

‘Oceans and Lakes’

Interuniversity Master of science in Marine and Lacustrine Science and Management



MICROPLASTIC INGESTION BY *NEOTRYGON INDICA* IN EASTERN SRI LANKA

Baseline assessment and development of a practical methodology for microplastic analysis in elasmobranchs

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Abstract

Microplastic (MP) pollution is an increasing threat to marine ecosystems, yet information on elasmobranch exposure remains limited. This study evaluated a practical and contamination-controlled methodology for the extraction of MPs from elasmobranch gastrointestinal tracts (GITs). The protocol was designed to be cost-efficient, safe and applicable for sample processing in laboratories with limited access to specialized infrastructure, while incorporating polymer validation through advanced analytical techniques when available. To evaluate the methodology, GITs from *Neotrygon indica* specimens (n = 24) from the East Coast of Sri Lanka were processed using potassium hydroxide digestion, density separation, filtration and micro-Fourier transform infrared spectroscopy (μ FTIR). MPs were detected with a frequency of occurrence (FO) of 66.7% in *N. indica*. Polyethylene terephthalate (PET) was the dominant polymer type (77.4%), and most detected particles measured below 100 μ m (64.0%). No significant relationship was observed between body size and MP abundance. Procedural blanks showed no confirmed contamination, while recovery experiments demonstrated a retrieval efficiency of 96%. Overall, this study demonstrates that reliable MP datasets can be generated through accessible sample-processing approaches when strict contamination control measures are applied. However, specialized equipment and laboratory infrastructure are required for polymer confirmation and the generation of high-quality, comparable data, which can be accessed through collaboration with specialized laboratories. The methodology provides a transferable framework for future MP research in elasmobranch species, particularly in regions where laboratory infrastructure may be limited.

Keywords: Gastrointestinal tract, μ FTIR, contamination control, marine pollution, polymer identification

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1. Introduction

From remote mountain ranges to the deepest ocean trenches, plastic pollution has become a global issue ([Habib et al., 2022](#)). The widespread use of plastics is driven by their durability, strength and versatility, qualities that have supported continued growth in global production across numerous industrial sectors. However, these same attributes contribute to their persistence in the environment, where they resist complete degradation and accumulate as pollutants ([Pasalari et al., 2025](#)).

1.1. Plastic pollution

Only a limited fraction of discarded plastics is recycled or incinerated, leaving the majority to accumulate in natural environments ([Geyer et al., 2017](#)). Marine ecosystems function as major sinks for synthetic debris ([Huang et al., 2022](#); [Sutton et al., 2016](#)), largely driven by inadequate waste infrastructure and unregulated dumping near riverine and coastal systems ([Ryan et al., 2009](#); [Wu et al., 2025](#)). It is estimated that approximately 80% of marine plastic pollution originates from terrestrial sources ([Andrady, 2011](#); [Bhuyan et al., 2020](#)).

The ubiquity of plastic litter in the oceans has made it a critical threat to aquatic ecosystem integrity, contributing to habitat degradation and ecological disruption at a global scale ([Jansen et al., 2024](#)). There is an urgent need to assess its effects across all marine compartments, including the biota that inhabit them ([Pasalari et al., 2025](#)).

Plastics are primarily composed of synthetic polymers, man-made chemical structures engineered in laboratory settings. A significant subset of these materials, known as thermoplastics, can be melted into commercial products and dominate the composition of plastic pollution ([Andrady, 2017](#)).

Plastic pollution occurs across a broad range of fragment sizes and therefore affects organisms at multiple levels of the marine food web ([Maes et al., 2020b](#)). Large debris can lead to problems such as entanglement, ghost fishing or intestinal blockage following ingestion, while smaller fragments such as microplastics (MP) can be consumed even by the smallest marine organisms and consequently cause chemical or physical harm ([Galli et al., 2023](#); [Maes et al., 2020a](#); [Maes et al., 2020b](#); [Fossi et al., 2018](#); [Lusher et al., 2015](#)).

Microplastics are defined as synthetic particles measuring between 0.1 μm and 5 mm ([Galli et al., 2023](#); [Germanov et al., 2018](#)). They are generally categorized as either primary or secondary particles. Primary MPs are intentionally manufactured for commercial and industrial applications, and are incorporated into products such as detergents, cosmetics and even insecticides in some countries ([Galli et al., 2023](#)). Secondary MPs originate from the fragmentation of larger synthetic objects that degrade under physical, biological and chemical forces such as ultraviolet radiation, wave action, wind and microbial activity ([Huang et al., 2022](#)). A key pathway for MPs to reach aquatic systems is through wastewater outflow ([Sutton et al., 2016](#); [Wu et al., 2025](#)). For example, synthetic fibers shed during domestic use often pass through treatment facilities that are unable to effectively filter them due to their material characteristics, allowing them to enter the environment with treated effluent ([Sutton et al., 2016](#)). Industrialized coastal zones have been identified as MP hotspots ([Wright et al.,](#)

2013). Five commodity thermoplastics are repeatedly identified in microplastic assemblages: polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET) (El-Sherif et al., 2022).

1.2. Effects of microplastics

Microplastics occur within a size range equal to sediment particles and planktonic organisms, which makes them readily available for ingestion by a wide variety of marine species (Wright et al., 2013). Following ingestion, physical and chemical damage can occur within exposed organisms (Galli et al., 2023; Maes et al., 2020a; Maes et al., 2020b; Fossi et al., 2018). These effects often start at lower trophic levels and continue through the food web via bioaccumulation (Maes et al., 2020b). Microplastics can act as vectors for contaminants, including persistent organic pollutants (POPs), and can reduce nutrient absorption or cause gastrointestinal blockage and mechanical damage (Germanov et al., 2018; Worm et al., 2017).

Plastics have a high affinity for POPs and their resistance to degradation contributes to the long-term environmental persistence of these pollutants (Habib et al., 2022). The hydrophobic surface of MPs facilitates the sorption of POPs from surrounding seawater and contamination levels can become several orders of magnitude higher than that of the ambient environment (Wright et al., 2013). In long-lived organisms, these contaminants may accumulate over time and disrupt biological functions including endocrine activity, growth and reproduction, and can additionally be transferred to offspring (Galli et al., 2023; Germanov et al., 2018; Maes et al., 2020b). Additional reported impacts include heightened immune responses, reduced feeding, weight loss, impaired growth, reduced fecundity and energy depletion (Valente et al., 2019).

Such effects have been documented in various seabirds, marine invertebrates and smaller fish species, yet data concerning large predatory taxa remains limited (Galli et al., 2023; Huang et al., 2022; Pasalari et al., 2025; Torres et al., 2024). Laboratory experiments demonstrate the potential scale of these impacts. In zebrafish (*Danio rerio*), exposure to polyethylene MPs caused dose-dependent behavioral abnormalities and increased toxicity with increasing concentrations (El-Sherif et al., 2022). Medaka fish (*Oryzias latipes*) exposed to polyethylene MPs combined with sorbed contaminants exhibited early signs of endocrine disruption, altered gene expression mediated by estrogen receptors, downregulation of target genes in females and abnormal germ cell proliferation in males (Rochman et al., 2013). Research on MP ingestion has focused predominantly on teleost fish, with relatively few studies investigating elasmobranchs (Pegado et al., 2021). These findings underline the relevance of expanding MP research to elasmobranchs, which occupy higher trophic levels as apex or mesopredators and may therefore represent key bioindicators for evaluating the presence and biological implications of MPs in marine ecosystems.

1.3. Methodological accessibility and standardization in microplastic research

Microplastic research is still a relatively new research area, and methodological variation remains an important challenge for generating reliable and comparable data. Different approaches to sample collection, digestion and particle identification can result in substantial differences in reported microplastic abundance and composition, making comparisons between studies difficult ([Hermsen et al., 2018](#)). Hermsen et al. (2018) therefore highlighted the need for standardized approaches and quality assurance criteria in microplastic research on marine biota. Although efforts toward methodological harmonization have increased, considerable variation remains in the methods applied across studies. This is particularly relevant for elasmobranchs, for which MP research remains underdeveloped. Existing studies have focused predominantly on sharks and pelagic rays, while benthic elasmobranchs remain comparatively understudied ([Pegado et al., 2021](#)). The limited and methodologically diverse datasets available for elasmobranchs therefore make it difficult to assess patterns of microplastic exposure across species and environments. Developing and applying consistent analytical approaches is consequently important not only for improving the reliability of individual studies, but also for enabling future comparisons and contributing to a broader understanding of microplastic exposure in elasmobranchs.

1.4. Microplastic research in marine fauna

Microplastic research in marine fauna commonly relies on gastrointestinal contents to assess ingestion and exposure. In larger organisms such as elasmobranchs, the gastrointestinal tract (GIT) is frequently selected as the target component, while whole specimens may be examined in smaller organisms such as bivalves and zooplankton. Examination of the complete GIT, from the esophagus to the vent, provides a more representative estimate of ingested microplastics than analyzing individual sections, as particles present further along the digestive tract would otherwise be missed ([Hermsen et al., 2018](#)). The GIT is also a widely used target component, facilitating comparison of microplastic occurrence across species and geographic regions.

This approach is particularly relevant to elasmobranchs, which share a relatively conserved digestive tract (Figure 1.) consisting of the oral cavity, pharynx, esophagus, stomach, spiral intestine and cloaca, despite some variation in spiral intestine morphology ([Buckling, 2015](#); [Leigh et al., 2017](#)). This anatomical similarity supports the broader application of GIT-based microplastic assessment across elasmobranch taxa, although dissection procedures may require adaptation to differences in body morphology. In rays and skates, for example, their dorsoventrally flattened body shape and gill position require a different approach than that typically used for sharks.

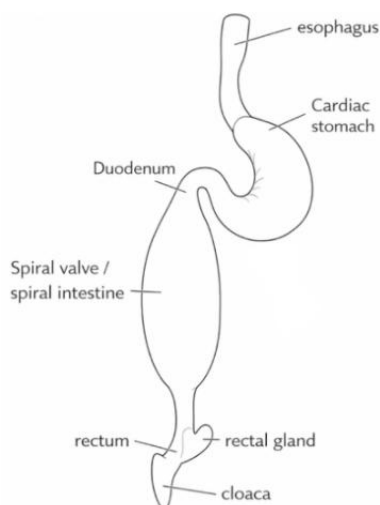


Figure 1. Schematic representation of the gastrointestinal tract Schematic representation of the gastrointestinal tract (GIT) of an elasmobranch, indicating the main anatomical structures examined during gastrointestinal tract dissections (Leigh et al., 2017).

Prior to particle analysis, biological material is generally digested to remove organic matter, which can obscure particles and increase the likelihood of missing microplastics, particularly smaller particles (Hermesen et al., 2018; Vandermeersch et al., 2015). The digestion method must therefore remove organic material while preserving the polymers of interest. Different approaches include acidic, enzymatic and alkaline treatments, with Dehaut et al. (2016) finding an adapted 10% potassium hydroxide (KOH) digestion to effectively digest biological material while maintaining the integrity of tested polymers. Following extraction, potential microplastics are commonly screened visually and, where possible, chemically confirmed using techniques such as FTIR or Raman spectroscopy. Chemical confirmation is important because visual identification can misclassify natural particles as microplastics, particularly as particle size decreases (Hermesen et al., 2018; Löder & Gerdts, 2015). Because of the high risk of contamination, all steps must be carried out under controlled measures described in appendix 1. Together, these steps provide a basis for more reliable and comparable microplastic assessments.

1.5. Selection of research species

Selecting an appropriate elasmobranch species for MP analysis requires careful consideration of practical and ecological factors. Ideally, the focal species should remain of manageable size at maturity to facilitate handling and processing. Very large filter-feeding taxa such as whale sharks (*Rhincodon typus*) or reef manta rays (*Morula alfredi*) are of scientific interest but their substantial body size greatly increases the logistical demands associated with sample collection and tissue processing. While larger species should not be excluded from future research, they require additional planning, resources and infrastructure.

To ensure statistically robust outcomes, the chosen species should also occur frequently enough within the study area to support adequate sample sizes. Access to reliable information on local shark, skate and ray populations is therefore essential when determining the feasibility and relevance of the study. Habitat use and migratory behavior are equally important. For studies assessing MP contamination within a defined geographic context, species with restricted spatial ranges or limited migratory behavior are preferred, as they provide a clearer representation of local environmental conditions (De Witte et al., 2022).

This study focuses on the Indian Ocean blue-spotted maskray (*Neotrygon indica*). The blue-spotted maskray was historically classified as a single species, *Neotrygon kuhlii*, with a distribution spanning the Indo-West Pacific. Molecular analyses have since identified significant genetic divergence within this complex, revealing the presence of cryptic species including *N. indica* (Pavan-Kumar et al., 2022). *Neotrygon indica* can be visually distinguished by its characteristic dorsal spot pattern, including numerous small ocellated blue spots, relatively few medium-sized ocellated blue spots and an absence of large ocellated blue spots. Numerous dark speckles and conspicuous occipital mark are also characteristic, although some overlap in spot patterns occurs among closely related species (Ranjith et al., 2018).

