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**CONTAMINAÇÃO POR RESÍDUOS PLÁSTICOS EM BOTO-CINZA (*Sotalia*
guianensis) NO LITORAL DO CEARÁ, BRASIL**

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Dissertação apresentada ao Programa de Pós-Graduação em Biodiversidade, da Universidade Federal Rural de Pernambuco, como requisito para obtenção do título Mestre em Biodiversidade.

Orientadora: Flávia Lucena-Frédou

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RESUMO

Os mamíferos marinhos, como representantes de topo da cadeia alimentar, são considerados espécies sentinelas e podem atuar como indicadores da saúde dos oceanos. Os resíduos plásticos estão amplamente distribuídos nos oceanos e já são reconhecidos como contaminantes perigosos. Uma vez ingeridos, estes resíduos podem causar diversos efeitos adversos à fauna, a depender do tamanho da partícula e da incidência. Este estudo teve como objetivo identificar e caracterizar a ingestão de plástico (macro - microplástico) em botos-cinza (*Sotalia guianensis*) do Atlântico Sudoeste Tropical, por meio da avaliação do conteúdo estomacal de uma amostragem retrospectiva de 10 anos. Os resíduos plásticos foram extraídos por digestão com solução de KOH e uma subamostra de partículas foi identificada pelo Laser Direto de Infravermelho. A maioria dos indivíduos avaliados (38 de 40) estava contaminada, os polímeros mais comuns identificados foram Poliuterano (PU), Politereftalato de etileno (PET) e Acetato vinila de Etileno (EVA). Os microplásticos (7.77 ± 1.25 partículas individuais⁻¹; média $\pm$ erro padrão) foram mais prevalentes do que as partículas plásticas maiores (0.15 ± 1.25 partículas individuais⁻¹). A variabilidade interanual não influenciou o tamanho ou o número de microplásticos ingeridos. No entanto, partículas menores foram detectadas durante as estações chuvosas. Houve correlação positiva entre a massa do conteúdo estomacal e o número de microplásticos, sugerindo contaminação por transferência trófica.

Palavras-chave: mamíferos marinhos, impactos antropogênicos, poluição plástica, atlântico tropical.

ABSTRACT

Marine mammals, as top marine predators, are considered sentinel species and may act as indicators of ocean health. Plastic residues are widely distributed in the oceans and are already recognised as hazardous contaminants. Once ingested, plastic residues can cause several adverse effects on wildlife, depending on the particle size and incidence. This study aimed to identify and characterise plastic ingestion (macro - microplastic) in the Guiana dolphins (*Sotalia guianensis*) from the Tropical Southwestern Atlantic by evaluating the stomach contents of a 10-year sampling retrospective. Plastic residues were extracted through KOH digestion and a subsample of particles was identified by LDIR Chemical Imaging System. Most of the individuals evaluated (38 out of 40) were contaminated, the most common polymers identified were PU, PET and EVA. Microplastics ($7.77 \pm \text{SE } 1.25$ particle individuals⁻¹) were more prevalent than larger plastic particles (0.15 ± 1.25 part. ind.⁻¹). The interannual variability did not influence either the size or number of microplastics ingested. However, smaller particles were detected during the rainy seasons. There was a positive correlation between the stomach content mass and the number of microplastics, suggesting contamination through trophic transfer.

Keywords: marine mammals, anthropogenic impacts, plastic pollution, sentinel species, Tropical Atlantic.

FUNDAMENTAÇÃO TEÓRICA

Lixo marinho é qualquer material sólido persistente, fabricado ou processado, descartado ou abandonado no ambiente marinho e costeiro (GOMIERO *et al.*, 2019). Por sua vez, o plástico é o principal componente do lixo marinho (DERRAIK, 2002; LAW, 2017), e tornou-se onipresente nos mais diversos habitats aquáticos, podendo representar até 95% dos resíduos que se acumulam nas linhas de costa, nas massas d'água superficiais e até mesmo no fundo do mar (BERGMAN *et al.*, 2015). Em caráter global, estima-se que 1,15 a 2,41 milhões de toneladas de plástico são transportadas anualmente dos rios para os oceanos, indicando, portanto, os sistemas fluviais como uma das mais significativas fontes de entrada dos resíduos nos oceanos (LEBRETON *et al.*, 2017).

A poluição por resíduos plásticos é reconhecida como uma grande ameaça à vida marinha e à conservação dos habitats oceânicos em todo o mundo. Durante a Conferência da Basileia em 2019, sediada na cidade de Genebra, aproximadamente 180 governos apontaram os plásticos como resíduos perigosos em decorrência da sua capacidade tóxica e de adsorção de outros poluentes (LIMA *et al.*, 2020). Microplásticos (MPs) compõem uma das menores e mais abundantes frações do lixo marinho (VAN SEBILLE *et al.*, 2015) e compreendem um conjunto muito heterogêneo de partículas que variam em tamanho, forma, cor, densidade e composição química baseada em diversos polímeros (GALGANI *et al.*, 2015). Resíduos plásticos no geral são classificados de acordo com o tamanho total da partícula, sendo agrupados em três categorias: macroplásticos (20 – 100 mm), mesoplásticos (5 – 20 mm) e microplásticos (1– 5000 µm) (BARNES *et al.*, 2009). Estes podem ainda ser divididos em duas categorias de acordo com a origem do material. MPs primários são produzidos em tamanho microscópico, para (i) serem utilizados como matéria prima para a fabricação da maior parte dos produtos feitos de plástico e (ii) para serem utilizados como agente abrasivo, principalmente na indústria de produtos cosméticos. Enquanto os MPs secundários são resultantes da fragmentação de plásticos de maiores dimensões descartados no meio ambiente (*e.g.* redes de pesca, sacolas, garrafas plásticas) (OLIVATTO *et al.*, 2018).

Aproximadamente, 2.250 espécies marinhas já foram documentadas como afetadas por detritos plásticos (TEKMAN *et al.*, 2019). Para mamíferos marinhos os impactos primários desse encontro decorrem de ingestão ou emaranhamento nos resíduos (GREGORY, 2009; WILCOX *et al.* 2015; ALEXIADOU *et al.*, 2019). No cenário global estima-se que 68% das espécies de cetáceos já se tornaram vítima dessas interações (EISFELD-PIERANTONIO *et al.*, 2022). Dentre as situações de comum reporte em mamíferos marinhos estão os emaranhamentos em petrechos de pesca, sendo as consequências dessas interações mais facilmente

compreendidas, uma vez que o comprometimento da locomoção pode afetar a capacidade de obtenção de alimento, levando a debilidade progressiva do indivíduo ou levar a morte imediata por asfixia (LAIST, 1997). Uma interação menos documentada, porém, com alto potencial de provocar efeitos adversos nos mamíferos marinhos é a ingestão de resíduos plásticos e tem suas consequências pouco compreendidas (SIMMONDS, 2017). Todavia, estudos apontam que a ingestão causa ulcerações e obstruções no trato gastrointestinal, ocasionando sensação de saciedade, bloqueio da digestão e fome levando à graves debilidades, além de inflamações e atuação como vetor de patógenos e/ou poluentes (FOSSI *et al.*, 2020).

