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Microplastic Occurrence in the Diet of Green Sea Turtles (*Chelonia mydas*) From the Coastal Region of São Paulo, Brazil

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ABSTRACT

Microplastics (MPs) represent the main vector of pollution in the marine environment. Originating from the slow degradation process of plastics into smaller particles, MPs vary in size between 1 and 5 mm. Their ability to adsorb and transport contaminants, disperse invasive organisms and be accidentally ingested by marine animals makes these particles an increasing threat to the health of coastal ecosystems and the conservation of marine life. Ingestion of MPs leads to digestive tract blockage, physiological alterations, changes in feeding behaviour or even total debilitation of the animal. This study analysed and quantified the presence, typology and colour of MPs in the gastrointestinal contents of 218 green sea turtle (*Chelonia mydas*) samples from the central–south and northern coasts of São Paulo. MPs with an average size between 3 and 4 mm, mostly secondary type and predominantly of white colour, were detected in 45.9% of the analysed samples. Proportionally, MPs were found in 75% of samples from the central–south coast, compared to 35% from the northern coast study region. This difference in occurrence is attributed to the higher anthropogenic impact from nearby port and industrial activities. This study enhances our understanding of plastic ingestion by marine fauna. It highlights the urgent need to implement strategies to prevent, reduce and mitigate plastic pollution and its detrimental effects on the coastal environment.

1 | Introduction

The ingestion of plastic debris by sea turtles is a growing global issue (Feitosa et al. 2024; Kushwaha et al. 2024). Marine plastic pollution affects sea turtles (Clukey et al. 2017; Nelms et al. 2016; Schuyler et al. 2014) and other aquatic fauna worldwide, either through the ingestion of macroplastics (Laist 1987; Lutz 1990; Santos et al. 2015; Nelms et al. 2016) or microplastics (MPs) (Foekema et al. 2013; Wright et al. 2013; Lusher et al. 2015;

Sun et al. 2017). Plastics account for approximately 80% of marine debris, with some areas reaching up to 95% (Moore 2008). It is, therefore, not surprising that marine life is impacted by plastic debris through entanglement and ingestion processes (Derraik 2002). In Brazil, data presented by Santos et al. (2015) estimate that 70% of juvenile turtles analysed for plastic ingestion along the coast contained debris, with average of 47.5 items per turtle. On the North Pacific Ocean, this proportion is even higher, with 83% of analysed sea turtles showing evidence

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of ingesting anthropogenic debris (Wedemeyer-Strombel et al. 2015). In the Great Barrier Reef, Australia, MPs and macroplastics were found in two green sea turtle specimens (Caron et al. 2018).

Approximately 20 distinct types of plastics are found worldwide, all characterised by their versatility, low cost, lightness, mechanical strength, durability and corrosion resistance, as well as their thermal and electrical insulation properties (Thompson et al. 2009). As a result, the use of these plastic polymers has grown exponentially in recent decades, with 50% of this material being used only once (Miller et al. 2017). Additionally, half of the plastic waste and fragments are not properly disposed of, leading to their transport into aquatic environments, often reaching the marine environment (Eriksen et al. 2014). All types of plastic resins are found in the oceans, but most plastics detected in the marine environment consist of polyethylene (PE) and polypropylene (PP) (Frias et al. 2010). An oceanographic model of floating debris dispersion estimated that, by 2014, at least 5.25 trillion plastic fragments were floating in the world's oceans, with a total approximate weight of 268,940 tons (Eriksen et al. 2014).

Once in the marine environment, the degradation process of plastic is slower due to the action of UV rays and photo-oxidation, resulting in the release of low molecular weight plastic fragments known as MPs (Miller et al. 2017), which is the focus of this study. MPs range in size from 1 (Browne et al. 2011) to 5 mm (Lusher et al. 2015) and originate from various types of plastic polymers or plastic resin pellets (Lehner et al. 2015). Their characteristics include low density, composition, persistence in the environment and small size, which contribute to their widespread and diverse dispersion. Studies have shown that this type of marine debris is most found in areas with intense anthropogenic activity; however, the presence of MPs has also been detected in areas without direct anthropogenic use, such as the polar regions, equatorial zones, coastal areas and deep-sea regions (Bergmann et al. 2015; Corradini et al. 2021). In a recent review of MP pollution in the marine environment, Kushwaha et al. (2024) noted that 80%–90% of MP pollution originates from land-based sources.

Among the major problems caused by MPs in the marine environment are (i) the ability to adsorb and transport contaminants, such as persistent organic pollutants (POPs), which are highly toxic (Rios et al. 2007); (ii) alterations in ecosystem dynamics; (iii) the dispersion of invasive organisms (Zhang et al. 2024) and (iv) issues related to accidental ingestion. Ingestion of MPs can lead to digestive tract blockages, physiological changes, alterations in feeding behaviour or even complete debilitation (Miller et al. 2017). MP particles have been detected in the digestive tracts of various animal groups, including birds, mammals, fish and invertebrates (Bergmann et al. 2015; Lusher et al. 2015; Wilcox et al. 2018). However, records of these particles in turtles are more recent (Caron et al. 2018; Santos et al. 2020). Due to their diverse diet, long lifespan and wide distribution from pelagic to benthic coastal areas, green sea turtles are in direct contact with marine pollutants generated by human activities (FAO 2009). According to Tourinho et al. (2010), analyses of the digestive contents of turtles revealed that 100% of individuals were contaminated in some way by anthropogenic solid waste.

Furthermore, the presence of MPs in the gastrointestinal contents of turtles has been shown to alter their gut microbiota, potentially compromising their health and increasing the risk of MP bioaccumulation within their bodies (Procopio et al. 2025; Biagi et al. 2021).

The Brazilian coastline is home to five recorded species of marine turtles: the leatherback turtle (*Dermochelys coriacea*), the hawksbill turtle (*Eretmochelys imbricata*), the olive ridley turtle (*Lepidochelys olivacea*), the loggerhead turtle (*Caretta caretta*) and the most abundant species, the green sea turtle (*Chelonia mydas*) (Marcovaldi and Dei Marcovaldi 1999). The *C. mydas* has the widest distribution ranging from temperate zones to the tropics mostly in coastal habitats, a region heavily impacted by anthropogenic activities (de Pádua Almeida et al. 2011; Chaousis et al. 2023). To contribute to understanding the environmental impacts of MP pollution, this study is aimed at quantifying and characterising these contaminants in the gastrointestinal content of specimens of *C. mydas* collected on the coast of the state of São Paulo, Brazil. The collected samples encompassed key areas, including the Porto de Santos region—known for its high concentration of MPs (Izar et al. 2019)—and the northern coast of the state, both of which are important feeding grounds for the species and are particularly vulnerable to environmental degradation. In doing so we (a) evaluate the frequency of occurrence of anthropogenic debris in the stomachs and intestines of green sea turtles between 2016 and 2018, (b) quantify and categorise MPs according to the manufacturing origin (primary or secondary) and (c) classify by colour categories. We hypothesise that specimens collected along the heavily occupied and densely populated portion of the central–south coast present a higher concentration of MPs than those sampled on the northern coast.

2 | Methods and Materials

2.1 | Study Sites and Sampling

Samples of stomach and intestine contents from green turtles (*C. mydas*) collected between 2016 and 2018 were provided by the Argonauta Institute for Coastal and Marine Conservation (IACCM) and the Laboratory of Biology, Ecology, and Physiology of Aquatic Organisms at the Institute of Biosciences, UNESP. The material used was obtained from samples of animals that had died and were collected for biometric analysis and necropsy along the coast of São Paulo. These regions included the municipalities of Ubatuba and Caraguatatuba on the northern coast and Praia Grande to Peruíbe on the central–southern coast of the state (Figure 1). Two hundred eighteen samples were analysed, with 159 provided by IACCM and 59 by the Institute of Biosciences, UNESP.

2.2 | Sample Processing and MP Extraction

The analysis of MP presence in fresh stomach and intestinal contents followed procedures used in other animal groups, such as whale (Lusher et al. 2015), due to the lack of specific methodologies for chelonians at the time of the analysis. The samples were separated into organic residues and marine

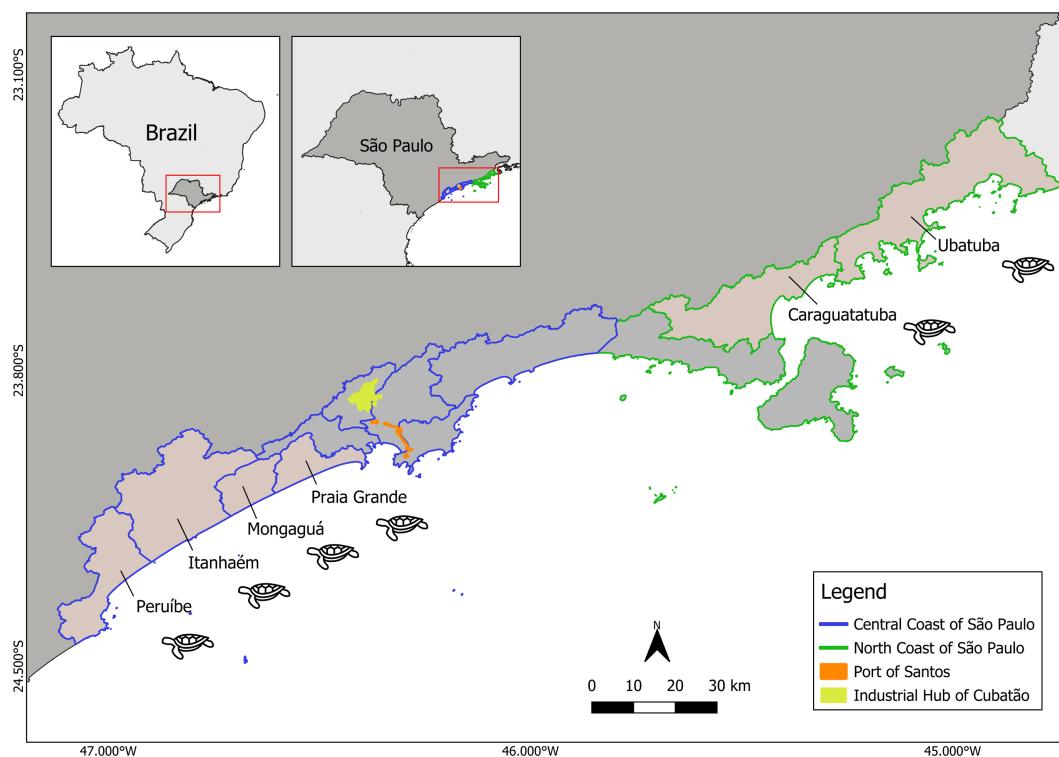


FIGURE 1 | Map of the São Paulo state coastline, highlighting the northern coast (Ubatuba and Caraguatatuba) in green and the central-southern coast (Praia Grande, Mongaguá, Itanhaém and Peruíbe) in blue. The Port of Santos region is marked in orange at the centre, while the Cubatão Industrial Complex is indicated in yellow. Turtle presence at sampling sites is also marked on the map.

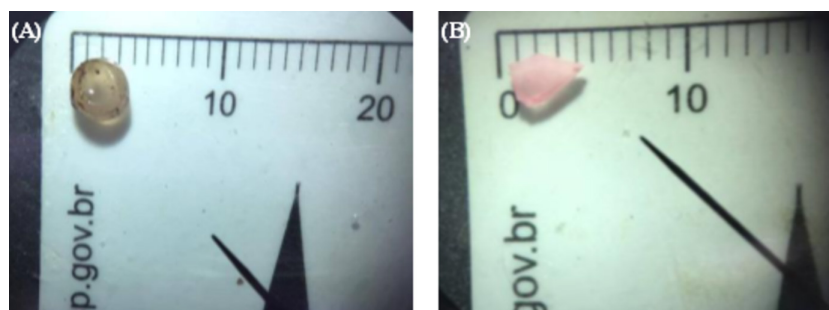


FIGURE 2 | Classification of microplastics by particle type: primary (A) and secondary (B) in the gastrointestinal contents of *C. mydas*. Depict examples of fragments MPs observed in the study.

debris and analysed with a stereomicroscope. Identified MPs were transferred to another Petri dish for size measurement and classification based on the type of plastic—whether primary or secondary (Figure 2). Primary MPs (Figure 2A) are typically derived from PP, PE and polystyrene, cylindrical in shape, low density and introduced into the marine environment through improper disposal or via ship transportation and port operations and were identified by their cylindrical shape (Andrady 2011), such as pellets (Lehner et al. 2015). Secondary MPs (Figure 2B), on the other hand, originate from the fragmentation of larger plastic particles—such as bags and PET bottles (do Sul et al. 2011)—due to environmental processes like photodegradation (exposure to sunlight), mechanical abrasion (e.g., friction with rocks and sand) and biological activity (e.g., the action of microorganisms) (Andrady 2011). Colour identification (e.g., white, black, red, blue, green, yellow, pink and orange) followed the terminology of Provencher

et al. 2018, which aids in standardising debris classification in marine megafauna, including turtles.

The samples were photographed for more accurate measurements using ImageJ software (Figure 3), a program used for image processing (Schneider et al. 2012). The images were calibrated using a precision ruler included in the photograph with a predefined scale (A) and subsequently cropped to allow the software to accurately measure the MPs (B).

2.3 | Statistical Analysis

Data on the number of samples, total MPs identified in stomach and intestinal contents, classification into primary and secondary types and colour categories were analysed using descriptive statistics, including mean, standard deviation and standard

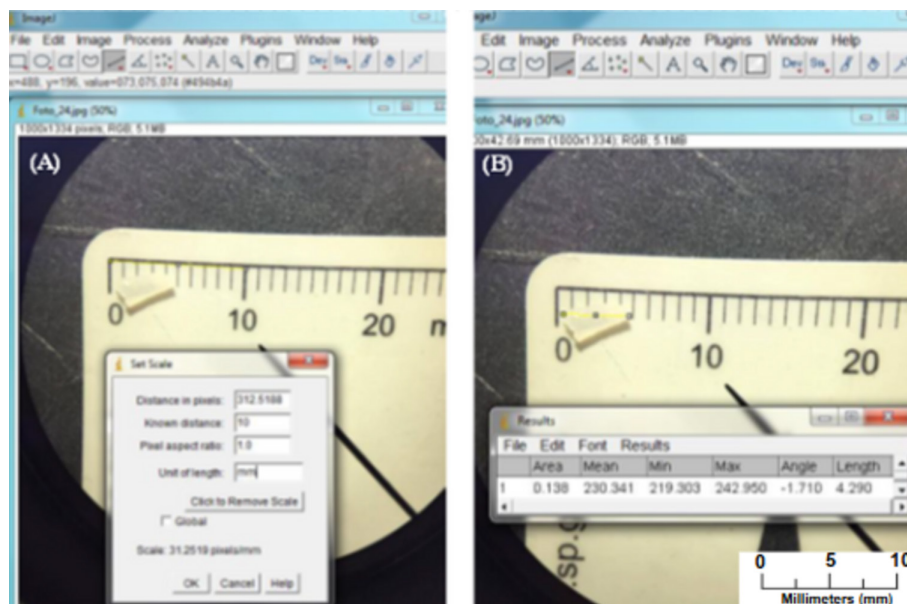


FIGURE 3 | (A) Calibration of a known area in millimetres using the ImageJ software and (B) measurement of the microplastic length and its estimated size, indicated by black rectangles. The microplastics analysed were extracted from the gastrointestinal contents of the turtles.

error, with the aid of SigmaStat software. Due to the nonparametric nature of the data, the Mann-Whitney test was used to compare the sample group, the northern and central-southern coasts, and the Kolmogorov-Smirnov (K-S) test was applied to assess data normality.

3 | Results

3.1 | MP Abundance and Shape

Two hundred eighteen samples were analysed, with 159 specimens from the northern coast and 59 samples from the central-southern coast. Of the samples analysed, 45.9% contained plastic debris smaller than 5 mm. When analysed by sampling region, the northern coast showed a $35\% \pm 4\%$ ($n=56$) occurrence of MPs. In the central-south region, primary and secondary MPs were present; however, only secondary MPs were found in the northern region. The frequency represents the total number of MPs in all samples (Figure 4).

On the central-southern coast, $75\% \pm 6\%$ of the samples contained MPs, with 6 classified as primary and 38 as secondary (Table 1).

Statistical analyses of the data were performed to correlate the number of MPs found with the number of samples. The Mann-Whitney test analysis showed that samples from the southern coast contained a significantly higher quantity of MPs compared to those from the northern coast, with results of $U=2844.500$ and $p=0.000008$. The concentration of MPs found in the intestinal samples of turtles from both regions was significantly higher than that found in the stomach content samples (Table 2).

The average size of the MPs was approximately 3.55 mm, with no significant differences between the two regions (Figure 5). The K-S normality test yielded $T=1.526$ and $p=0.159$.

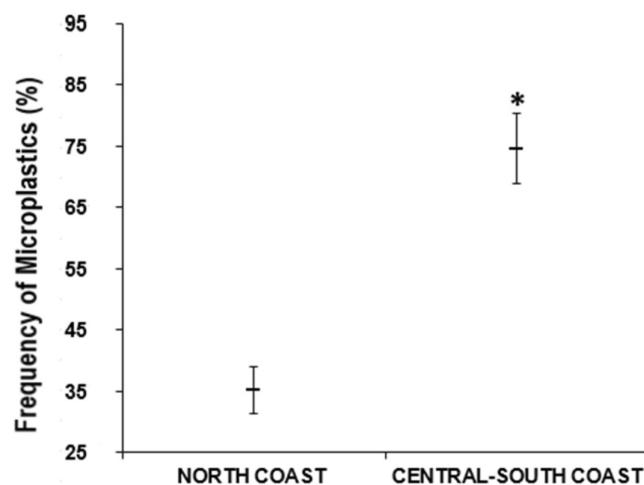


FIGURE 4 | Frequency (percentage) of microplastics found in each coastal region of São Paulo state. * indicates a statistically significant difference between regions.

TABLE 1 | Total number of samples by collection area and type of microplastic particle.

	Total	North coast	Central-south coast
N =total	218	159	59
Samples with microplastic	100	56	44
Samples with primary microplastic	6	0	6
Samples with the second microplastic	94	56	38

3.2 | MP Colours

Regarding the colouration of the MPs, the statistical analysis showed no significant differences, except for white MPs, which were detected in greater numbers (43 specimens) in the central-southern region compared to 24 specimens from the northern coast (Figure 6).

The characteristics found in this study provide important insights into how the sources of MPs in the marine environment and the ingestion of these particles may affect chelonians, specifically *C. mydas*.

4 | Discussion

Urbanised coastal regions near industrial centres show a high concentration of various pollutants, including MPs (Meera et al. 2022). In a recent review of MP pollution in the marine environment, Kushwaha et al. 2024 noted that, although MPs are found in various aquatic environments, 80%–90% of MP pollution originates from land-based sources. The results of this study highlight the concentration of MPs in green sea turtle feeding areas in the state of São Paulo, a region heavily impacted by anthropogenic pollution (Santana et al. 2016). Our findings were also in line with other studies conducted along the Brazilian coast and worldwide. The study by Jaubet et al. 2021, for example, showed that areas of Mar del Plata in Argentina have a high concentration of MPs. This contaminant was also detected along the coast of South Africa (Ryan et al. 2018). In Ecuador, water and sediment samples from the mangroves and sandy beaches of the Salado Estuary in Guayas contained MPs (Arteaga et al. 2024). In most of the reported studies, the average size of the particles found ranged from 3 to 4 mm, corroborating the previous study

TABLE 2 | Percentage (%) of microplastics (MPs) present in the stomach and intestines of *Chelonia mydas*.