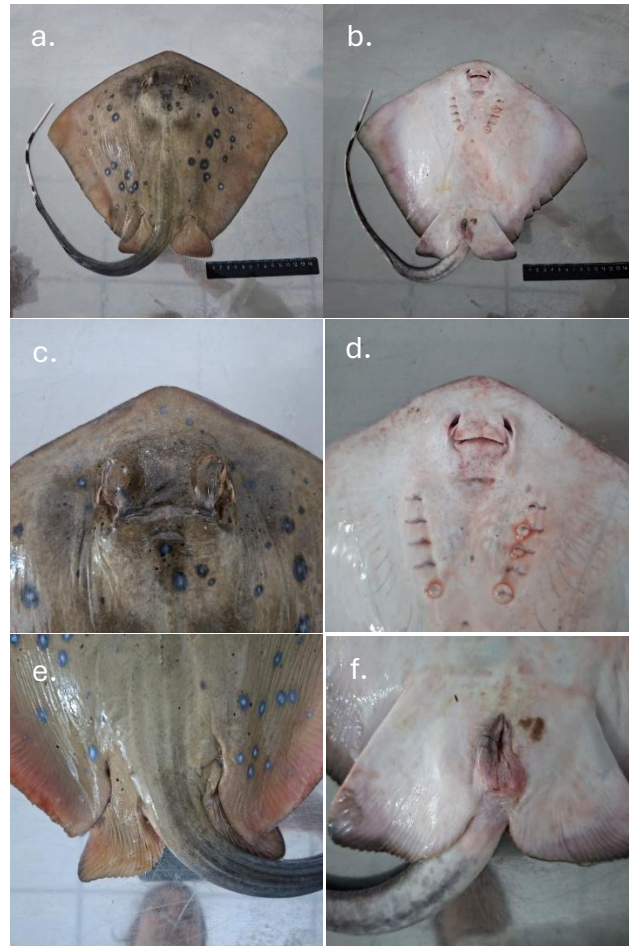


Figure 2. External morphological characteristics of a *Neotrygon indica*: Representative female *Neotrygon indica* specimen (29_NI_13.11.2025) included in this study. (a) Dorsal view of the complete specimen showing overall body morphology and disc shape. (b) Ventral view of the complete specimen. (c) Dorsal close-up of the cephalic region showing the characteristic facial mask pattern and blue spotting. (d) Dorsal close-up of the posterior disc and tail base region. (e) Ventral close-up of the cephalic region displaying the mouth and gill slits. (f) Ventral close-up of the cloacal region; the absence of claspers indicates a female individual.

Blue-spotted maskrays occupy a wide range of habitats spanning from the intertidal zone to depths of approximately 90 m, including sandy and muddy substrates as well as rocky and coral reef environments. Common across the Indo-West Pacific, this species constitutes a notable component of fisheries in several regions within this area. Individuals have been reported moving onto reef flats and lagoon areas during high tide, although they are more typically observed in deeper sandy areas near rocky reef structures ([Ranjith et al., 2018](#)).

As a benthic mesopredator closely associated with coastal sediments, *N. indica* may provide insight into sediment-associated MP exposure pathways within tropical nearshore ecosystems. Because benthic environments are considered major sinks for marine MPs ([Mason et al., 2022](#)), this species may serve as a useful bioindicator for localized contamination patterns.

Although research on *N. indica* remains limited and several data gaps persist, the species presents a suitable model for baseline MP assessments in this region. Its moderate size, frequent occurrence in catches landed in the region and association with coastal environments make it a practical candidate ([Fernando and Stewart, 2021](#) and [Ranjith et al., 2018](#)). Additionally, its limited spatial range in comparison with highly migratory elasmobranchs allows for a more accurate representation of MP contamination specific to the East Coast of Sri Lanka. As a result, *N. indica* provides a valuable foundation for evaluating both methodological feasibility and MP occurrence within elasmobranch gastrointestinal tracts (GIT) in areas with restricted laboratory resources.

1.6. Research area

Given the need to assess MP exposure in a coastal species with limited migratory behavior, the research focuses on populations of *N. indica* inhabiting the nearshore waters of Sri Lanka. This region presents an especially relevant context for MP contamination studies, as local environmental conditions, waste management pressures and fisheries interactions intersect to influence elasmobranch exposure.

Sri Lanka is an island nation in the Indian Ocean with over 1340 km of coastline and a network of 103 rivers that transport considerable quantities of land-based debris into the surrounding seas ([Katupotha and Gamage, 2021](#); [Guruge et al., 2025](#)). Much of the coastline consists of sandy beaches influenced by seasonal monsoon cycles, making them particularly susceptible to the accumulation of marine debris ([Jang et al., 2018](#)). These coastal areas support highly diverse nearshore ecosystems and are central to the tourism sector, which accounts for approximately 10% to the national service economy ([Jang et al., 2018](#)). Despite this economic value, Sri Lanka ranked fifth globally among countries discharging plastic debris into the oceans ([Jambeck et al., 2015](#)). Strong monsoonal wind systems further shape the distribution and concentration of marine debris along the coastal zone ([Guruge et al., 2025](#)).

Sri Lanka's extensive small-scale fisheries further highlight the relevance of the region for elasmobranch-focused MP research. The country's Exclusive Economic Zone covers more than 517,000 km² and supports extensive small-scale fisheries where gillnets dominate due to their low cost, ease of use and efficiency. Because this gear type frequently captures non-target species,

including elasmobranchs ([Fernando and Stewart, 2021](#)), fisheries landings provide practical opportunistic specimen collection without targeted capture for scientific purposes. Of the country's 21.4 million inhabitants, 27% reside along its 1,770 km coastline, and fish products account for 50% of total animal protein consumed nationally ([Fernando and Stewart, 2021](#)).

While tuna and billfish represent the most profitable targets, the Sri Lankan gillnet fleet routinely captures a diverse assemblage of non-target species including marine mammals, turtles and elasmobranchs ([Fernando and Stewart, 2021](#)). Some species must be discarded due to legal restrictions, but elasmobranchs are frequently retained and sold in response to domestic and international market demand for their meat and derived products ([Fernando and Stewart, 2021](#)). As a result, fisheries activity simultaneously supports livelihoods while placing additional stress on vulnerable elasmobranch populations.

The combination of Sri Lanka's high plastic discharge, ecological sensitivity and frequent fisheries interactions with elasmobranchs, position the region as a highly relevant site for assessing MP exposure in benthic rays. This context provides both the scientific justification and practical necessity for establishing reliable, low-cost and adaptable analytical protocols that can be applied within regions where laboratory capacity may be limited. Consequently, this study aims to evaluate MP occurrence in *N. indica* while simultaneously assessing the applicability of a practical and contamination-controlled methodology for elasmobranch GIT analysis under resource-limited conditions.

1.7. Study aims and hypotheses

This study aimed to develop and evaluate a practical methodology for the extraction and identification of MPs from elasmobranch GIT. Particular attention was given to contamination control throughout the process, while also ensuring that the methodology for the extraction remained cost-efficient, safe and feasible for laboratories with limited access to specialized equipment (full protocol in Appendix 1). In addition to methodological development, the study investigated the occurrence of MPs within examined elasmobranch specimens and explored whether specimen size influences MP abundance within the GIT. Based on previous studies reporting widespread microplastic ingestion in marine organisms ([Hermsen et al., 2018b](#); [Lusher et al., 2015](#) and [Wright et al., 2013](#)), it was hypothesized that MPs would be present in the majority of examined GIT. Furthermore, it was expected that larger specimens would contain greater abundance of MPs due to increased food intake and longer potential exposure times.

2. Methodology

Schematic overview of the methodology for the extraction and identification of microplastics from elasmobranch

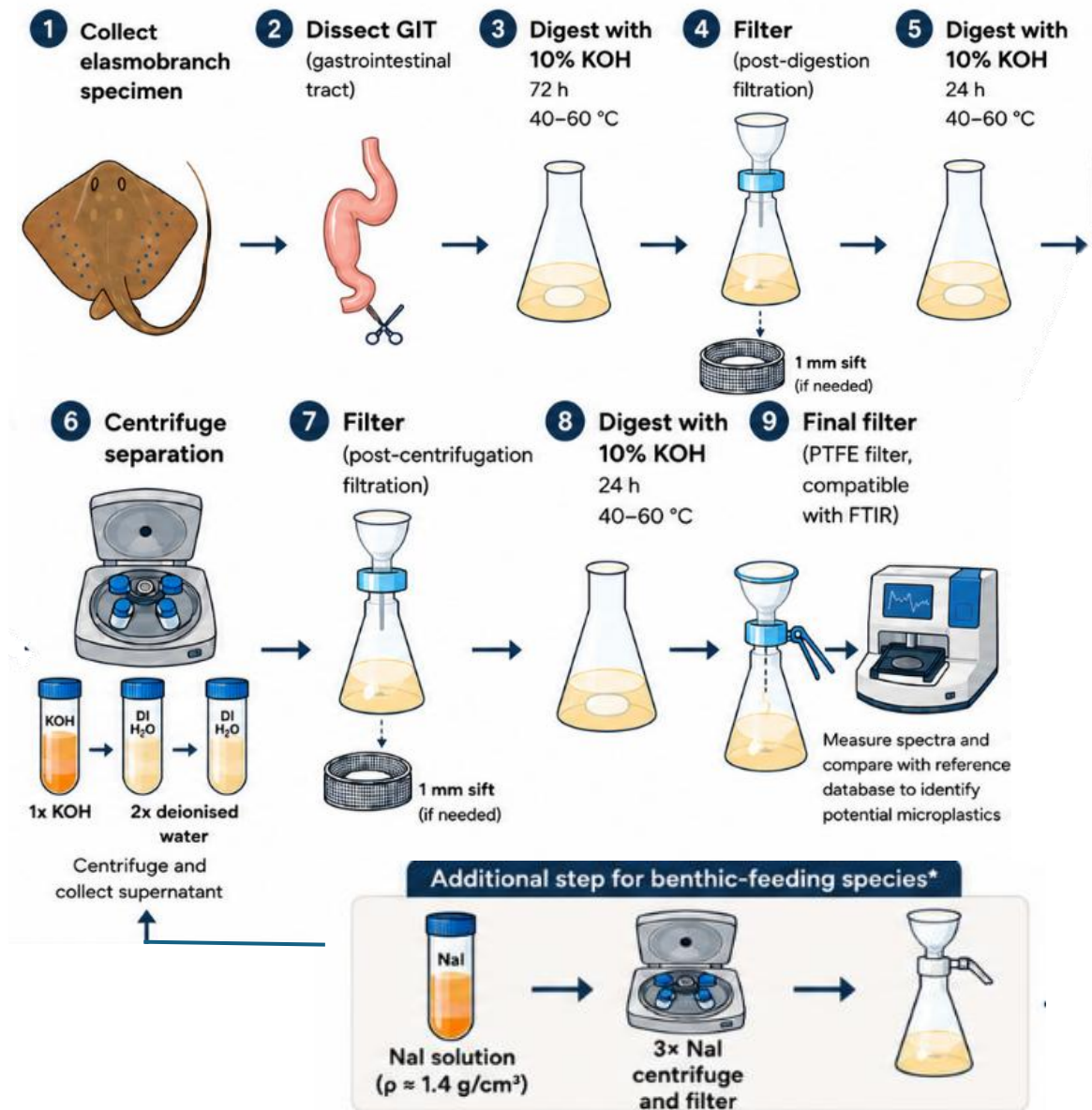


Figure 3. Schematic overview of the methodology employed in this study for the extraction and identification of microplastics from elasmobranch gastrointestinal tract samples. Image generated using ChatGPT.

2.1. Sample collection

Between September and November 2025, a total of 30 elasmobranch specimens were collected, of which 24 were identified as *Neotrygon indica*. The *N. indica* specimens were used for exploratory statistical analyses, while the additional five ray specimens were included to provide preliminary observations on the applicability of the developed methodology across different taxa and size classes. These additional specimens comprised one honeycomb stingray (*Himantura uarnak*), one broad cowtail ray (*Pastinachus ater*), one spinetail devil ray (*Mobula mobular*), one long-tailed butterfly ray (*Gymnura poecilura*) and two Jenkins' whipsnakes (*Pateobatis jenkinsii*) (see Appendix 2).

Specimens were collected opportunistically at local landing sites, primarily as bycatch from ongoing fisheries activities, with some individuals originating from targeted catches. Sampling was conducted along the East Coast of Sri Lanka in the vicinity of the Blue Resources Trust research station. The locations of sample collecting consist of three local landing sites; Pethalai landing site (7°93'19"N – 81°55'73"E), Valaichchenai harbor (7° 55' 37" N – 81° 31' 47" E) and Kalkudah landing site (7°43'0"N - 81°35'0"E). Fishing techniques and vessel types were recorded, together with observations of plastic material that was found at the landing sites (see Appendix 2). The exact catch locations were not available and could therefore only be approximated.

Following collection, specimens were transported to the Blue Resources Trust laboratory and stored at -20°C until sample processing, to avoid deterioration.

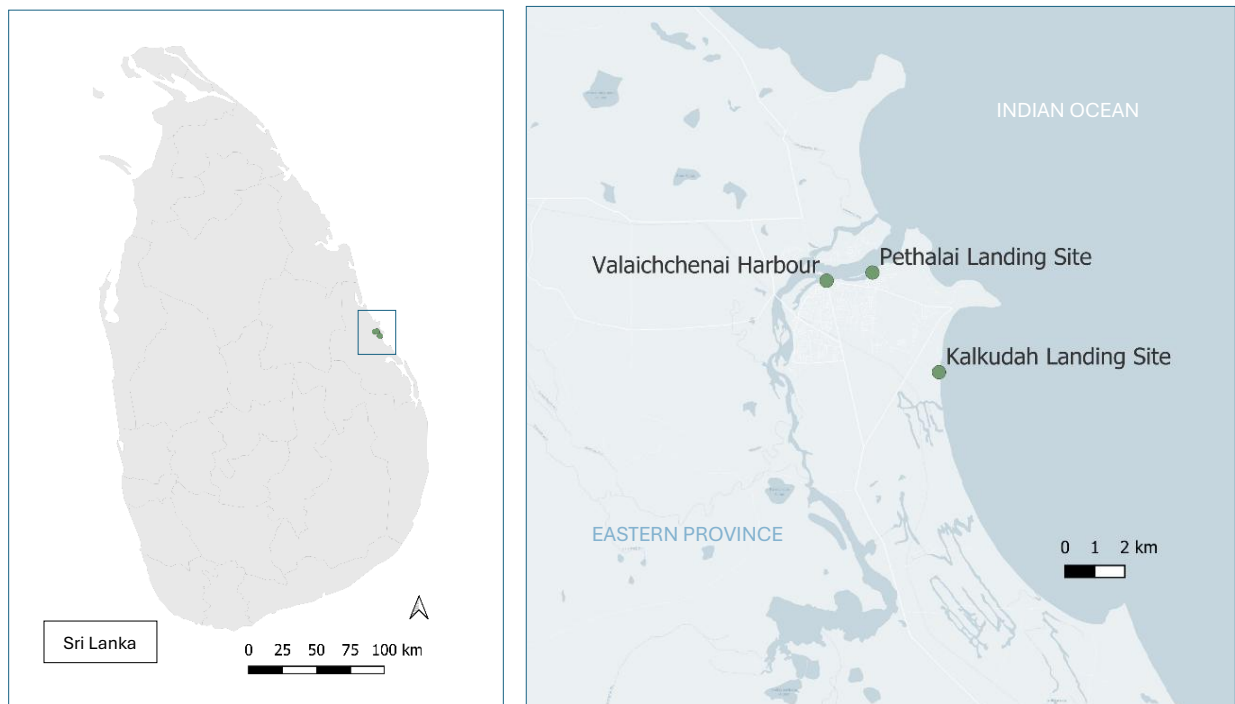


Figure 4. Location of the study area and sampled landing sites in Sri Lanka Detailed map of the study area showing the locations of Valaichchenai Harbour, Pethalai Landing Site, and Kalkudah Landing Site on the east coast of Sri Lanka.

2.2. Sample preparation

All samples were thawed at room temperature prior to the dissection process. Morphometric measurements, including total body width and height of the specimen were recorded, along with the wet weight of the gastrointestinal tract (GIT) (see annex 2).

To minimize external contamination, specimens were with filtered water rinsed prior to dissection, following recommendations for exterior contamination removal in MP biota studies ([Hermesen et al., 2018](#)). To facilitate efficient GIT removal and minimize the loss of GIT contents, dissection was performed from the anus towards the gill region. This direction provided immediate access to the rectum, which was clamped after opening the body cavity and before removing the GIT.

The GIT was removed from the cloaca to the most proximal accessible section of the esophagus underneath the gills. The extracted tissue was weighed to determine its wet weight and immediately transferred to an appropriately sized Erlenmeyer flask or, when glassware was unavailable, wrapped in aluminum foil. Storage in a glass container that could later be used for the digestion process was preferred, as it minimizes sample handling and reduces contamination risk. All samples were stored individually and covered to prevent airborne contamination. Samples were handled as quickly as possible throughout processing to further reduce contamination risk ([Hermesen et al., 2018](#)).

2.3. Contamination control and recovery assessment

To monitor potential contamination introduced during laboratory processing, negative procedural blanks were processed alongside the biological samples throughout the analytical workflow. With every batch of samples that was processed, procedural blanks were subjected to the same handling, incubation and filtration steps to assess potential contamination originating from laboratory reagents, equipment or sample processing procedures.

Airborne contamination was monitored using air blanks exposed within the laboratory during sample processing.

To assess the recovery efficiency of the methodology, positive procedural controls were conducted by intentionally introducing a known number of polyethylene terephthalate (PET) and polyethylene (PE) particles into selected GIT samples prior to digestion and filtration. These particles were subsequently tracked throughout the complete analytical procedure to evaluate potential particle loss during digestion, centrifugation, sieving and filtration steps.

2.4. Digestion

Organic material was digested using a 10% (w/v) potassium hydroxide (KOH) solution at a solution-to-tissue volume ratio of approximately 3:1. Samples were incubated at 40–60 °C for 72 hours in an oven to accelerate the digestion while maintaining the integrity of potential microplastic particles ([Rochman et al., 2015](#); [Thiele et al., 2019](#); [Wesch et al., 2016](#)). Samples were gently mixed once a day to help the tissue breakdown.

2.5. Microplastic extraction and filtration

Following digestion, the solution was vacuum-filtered using Whatman™ cellulose nitrate membrane filters (8 µm pore size, 45 mm diameter) with a Fisherbrand FB70155 vacuum pump. Due to the high sediment content, the filters frequently clogged, reducing filtration efficiency and requiring several replacements per sample. In addition, residual KOH caused partial degradation of the cellulose nitrate filters. In some cases, degradation occurred before filtration was complete, limiting subsequent rinsing with filtered water. After filtration, the filters were folded and stored in aluminum foil for transport and storage. Residual KOH also caused the filters to adhere to the aluminum foil, complicating their removal during subsequent processing.

Subsequent processing was conducted at the BLUEGent laboratory at Ghent University, Belgium, to further separate potential MPs and determine their polymer composition.

To separate the potential MPs from non-synthetic particles, the cellulose nitrate membrane filters were subjected to a secondary digestion using 150 mL of 10% KOH per sample at 60 °C until the filters were fully dissolved. The resulting solution was then centrifuged at 3500 rpm for 5 minutes at 20 °C using a Thermo Scientific Megafuge 40R Refrigerated Centrifuge to separate heavier material from the supernatant. The supernatant was carefully decanted and filtered, while the remaining sediment and heavier material were retained for further centrifugation. After each filtration step, all glassware was thoroughly rinsed with distilled water to recover any remaining particles. The centrifugation, decanting, rinsing and filtration steps were repeated twice using distilled water to maximize microplastic recovery while reducing residual sediment. Subsequently, density separation was performed using a sodium iodide Sigma-Aldrich™ (Nal) solution brought to a density of 1,4 g/cm³. The centrifugation and filtration steps were repeated three times with Nal to exploit density differences, allowing microplastic particles with a lower density to remain in the supernatant that was filtered while heavier inorganic material settled. The remaining sediment fraction was discarded after completion.

The obtained filters, containing the isolated microplastic fraction, were subjected to a final digestion using 10% KOH at 60 °C for a minimum of 24 hours to ensure complete removal of residual organic material.

In order to perform chemical identification, the samples were subjected to a final filtration using Omnipore™ polytetrafluoroethylene (PTFE) membrane filters (10.0 µm, 25 mm diameter), which are compatible with micro-Fourier transform infrared (µFTIR) spectroscopy analyses. To reduce clogging of the PTFE filters, samples were first subjected to sequential sieving to remove larger residual particles. Despite the KOH digestion, some larger organic remains, such as shrimp carapace fragments, persisted within the samples and could obstruct filtration or interfere with spectroscopic detection of potential MPs. Samples were first passed through a 1 mm stainless steel sieve to remove large residual material. Material retained on the 1 mm sieve was rinsed with filtered water and kept for visual inspection. Subsequently, the remaining fraction was subdivided using 100 µm and 35 µm sieves. The 35–100 µm fraction was filtered separately from the >100 µm fraction due to the high presence of very fine particulate matter within the samples. Although the 35–100 µm

fraction was not analyzed in the present study due to time constraints, it was stored for potential future research purposes. Because the lower detection limit of the μ FTIR system was approximately 30 μ m, particles smaller than 35 μ m were not retained for further analysis.

The PTFE filters were subsequently dried for 24 hours prior to spectroscopic analysis.

2.6. Polymer identification

The Omnipore PTFE membrane filters with suspected microplastics were further analyzed using μ FTIR to validate the results and determine the polymer composition.

Spectral analysis was performed using a Thermo Scientific Nicolet iN10 micro-Fourier transform infrared spectroscope operated in transmission mode. Spectra were collected within the spectral range of 4000–1300 cm^{-1} using a cooled detector. Infrared spectra obtained from observed particles were compared against reference spectra available within the GhenToxLab OMNIC Picta database associated with the Nicolet iN10 microscopy software. Polymer identification was accepted when spectral matches exceeded 80% similarity, after which a representative subset of spectra was visually inspected to verify the reliability of the automated classifications.

2.7. Data analysis

Statistical analyses and graphical visualizations were performed using the statistical software R (R version 4.6.0) within the RStudio environment. Data organizations and preprocessing were conducted using the packages tidyverse (v2.0.0; Wickham et al., 2019), janitor (v2.2.1; Firke, 2024), ggpubr (v0.6.3; Kassambara, 2026), patchwork (v1.3.2; Pedersen, 2025), rstatix (v0.7.3; Kassambara, 2025) and vegan (v2.7-3; Oksanen et al., 2026).

Descriptive statistics were calculated for MP abundance, polymer composition and particle dimensions. The frequency of occurrence (FO%) of the MPs was calculated as the percentage of GIT containing at least one suggested MP particle relative to the total number of specimens analyzed. Microplastic abundance was expressed in two different ways: as the total number of plastics per individual and as the number of particles standardized to GIT weight (items g^{-1} GIT).

Normality was evaluated using the Shapiro-Wilk normality test followed by non-parametric statistical approaches. Relationships between morphometric variables and MP abundance were assessed using Spearman's rank correlation tests. Correlation analyses were performed for a combined body size metric derived from body width and body height measures.

To evaluate the MP size distribution and potential ecological relevance, three size classes were defined: <100 μ m, 100-500 μ m and >500 μ m, allowing evaluation of particle size distribution patterns and potential ecological relevance. Polymer composition was summarized as relative percentages of the total number of identified particles.

Data visualization included histograms, scatterplots and bar plots to illustrate particle abundance, size distribution and polymer composition. Statistical significance was evaluated at $\alpha = 0,05$ throughout all analyses.

3. Results

3.1. Microplastic occurrence in *Neotrygon indica*

A total of 24 gastrointestinal tract (GIT) samples of *Neotrygon indica* were processed and analyzed to assess the presence of microplastics (MPs). Particles were detected in 16 out of 24 individuals, corresponding to an occurrence frequency of 66.7%. In total, 186 suspected MP particles were confirmed through micro-Fourier transform infrared (μ FTIR) spectroscopy (μ FTIR) analysis with a minimal match of 80%.

The number of particles identified per individual varied considerably. Microplastic abundance ranged from 0 to 89 particles per individual, with a mean abundance of 7.79 ± 18.22 particles per GIT. When standardized to GIT weight, MP abundance showed a range of 0 to $1.63 \text{ items g}^{-1} \text{ GIT}$, with a mean value of $0.18 \text{ items g}^{-1} \text{ GIT}$ (see Figure 5.).