Entre as espécies de cetáceos, a ingestão de lixo marinho foi documentada em, pelo menos, 50 espécies, isso representa 56% do número total de cetáceos (BAULCH; PERRY, 2014; KÜHN *et al.*, 2015; FOSSI *et al.*, 2018; PADULA *et al.*, 2023). Do material ingerido, 46% da composição eram itens plásticos (BAULCH; PERRY, 2014). Entretanto, estudos que avaliam especificamente a contaminação por microplásticos, em cetáceos ainda são escassos (LUSHER, 2018; FOSSI, 2020).

O Brasil é o quarto maior produtor de resíduos plásticos do mundo (DE AGUIAR., 2020) e um importante representante da megadiversidade, com 51 espécies de mamíferos marinhos (COSTA *et al.*, 2005). Associado a esse cenário, os países em desenvolvimento possuem estratégias de gestão de resíduos pouco eficientes (MARGALLO *et al.*, 2019). Embora a mortalidade como consequência da ingestão de detritos tenha sido observada em boto-cinza (*Sotalia guianensis*), golfinho-de-dentes-rugosos (*Steno bredanensis*), baleias-bicudas (Zifídeos) e peixes-bois-marinho (*Trichechus manatus*) (Aquasis, dados não publicados, MEIRELLES *et al.*, 2007; ATTADEMO *et al.*, 2015), não há estudos dedicados exclusivamente à documentação da ingestão de MPs no grupo (CASTRO; SILVA; ARAÚJO, 2018) com a aplicação de protocolos padronizados com controle de qualidade utilizando técnicas de digestão química.

Pesquisas sobre contaminação por microplásticos em cetáceos podem reforçar o uso de mamíferos marinhos como bioindicadores, uma vez que esses animais podem fornecer indicativos da saúde do ecossistema, tendo em vista o seu alto nível trófico, a longa expectativa de vida, a presença de estoques de gordura com capacidade de acúmulo de poluentes, além desse grupo possuir vários representantes costeiros (BOSSART, 2011). Os resultados de estudos no tema proposto tem o potencial de melhorar a avaliação e gestão dos impactos oriundos de resíduos plásticos. Adicionalmente, a demonstração dos efeitos da poluição plástica sobre a megafauna carismática a ser avaliada nessa pesquisa pode alertar a sociedade para os impactos antrópicos no ambiente marinho (ZANTIS *et al.*, 2021).

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**Microplastic ingestion by Guiana dolphins (*Sotalia guianensis*) on the Brazilian coast:
does temporal variability matter?**

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ABSTRACT

Marine mammals, as top marine predators, are considered sentinel species and may act as indicators of ocean health. Plastic residues are widely distributed in the oceans and are already recognised as hazardous contaminants. Once ingested, plastic residues can cause several adverse effects on wildlife, depending on the particle size and incidence. This study aimed to identify and characterise plastic ingestion (macro - microplastic) in the Guiana dolphins (*Sotalia guianensis*) from the Tropical Southwestern Atlantic by evaluating the stomach contents of a 10-year sampling retrospective. Plastic residues were extracted through KOH digestion and a subsample of particles was identified by LDIR Chemical Imaging System. Most of the individuals evaluated (38 out of 40) were contaminated, the most common polymers identified were PU, PET and EVA. Microplastics ($7.77 \pm \text{SE } 1.25$ particle individuals⁻¹) were more prevalent than larger plastic particles (0.15 ± 1.25 part. ind.⁻¹). The interannual variability did not influence either the size or number of MPs ingested. However, smaller particles were detected during the rainy seasons. There was a positive correlation between the stomach content mass and the number of microplastics, suggesting contamination through trophic transfer.

Keywords: marine mammals, anthropogenic impacts, plastic pollution, sentinel species, Tropical Atlantic.

HIGHLIGHTS

The ingestion of microplastics prevailed over larger size fractions;

Seasonality influenced the size of microplastics detected in *S. guianensis*;

The stomach content mass was positively correlated with the number of microplastics;

S. guianensis is a suitable indicator species in the Tropical Western Atlantic.

INTRODUCTION

Highlighted by their charismatic appearance and cosmopolitan distribution, marine mammals compose a diverse and heavily impacted by anthropogenic activities group (Avila et al., 2018). In addition, they play an important role in ecosystem structures and functionality due to their high trophic status and metabolic rates (Roman et al., 2010). Marine mammals are also considered sentinels species since they can act as bioindicators providing early warnings about anthropogenic impacts (Tabor & Aguirre 2004; Bossart, 2011; Hazen et al., 2019; Fossi et al., 2020). Hunting (Hovelsrud et al., 2008), bycatch (Hamilton & Baker, 2019), hydrocarbon exploration (Gales et al., 2003; Helm et al., 2014; Bröker, 2019), persistent organic pollutants (Hall et al., 2017), habitat degradation (Brakes & Dall, 2016), and marine litter (Panti et al., 2019) are some of the significant threats for this group. Plastic residues became a major environmental issue because of their widespread use associated with poor management (Worm et al., 2017; Sharma & Chatterjee, 2017), making them ubiquitous in rivers, coastal areas, and ocean basins (Borrelle et al., 2017; Morales-Caselles et al., 2021).

Once in the environment, plastic particles are classified based on their size as macroplastics (20 – 100 mm), mesoplastics (5 – 20 mm), and microplastics (1– 5000 µm) (Barnes et al., 2009). Considering their abundance, small size (Betts, 2008; Ferreira et al., 2019a), and the colonisation of the particles by microorganisms (Galloway et al., 2017), microplastics (MPs) can be easily ingested by marine biota. Among cetaceans, ingestion of marine litter has been documented in at least 50 species, representing 56% of the diversity of this infraorder (Baulch & Perry, 2014; Kühn et al., 2015; Fossi et al., 2018; Padula et al., 2023). From the ingested material, 46% of the composition was plastic items (Baulch & Perry, 2014). Regarding microplastic ingestion, studies are still scarce (Lusher et al., 2018; Fossi et al., 2020), which reinforces the relevance of investigating what these sentinel species can tell us about plastic pollution and its effects.