	North coast	Central-south coast
Stomach (%)	8 ± 2	6 ± 1
Intestine (%)	92 ± 4	94 ± 5

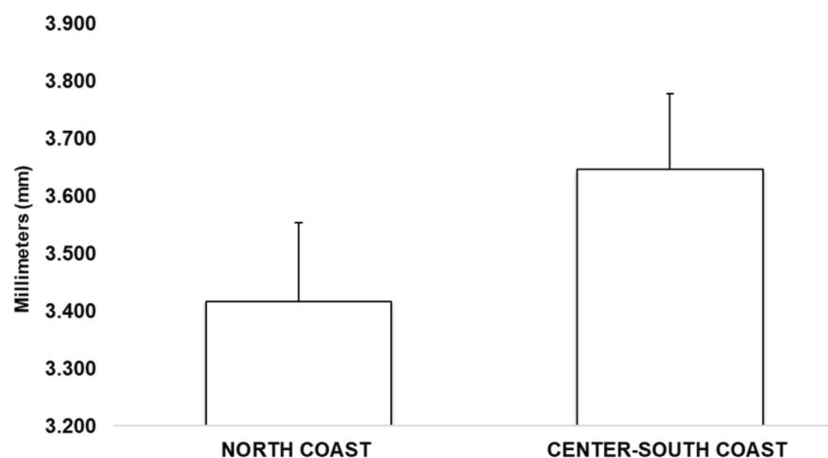


FIGURE 5 | Average size of microplastics in the stomachs and intestines of *C. mydas* stranded on the northern and central-southern coasts of the state of São Paulo, Brazil.

by Eriksen et al. (2014), who estimated the average size of polymer particles in the South Atlantic Ocean to be between 1.01 and 4.75 mm. The presence and size of MPs are consistent with our findings from the 2016–2018 period, suggesting a potential trend not only in the Brazilian region but also globally.

It is important to note that at the time this present study was conducted, there were no specific methodologies for quantifying MPs in the organic contents of turtles. Therefore, the methods used did not allow for the analysis of particles smaller than 1 mm, which may have led to an underestimation of the actual concentration of MPs present in the stomach and intestines of these animals. Additionally, due to necropsies being conducted by different collaborators, it was not possible to determine the exact location within the digestive tract where the MPs were found. Nevertheless, the overall data indicate that, regardless of the region of the world, turtles may be at risk due to the ingestion and presence of MPs. Recent studies show that plastic fragments and industrial pellets constituted the largest proportion of marine debris recorded from turtles in the Arabian Gulf (AlMusallami et al. 2024). Contamination by MPs has also been reported in the nests of green sea turtles on Redang Island (Malaysia). This contamination poses a potential risk to turtle egg incubation by altering nest temperatures; given their extensive exposure to marine plastic pollution, green sea turtles *C. mydas* are regarded as suitable bioindicators of marine plastic pollution (Aranda et al. 2024).

In comparison to the studied regions, our data show that the proportion of MPs in the stomach and intestinal contents of *C. mydas* from the central-south coast was significantly higher than in samples from the northern coast. The hypothesis explored in this study suggests that the sampling area may have influenced these results, due to the considerable difference in population size and the diversity of activities in the coastal zone between the central-south coast, with approximately 1,718, 894 inhabitants, and the northern coast, with 209,372 inhabitants (IBGE 2017).

Research suggests that MP ingestion by *C. mydas* may be linked to the odour emitted by these particles in the water (Petry et al. 2021). In the marine environment, MPs release odour

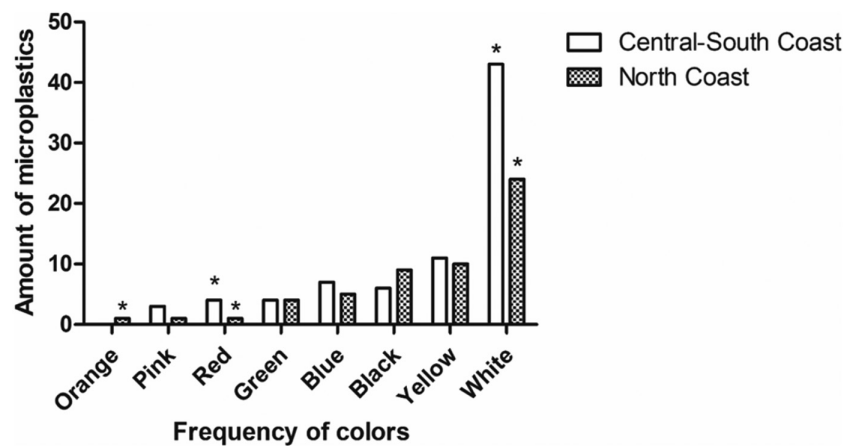


FIGURE 6 | Colour categories of microplastics and their frequency of occurrence in stomachs and intestines of *C. mydas* stranded on the north and south-central coast of São Paulo state, Brazil.

molecules like those used by marine predators to locate their prey, which could attract turtles (Petry et al. 2021). Some hypotheses suggest that turtles may confuse this odour with that of their prey, leading to the ingestion of MPs, as evidenced by their presence in the digestive tract of these species (Pfäller et al. 2020). In addition to the issues associated with the direct ingestion of MPs, there is also the possibility that turtles consume these particles indirectly. For example, studies indicate that species such as *Cyanea capillata*, *Cyanea lamarckii*, *Cosmetira pilosella*, *Pelagia noctiluca* and *Cassiopea xamachana* have ingested MPs (Macali et al. 2018; Iliff et al. 2020; Devereux et al. 2021; Rapp et al. 2021). Within the food chain, this contaminant could reach the digestive tract of turtles, as they predominantly feed on jellyfish daily (Heaslip et al. 2012). Other studies report the possibility of MPs becoming entangled in the tentacles of cnidarians or being assimilated during their pulsation (Ohdera et al. 2018). In such cases, these particles could also reach the stomach and intestines of *C. mydas* if they ingest these animals.

According to the results of this study, MPs were found predominantly in the intestines rather than the stomachs of *C. mydas*. These findings are consistent with a study by Duncan et al. 2019, conducted on a population of the same species in the Mediterranean Sea. They observed that 100% of intestinal samples contained MPs, whereas only 33% were found in the stomach. The tendency for MPs to be in the intestinal portion of these animals may occur because these particles tend to accumulate and remain longer in the intestine, where food transit is slower (Casale et al. 2016). Another hypothesis is that these particles were ingested as fragments of macroplastic and, through the digestive process, reached the intestine as MPs (Ugwu et al. 2021). The predominance of MPs in the intestine has also been observed in other animal groups, such as fish and birds (Rezania et al. 2018; Provencher et al. 2018).

Regarding the typology, samples from the central-south coast contained 10.2% of primary MPs ($n=6$), while those from the northern coast consisted exclusively of secondary MPs (100%, $n=56$). This difference may be attributed to a higher anthropogenic impact in the central-south region, possibly due to the presence of the Port of Santos and associated port activities, responsible for transporting 634.000 tons of plastic pellets across

Brazil. A previous study (Taniguchi et al. 2016) indicated an inverse correlation between the density of pellets and the distance from the Port of Santos, suggesting that proximity to the port influences the presence of these particles. Alternatively, the origin of the pellets (primary MPs) found in the stomach and intestinal contents of samples from the central-south coast may be linked to circulation patterns in the Santos-São Vicente Estuary and the currents of the channel, which facilitate the concentration of these pellets in the region (Parra et al. 2025). The presence of plastic industries in the Cubatão industrial complex (Ferreira et al. 2021), located in the Santos Estuary, may also have contributed to the large number of pellets found in the stomach and intestinal contents of animals from the Southern São Paulo coast. These particles have also been reported in various coastal regions worldwide, such as the Bahía Blanca Estuary in Argentina (Arias et al. 2023), the Avon-Heathcote Estuary in New Zealand (Hunter et al. 2022), and even near the equator, in the estuaries of the Amazon (Rodrigues et al. 2024). This data demonstrates that the dispersion of these particles is widespread across the oceans, including areas where turtles feed and complete their life cycle. Multiple sources are likely contributing to MP pollution, highlighting the need for improved urban and coastal waste management to minimise the introduction of this pollutant into the ecosystem (Arteaga et al. 2024).

Lastly, but also importantly, our findings point that MP colours may also be associated with their origin. On the central-south coast, a higher number of white MPs were found compared to the northern coast of São Paulo. This is likely due to the high concentration of pellets in the Santos-São Vicente Estuary region (Ferreira et al. 2021). A similar pattern of MPS colour abundance was found at the Southern Brazil coast with predominance of white and transparent debris (Petry et al. 2021). However, it is important to consider that plastics in the marine environment tend to discolour, bleach or oxidise owing to time in the water, solar exposure and fragmentation (Arteaga et al. 2024). Lighter colours are like the turtles' prey (Schuyler et al. 2014), such as jellyfish, algae, small crustaceans and molluscs. Therefore, the large presence of plastic in the environment may confuse turtles during their food search (Petry et al. 2021). Another important point to note is that the MPs found in samples from the northern coast exhibited a wide range of colours relative to the number of samples containing

MPs. This might be related to the presence of larger plastic debris in the region, which underwent weathering and broke down into smaller particles, which could then be found as MPs. Further studies should explore the relationship between the colour and origin of MPs and their ingestion by green sea turtles, as this may lead to confusion with their natural prey and exacerbate the environmental and ecological risks associated with MP contamination. Furthermore, future studies and continued quantification of MPs in the gastrointestinal contents of turtles may help track temporal fluctuations in the presence of these particles and potentially establish turtles as bioindicators of environmental MP pollution. Additionally, analysing the composition of the MPs found could enable meaningful comparisons over time. These insights could inform conservation strategies and guide policy decisions aimed at mitigating plastic pollution in marine ecosystems.

5 | Conclusion

Assessing the abundance of MPs and determining their colour, shape and size in green sea turtles (*C. mydas*) is crucial for understanding the extent of exposure to this pollutant and the potential consequences for the species survival. This study demonstrated that a significant proportion of gastrointestinal content samples from green sea turtles along the São Paulo coastline revealed the presence of MPs, highlighting the intense anthropogenic influence in the region. Our results constitute important baseline data to understand the impacts of MPs on *C. mydas* in coastal waters. We observed that stomach and intestinal content samples from green sea turtles were mainly composed of secondary MPs with 3.5 mm on average and a predominance of white debris mostly found in the intestine contents.

Although scientific interest in this topic has increased in recent years, substantial gaps remain regarding the real impacts of these particles on marine biodiversity, particularly among turtles. Finally, these findings highlight the need for increased organised efforts for plastic pollution mitigation and reduction into the marine environment. For instance, future studies could explore the relationship between the specific locations of sample collection and the intensity of anthropogenic influence. Investigating the distribution of these particles within the bodies of turtles also represents a promising approach for future research. This highlights the need for further investigation to ensure the sustainability of this endangered species.

Research on MPs is crucial for understanding marine pollution and its impact on sea turtles. By investigating the presence and effects of MPs in turtles, we can assess the extent of contamination and identify potential risks to their health and survival. These studies are vital for developing effective conservation strategies and addressing the broader issue of marine plastic pollution.

Author Contributions

Juliana R. da Costa: writing – original draft, investigation, formal analysis, data curation. **Débora M. de Freitas:** writing – review and editing, writing – original draft, supervision, conceptualization.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Figshare at <https://figshare.com/account/home>, reference number <https://doi.org/10.6084/m9.figshare.29064443>.

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