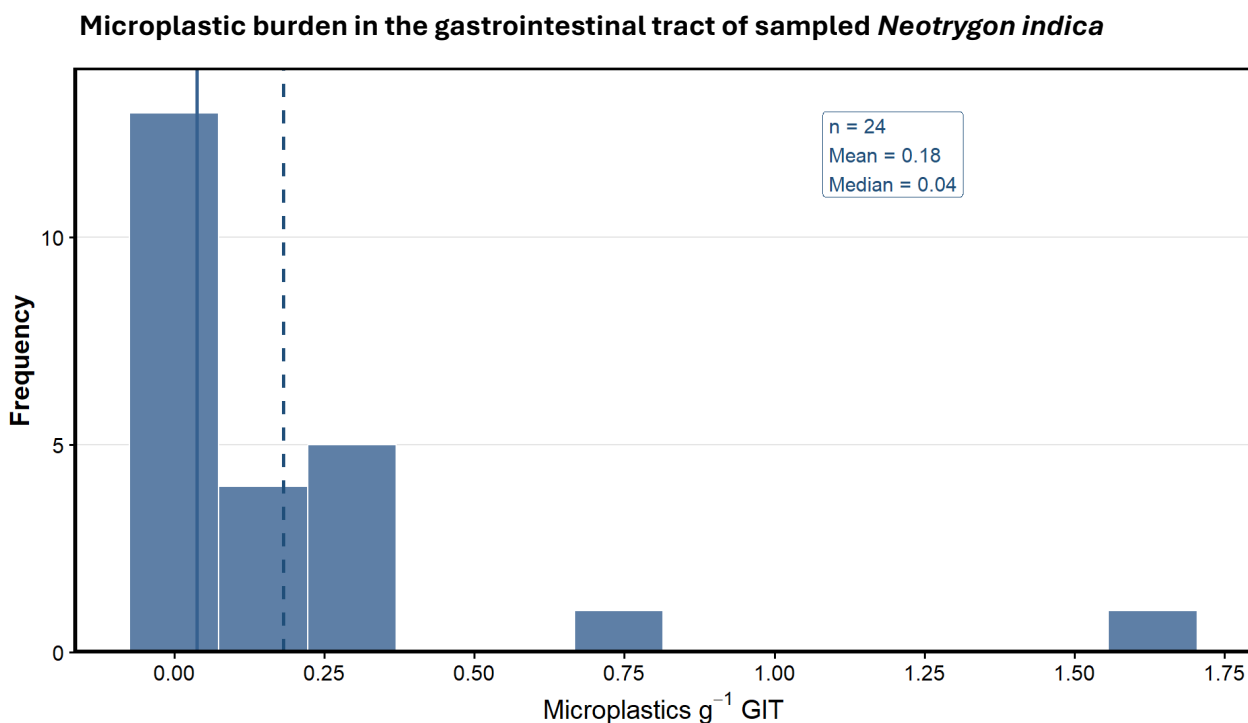


Figure 5. Microplastic burden in the gastrointestinal tract of sampled *Neotrygon indica*: frequency distribution of microplastic abundance expressed as the number of particles per gram wet weight of the gastrointestinal tracts ($\text{MPs g}^{-1} \text{ GIT}$). The frequency represents the number of individuals within each microplastic abundance range.

The distribution pattern of MP abundance per individual was strongly right-skewed due to the presence of several highly contaminated individuals (Figure 5.). Shapiro-Wilk normality testing confirmed that the data was not normally distributed ($W = 0.44$; $p < 0.001$). Similarly, MP abundance standardized by GIT weight was also non-normally distributed ($W = 0.55$; $p < 0.001$).

3.2. Polymer composition

Among the 186 identified particles, four polymer types were found: polyethylene terephthalate (PET), polystyrene (PS), polyethylene (PE) and polypropylene (PP). The dominant polymer type within the samples was PET, accounting for 77.4% of all identified particles. This finding was consistent across the majority of positive samples. Polyethylene particles were encountered less frequently, but included some of the largest particles identified in the dataset (see Figure 7.).

Polymer composition of microplastics detected in the gastrointestinal tract of *Neotrygon indica*

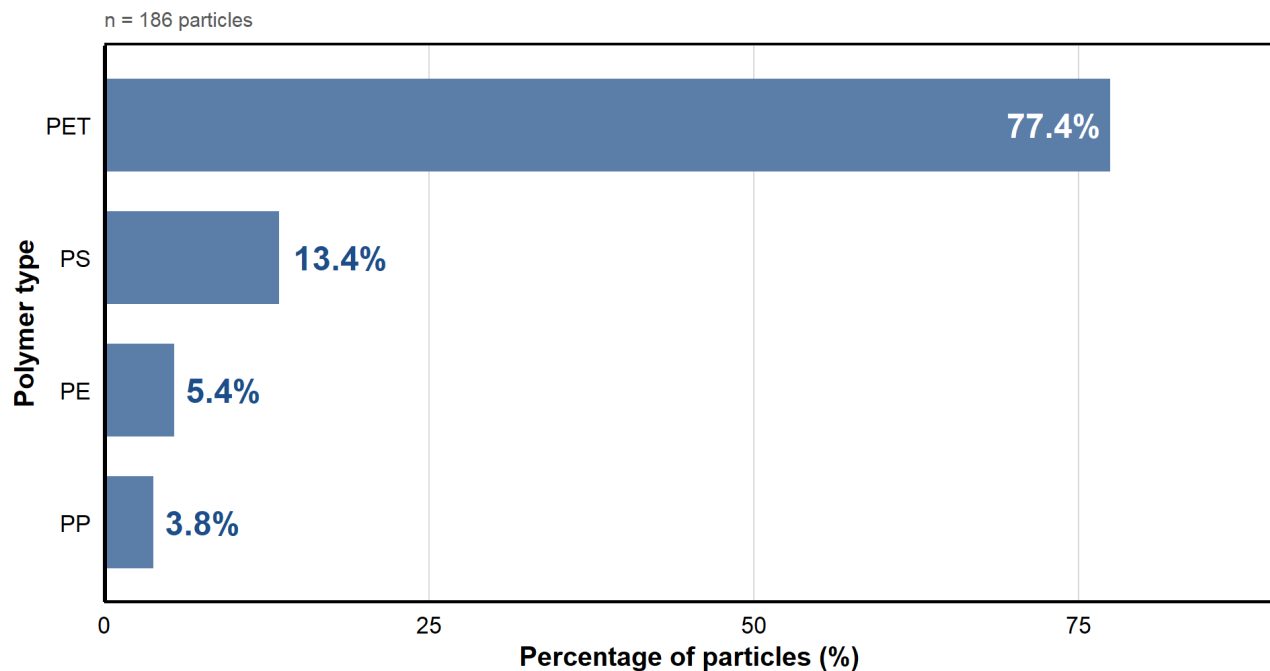


Figure 6. Polymer composition of microplastics detected in the gastrointestinal tract of *Neotrygon indica*: Polymer composition of identified microplastic particles following an 80% match threshold. The main categories included polyethylene terephthalate (PET), polystyrene (PS), polyethylene (PE), and polypropylene (PP).

3.3. Particles size distribution

Particle size varied substantially among samples with lengths ranging from 30.1 μm to 6352.4 μm . The median particle length was 82.8 μm with a mean length of 148.9 μm . Width measurements ranged from 12.1 μm to 400.5 μm .

To evaluate the potential ecological relevance of particle size, three size classes were defined: <100 μm , 100–500 μm and >500 μm . The majority of detected particles were smaller than 100 μm , thus representing the dominant size fraction within the samples. Particles between 100 and 500 μm accounted for 63 particles within the GIT of *N. indica*, while particles belonging the largest size class rarely occurred (see Figure 7.).

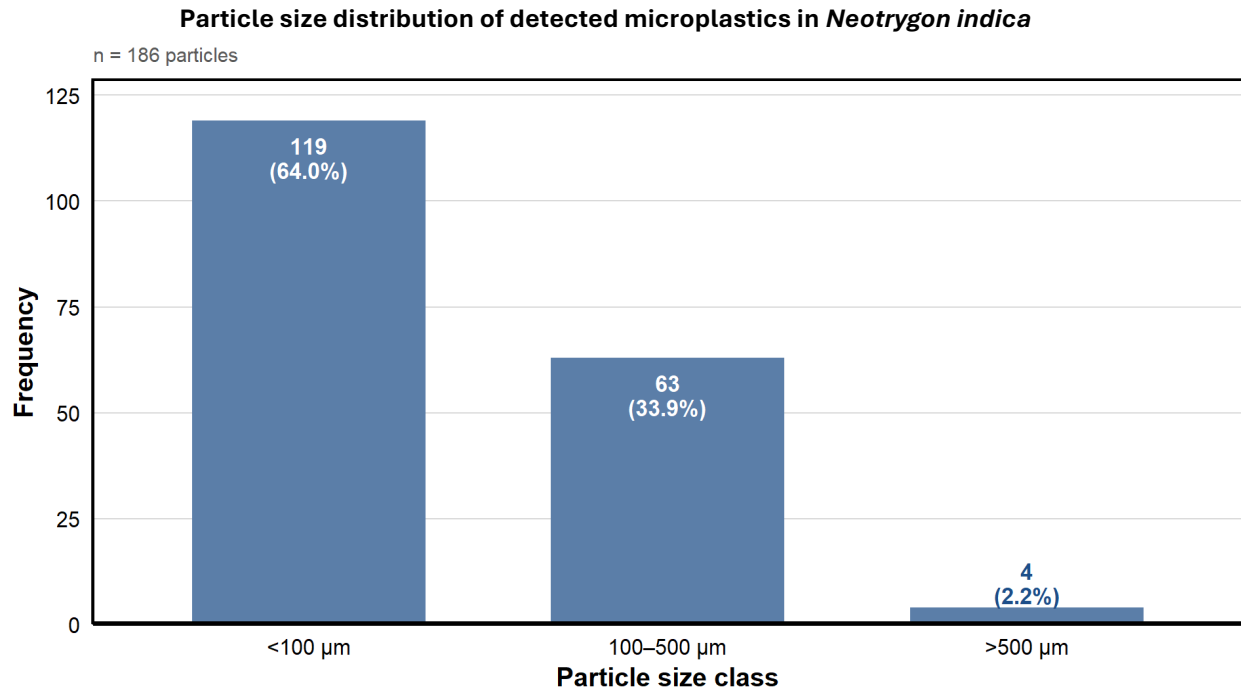


Figure 7. Particle size distribution of detected microplastics in *Neotrygon indica*: number of particles within three size classes among the 186 particles detected in *N. indica* gastrointestinal tracts. Percentages indicate the relative proportion of particles within each size class.

3.4. Body size and microplastic abundance

Body width (350.83 ± 25.21 mm) showed no significant relationship with the number of MPs detected per individual (Spearman's $\rho = 0.12$, $p = 0.56$). Similarly, when body size was expressed as a combined metric calculated as body width $\times$ body height, no significant relationship with MP abundance was observed ($\rho = 0.098$, $p = 0.65$; Figure 8).

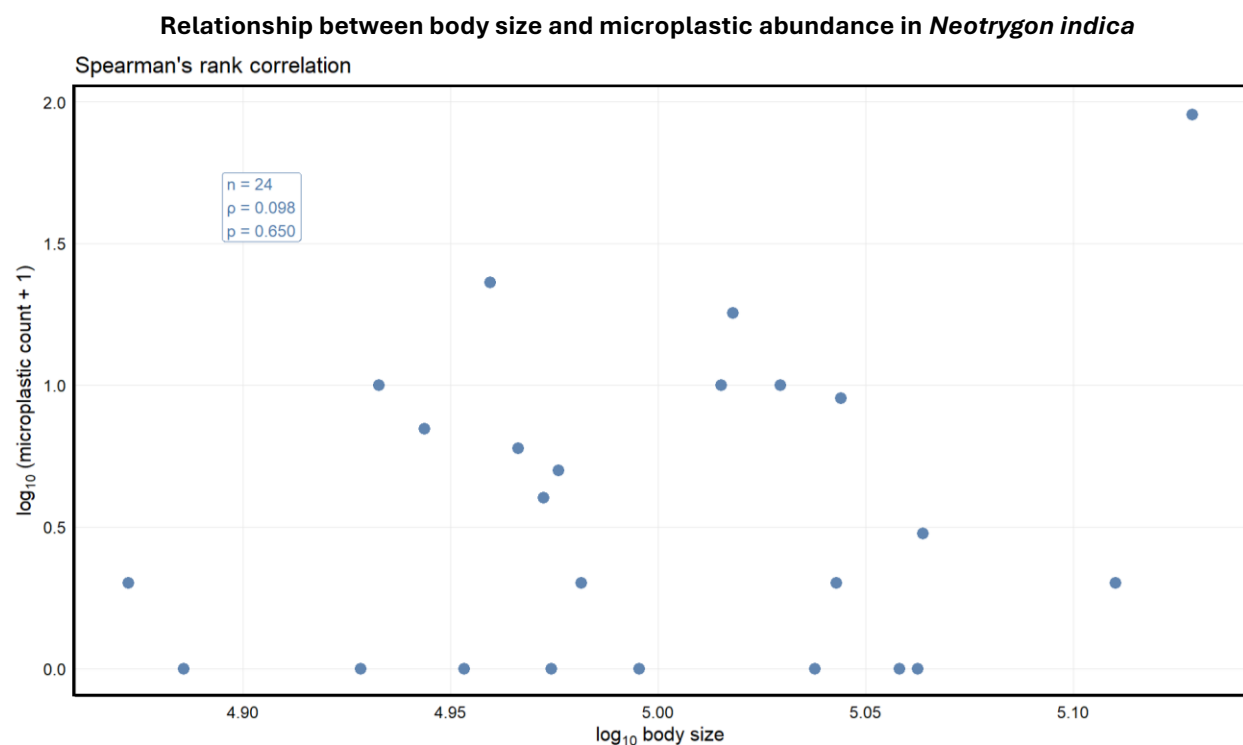


Figure 8. Relationship between body size and microplastic abundance in *Neotrygon indica*: microplastic abundance is shown across specimen body sizes, with Spearman's rank correlation analysis results indicated.

Further a repeated analysis excluding the outlier likewise revealed no significant relationship between body size and MP abundance ($\rho = -0,028$, $p = 0,898$).

3.5. Contamination control and recovery assessment

No MP particles were detected in the procedural blanks after applying the final FTIR identification threshold of 80% spectral similarity.

Table 1. Procedural and air blank analysis: Micro-Fourier transform infrared (μ FTIR) identification results for procedural and air blanks, including identified components, spectral match percentage, area and particle dimensions. A spectral match of $\geq 80\%$ was considered a positive identification of a microplastic particle.

Blank ID	Identified Component	Match %	Area %	Length ($\hat{\text{A}}\mu\text{m}$)	Width ($\hat{\text{A}}\mu\text{m}$)
BLANCO_1_25.09.2025	0	0	0	0	0
BLANCO_2_10.10.2025	Polystyrene	75.01	0.13	75.3	48.9
BLANCO_3_07.11.2025	0	0	0	0	0
BLANCO_4_13.11.2025	Polyethylene Terephthalate	76.64	0.01	48.2	28.4
BLANCO_AIR_13.11.2025	Polypropylene	82.72	0.38	140.0	84.0
BLANCO_AIR_13.11.2025	Polystyrene	80.40	0.37	225.8	50.2
BLANCO_AIR_13.11.2025	Polypropylene	89.78	0.34	316.6	33.3

Air blanks contained several airborne particles identified as polypropylene (PP) and polystyrene (PS).

Recovery experiments demonstrated a retrieval efficiency of 96%, with 24 of 25 experimentally added particles successfully recovered following the complete analytical procedure.

Table 2. Positive procedural control recovery of PET and PE particles: *Recovery of PET and PE particles used as positive procedural controls, including particles added, particles retrieved and recovery percentage for each sample.*

Sample ID	PET added (n)	PET retrieved (n)	PET recovery (%)	PE added (n)	PE retrieved (n)	PE recovery (%)
02_NI_25.09.2025	2	2	100	0	0	–
04_NI_10.10.2025	1	1	100	1	1	100
06_NI_10.10.2025	0	0	–	2	2	100
08_NI_10.10.2025	0	0	–	1	1	100
09_NI_10.10.2025	2	2	100	2	2	100
12_NI_10.10.2025	1	0	0	2	2	100
14_NI_07.11.2025	2	2	100	0	0	–
15_NI_07.11.2025	0	0	–	3	3	100
16_NI_07.11.2025	1	1	100	1	1	100
24_NI_13.11.2025	0	0	–	3	3	100
26_NI_13.11.2025	1	1	100	2	2	100
29_NI_13.11.2025	1	1	100	1	1	100

4. Discussion

The present study combined methodological evaluation with an initial assessment of microplastic (MP) occurrence in *Neotrygon indica* from the east side of Sri Lanka, providing a standardized methodology and baseline information on MP occurrence within the analyzed specimens.

4.1. Methodological applicability

The results demonstrate that the extraction and processing of elasmobranch GIT samples for MP analysis can be performed using relatively low-cost procedures under resource-limited laboratory conditions. The digestion, filtration and sample-cleaning steps used in this study did not require highly specialized analytical infrastructure, indicating that these initial stages of MP analysis can be implemented in laboratories where access to advanced equipment is limited. This is particularly relevant for studies conducted in regions where specialized analytical facilities are not readily available, as samples can undergo these initial processing steps locally before being transferred to a more specialized laboratory for further analysis.

In this study, chemical identification of the recovered particles was performed through collaboration with a specialized laboratory using μ FTIR spectroscopy. Although this step does require specialized

equipment and expertise, it does not necessarily need to be available at the location where sample collection and initial processing are conducted. Collaboration with, or outsourcing to, specialized laboratories may therefore provide a practical approach for obtaining polymer-level identification while allowing the more accessible stages of MP extraction and preparation to be conducted locally. This distinction is important when considering the applicability of standardized MP methodologies in resource-limited settings, as the absence of advanced analytical equipment should not necessarily prevent data collection during sample processing.

The inclusion of both procedural blanks and recovery controls strengthened the assessment of methodological reliability. No MPs with a spectral match above 80% were detected in the procedural blanks after undergoing the complete digestion and filtration protocols, indicating that no detectable contamination was introduced during these processing steps.

Air blanks provide an important indication of the potential of airborne contamination, particularly in laboratory settings without specialized clean-air facilities. In the present study, MP contamination was detected in the air blanks, which were intentionally exposed to the laboratory environment throughout the processing of a complete sample batch. Because the air blanks were exposed for substantially longer periods than the samples and the latter were kept covered whenever possible, these results likely represent a conservative estimate of the potential airborne contamination rather than direct evidence of contamination of the analyzed samples. Nevertheless, the detection of MPs in the air blanks highlights the importance of consistently applying contamination-control measures (see Appendix 1).

The positive recovery controls further supported the applicability of this methodology. Artificially introduced polyethylene terephthalate (PET) and polyethylene (PE) particles were successfully retrieved, with only a single PET particle not recovered from one specimen. This indicated a high recovery efficiency, while simultaneously suggesting that some degree of underestimation of total particle abundance remained possible. Such losses are difficult to avoid entirely in MP studies due to repeated handling, filtration and transfer steps. Nevertheless, the overall recovery success demonstrated that the protocol was sufficiently robust for baseline assessment of MP occurrence in elasmobranch GIT.

The relatively conserved digestive tract morphology of elasmobranch suggests that a similar basic methodological approach could be applied across taxa ([Buckling, 2015](#); [Leigh et al., 2017](#)). However, methodological requirements may vary according to feeding ecology and the type of material ingested. In this study, the substantial sediment content in the GITs of *N. indica*, a benthic feeder, resulted in high inorganic loads and required additional centrifugation and sieving steps before filtration and FTIR analysis. Sediment particles can also overlap with MPs in size, making their separation particularly challenging ([Van Melkebeke et al., 2020](#)). This suggests that adaptations to the methodology may depend more strongly on feeding strategy and associated sediment ingestion than on differences in digestive morphology. Further comparative research across elasmobranch species with different feeding ecologies is needed to assess the broader applicability of the approach.

4.2. Microplastic occurrence in *Neotrygon indica*

Microplastics were detected with a frequency of occurrence (FO) of 66.7% in the analyzed specimens, indicating that exposure to plastic pollution is common for *N. indica* individuals inhabiting the coastal waters of eastern Sri Lanka. This supports the hypothesis that MPs would be present in the majority of examined GIT. However, the abundance of particles per individual remained relatively low. The observed FO falls within the broad range reported in previous elasmobranch studies, where MP occurrence varied considerably between species and regions. Ranging from 6.4% in pelagic sharks ([De Oliveira Soares Espinosa et al., 2025](#)) to 100% in tropical species sampled in Malaysia ([Matupang et al., 2023](#)) and porbeagle sharks (*Lamna nasus*) from the North East Atlantic ([Maes et al., 2020b](#)). In blue sharks (*Prionace glauca*) from the eastern tropical Pacific Ocean an FO of 39.1% has been reported ([Huang et al., 2022](#)). Such variability likely reflects the differences in environmental exposure, feeding ecology, sample processing methods and contamination control protocols between studies.

No significant correlation was observed between body size and MP abundance. This contrasts with the initial hypothesis that larger individuals would contain greater MP abundances due to increased food intake and longer potential exposure time. This suggests that larger individuals did not necessarily accumulate more MPs than smaller individuals within the analyzed population.

The relationship between body size and MP abundance within the GIT remains debated, with studies reporting both positive relationships as well as the absence of size-related patterns. Several studies have found that MP abundance does not necessarily increase with fish size ([Foekema et al., 2013](#)), whereas other studies have reported higher MP loads in larger elasmobranch individuals ([Matupang et al., 2023](#)). This variation suggests that body size alone may not be a consistent predictor of MP abundance across species.

Several explanations may underly this absence of correlation. MP ingestion may primarily reflect recent feeding activity rather than long-term accumulation, particularly when particles are rapidly egested from the digestive tract ([Lusher et al., 2013](#); [Rochman et al. 2019](#)). Furthermore, exposure likely depends on local prey availability, sediment conditions and individual feeding behavior rather than body size only. Although larger individuals generally require higher food intake, which theoretically increases exposure probability ([Bond et al., 2018](#); [Matupang et al., 2023](#)), this effect may be outweighed by differences in habitat use, prey composition and short-term feeding conditions. Feeding strategy and habitat use are considered important factors influencing MP exposure, as benthic species feeding close to sediments may encounter higher concentrations of sediment-associated MPs when compared to pelagic species ([Mason et al., 2022](#)). Similarly, Maes et al. ([2020b](#)) reported no statistically significant relationship between MP abundance and biological condition indices in porbeagle sharks, suggesting that MP accumulation may not follow straightforward biological patterns across elasmobranch species.

The majority of detected plastic particles consisted of relatively small size classes, with approximately 64% measuring below 100 µm and only a small portion exceeding 500 µm. Particle size is an important factor influencing the ecological relevance of MP exposure, as smaller particles

have a higher surface area-to-volume ratio and may interact more readily with surrounding contaminants and biological tissues ([Koelmans et al., 2013](#); [Rochman, 2015](#)). Furthermore, particles at the lower end of the MP size spectrum, particularly those in the micrometer-to-nanometer size range, have a greater potential for uptake across biological barriers and translocation beyond the GIT ([Rochman et al., 2019](#)). However, the present study was limited by the detection range of the Thermo Scientific Nicolet iN10 μ FTIR spectroscope with the lower boarder being 30 μ m. Because of this, particles not retained by the sift with the 35 μ m grid size were not retained for polymer identification. Consequently, the smallest particle fractions were not fully captured, resulting in an underestimation of MP abundance and limiting the assessment of their relative contribution to any potential biological effects. Although this study focused exclusively on gastrointestinal contents, the observed size distribution provides valuable baseline information on MP exposure in *N. indica* and highlights the need for future research including smaller particle fractions.

The predominance of smaller particles within the analyzed fraction was notable, particularly as a sieving step was incorporated into the methodology to reduce clogging of the Omnipore PTFE membrane filters during filtration, which may have resulted in the loss of some smaller particles. Despite this loss, smaller particles represented the largest proportion of detected MPs. Furthermore, the 35–100 μ m size fraction was excluded from FTIR analysis due to time constraints and was therefore not included in the final dataset. Consequently, the abundance of smaller MPs in the examined specimens is likely underestimated.

4.3. Polymer composition and environmental context

Polymer identification revealed a strong dominance of PET, followed by PS, PE and PP. The predominance of PET may reflect both widespread global use of this polymer as well as regional patterns of plastic consumption. Polyethylene terephthalate is commonly associated with beverage bottles, synthetic textiles and other consumer products ([Rochman et al., 2019](#)). In addition, its relatively high density compared to seawater increases its potential to accumulate within the sediment ([Rochman et al., 2019](#)). Since *N. indica* is a benthic species that forages close to or within sedimentary habitats, the high proportion of PET observed in the study may reflect the composition of plastics accumulating on the seafloor.

The observed polymer composition is also consistent with known sources of plastic pollution in Sri Lanka as coastal and aquatic ecosystems are affected by multiple anthropogenic activities.

The occurrence of PE and PP, despite their lower densities and consequently higher expected buoyancy, may similarly be explained by environmental aging and biofouling processes. Although these polymers initially tend to float, biofouling and attachment of organic material can increase particle density and facilitate sinking to the seafloor ([Mason et al., 2022](#); [Van Melkebeke et al., 2020](#)). The majority of marine MP are believed to ultimately accumulate within benthic environments due to these processes. Consequently, benthic species such as *N. indica* may be particularly exposed to MPs that have undergone prolonged environmental transport and aging.