Brazil is the world's fourth largest producer of plastic waste (De Aguiar et al., 2020); associated with inadequate solid waste management, it may be a powerful threat to marine biota. In the Southwestern Tropical Atlantic, the Brazilian coast is home to 43 cetacean species (ICMBIO, 2018). Among this list, the Guiana dolphin (*Sotalia guianensis*) is a near-threatened cetacean (Secchi et al., 2018) that stands out due to its common presence on most of the Brazilian coast (Carvalho & Meirelles, 2020). This species is susceptible to several anthropogenic impacts, with a high rate of strandings (Domit et al., 2021).

The Guiana dolphin is a small cetacean that preferentially inhabits sheltered areas in coastal and estuarine waters and even port areas (Cunha et al., 2020) and has an estimated life

expectancy of 30 years (Rosas & Monteiro-Filho, 2002). The basis of its diet is composed of coastal demersal fishes, cephalopods and crustaceans (Santos et al., 2002; Gurjão et al., 2003; Pansard, 2010). The Guiana dolphin is exposed to several anthropogenic impacts as a coastal species, especially in highly urbanised regions. Latent threats to the species are i) accidental capture in fishing nets, ii) exposure to contaminants and vessel traffic, iii) noise pollution and iv) habitat loss (Di Benedetto & Ramos, 2014; Salgado et al., 2018; Schiavetti et al., 2020). Within Brazilian waters, they are considered vulnerable to extinction (ICMBIO, 2018), which emphasises the need for immediate implementation of conservation actions.

Although most scientific investigations on plastic contamination in the Brazilian ecosystem (46%) between 2009 and 2017 have focused on analyses of microplastics associated with biota (Castro et al., 2018), to our knowledge, this species has only been investigated for larger anthropogenic debris through naked-eye inspection (Di Benedetto & Ramos, 2014; Salgado et al., 2018). Moreover, *S. guianensis* has not yet been investigated for plastic contamination by applying chemical digestion, quality assurance and quality control protocols aligned with a size detection down to the millilitre particles.

Therefore, this study aims to (i) quantify and characterise (size, shape and colour) plastic debris (from macro to microplastic) contamination in the stomachs of stranded *S. guianensis* on the Northeastern Brazilian coast (Southwestern Tropical Atlantic), (ii) evaluate whether individual's characteristics are linked to contamination and (iii) investigate temporal variability (seasonal and interannual), based on a 10-year sampling retrospective.

METHODS

STUDY AREA

The samples (stranded individuals) were collected along the 573 km of the Ceará state (Northeastern Brazil), which is located on the Tropical Southwestern Atlantic (Fig. 1). The Ceará state has an estimated population size of 9,240,580, with a greater concentration in its capital (Fortaleza) located on the coastal line (population size: 2,447,409 in 2010 and 2,703,391 in 2020) and its metropolitan region (IBGE, 2021).

The study area is tropical (26 to 32°C) and characterised by an irregular rainy season ruled by the Intertropical Convergence Zone migration, with the highest volume (90%) of annual precipitation occurring in the first semester (January to June), whereas the second semester (July to December) is marked by the dry season combined with the presence of strong trade winds (up to 4 m/s) (Campos et al., 2003).

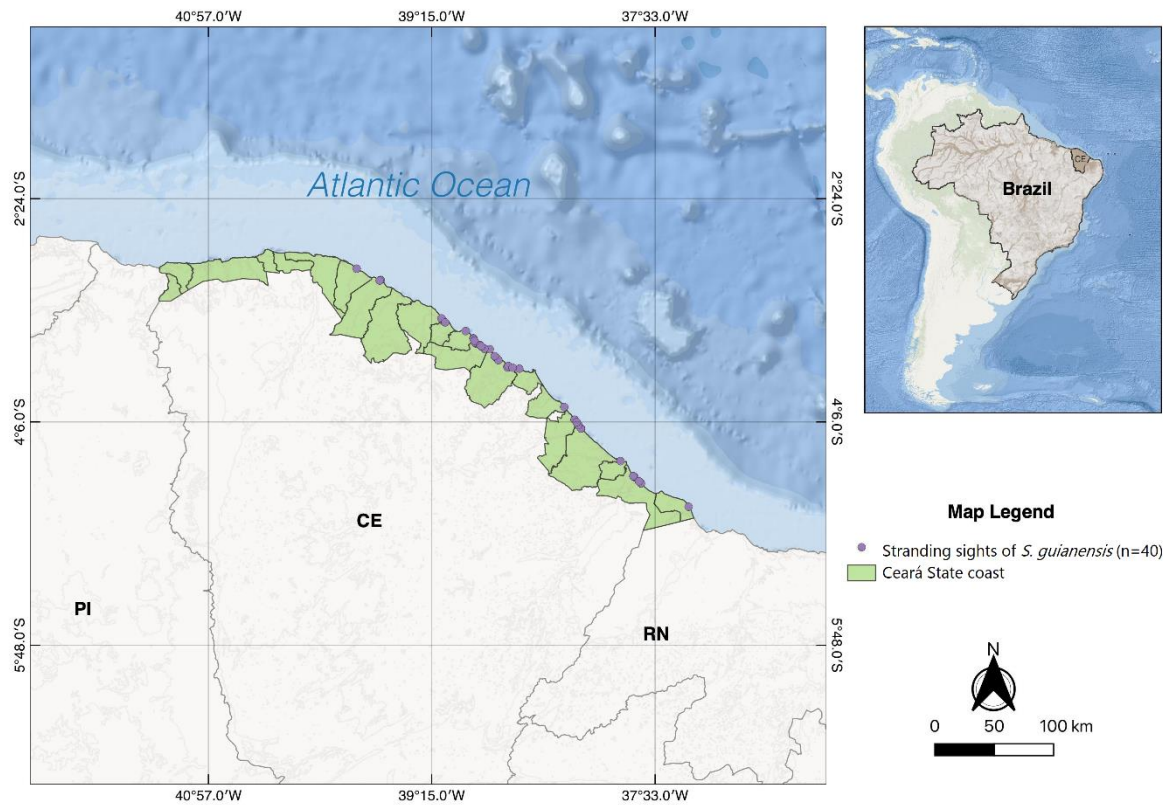


Figure 1: Distribution of stranding sights of *S. guianensis* along the study area on the Northeastern Brazilian coast in the Tropical Southwestern Atlantic. PI (Piauí state); CE (Ceará state); RN (Rio Grande do Norte state).

SAMPLING

During a decade, from 2011 to 2021, carcasses of *S. guianensis* were recovered (Fig. S1) during rescuing activities and beach monitoring on the coastline of Ceará state by Aquasis, a non-governmental organisation (NGO) with efforts to promote research and actions focusing on the conservation of endangered species in Ceará state (S1). Rescues were opportunistic since they depended on calls from the local community. For this study, a total of 40 individuals were used; among them, 23 were full stomachs, and 17 were stomach contents only (Table S1).

Whenever a dead individual of *S. guianensis* was found, basic information was collected for registering the stranding: geographic coordinates, date, season, total length, and stranding code according to Geraci & Lounsbury (2005) (1- alive, 2 - freshly dead, 3 – decomposed, 4 - advanced decomposition, 5 – mummified). At the laboratory or in the field, the entire stomach or stomach contents were removed from the body cavity. To avoid stomach contents leaking, the oesophagus' terminal portion and the initial portion of the duodenum were tied using a cotton rope, and then the stomach was cut away using a scalpel (Pugliares et al., 2007).

Once collected, samples were stored in plastic bags and frozen (-20 °C) until further analysis. The stomach contents were stored without the stomach chambers for specimens that

went through necropsy. For individuals inappropriate to undergo necropsy (Code 4), stomach or stomach contents were removed in the field using a scalpel. When the specimen was in an advanced stage of decomposition (Code 4), at least the total length was measured or estimated to access the age class of the specimen, following Rosas & Monteiro-Filho (2002), which state that individual males reach sexual maturity at 170 cm and females at 165 cm of total length.

LABORATORY PROCEDURES FOR MICROPLASTIC EXTRACTION

The protocol applied for microplastic extraction in the *S. guianensis* samples was adapted from Lusher & Hernandez-Milian (2018) and Justino et al. (2021). Firstly, samples were removed from the freezer, kept inside the bags on covered metal trays, and maintained at room temperature (30 °C) for approximately 14 hours for thawing. Once thawed, the external stomach surface was rinsed with filtered (cellulose fibre filter, 8 µm pore size, Whatman GR 40) distilled water to remove any particles attached and weighed (10^{-1} g). Stomach chambers were cut off using a scalpel and were then inverted directly on the beaker. The excess attached to the mucosa was rinsed out into the beaker using filtered distilled water. When the sample had no stomach chambers and represented the stomach contents solely, they were transferred to the beaker and then weighed.

Chemical digestion was used to extract microplastic particles from the samples. Stomach contents were digested in a filtered (cellulose fibre filter, 8 µm pore size, Whatman GR 40) 10% KOH solution (Lusher & Hernandez-Milian, 2018; Zhu et al., 2018; Moore et al., 2022) in a volume three times greater than the sample and kept in an oven (60 °C) for 24 hours (Justino et al., 2021). Beakers were covered with glass lids, and the solution was mixed two times during the process, using a glass stick, to homogenise the solution. If the sample had large bone parts, such as the skull and vertebral column, they were removed from the sample after the digestion step using steel forceps.

After digestion, samples were filtrated onto a cellulose fibre filter (8 µm pore size) using a vacuum pump system. Filters were transferred to covered Petri dishes and oven-dried at 60 °C. After 24 h, the samples were observed under a stereomicroscope (Zeiss Stemi 508, using 40-50x magnification) coupled with a device camera (Axiocam 105 Color), with a detection limit of 20 µm. Potential microplastic particles were photographed, measured (longest particle axis; Zeiss ZEN 3.2), counted, and characterised according to shape [fibres (filamentous particles), fragments (thick particles with an irregular shape), film (flat particles with an irregular shape), foam (soft particles with an irregular shape) and beads (spherical particles)] and colours in black, blue, green, red, and white (Lusher et al., 2017). Two observers conducted the inspection separately to avoid under or overestimating particles.

A sub-sample of 7% (22 particles) of the detected particles had the polymer composition investigated through Laser Direct Infrared analysis (LDIR). The absorbance of the suspected microplastics was obtained using the Agilent 8700 LDIR Chemical Imaging System and compared with the reference spectra of polymers from the Microplastics Starter 1.0 library. Each spectral curve resulted from at least ten scans performed in the wavelength ranging from 1800 to 975 cm^{-1} (Ourgaud et al., 2022). Then, the specific polymer was asserted when the analysed particle registered above 60% of similarity with the reference spectrum.

QUALITY ASSURANCE AND QUALITY CONTROL

During the field samplings, precautionary measurements to avoid airborne MP contamination were not implemented. At the laboratory, workstations and equipment were cleaned with filtered 70% ethanol to prevent contamination and rinsed out with filtered distilled water before the analysis. Additionally, the KOH solution was prepared using filtered distilled water, and the solution was also filtered before the digestion (8 μm pore size: Whatman GR 40). During laboratory procedures, 100% cotton lab coats, facemasks, and latex gloves were worn. In addition, a procedural blank (beaker filled with 50 ml of 10% KOH solution) was implemented for each individual sample, and blanks were submitted to the same methodological steps as samples. The use of the room where the analysis took place was restricted to two or three people involved in the procedures.

DATA ANALYSIS

Since the data did not meet parametric assumptions, Wilcox tests were applied to determine whether there were any differences in the number and size of microplastic particles detected according to the season. In addition, we tested whether the number and size of detected particles were correlated with sampling years, stomach content mass (g), and total length of specimens using the Spearman correlation test. All analyses were carried out using R 3.6 (R Core Team, 2020) with a 5% significance level.

RESULTS

In total, 317 suspected plastic particles were detected in the 40 Guiana dolphins analysed (Fig. 2), with a frequency of occurrence of 95% (38 out of 40 samples). Most of the suspected plastic particles detected were microplastics (311 particles $< 5 \text{ mm}$), while three were mesoplastics (5–20 mm), and three were macroplastics (20 - 100 mm). Considering the number of suspected MP particles solely, the general mean was 7.77 ± 1.25 particle individuals⁻¹ ($\pm$ standard error) (Fig. 3). Our results revealed that the size of detected MPs ranged from 0.018 to 4.24 mm, averaging $0.36 \pm 0.03 \text{ mm}$ per individual (Fig. 4).

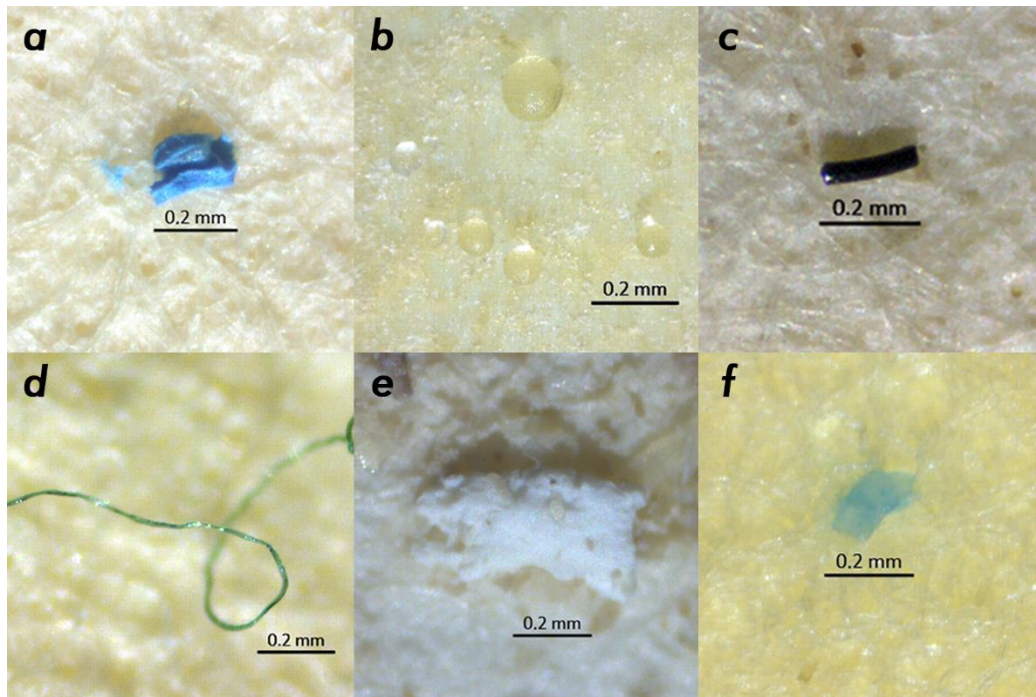


Figure 2 - Microplastic particles extracted from *S. guianensis* in the Southwestern Atlantic (a) fragment, (b) beads, (c) fragment, (d) filament, (e) foam, and (f) film.

Regarding the number of detected particles, ingestion was not significantly different between seasons ($W= 144$; $p= 0.292$). Furthermore, there was no correlation with the number of suspected MPs over the sampling years nor with the dolphin's total length (Table S2 and Fig. 4). On the other hand, the particles were smaller during the rainy season (0.30 ± 0.04 mm) than in the dry season (0.40 ± 0.04 mm) ($W= 13712$; $p= 0.021$) (Table S2 and Fig. 4).

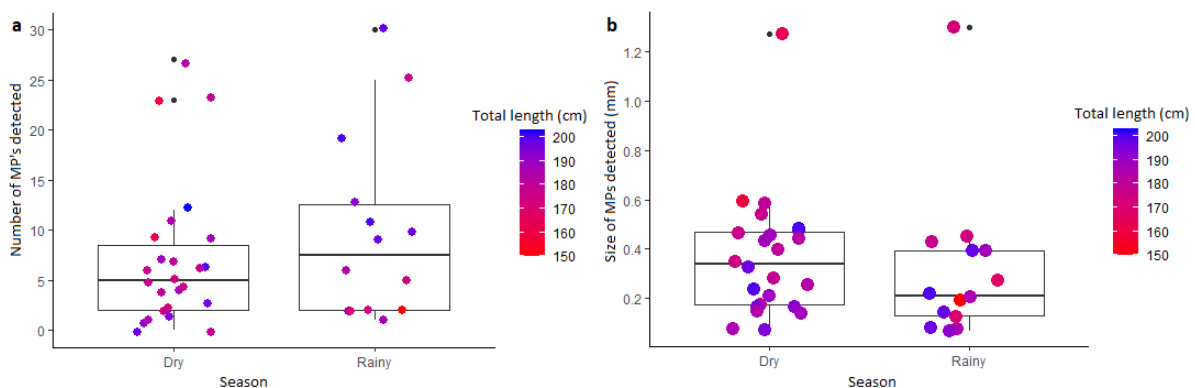


Figure 3 - (a) Number and (b) size of detected microplastics (individual⁻¹) in *S. guianensis* from the Northeastern Brazilian coast (Ceará state) in the Tropical Southwestern Atlantic, according to the seasons and total length of the specimens (cm).

The Spearman correlation analysis showed a positive moderate relationship ($\rho = 0.42$; $p = 0.006$) between the number of MPs detected and the stomach content weight (Table S3). On the other hand, there was no correlation between the number of particles detected regarding the sampling year and the total length of specimens (both $\rho = 0.17$; $p \geq 0.05$) (Fig. 4).

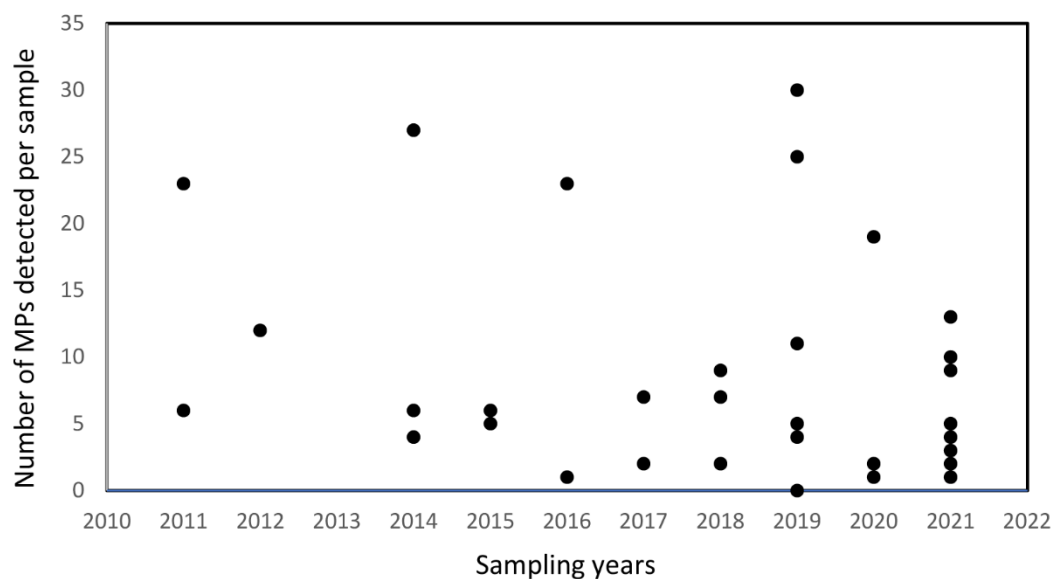


Figure 4 - Total number of microplastic particles extracted from each sample (*S. guianensis*) over a decade (2011 – 2021) in the Northeastern Brazilian coast. No significant difference over the years (p -value = 0.286; $\rho = 0.173$).

Among the different suspected microplastic shapes detected in the dolphins, fragments (57.2%) were predominant, followed by filaments (15.8%), foam (10.3%), films (9.6%), and beads (7.1%) (Fig. 6). White and black were the most abundant colour (39.5%), followed by blue (13.2%), green (4.2%), and red (3.5%) (Fig. 5).

From the analysed subsample (7%; 22 of the 317 detected particles), 55% (i.e. 12 particles) were successfully identified as plastic polymers, 10% (i.e. 2 particles) were biopolymers, and 35% (i.e. 8 particles) did not match the cutoff point (below 60% of similarity with the reference spectra) (Fig. 7). Regarding the identified plastic polymers, polyurethane (PU), polyethylene terephthalate (PET), and ethylene-vinyl acetate (EVA) were the most prevalent (18% each), followed by styrene-butadiene rubber (SBR), polypropylene (PP), polyamide (PA), acrylonitrile butadiene styrene (ABS) and high-density polyethylene (HDPE) (9% each) (Fig. 6).

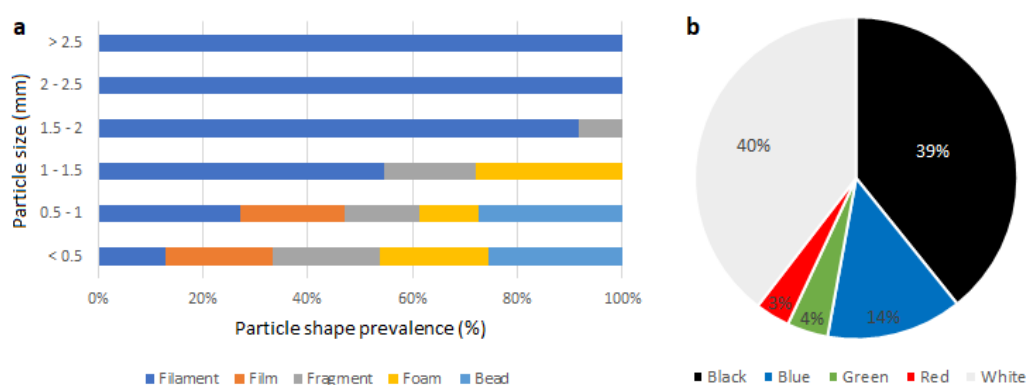


Figure 5 - (a) Size distribution of the detected microplastic shapes and (b) proportion of different colours of microplastics ingested by *S. guianensis* from Northeastern Brazilian coast (Ceará state) in the Tropical Southwestern Atlantic.

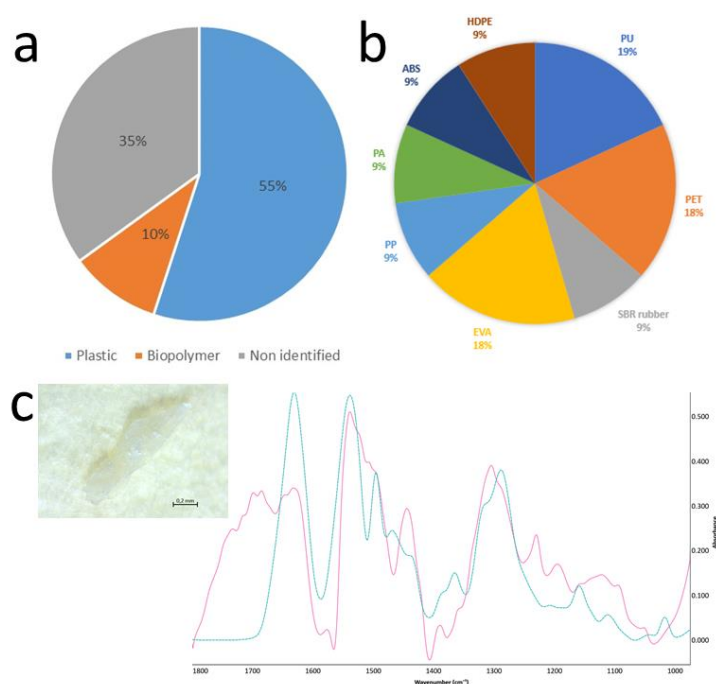


Figure 6 - Microplastic polymers extracted from *S. guianensis* identified by the LDIR analysis: (a) particle composition, (b) plastic polymers composition, (c) PA (polyamide) white fragment (pink line: particle spectrum; blue line: reference spectrum).

DISCUSSION

MPs are widely distributed in different aquatic ecosystems and raise concerns about their potential as pollutants (Battaglia et al., 2020). Larger plastics might represent a greater concern when ingested by marine biota due to the cause of potential injuries and blocking of the digestive tract (Derraik, 2002). However, the smaller size fractions (MPs) represent the majority of plastic particles detected in our samples, likewise as observed by Lusher (2018).

Comparison between our results for MPs with other populations of *S. guianensis* was not possible because, to our knowledge, this is the first study to document exposure to MPs in this species following up-to-date recommendations (Woodal et al., 2015; Lusher et al., 2017; Provencher et al., 2017). Two previous studies have investigated mesoplastic ingestion in this species (Di Benedetto and Ramos 2014; Padula et al., 2023). The frequency of occurrence of mesoplastics was comparable to our data (FO%= 5%), in addition, Padula and collaborators (2023) also reported marine debris down to 1 mm through sieving but did not measure the MP particles. Moreover, there is no data available on MP contamination in small cetaceans from coastal waters of South America that would allow extrapolations for comparisons and correlations in the region. Worldwide, most of the studies on microplastic contamination in marine mammals were conducted on carcasses stranded on European coastlines (Zantis et al., 2021). Furthermore, the sample size for each species is usually small, which can hinder comparisons and induce type II errors (Underwood et al., 2017).

Some studies have reported MP contamination in cetaceans from Europe, China, and North American coastal waters, where the number of detected particles varied greatly. While we detected 1 to 30 MPs per sample (sample size = 40; 8 µm pore size), Zhu et al. (2018) detected 2 to 45 MPs per individual of *Sousa chinensis* (3; 5 µm, respectively), Battaglia et al. (2020) extracted 67 to 304 MPs from *Tursiops truncatus* (7; 0.45 µm, respectively) and Hernandez-Gonzalez et al. (2018) detected 3 to 41 MPs in *Delphinus delphis* (sample size = 35; sieving down to 355 µm). These expressive differences in the number of detected particles are probably related to the different methods of MP extraction used associated with the sample type (stomach/intestine), or both (Hart et al., 2022). Furthermore, the different feeding strategies of the target species as well as the abundance of MPs in the environment of the study area may influence the differences observed (Di Benedetto & Ramos, 2014; Ferreira et al., 2019b; Justino et al., 2021).

However, the mean number of MPs per individual (7.77 ± 1.25 particles) was quite similar to those observed in several marine mammal species (mean = 5.5 ± 2.7 particles), including the *L. acutus*, *T. truncatus*, *P. phocoena*, *G. griseus*, *S. coeruleoalba*, and *L. albirostris* (Nelms et al. 2019). Similar findings may be related as the species share some characteristics: they are all small odontocetes, inhabit coastal and shallow waters, have a similar pattern of a diverse diet composed of fish and cephalopods, and are raptorial feeders (Hocking et al., 2017).

In agreement with Hernandez-Gonzalez. (2018) and Battaglia et al. (2020), the individual's total length did not correlate with the number of MPs detected in the stomach content, suggesting that there is no difference in the foraging process between juveniles and

adults (Rodrigues et al., 2019). However, we identified a positive correlation between the number of particles and the stomach contents mass (g). This finding, associated with the sparse ingestion of macro and mesoplastics by *S. guianensis*, supports the hypothesis that individuals are less susceptible to intentionally/actively ingesting plastic particles, which suggests that plastic contamination is more prone to occur through trophic transfer (Farrel & Nelson, 2013) or by accidental ingestion of the contaminant during the foraging process (Takada & Karapanagioti, 2019; Roch et al., 2020).

Moreover, the small size of detected MPs indicates that the major source of contamination might be the trophic transfer from the prey (Moore et al. 2020) since odontocetes do not feed using a filtering method contrarily to the baleen whales. This hypothesis is strengthened by data from Dantas et al. (2020), who documented MP contamination (average of 1 particle individual⁻¹; size not reported) in four coastal fishes (*Opisthonema oglinum*, *Conodon nobilis*, *Chloroscombrus chrysurus*, *Pomadasys corvinaeformis*), which compose the diet of *S. guianensis* (Pansard 2010; Campos 2012; Rodrigues et al. 2019), in the same study areas as ours.

MPs from the continent are usually retained along riverbanks and therefore exposed to weathering (mainly photooxidation), resulting in the potential breakdown into smaller pieces (Gewert et al., 2015; Enfrin et al., 2020.). During the rainy season when runoff increases, there is a higher MP input to the sea, specifically among smaller fractions (Lima et al., 2014; Lebreton et al., 2017). The transboundary nature of plastic pollution and the fragmentation of these particles could explain the association we found between the smaller particles being detected in the dolphins' stomachs during the rainy season. In addition, during this seasonal period (April and May), the Brazilian current flows to the adjacent surface coastal waters, mainly influencing the study area's western portion (Dossa et al., 2021) and may transport MPs from allochthonous sources.

Different MP shapes are found in the environment of our study area, and the most abundant shapes are fibres and fragments (Garcia et al., 2019; Nolasco et al., 2022). Most studies on Cetacea also found fibres to be the most abundant shape (Zantis et al., 2021; Lusher et al., 2017). In our case, most detected MPs were fragments (57.2%), agreeing with Moore et al. (2020), who identified half of the MPs in Beluga whales as fragments. The same was observed in pinniped samples (Zantis et al., 2021).

Even though plastic production and inputs into the oceans are increasing (Geyer et al., Isobe et al., 2019; 2017; Galgani et al., 2021), our results show no increase in the number of ingested MPs over the last decade (between 2011 and 2021), despite the considerable

population growth of the main city in our study area, which increased the population roughly 10% over this period (IBGE, 2021). Interestingly, a 15-year survey on stranded cetaceans conducted in the Irish coastal waters did not evince any temporal trend in MPs contamination (Lusher et al., 2018). Midterm monitoring (~10 years) of beached plastics (macro and mesoplastics) from different ocean basins indicated an absence of accumulation trend (Schulz et al., 2013; Hildalgo-Ruz et al., 2018; Walther et al., 2018). Coastlines are one of the main reservoirs of land-based plastics that are constantly resuspended and transported to coastal areas (Onink et al., 2021).

Though the adverse effects of MP ingestion by marine mammals are not well described *per se*, it is known that these animals are exposed to the toxicological effects of endocrine disruptor chemicals (Fossi & Marsili, 2003), which are found in plastic particles as chemical additives (Rani et al., 2015). The variety of polymers identified in this study raises concerns, as each polymer may be associated with different chemical pollutants (Nabi et al., 2022) and consequently with different toxic effects. The lack of an apparent increase in MP concentrations in *S. guianensis* samples over time can be seen as a positive sign, indicating that the pressure from MP contamination has not increased during the last decade on this particular species. Although the findings of this study have not been related to the cause of death of individuals, it is strongly recommended to investigate the effect of microplastics as chemical pollutants since MPs have a high adsorptive potential for persistent organic pollutants (POPs) and heavy metals and pathogens available in the environment (Liu et al., 2022; Pedrotti et al., 2022).

We would like to point out that our results need to be considered with care since our study has certain limitations. The subsample used for MP identification was relatively low due to time limitations and logistical issues. It is, therefore, not recommended to generalise the results from the subsample to the total samples but rather to consider them as an exploratory snapshot. We consider this acceptable since our study aimed not to investigate in detail the polymer composition and the sources of the MP contamination but to give a first description of the presence of MP particles in *S. guianensis* as well as insights into temporal variability and size distribution. Furthermore, while we cannot confirm that the 35% of unidentified particles in the subsample were plastics, it should be considered that the particles were strongly weathered, hampering identification and that the LDIR is a relatively new technique with a still limited number of reference spectra, which will increase in the future.

