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# The sea anemone *Bunodosoma cangicum* as a potential biomonitor for microplastics contamination on the Brazilian Amazon coast☆

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## ABSTRACT

This study reports for the first time the ingestion of meso- (5.01–25 mm) and microplastics (1 µm–5 mm) by the sea anemone *Bunodosoma cangicum*, the most abundant actiniarian species on the Amazon coast. At three sites on the coast of Pará, Brazil, anemones were collected from beachrocks in the intertidal zone (30 at each site), measured (pedal disc diameter, mm) and weighed (wet weight, g). The contents of the gastrovascular cavity were extracted and analyzed under a stereoscope. The recovered plastic particles were characterized by Fourier Transform Infrared (FTIR) spectroscopy. Overall, 139 microplastic and 2 mesoplastic items were identified in 68 individuals (75.6%) among the 90 examined, with a mean of 1.6 (±1.5) items per individual. Plastic fibers comprised about 84% of the ingested plastics, followed by fragments (~12%) and films (~4%). Particle diameters ranged from 0.10 to 9.17 mm (1.57 ± 1.23 mm). A weak positive correlation was found between the weight of anemones and the number of plastic particles in the gastrovascular cavity ($p = 0.03$) and between the number of prey items and the number of plastic particles in the gastrovascular cavity ($p < 0.01$). The main polymers identified by FTIR analysis were polyethylene terephthalate (PET), polypropylene (PP), polyamide (PA), polyurethane (PU), polyethylene (PE), acrylonitrile butadiene styrene (ABS), polystyrene (PS) and rayon. Sea anemones ingested significantly more plastic debris at the most urbanized and populous sampling sites. This study provides the first evidence of microplastics contamination of marine invertebrates from the Amazon coast. Abundant species such as *B. cangicum* have the potential to monitor the levels of plastic contamination in the region. Our results support this potential, as the species showed a high frequency of plastic ingestion and allowed detection of plastic contamination even in the best-preserved area where anemones were collected.

## 1. Introduction

Plastics are synthetic organic polymers derived from diverse monomers commonly extracted from oil or gas (Güven et al., 2017). The low cost and high versatility of these materials has led to a continual increase in their production, which in 2018 reached 359 million tons (Andrady and Neal, 2009; PlasticsEurope, 2019). High production of these non-biodegradable materials and inadequate waste management make plastic a serious environmental problem. Today, plastic debris is widespread in the world's oceans and coasts, from preserved to highly impacted environments, and comprises over 80% of all marine debris (Barnes et al., 2009; Bellas et al., 2016).

In the marine environment, plastic items are degraded primarily through solar UV radiation-induced photo-oxidation reactions and physical abrasion. Slow thermal oxidation may also occur in conjunction with photo-oxidation of plastic materials, especially on beaches. Hydrolysis and biodegradation can also degrade plastics in the oceans, but these processes are orders of magnitude slower than the main agents (Barnes et al., 2009; Andrady, 2011). These

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degradation processes give rise to meso- (5.01–25 mm), micro- (1 μm–5 mm) and nanoplastics (<1 μm) (GESAMP, 2019). Microplastics (MPs) can be classified into primary microplastics, which are intentionally manufactured in microscopic size (e.g., plastic microspheres used in cosmetics and cleaning products), or secondary microplastics, derived from the fragmentation of larger plastics (Cole et al., 2011). In 2014 alone, an estimated 93 to 236 thousand metric tons of microplastics were deposited in the oceans (Sebille et al., 2015). These tiny size fractions of debris are a major concern, since they are potentially bioavailable to a wide variety of invertebrates from different trophic levels and thus may impact the entire trophic web through bioaccumulation and biomagnification (Browne et al., 2008; Thompson et al., 2009; Carbery et al., 2018).

Accumulation of ingested plastic particles in organisms can result in physical damage such as abrasion and blockage of the digestive tract, which can lead to starvation, reduction of reproductive capacity, limited predator avoidance and impairment of feeding capacity (Gregory, 2009; Wright et al., 2013). Ingestion of plastic can also expose the marine biota to chemical pollutants. Plastics can release additives present in their composition (such as phthalates, organotins and bisphenol-A) and hydrophobic chemicals that are adsorbed from surrounding seawater (such as alkylbenzenes, chlorinated hydrocarbons, polycyclic aromatic hydrocarbons and polychlorinated biphenyls). These substances can interact with important biomolecules inside cells and disrupt the endocrine system (Teuten et al., 2009).

Sea anemones are sessile organisms and generally polyphagous and opportunistic feeders (Shick, 1991) and may be particularly affected by microscopic plastic particles consumption, which makes them an excellent potential study object to monitor microplastics contamination. Studies of the consumption of plastic debris by anthozoans are still scarce. Experiments show that scleractinian corals can directly ingest plastic particles suspended in the water (Hall et al., 2015; Allen et al., 2017; Hankins et al., 2018). Plastic microfibers were found in the gastrovascular cavity of two deep-sea anthozoans from the southwest Indian Ocean, one belonging to subclass Hexacorallia and the other to Octocorallia (Taylor et al., 2016). