marine habitats, and thus in turn are home to a large number of unique and and endemic species. The tropical rainforests, mangrove swamps, and coral reefs support an extraordinary array of flora and fauna and hence play a vital role in maintaining regional and global biodiversity. The islands also serve as crucial nesting and breeding sites for various marine species, including sea turtles and several avian species. Also, the surrounding waters are home to various marine life and provide a complex and interconnected ecosystem. The intricate balance of these habitats ensures the sustainability of marine and terrestrial ecosystems, contributing to ecological resilience and protection against natural disasters like tsunamis and storm surges. Furthermore, the islands' conservation significance extends beyond their terrestrial borders, as the rich marine biodiversity supports the livelihoods of local communities through fishing and ecotourism. The islands also happen to be a considerable contributor to Indian shark landings (Karnad, Sutaria, and Jabado 2020).

2.2 Sampled species and their known habitats

Table 1: Sampled species and their known habitats

Pelagic	**Reef**	**Demersal**
Bull Shark *Carcharhinus leucas*	**Black Tip Shark** *Carcharhinus melanopterus*	**Nurse Shark** *Nebrius ferrugineus*
Scalloped Hammerhead Shark *Sphyrna lewini*	**Grey Reef Shark** *Carcharhinus amblyrhynchos*	**Slender Weasel Shark** *Paragaleus Randalli*
Shortfin Mako Shark *Isurus oxyrinchus*	**Silver Tip Shark** *Carcharhinus albimarginatus*	
Tiger Shark *Galeocerdo cuvier*		
Pelagic Thresher Shark *Alopias pelagicus*		
White Tip Shark *Carcharhinus longimanus*		

2.3 Sample Site

Landed shark individuals were sampled from the fish landing centre in Junglighat, Port Blair, Andaman and Nicobar Islands, India.

Primary morphometric data such as the length and weight were collected post-species identification and were followed by dissecting the individual. The sex of the shark was determined by the presence or absence of anal claspers whereas age was broadly determined by the hardness of the claspers (Bargione et al. 2019).

The individuals were then dissected for the collection of:

1. Gut content which was flushed out from the intestines
2. Gut tissue, primarily the anal end of the intestine
3. Muscle tissue, primarily white muscle from the dorsal fin.

The tissues and content samples collected were placed in 15ml vials and were stored at -20 degrees while in the field for preservation.

2.4 Sample Processing

The collected samples were thawed at room temperature and weighed in the lab before performing any of the following procedures. Tissue samples were chopped into fine pieces to aid in the digestion procedures to follow

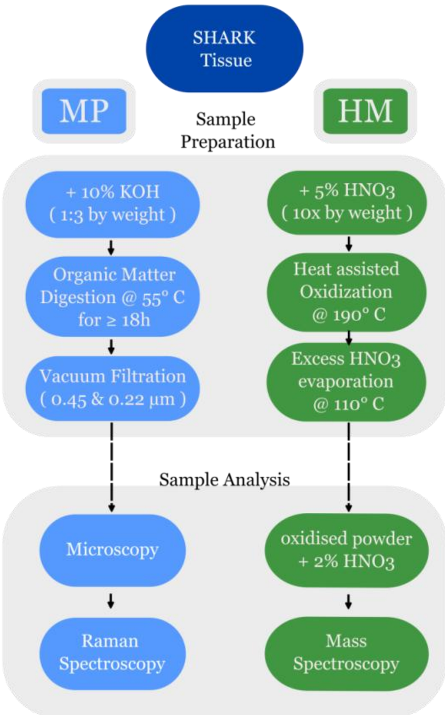

Figure 1: Flow chart depicting the sample processing procedure

2.4.1 Digestion

For MP analysis, organic matter digestion was performed by using 10 % KOH solution in a 1: 3 ratio of the sample weight, at 55 degrees Celsius for at least 18 hours.
For HM analysis, oxidation was carried out using 5% Nitric acid overnight, after which the samples were placed in a hot air oven at 200 degrees Celsius for 10 minutes to ensure proper digestion. Excess nitric acid was then evaporated at 100-110 degrees Celsius.
Procedural Blanks were prepared with KOH, Nitric Acid, and Purified water, to account for possible contamination through the reagents.

2.4.2 Filtration (for MP analysis only)

The digested mixes were diluted with purified water up to 50 ml approximately. The diluted mix was then filtered redundantly (filtered twice for the same pore size) using filter papers of pore size 0.45 and 0.22 µm (Merck Durapore, Bangalore, India) in a vacuum filtration setup. The vacuum pressure varied between 400-700 mm Hg. The beakers and the filtration setup were covered with aluminium foil throughout to prevent airborne contamination. Post-filtration, the filter papers were moved to clean and labelled Petri-dishes and air-dried before further analysis.

2.4.3 Identification (for MP analysis only)

The dried filter papers were then taken for visual assessment under a microscope. The MP pieces were then counted and categorised based on colour, and shape. A representative subset of the analyzed samples was taken for Spectroscopic analysis to identify and categorize the MPs based on their polymer type.

The particle was considered:

1. a fibre if it had a uniform width throughout the length, with length being greater than the width.
2. a pellet if was spherical in shape with rounded off edges.
3. a film if it was shaped in a sheet like fashion.
4. a fragment if it had a irregular shape. Clumps of fibres were also considered in the same category.

2.4.4 For analyzing Heavy Metal Concentration

Oxidised sample mixes were dried and homogenized as a powder, post which they were stored in 2% nitric acid and taken for ICP-MS

3. Results

3.1 Micro-plastic Analysis

3.1.1 MP Quantification

Films and pellets were observed to occur only in white/ transparent colours while fragments and fibres showed a wider colour variation. Despite displaying the colour above variation, white/transparent remained one of the most common colours to be observed, closely followed by blue, with black and red coming in close thirds in the fibres and fragment categories respectively (*Figure 2*).

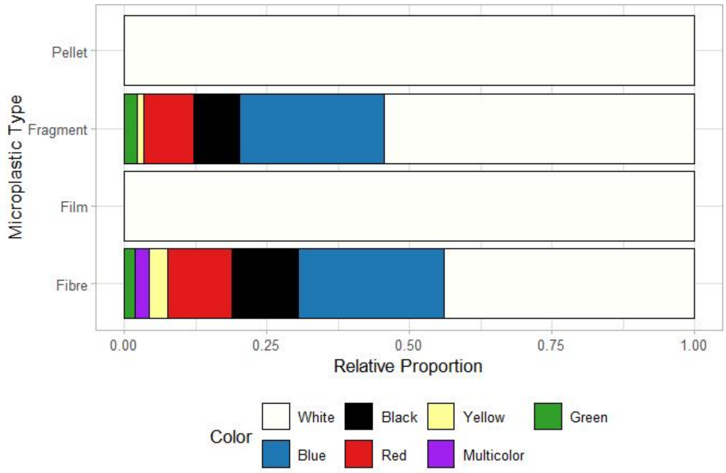

Figure 2: Relative proportion of MP color in observed MP types

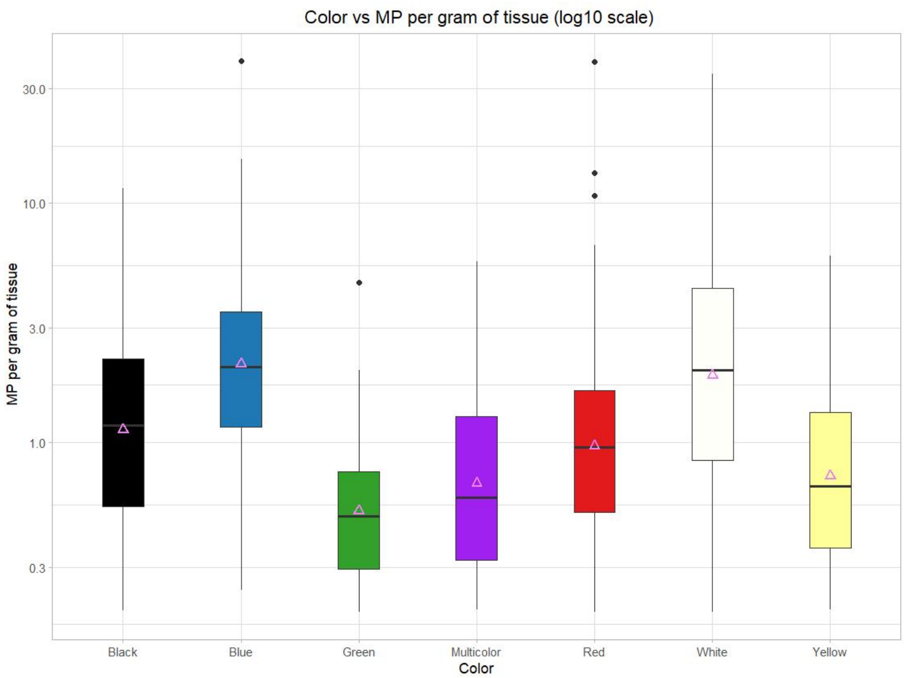

Figure 3: MP detected per gram across colors

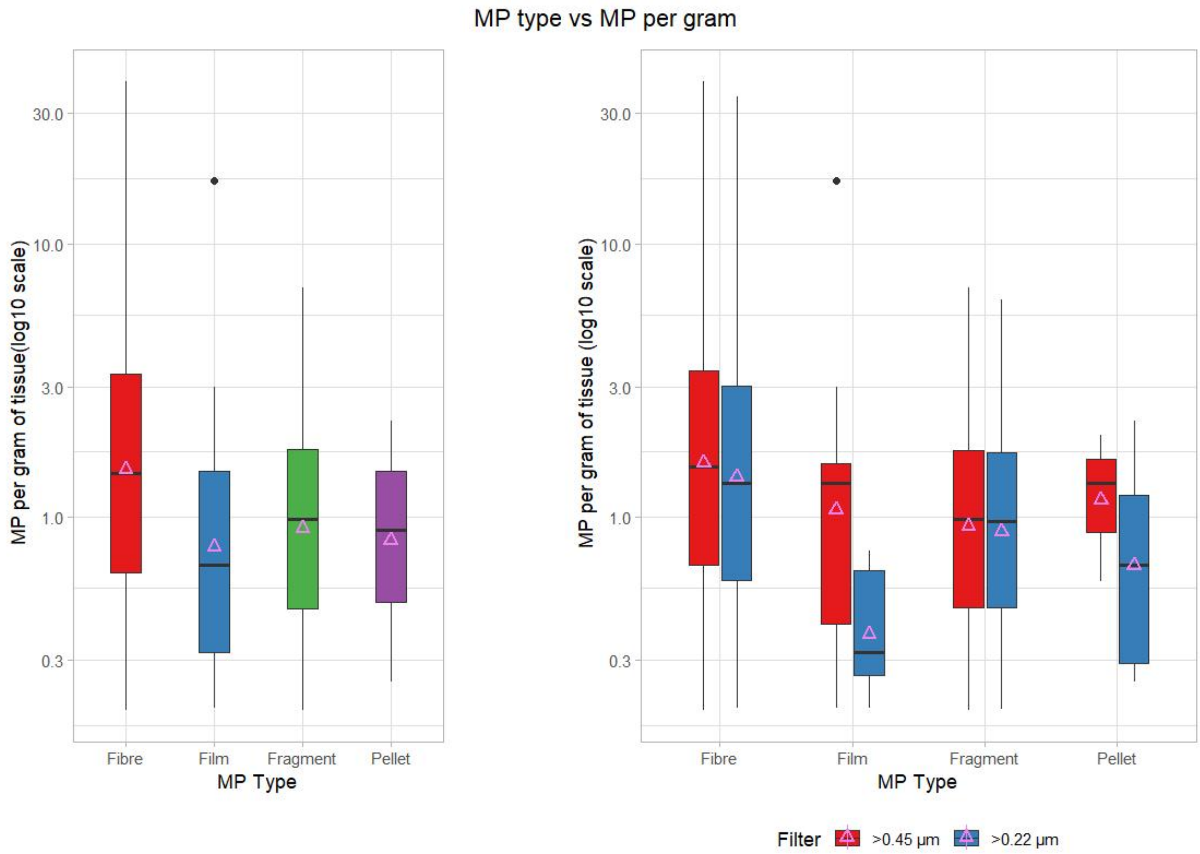

Figure 4: (Left) MP per gram of tissue across MP type; (Right) MP per gram of tissue across MP type and size class

Wilcoxon Rank sum test was carried out to infer pairwise significant differences in the MP concentrations across the different observed colours. Significant differences were observed among the following pairs of colours: Blue-Black ($p<0.001$), Green-Black ($p<0.001$), Green-Blue ($p<0.001$), Multicolor-Black ($p=0.03$), Multicolor-Blue ($p<0.001$), Red-Blue ($p<0.001$), Red-Green ($p=0.0024$), White-Black ($p=0.001$), White-Green ($p<0.001$), White-Multicolor ($p<0.001$), White-Red ($p<0.001$), Yellow-Blue ($p<0.001$), Yellow-White ($p<0.001$), whereas the rest of the pairs were not found to have significant differences. The same test was performed to infer differences between the observed MP types, with the fragment-fibre ($p<0.001$) and film-fibre($p=0.01$) pairs having significant differences. *(Figures 3 & 4)*

No significant difference was observed when the analysis was conducted to compare MP concentrations between the two age classes. Within the juvenile age class, significant difference was found between fragment-fibre pair across the size classes (Pairwise Wilcox, $p<0.001$ for 0.45 µm, $p=0.004$ for 0.22 µm), whereas the adult age class had significant differences between the film-fibre ($p= 0.02$) pair for the 0.22 µm size class (Figure 5)

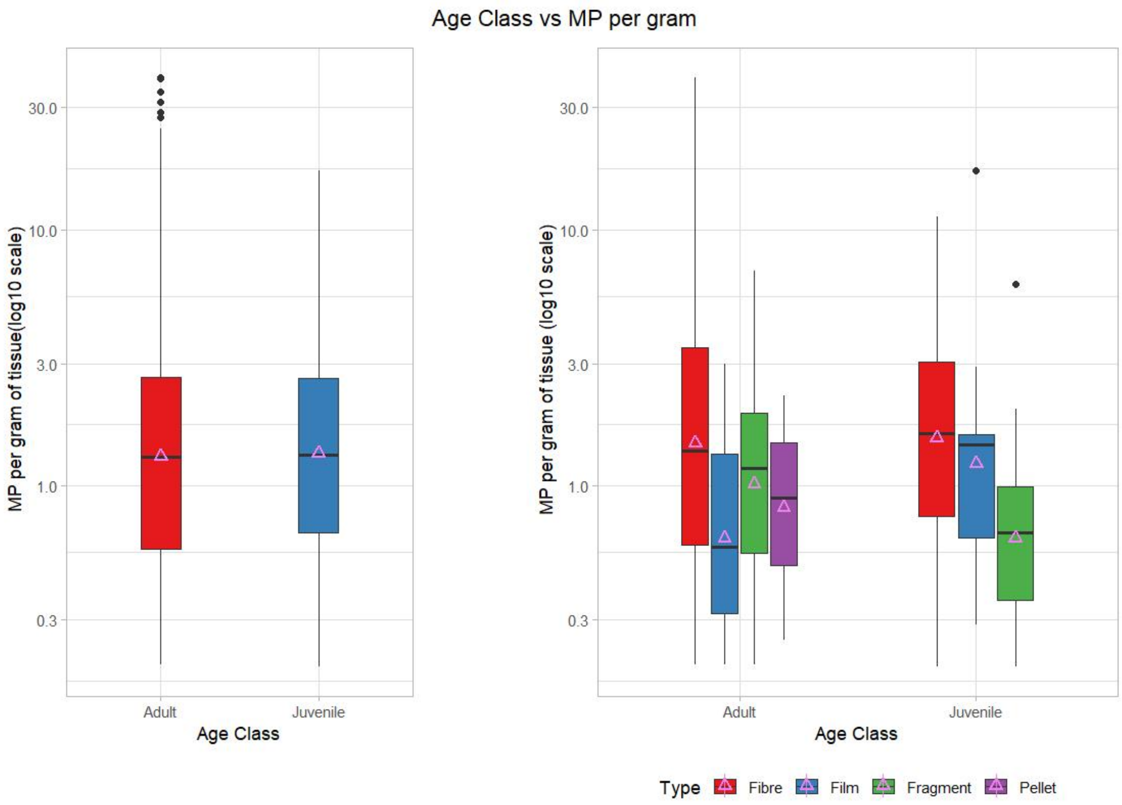

*Figure 5: (*Left*)* MP per gram of tissue detected across age classes (Right) MP per gram detected across the age classes and MP types

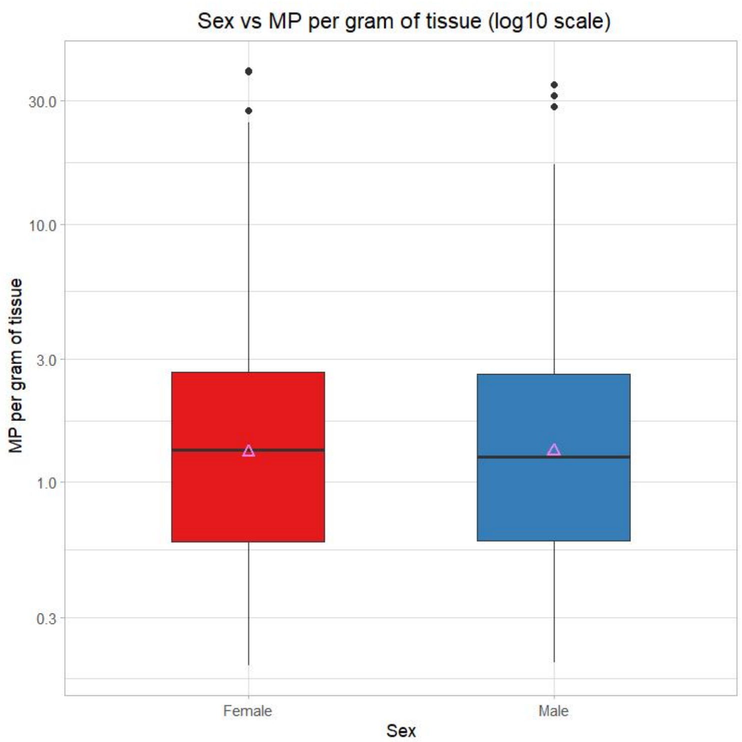

Figure 6: MP per gram of tissue across sexes

No significant difference was observed between the two sexes among the sampled individuals.(*Figure 6)*

3.1.2 MP Assimilation

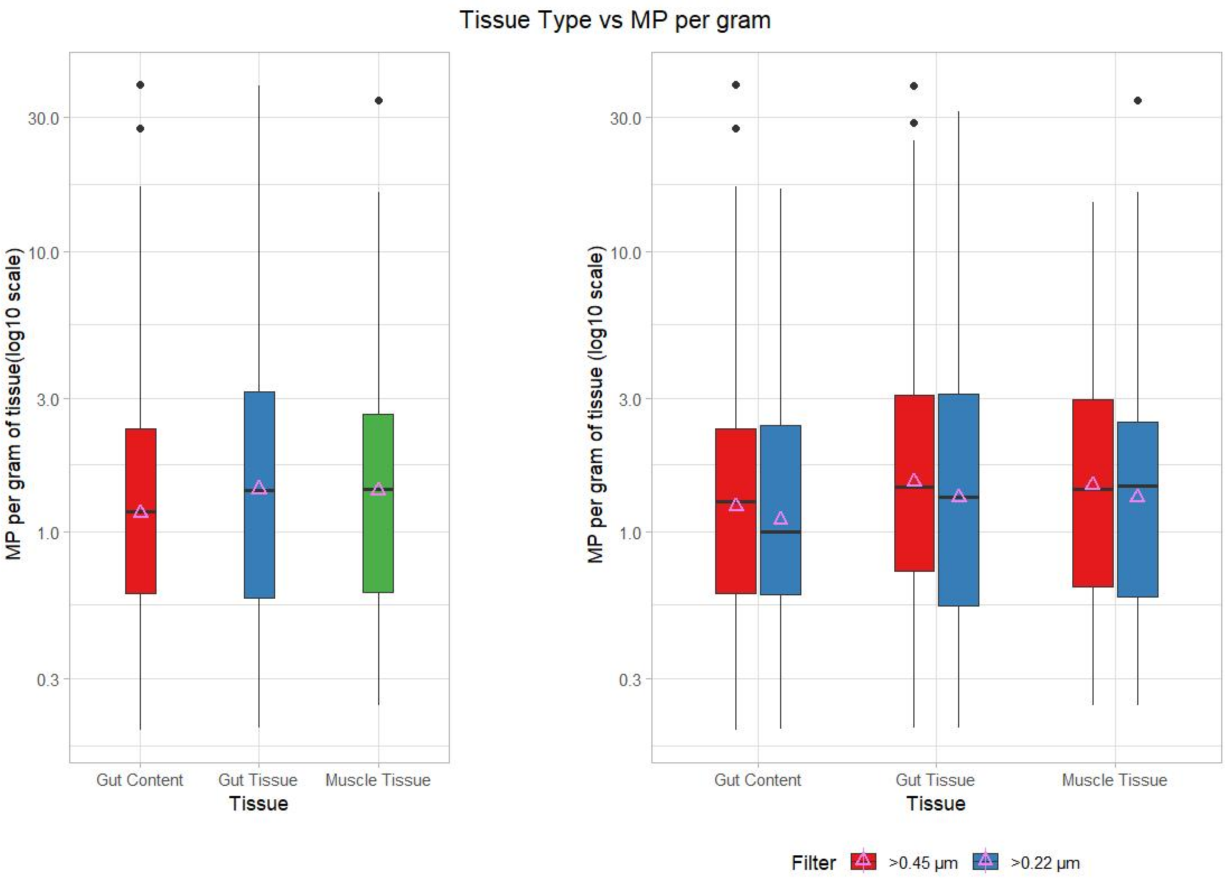

Figure 7: (Left) MP per gram of tissue for across tissue types (Right) MP per gram of tissue across tissue types and MP size classes

No significant difference in MP accumulation was observed across the sampled tissue types. However, significant difference was observed between the film-fibre MP type pair for 0.22 µm size class within the gut content samples ($p=0.04$) as well as among the fragment-fibre pair across size classes ($p=0.02$ for 0.45 µm, $p=0.02$ for 0.22 µm) among the gut tissue samples.

3.1.3 Habitat-Specific Accumulation of MPs

Kruskal Wallis Rank Sum test was conducted to check for significant differences in MP accumulation patterns across different habitats post which Wilcoxon Rank sum test was performed to identify the groups with significant differences. Significant difference was observed between the reef and demersal habitats ($p=0.019$) while the other pairs pelagic-demersal ($p>0.05$) and reef-pelagic ($p>0.05$) were not found to be significant.

Among the reef dwelling individuals, significant difference was observed between the fragment-fibre pairs across the size classes ($p=0.007$ for 0.45 µm, $p=0.06$ for 0.22 µm) and film-fibre pair (p=0.01 for 0.22 µm) while the rest of the MP type pairs were not found to have significant differences. *(Figure 8)*

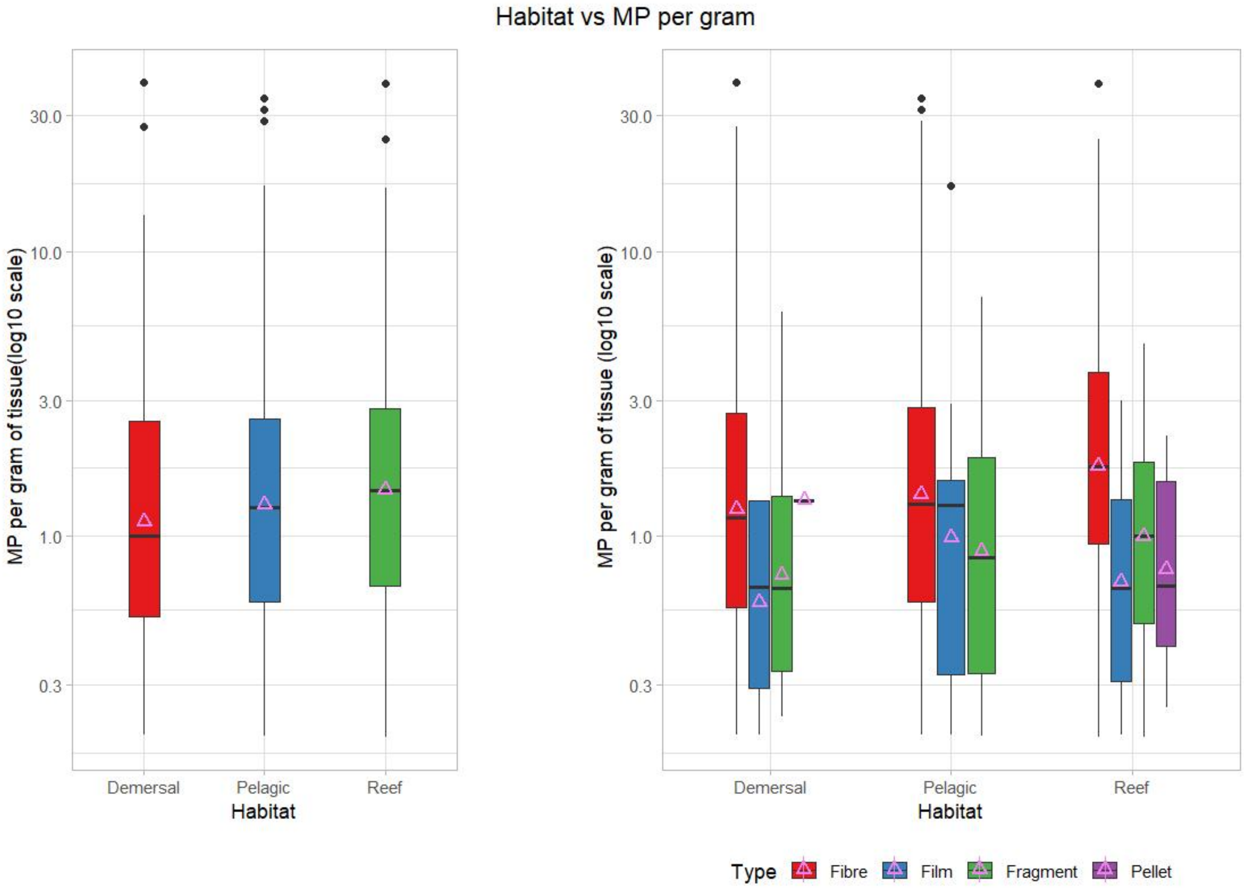

Figure 8: (Left) MP per gram of tissue across habitats (Right) MP per gram of tissue across habitats and MP types

Due to multiple logistical reasons, spectroscopic analysis for micro-plastics and ICP-MS for deciphering heavy metal concentrations was not possible in the stipulated time for the project.

4. Discussion

White as a colour and fibres as an MP type were the most commonly encountered. A significant difference was observed between the two most abundant colours - white/transparent and blue, along with multiple other pairwise differences among the colours. These differences can be attributed to variances between the sources of these plastics, the degradation processesas well the environmental conditions. For example, multiple MPs were possibly observed to be multicoloured for the very reason of losing their dyes, which can possibly be attributed to photo-degradation, thereby transforming them into colours other than the source (Zhao et al. 2022). This could partly explain the abundance of the white/tranparent colours. Particular colours like white/transparent and blue could be coming up front in the degradation of fishing lines, most of which were anecdotally observed to be of these colours during the sampling period. The same could be the reason why these colours dominate the fibre and fragment MP types as well. The relatively low occurrence of the film and pellet MP types could be associated with the ban on single-use plastics in the islands, restricting the discarding of the same into the ocean(*Towards making A&N Islands 'Plastic Free' PBMC warns stakeholders to refrain from using/selling single use plastic products :: The Daily Telegrams*). This would imply that the MP contaminants in the fish being landed in the Andamans are primarily fishery-related which provides a greater reason to pay attention to the contamination patterns and how they get affected by other demographic, environmental as well as anthropogenic factors.

Interestingly, there were not any significant differences in MP contamination levels across age, sex, or tissue types of the sampled sharks. However, trends in the mean MP content per gram did hint towards the possibility of the contaminants getting accumulated over age and being assimilated across different tissues. Statistical analysis, however, despite the trends, suggests that the shark populations of the Andamans may not be influenced by these factors. However, these seemingly insignificant differences should not be interpreted absolutely, as the sample sizes were fairly small. Hence, further studies must establish the presence/absence of such occurrence patterns with larger sample sizes and with more comprehensive sampling strategies.

Significant difference was observed between certain types of MPs in reef-dwelling species as well as across the reef and demersal habitats. This suggests the possibility of coral reefs and reef-dwelling species being differently susceptible to certain kinds of MPs as compared to species spending most of their time close to the benthos. Moreover, the differences can be attributed to the difference and variations in feeding behaviour, habitat use as well as exposure pathways where the physical properties of the MPs could also potentially have a role to play.

Overall this study contributes to the body of research and knowledge on MP contamination in marine ecosystems. Quantifying contaminant levels, identifying contaminant accumulation patterns across demographics of affected species as well as identifying habitat-specific vulnerabilities, provides valuable insights for possible conservation efforts and possible contaminant mitigation strategies specific to the ecological setting of the Andaman and Nicobar Islands, India.

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