The observed polymer composition may additionally reflect regional waste management patterns in Sri Lanka. Plastic consumption and disposal have increased substantially during recent decades, while recycling infrastructure remains limited for several polymer types. In particular PET and PS are associated with recycling challenges due to contamination, low economic value and inadequate processing infrastructure ([Jayasinghe et al., 2023](#)). Marine debris surveys along the Sri Lanka coastline have similarly reported plastics as the dominant type of debris, with packaging material representing the largest source category. Previous studies on Sri Lankan beaches also documented the presence of PET bottles and plastic packaging transported by regional ocean currents, particularly along the eastern coastline ([Jang et al., 2018](#)). The polymer composition observed in the present study therefore appears consistent with both regional plastic consumption patterns and known debris accumulation processes.

However, the detected polymer composition should not automatically be interpreted as a direct representation of environmental polymer abundance patterns, as this observed patterns might be influenced by other factors such as ingestion pathways, particle retention and methodological factors.

Particle morphology was not systematically classified in the present study, although shape may strongly influence transport dynamics, biofouling behavior and ingestion probability.

4.4. Potential ecological implications

Although the relatively low number of particles identified per specimen suggest limited risk of physical blockage within the digestive tract, the potential chemical implications of MP ingestion should not be underestimated. Plastics contain a wide range of additives including plasticizers, stabilizers, flame retardants and colorants ([Rochman et al., 2019](#)). In addition, MPs can absorb persistent organic pollutants and trace metals from surrounding seawater and sediment ([Rochman, 2015](#)). Consequently, MPs may function as vectors, facilitating contaminant transfer into marine organisms.

The ecological implications of this process are complex and likely depend on polymer type, particle size and environmental conditions. Some polymers, such as PE and PP, have relatively high sorption capacities for certain organic pollutants, whereas others such as polyvinyl chloride (PVC) and PS may themselves contain potentially hazardous monomers or additives ([Rochman, 2015](#)). The predominance of small particles identified in the present study may further increase toxicological relevance due to the greater surface area-to-volume ratio of smaller MPs and their increased potential for translocation into tissues ([Rochman et al., 2019](#)). However, the actual physiological consequences for *N. indica* remain unknown, as these were beyond the scope of this study, which did not assess tissue accumulation, biomarker responses or contaminant transfer.

Elasmobranchs may be particularly vulnerable to chronic contaminant exposure due to their relatively slow growth, long life spans and low reproductive rates ([Pavan-Kumar et al., 2022](#)). Even sublethal effects on immune function, endocrine regulation or reproduction could therefore have ecological consequences at the population level ([Galli et al., 2023](#); [Germanov et al., 2018](#)). At

present, however, the direct effects of MP exposure on rays and sharks remain poorly understood, highlighting the need for further ecotoxicological research ([Galli et al., 2023](#); [Huang et al., 2022](#); [Pasalari et al., 2025](#); [Torres et al., 2024](#)).

4.5. Applicability across species

Although the primary focus of this study was *N. indica*, additional ray species were opportunistically processed to evaluate whether the developed methodology could be applied across taxa with differing body size and ecological characteristics. These entailed the honeycomb stingray (*Himantura uarnak*), broad cowtail ray (*Pastinachus ater*), spinetail devil ray (*Mobula mobular*), long-tailed butterfly ray (*Gymnura poecilura*) and Jenkins' whiplay (*Pateobatis jenkinsii*). Due to the limited number of specimens per species, these observations cannot be interpreted statistically and should therefore be regarded as preliminary methodological observations rather than species-specific trends.

Nevertheless, substantial variations in MP abundance were observed among species. Particularly notable were the high particle counts detected for both *P. jenkinsii* specimens, which contained considerably larger numbers of PE and PP particles compared to *N. indica*. These differences may partly reflect variation in feeding ecology, as the species examined encompassed different feeding strategies, including filter feeding in *M. mobular* and benthic feeding in the other species, with GIT contents ranging from sand, crushed shells and shrimp remains. While these findings may partially reflect stochastic variation associated with low sample size, ecological differences such as feeding behavior, habitat use and sediment interaction may also contribute to differences in MP exposure between ray species. These preliminary observations support the need for broader comparative studies across elasmobranch taxa occupying different ecological niches.

An important methodological observation emerged during the processing of the *M. mobular* specimen. This individual represented the largest specimen included in this study with a body width of 1643 mm, body height of 705 mm and GIT wet weight of 1147 g. The standard digestion protocol, which was successfully applied to the other smaller species, did not achieve a comparable digestion efficiency for *M. mobular*. Due to time constraints, additional optimization experiments could not be conducted. However, the incomplete digestion suggested that larger-bodied elasmobranchs may require methodological adjustments, such as longer digestion times, increased reagent volumes relative to tissue mass, or repeated digestion steps.

These observations highlight important logistical and methodological considerations for future studies targeting larger elasmobranchs. Processing large-bodied species requires substantially larger glassware volumes, increased laboratory space and additional handling capacity during dissection and digestion. Furthermore, transporting large specimens into contamination-controlled working environments presents practical challenges, particularly in remote or resource-limited laboratory settings. Although the methodology developed in this study appears broadly applicable to rays and potentially other elasmobranchs, further optimization for large species remains necessary.

4.6. Limitations and future research directions

Several limitations should be acknowledged. Due to logistic constraints, this study focused primarily on a single species from a relatively restricted geographic region and did not include seasonal or spatial comparisons. Sample numbers were uneven across sampling periods, limiting the ability to evaluate temporal variability such as potential monsoon-related changes in MP exposure. This is particularly relevant in Sri Lanka where monsoonal systems strongly influence marine debris transport and coastal accumulation patterns ([Jang et al., 2018](#)). Future studies should therefore include broader temporal sampling to assess whether seasonal hydrodynamic changes influence MP exposure in benthic elasmobranchs.

Additional research should also expand the range of species and habitats investigated. Comparative studies between benthic and pelagic elasmobranchs may help clarify how feeding ecology influences MP exposure and whether methodological adaptations are required for species occupying different ecological niches. On a global scale, comparative studies across geographic regions could help identify spatial patterns in MP exposure and reveal how environmental conditions and pollution sources contribute to regional differences.

Methodological refinement remains another important research direction. The incorporation of an initial sieving step following digestion may improve filtration efficiency and reduce clogging in sediment-rich samples.

Finally, future research should move beyond occurrence data alone. The toxicological implications of MP ingestion in elasmobranchs remain largely unresolved. Ethical investigations into tissue translocation, chemical transfer, physiological stress and reproductive impacts are needed to better understand how chronic MP exposure may affect these ecologically important species. Overall, the present study provides a practical methodological framework for MP analysis in elasmobranch GIT while simultaneously contributing baseline data in *N. indica* from Sri Lanka.

5. Conclusion

This study successfully developed and applied a contamination-controlled methodology for the extraction of microplastics (MP) from elasmobranch gastrointestinal tracts (GIT) under resource-limited laboratory conditions. While sample collection and preparation were designed to be feasible in basic laboratory settings, final polymer identification was conducted in collaboration with a specialized laboratory using micro-Fourier transform infrared (μ FTIR) spectroscopy. The protocol proved capable of generating reliable MP occurrence data while relying on accessible materials and procedures. It demonstrated that meaningful MP data research can be conducted outside of highly specialized laboratory environments when strict contamination prevention measures are implemented.

Microplastics were detected in the majority *Neotrygon indica* specimens, with particles classified as MPs when their spectra showed a match of $\geq 80\%$ with reference polymer spectra in the spectral

database. This indicates that plastic exposure may be widespread within benthic coastal habitats along the East Coast of Sri Lanka. The dominance of polyethylene terephthalate (PET) particles and the prevalence of small particle size classes suggest that sediment-associated MPs represent an important exposure pathway for this species. Although particle abundances were relatively low in most individuals and no relationship between body size and MP abundance was observed, the predominance of smaller particles may still be ecologically relevant due to their higher potential for contaminant sorption and tissue translocation.

The study additionally highlighted the importance of incorporating species ecology traits when developing MP extraction protocols. The substantial sediment load present within the GIT of *N. indica* caused significant filtration challenges, indicating that benthic elasmobranch may require additional purification or separation steps compared to the pelagic species. Preliminary observations from other ray species further suggest that feeding behavior and habitat use strongly influence both MP exposure and methodological requirements. In particular, the incomplete digestion observed in *Mobula mobular* specimen indicates that larger-bodied elasmobranchs may require modified digestion conditions and increased logistical capacity.

Despite several limitations, including restricted sample size, limited spatial and temporal coverage and the absence of toxicological analyses, this study provides valuable initial data on MP occurrence in *N. indica* while additionally establishing a practical methodological framework for future elasmobranch research. Future studies should expand sampling across species, regions and seasons while further investigating the physiological and ecological consequences of chronic MP exposure in elasmobranchs. Continued methodological refinement, particularly for sediment-rich and larger-bodied species, will remain essential for improving data comparability and advancing our understanding of MP within marine food webs.

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7. References

- Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, 62(8), 1596–1605. <https://doi.org/10.1016/j.marpolbul.2011.05.030>
- Bhuyan, S., et al. (2021). Plastics in marine ecosystem: A review of their sources and pollution conduits. *Regional Studies in Marine Science*, 41, 101539. <https://doi.org/10.1016/j.rsma.2020.101539>
- Bond, M. E., Valentin-Albanese, J., Babcock, E. A., Hussey, N. E., Heithaus, M. R., & Chapman, D. D. (2018). The trophic ecology of Caribbean reef sharks (*Carcharhinus perezi*) relative to other large teleost predators on an isolated coral atoll. *Marine Biology*, 165(4). <https://doi.org/10.1007/s00227-018-3322-2>
- Bucking, C. (2015). Feeding and digestion in elasmobranchs: Tying diet and physiology together. In *Fish physiology* (pp. 347–394). <https://doi.org/10.1016/b978-0-12-801286-4.00006-x>
- Dehaut, A., Cassone, A., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivi re, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, 215, 223–233. <https://doi.org/10.1016/j.envpol.2016.05.018>
- De Oliveira Soares Espinosa, L., Lacerda, A. L., Oddone, M. C., Kessler, F., & Proietti, M. C. (2025). Interaction of elasmobranchs with litter in South Brazil: ingestion and oviposition patterns. *Marine Pollution Bulletin*, 216, 117992. <https://doi.org/10.1016/j.marpolbul.2025.117992>
- De Witte, B.; Catarino, A.I.; Vandecasteele, L.; Dekimpe, M.; Meyers, N.; Deloof, D.; Pint, S.; Hostens, K.; Everaert, G.; Torrelee, E. (2022). Fish gastrointestinal sampling protocol for microplastics extraction and quantification. *ILVO/VLIZ: Ostend*. 4 pp. <https://dx.doi.org/10.48470/6>
- El-Sherif, D. M., Eloffy, M. G., Elmesery, A., Abouzid, M., Gad, M., El-Seedi, H. R., Brinkmann, M., Wang, K., & Naggar, Y. A. (2022). Environmental risk, toxicity, and biodegradation of polyethylene: a review. *Environmental Science and Pollution Research*, 29(54), 81166–81182. <https://doi.org/10.1007/s11356-022-23382-1>
- Fernando, D., & Stewart, J. D. (2021). High bycatch rates of manta and devil rays in the “small-scale” artisanal fisheries of Sri Lanka. *PeerJ*, 9, e11994. <https://doi.org/10.7717/peerj.11994>
- Foekema, E. M., De Gruijter, C., Mergia, M. T., Van Franeker, J. A., Murk, A. J., & Koelmans, A. A. (2013). Plastic in North Sea fish. *Environmental Science & Technology*, 47(15), 8818–8824. <https://doi.org/10.1021/es400931b>
- Fossi, M. C., Ped , C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., & Bains, M. (2018). Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. *Environmental Pollution*, 237, 1023–1040. <https://doi.org/10.1016/j.envpol.2017.11.019>