CONCLUSION

Our study promoted unprecedented findings of contamination by MPs for cetaceans, with emphasis on the Guiana dolphin, in South America and opened an important space for

undefined questions about the origin of the contamination of these animals. In addition, it reinforces the title and use of the species as a sentinel of the seas, since through the investigation of stomach contents of dead individuals, it was possible to verify the contamination along the entire Ceará state coast, which can be further expanded to the Tropical Western Atlantic, due to the species distribution range.

Our study found that the presence of MP in this species is disturbing; however, we expect no major health effects at this concentration. The low frequency of occurrence of macro to mesoplastics and the fact that the contamination seems to come from the trophic transfer (and not from trophic transfer associated with active ingestion, which would lead to higher concentrations) further indicates that the impact from POPs associated with MPs on this species is rather low.

Furthermore, we recommend continuous assessment and monitoring of plastic pollution and its impacts on this threatened marine mammal species since its findings may provide important subsidies for designing mitigation action plans for species conservation. We also encourage expanding the study area for future research to compare the degree of contamination along different coastal ecosystems and habitats.

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CONSIDERAÇÕES FINAIS

Embora os achados deste estudo não tenham sido relacionados à causa da morte de indivíduos, é altamente recomendável investigar o efeito dos microplásticos como poluentes químicos, uma vez que os MPs têm um alto potencial de adsorção de poluentes orgânicos persistentes (POPs), metais pesados e patógenos disponíveis no ambiente (Liu et al., 2022; Pedrotti et al., 2022). Nosso estudo indica que a presença de microplásticos nesta espécie desperta um alerta; no entanto, efeitos adversos para a saúde dos animais não são esperados nessas concentrações. Além disso, recomendamos a avaliação e monitoramento contínuo da poluição plástica e seus impactos sobre esta espécie ameaçada de mamífero marinho, pois os resultados podem fornecer subsídios importantes para a elaboração de planos de ação de mitigação para a conservação da espécie. Também encorajamos a expansão da área de estudo para pesquisas futuras para comparações do grau de contaminação ao longo de diferentes ecossistemas e habitats costeiros.

SUPPLEMENTARY MATERIAL

Microplastic ingestion by Guiana dolphins (*Sotalia guianensis*) on the Brazilian coast: does temporal variability matter?

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Figure captions

Figure S1 - Sample collection from a Guiana dolphin stranded dead in an advanced stage of decomposition

Table captions

Table S1 – Summary of *Sotalia guianensis* specimens sampled in the study. FMR (Fortaleza's Metropolitan Region); Eastern and Western monitoring sectors according to the FMR.

Table S2 - Summary of the Wilcox tests for the microplastics detected in the stomach contents of *S. guianensis*. *df* (degrees of freedom); *W* (W-values); *ns* (not significant); * ($p < 0.05$).

Table S3 - Summary Spearman's rank correlation for the number of microplastics (individual¹) detected in the stomach contents of *S. guianensis* with explanatory variables. *ns* (not significant); * ($p < 0.05$).



Figure S1 - Sample collection from a Guiana dolphin (*Sotalia guianensis*) stranded dead in an advanced stage (code 4) of decomposition.

Table S1 – Summary of *Sotalia guianensis* specimens sampled in the northeastern Brazilian coast (Ceará state). FMR (Fortaleza's Metropolitan Region); Eastern and Western monitoring sectors according to the FMR. * Presence of Stomach chambers.

ID	Sector	Season	Sex	Total length (cm)	Stomach content (g)	Year	Stomach Chambers
497	Eastern	Rainy	Female	185	557	2011	*
504	Eastern	Dry	Male	159	441	2011	*
561	Eastern	Dry	Male	203	1255	2012	*
634	Eastern	Dry	Male	180	504	2014	
650	FMR	Dry	Male	200	401	2014	
651	Eastern	Dry	Male	183	824	2014	*
654	Eastern	Dry	Male	177	670	2014	*
700	Western	Dry	Male	180	918	2015	
708	FMR	Dry	Male	178	160	2015	
740	Eastern	Dry	Male	180	1223	2016	*
758	FMR	Rainy	Unknow	174	197	2017	*
750	Eastern	Dry	Male	192	62	2016	
778	Eastern	Dry	Male	182	950	2017	*
794	Eastern	Rainy	Male	198	1139	2018	*
795	Eastern	Rainy	Unknow	150	206	2018	*
813	FMR	Dry	Female	165	778	2018	
846	Western	Rainy	Female	198	297	2019	*
853	Western	Rainy	Male	177	816	2019	*
855	Eastern	Rainy	Male	173	458	2019	
886	RMF	Dry	Unknow	188	872	2019	
867	FMR	Dry	Female	197	224	2019	*

877	FMR	Dry	Male	176	865	2019	*
878	FMR	Dry	Male	185	101	2019	
887	FMR	Dry	Female	178	64	2019	
891	FMR	Rainy	Female	200	390	2020	*
913	FMR	Dry	Female	169	460	2020	*
924	FMR	Dry	Male	167	20	2020	*
927	FMR	Dry	Male	195	139	2020	*
937	Western	Rainy	Male	188	237	2021	*
938	Eastern	Rainy	Female	188	1124	2021	*
940	FMR	Rainy	Unknow	170	56	2021	
942	FMR	Rainy	Male	192	117	2021	*
943	FMR	Rainy	Male	197	1250	2021	*
947	FMR	Rainy	Male	200	483	2021	
956	FMR	Dry	Unknow	188	460	2021	
960	Eastern	Dry	Unknow	185	883	2021	*
961	FMR	Dry	Male	196	169	2021	
962	FMR	Dry	Male	189	186	2021	
963	FMR	Dry	Male	184	81	2021	
968	Western	Dry	Male	175	164	2021	

Table S2 - Summary of the Wilcox tests for the microplastics detected in the stomach contents of *S. guianensis*. *df* (degrees of freedom); *W* (W-values); *ns* (not significant); * ($p < 0.05$).

Microplastics detected	<i>df</i>	<i>W</i>	<i>p</i> -value	
Number				
Season	1	144.5	0.292	<i>ns</i>
Size				
Season	1	13712	0.021	*

Table S3 - Summary Spearman's rank correlation for the number of microplastics (individual¹) detected in the stomach contents of *S. guianensis* with explanatory variables. *ns* (not significant); * ($p < 0.05$).

Microplastics detected	<i>S</i>	<i>p</i> -value	ρ	
Food contents	6131.1	0.006	0.425	*
Year	8816.9	0.286	0.173	<i>ns</i>
Total length	8778.7	0.276	0.176	<i>ns</i>