- Galli, M., Garcia, T. O., Bains, M., R, J. U., Ramírez-Macías, D., Vitoria-Gómora, L., Panti, C., Martellini, T., Cincinelli, A., & Fossi, M. C. (2023). Microplastic occurrence and phthalate ester levels in neuston samples and skin biopsies of filter-feeding megafauna from La Paz Bay (Mexico). *Marine Pollution Bulletin*, 192, 115086. <https://doi.org/10.1016/j.marpolbul.2023.115086>
- Germanov, E. S., Marshall, A. D., Bejder, L., Fossi, M. C., & Loneragan, N. R. (2018). Microplastics: No Small Problem for Filter-Feeding Megafauna. *Trends in Ecology & Evolution*, 33(4), 227–232. <https://doi.org/10.1016/j.tree.2018.01.005>
- Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, 3(7). <https://doi.org/10.1126/sciadv.1700782>
- Guruge, K., Siriwardhana, S., Bandara, T., Suwandhahannadi, W., Jayarathna, W., Hewathilake, H., & Kumara, P. (2025). Assessment of marine debris and plastic pollution along the western coast of Sri Lanka. *Regional Studies in Marine Science*, 86, 104176. <https://doi.org/10.1016/j.rsma.2025.104176>
- Habib, R. Z., Aldhanhani, J. a. K., Ali, A. H., Ghebremedhin, F., Elakashlan, M., Mesfun, M., Kittaneh, W., Kindi, R. A., & Thiemann, T. (2022). Trends of microplastic abundance in personal care products in the United Arab Emirates over the period of 3 years (2018–2020). *Environmental Science and Pollution Research*, 29(59), 89614–89624. <https://doi.org/10.1007/s11356-022-21773-y>
- Hermesen, E., Mintenig, S. M., Besseling, E., & Koelmans, A. A. (2018b). Quality Criteria for the analysis of microplastic in biota samples: A Critical review. *Environmental Science & Technology*, 52(18), 10230–10240. <https://doi.org/10.1021/acs.est.8b01611>
- Huang, X., Gao, H., Li, Z., Wu, F., Gong, Y., & Li, Y. (2022). Microplastic contamination and risk assessment in blue shark (*Prionace glauca*) from the eastern tropical Pacific Ocean. *Marine Pollution Bulletin*, 184, 114138. <https://doi.org/10.1016/j.marpolbul.2022.114138>
- Jang, Y. C., Ranatunga, R., Mok, J. Y., Kim, K. S., Hong, S. Y., Choi, Y. R., & Gunasekara, A. (2018). Composition and abundance of marine debris stranded on the beaches of Sri Lanka: Results from the first island-wide survey. *Marine Pollution Bulletin*, 128, 126–131. <https://doi.org/10.1016/j.marpolbul.2018.01.018>
- Jansen, M. A. K., Andrady, A. L., Bornman, J. F., Aucamp, P. J., Bais, A. F., Banaszak, A. T., Barnes, P. W., Bernhard, G. H., Bruckman, L. S., Busquets, R., et al. (2024). Plastics in the environment in the context of UV radiation, climate change and the Montreal Protocol: UNEP Environmental Effects Assessment Panel, Update 2023. *Photochemical & Photobiological Sciences*, 23(4), 629–650. <https://doi.org/10.1007/s43630-024-00552-3>
- Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, 347(6223), 768–771. <https://doi.org/10.1126/science.1260352>

- Jayasinghe, R. R., Abeyrathna, W. P., Jayasingha, K. R., Hendawitharana, M. P., Bandara, T. S., Liyanage, C. L., & Williams, K. S. (2023). Exploring the plastic collection and recycling trends in Sri Lanka. *Recycling*, 8(6), 94. <https://doi.org/10.3390/recycling8060099>
- Katupotha, J., & Gamage, S. (2021). UNDERSTANDING THE RIVER BASIN CLASSIFICATION OF SRI LANKA. *WILDLANKA*, 25. <http://dr.lib.sjp.ac.lk/handle/123456789/9130>
- Koelmans, A. A., Besseling, E., Wegner, A., & Foekema, E. M. (2013). Plastic as a Carrier of POPs to Aquatic Organisms: A Model Analysis. *Environmental Science & Technology*, 47(14), 7812–7820. <https://doi.org/10.1021/es401169n>
- Leigh, S. C., Papastamatiou, Y., & German, D. P. (2017b). The nutritional physiology of sharks. *Reviews in Fish Biology and Fisheries*, 27(3), 561–585. <https://doi.org/10.1007/s11160-017-9481-3>
- Löder, M. G. J., & Gerdt, G. (2015). Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal. In *Marine Anthropogenic Litter* (pp. 201–227). https://doi.org/10.1007/978-3-319-16510-3_8
- Lusher, A., McHugh, M., & Thompson, R. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin*, 67(1–2), 94–99. <https://doi.org/10.1016/j.marpolbul.2012.11.028>
- Lusher, A., Tirelli, V., O'Connor, I., & Officer, R. (2015). Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. *Scientific Reports*, 5(1). <https://doi.org/10.1038/srep14947>
- Maes, T., Barry, J., Stenton, C., Roberts, E., Hicks, R., Bignell, J., et al. (2020a). The world is your oyster: low-dose, long-term microplastic exposure of juvenile oysters. *Heliyon* 6(1), e03103. <https://doi.org/10.1016/j.heliyon.2019.e03103>
- Maes, T., Van Diemen de Jel, J., Vethaak, A. D., Desender, M., Bendall, V. A., Van Velzen, M., & Leslie, H. A. (2020b). You Are What You Eat, Microplastics in Porbeagle Sharks From the North East Atlantic: Method Development and Analysis in Spiral Valve Content and Tissue. *Frontiers in Marine Science*, 7. <https://doi.org/10.3389/fmars.2020.00273>
- Mason, V. G., Skov, M. W., Hiddink, J. G., & Walton, M. (2022). Microplastics alter multiple biological processes of marine benthic fauna. *The Science of the Total Environment*, 845, 157362. <https://doi.org/10.1016/j.scitotenv.2022.157364>
- Matupang, D. M., Zulkifli, H. I., Arnold, J., Lazim, A. M., Ghaffar, M. A., & Musa, S. M. (2023). Tropical sharks feasting on and swimming through microplastics: First evidence from Malaysia. *Marine Pollution Bulletin*, 189, 114762. <https://doi.org/10.1016/j.marpolbul.2023.114762>

- Pasalari, M., Esmaeili, H. R., Keshavarzi, B., Busquets, R., Abbasi, S., & Momeni, M. (2025). Microplastic footprints in sharks and rays: First assessment of microplastic pollution in two cartilaginous fishes, hardnose shark and whitespotted whiptail. *Marine Pollution Bulletin*, 212, 117350. <https://doi.org/10.1016/j.marpolbul.2024.117350>
- Pavan-Kumar, A., Singh, S., Mishra, A., Suman, S., Gireesh-Babu, P., Chaudhari, A., Shen, K., & Borsa, P. (2022). Characterization of mitochondrial genome of Indian Ocean blue-spotted maskray, *Neotrygon indica* and its phylogenetic relationship within Dasyatidae Family. *International Journal of Biological Macromolecules*, 223(Pt A), 458–467. <https://doi.org/10.1016/j.ijbiomac.2022.10.277>
- Pegado, T., Brabo, L., Schmid, K., Sarti, F., Gava, T. T., Nunes, J., Chelazzi, D., Cincinelli, A., & Giarrizzo, T. (2021). Ingestion of microplastics by *Hypanus guttatus* stingrays in the Western Atlantic Ocean (Brazilian Amazon Coast). *Marine Pollution Bulletin*, 162, 111799. <https://doi.org/10.1016/j.marpolbul.2020.111799>
- Prata, J. C., Da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2018). Methods for sampling and detection of microplastics in water and sediment: A critical review. *TrAC Trends in Analytical Chemistry*, 110, 150–159. <https://doi.org/10.1016/j.trac.2018.10.029>
- Ranjith, L., Saravanan, R., Kalidas, C., Kavitha, M., Ramkumar, S., Joshi, K. K., & Manojkumar, P. P. (2018). Morphological Deformities in *Neotrygon kuhlii* (Muller & Henle, 1841) from Gulf of Mannar, Bay of Bengal, India. *Thalassas an International Journal of Marine Sciences*, 35(1), 49–56. <https://doi.org/10.1007/s41208-018-0083-0>
- Rochman, C. M., Hoh, E., Kurobe, T., & Teh, S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific Reports*, 3(1), 3263. <https://doi.org/10.1038/srep03263>
- Rochman, C. M. (2015). The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. In *Marine Anthropogenic Litter* (pp. 117–140). https://doi.org/10.1007/978-3-319-16510-3_19
- Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S. B., Wu, T., Santoro, S., Werbowksi, L. M., . . . Hung, C. (2019). Rethinking microplastics as a diverse contaminant suite. *Environmental Toxicology and Chemistry*, 38(4), 703–711. <https://doi.org/10.1002/etc.4379>
- Ryan, P. G., Moore, C. J., Van Franeker, J. A., & Moloney, C. L. (2009). Monitoring the abundance of plastic debris in the marine environment. *Philosophical Transactions Of The Royal Society B Biological Sciences*, 364(1526), 1999–2012. <https://doi.org/10.1098/rstb.2008.0207>

- Schirinzi, G. F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., Farré, M., Barceló, D., Fossi, M. C., Andaloro, F., & Romeo, T. (2020). A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea. *Journal Of Hazardous Materials*, 397, 122794. <https://doi.org/10.1016/j.jhazmat.2020.122794>
- Sutton, R., Mason, S. A., Stanek, S. K., Willis-Norton, E., Wren, I. F., & Box, C. (2016). Microplastic contamination in the San Francisco Bay, California, USA. *Marine Pollution Bulletin*, 109(1), 230–235. <https://doi.org/10.1016/j.marpolbul.2016.05.077>
- Thiele, C. J., Hudson, M. D., & Russell, A. E. (2019). Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Marine Pollution Bulletin*, 142, 384–393. <https://doi.org/10.1016/j.marpolbul.2019.03.003>
- Torres, S., Compa, M., Box, A., Pinya, S., & Sureda, A. (2024). Presence and Potential Effects of Microplastics in the Digestive Tract of Two Small Species of Shark from the Balearic Islands. *Fishes*, 9(2), 55. <https://doi.org/10.3390/fishes9020055>
- Valente, T., Sbrana, A., Scacco, U., Jacomini, C., Bianchi, J., Palazzo, L., De Lucia, G. A., Silvestri, C., & Matiddi, M. (2019). Exploring microplastic ingestion by three deep-water elasmobranch species: A case study from the Tyrrhenian Sea. *Environmental Pollution*, 253, 342–350. <https://doi.org/10.1016/j.envpol.2019.07.001>
- Vandermeersch, G., Van Cauwenberghe, L., Janssen, C. R., Marques, A., Granby, K., Fait, G., Kotterman, M. J., Diogène, J., Bekaert, K., Robbens, J., & Devriese, L. (2015). A critical view on microplastic quantification in aquatic organisms. *Environmental Research*, 143(Pt B), 46–55. <https://doi.org/10.1016/j.envres.2015.07.016>
- Van Melkebeke, M., Janssen, C., & De Meester, S. (2020). Characteristics and sinking behavior of typical microplastics including the potential effect of biofouling: Implications for remediation. *Environmental Science & Technology*, 54(14), 8668–8680. <https://doi.org/10.1021/acs.est.9b07380>
- Wesch, C., Barthel, A., Braun, U., Klein, R., & Paulus, M. (2016). No microplastics in benthic eelpout (*Zoarces viviparus*): An urgent need for spectroscopic analyses in microplastic detection. *Environmental Research*, 148, 36–38. <https://doi.org/10.1016/j.envres.2016.03.017>
- Worm, B., Lotze, H. K., Jubinville, I., Wilcox, C., & Jambeck, J. (2017). Plastic as a Persistent Marine Pollutant. *Annual Review Of Environment And Resources*, 42(1), 1–26. <https://doi.org/10.1146/annurev-environ-102016-060700>

- Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, 178, 483–492. <https://doi.org/10.1016/j.envpol.2013.02.031>
- Wu, B., Yu, H., Lei, P., He, J., Yi, J., Wu, W., Wang, H., Yang, Q., Zeng, G., & Sun, D. (2025). Microplastics in aquatic ecosystems: Detection, source tracing, and sustainable management strategies. *Ecotoxicology and Environmental Safety*, 291, 117883. <https://doi.org/10.1016/j.ecoenv.2025.117883>

ELASMOBRANCH GASTROINTESTINAL TRACT PROTOCOL FOR MICROPLASTIC ANALYSIS AND QUANTIFICATION

To ensure consistent and comparable results across different species, geographic regions, and time periods, a standardized approach to microplastic (MP) analysis is essential. The presented protocol has been developed with practicality, affordability and accessibility in mind. It is designed to be applicable in both well-equipped laboratories and resource-limited research settings while maintaining analytical reliability and minimizing contamination risks.

The protocol combines contamination-controlled dissection procedures, potassium hydroxide (KOH) digestion, filtration and purification steps, and polymer verification using micro-Fourier transform infrared spectroscopy (μ FTIR). Particular attention is given to contamination prevention, as MP analysis is highly susceptible to background contamination from laboratory materials, airborne fibers and handling procedures. The recommended protocol, based on the guidelines provided by De Witte et al. (2022), includes several key measures.

Avoidance of background contamination

One of the greatest challenges in MP research is the prevention of external contamination. During sample collection, dissection, digestion and analysis, airborne fibers and plastic particles may unintentionally contaminate samples, leading to an overestimation of MP abundance. To minimize this risk, strict contamination prevention measures should be implemented throughout all stages of sample processing.

- Specimens should not be dissected on the field to minimize contamination risks associated with uncontrolled field conditions; following quality criteria by Hermesen et al. (2018).
- Potential sources of contamination within the laboratory environment, along with their colors and the types of synthetic materials they are made of, should be documented.
- Air movement within the laboratory should be minimized during processing. When available a clean air workstation with fume hood should be used.
- Samples should remain covered whenever possible to reduce exposure to airborne contamination.
- Individuals handling the samples should wear non-synthetic clothing, preferably also covered by a 100% cotton laboratory coat and avoid garments that easily shed fibers, such as fleece.
- All laboratory surfaces should be cleaned thoroughly with 70% ethanol or 70% isopropyl alcohol before processing each specimen.

- Glass and metal equipment should be used whenever possible, while the use of plastic materials should be minimized.
- All glassware and equipment should be rinsed thoroughly with filtered water prior to use. Where appropriate, equipment may additionally be cleaned using ethanol or isopropyl alcohol.
- Procedural blanks and airborne contamination controls should be processed alongside biological samples to monitor contamination originating from laboratory air, reagents or equipment (De Witte et al., 2022; Galli et al., 2023).

Dissection of the specimen

The following dissection protocol is based on the sampling guidelines outlined by De Witte et al. (2022). It needs to be followed with all precautions in mind to avoid background contamination. This approach ensures that gastrointestinal tract (GIT) samples can serve as a reliable matrix for MP monitoring, supporting comparative studies across different regions and time periods.

1. For each specimen, record the sampling location and date, along with species identification and basic morphometric data, including total length and width.
2. Prior to GIT sampling, inspect the storage container for any signs of MP contamination, and keep it closed until use to minimize exposure.
3. Prior to dissection, rinse the exterior of the specimen thoroughly using filtered water to remove adhering contaminants.
4. Dissect the specimen from the cloacal region towards the gill area and carefully remove the entire GIT, including the stomach and intestine.
5. Determine the wet weight of the extracted GIT and transfer the tissue immediately into a clean glass Erlenmeyer flask or wrap it in aluminum foil if glassware is unavailable. Whenever possible, use the same glass container for subsequent digestion to minimize sample handling.
6. Store samples individually and keep them covered at all times to reduce contamination risk.
7. If digestion cannot be initiated immediately, store samples at -20°C until further processing.

Digestion of the gastrointestinal tracts

This protocol builds on the criteria worked out in the evaluation by Thiele et al. (2019). The method is cost-effective to allow implementation even in studies with limited budgets. Additionally, minimizing the number of procedural steps is essential to reduce the risk of background contamination and enhance efficiency of workflow. Similar to the dissection protocol, here one also needs to follow all precautions to avoid background contamination.

1. Tissue digestion is carried out using 10% (w/w) KOH, added to each flask, exceeding three times the volume of the digestive tract. The flasks are covered with aluminum (Al) foil to avoid contamination. The upper surface of the flask should be kept clean to prevent corrosion of the aluminum foil and potential contamination of the sample.
2. Incubate samples at 40–60°C for 72 hours. Gently mix samples once daily to facilitate tissue breakdown.
3. Following digestion, inspect the sample visually to confirm that soft tissues have been sufficiently degraded. For larger specimens or samples containing large amounts of organic material, additional digestion time may be required.
4. If necessary due to big particles, sift the digested sample to prevent clogging of the filters.

Microplastic extraction and filtration

1. Filter the digested solution using cellulose nitrate membrane filters.
2. Following initial filtration, transfer the filters to clean glass containers and subject them to a secondary digestion using approximately 150 mL of 10% KOH at 40-60°C until the cellulose nitrate filters have dissolved completely.
3. Centrifuge the resulting solution to separate sediment from the supernatant. Carefully decant the supernatant and filter it. Repeat the centrifugation, rinsing and filtration procedure twice using distilled water.
4. For benthic species perform density separation using sodium iodide (NaI; density approximately 1.4 g cm^{-3}). Repeat centrifugation and filtration three times to separate lower-density MPs from heavier inorganic material.
5. Discard the remaining sediment fraction after completion of density separation.
6. Subject the recovered material to a final digestion using 10% KOH at 60°C for a minimum of 24 hours.
7. Filter the final solution onto Omnipore PTFE membrane filters (10 μm pore size, 25 mm diameter) suitable for μFTIR analysis.

8. Dry the filters for 24 hours before spectroscopic analysis.

Protocol for microplastic identification

Following filtration and drying, the recovered particles should be examined visually using a stereomicroscope or comparable optical system. Where possible, particle characteristics such as size, shape and color should be recorded.

Although visual inspection provides valuable information on particle morphology, visual identification alone is insufficient for reliable polymer determination and may lead to misclassification. Therefore, polymer verification using spectroscopic techniques is strongly recommended.

Suitable methods include Fourier-transform infrared spectroscopy (FTIR), micro-Fourier transform infrared spectroscopy (μ FTIR) or Raman spectroscopy, depending on equipment availability and particle size. These techniques allow the determination of the chemical composition of suspected particles through comparison with reference spectra.

For each confirmed microplastic particle, polymer type and particle dimensions should be recorded. Additional information on particle shape and color should also be documented when visible, as these characteristics may provide valuable information regarding potential sources and environmental transport pathways

References

- De Witte, B.; Catarino, A.I.; Vandecasteele, L.; Dekimpe, M.; Meyers, N.; Deloof, D.; Pint, S.; Hostens, K.; Everaert, G.; Torrelee, E. (2022). Fish gastrointestinal sampling protocol for microplastics extraction and quantification. *ILVO/VLIZ: Ostend*. 4 pp. <https://dx.doi.org/10.48470/6>
- Galli, M., Garcia, T. O., Bains, M., R, J. U., Ramírez-Macías, D., Vitoria-Gómora, L., Panti, C., Martellini, T., Cincinelli, A., & Fossi, M. C. (2023). Microplastic occurrence and phthalate ester levels in neuston samples and skin biopsies of filter-feeding megafauna from La Paz Bay (Mexico). *Marine Pollution Bulletin*, 192, 115086. <https://doi.org/10.1016/j.marpolbul.2023.115086>
- Hermesen, E., Mintenig, S. M., Besseling, E., & Koelmans, A. A. (2018b). Quality Criteria for the analysis of microplastic in biota samples: A Critical review. *Environmental Science & Technology*, 52(18), 10230–10240. <https://doi.org/10.1021/acs.est.8b01611>
- Thiele, C. J., Hudson, M. D., & Russell, A. E. (2019). Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Marine Pollution Bulletin*, 142, 384–393. <https://doi.org/10.1016/j.marpolbul.2019.03.003>

Annex 2 – table: metadata specimen

Annex 2: Specimen metadata, morphometric characteristics and confirmed microplastic polymer counts for all analyzed elasmobranch gastrointestinal tract samples

Sample ID	Collecting date	Collecting location	Latitude	Longitude	Species	Sex	Width [mm]	Height [mm]	ww GIT [g]	Catch method	Boat type	PET	PP	PS	PE
1_NI_25.09.2025	25-9-2025	Pethalai landing site	7°55'45"	81°32'37"	<i>Neotrygon indica</i>	F	379	340	66.98	bottem set gillnet	OFRP fishing boat	1	0	0	0
2_NI_25.09.2025	25-9-2025	Pethalai landing site	7°55'45"	81°32'37"	<i>Neotrygon indica</i>	M	345	272	35.22	bottem set gillnet	OFRP fishing boat	2	1	0	0
3_NI_25.09.2025	25-9-2025	Pethalai landing site	7°55'45"	81°32'37"	<i>Neotrygon indica</i>	F	357	292	52.93	bottem set gillnet	OFRP fishing boat	0	3	14	0
4_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	320	265	20.69	bottem set gillnet	IMUL fishing boat	0	0	0	0
5_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	310	248	25.47	bottem set gillnet	IMUL fishing boat	0	0	0	0
6_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	379	292	34.17	bottem set gillnet	IMUL fishing boat	8	0	0	0
7_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	370	313	41.07	bottem set gillnet	IMUL fishing boat	2	0	0	0
8_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	351	295	38.20	bottem set gillnet	IMUL fishing boat	9	0	0	0
9_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	338	280	32.80	bottem set gillnet	IMUL fishing boat	4	0	0	0
10_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	334	263	26.00	bottem set gillnet	IMUL fishing boat	6	0	0	0
11_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	334	277	31.46	bottem set gillnet	IMUL fishing boat	5	0	0	0
12_NI_10.10.2025	10-10-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	332	258	28.65	bottem set gillnet	IMUL fishing boat	2	1	0	6
13_NI_14.10.2025	14-10-2025	Pethalai landing site	7°55'45"	81°32'37"	<i>Neotrygon indica</i>	M	323	278	46.17	bottem set gillnet	OFRP fishing boat	0	0	0	0
14_NI_7.11.2025	7-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	F	405	332	54.60	bottem set gillnet	IMUL fishing boat	78	1	9	1
15_NI_7.11.2025	7-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	368	300	56.71	bottem set gillnet	IMUL fishing boat	0	0	0	1
16_NI_7.11.2025	7-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	364	294	48.25	bottem set gillnet	IMUL fishing boat	4	1	2	2
17_NI_7.11.2025	7-11-2025	Pethalai landing site	7°55'45"	81°32'37"	<i>Neotrygon indica</i>	F	303	246	37.04	bottem set gillnet	OFRP fishing boat	0	0	0	0
18_MM_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Mobula mobular</i>	M	1643	705	1147.00	bottem set gillnet	IMUL fishing boat	2	0	0	2

Annex 2 – table: metadata specimen

19_PJ_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Pateobatis jenkinsii</i>	M	374	326	39.84	bottem set gillnet	IMUL fishing boat	1	155	0	466
20_PJ_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Pateobatis jenkinsii</i>	F	468	415	87.18	bottem set gillnet	IMUL fishing boat	6	1	4	4
21_HU_13.11.2025	13-11-2025	Kalkudah landing site	7°53'57"	81°33'49"	<i>Himantura uarnak</i>	M	344	327	34.00	bottem set gillnet	OFRP fishing boat	4	0	1	465
22_GP_13.11.2025	13-11-2025	Kalkudah landing site	7°53'57"	81°33'49"	<i>Gymnura poecilura</i>	M	512	265	25.98	bottem set gillnet	OFRP fishing boat	0	0	0	1
23_PA_13.11.2025	13-11-2025	Kalkudah landing site	7°53'57"	81°33'49"	<i>Pastinachus ater</i>	M	505	403	21.00	bottem set gillnet	OFRP fishing boat	1	0	0	0
24_NI_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	371	294	37.27	bottem set gillnet	IMUL fishing boat	0	0	0	0
25_NI_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	376	304	51.29	bottem set gillnet	IMUL fishing boat	0	0	0	0
26_NI_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	340	268	30.52	bottem set gillnet	IMUL fishing boat	22		0	0
27_NI_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	346	277	40.76	bottem set gillnet	IMUL fishing boat	0	0	0	0
28_NI_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	M	346	286	52.65	bottem set gillnet	IMUL fishing boat	0	0	0	0
29_NI_13.11.2025	13-11-2025	Valaichchenai harbour	7° 55' 37"	81° 31' 47"	<i>Neotrygon indica</i>	F	385	300	47.69	bottem set gillnet	IMUL fishing boat	0	0	0	0
30_NI_21.11.2025	21-11-2025	Pethalai landing site	7°55'45"	81°32'37"	<i>Neotrygon indica</i>	M	344	274	53.05	bottem set gillnet	OFRP fishing boat	0	0	0	0

Table A2. Metadata and analytical results for the (30n) elasmobranch specimens included in this study. Information includes collection date, landing site, species identification, sex, morphometric measurements (body width and height), wet gastrointestinal tract weight (ww GIT), fishing method, vessel type and the number of confirmed (with a >80% match) microplastic particles per polymer category determined by μ FTIR spectroscopy. Polymer categories comprise polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS) and polyethylene (PE). IMUL = inboard engine multi-day fishing vessel; OFRP = outboard fiberglass reinforced plastic fishing vessel used for single-day fishing trips.

Use of generative AI

ChatGPT was used as a supplementary tool during the preparation of this report, primarily for language editing and paraphrasing. For rewriting text, the following prompt was used: “Can you rewrite this sentence as if you were writing a scientific report. It should be academic but still easy to understand.” ChatGPT was also employed for grammar and language checks.

During the statistical analysis, ChatGPT was used to assist with resolving errors in R scripts and adapting graphical visualizations to improve their presentation value.

Additionally, ChatGPT was used to assist in creating the schematic visualization of the methodology. The methodology and its required components were described, and original visuals of the materials used were provided as references. ChatGPT was used to assist in organizing these elements into a coherent visual representation.

All generated responses were critically evaluated and adapted where appropriate. Use of ChatGPT was strictly restricted to a supportive tool and did not replace the author's interpretation of the data, methodological decisions or scientific conclusions.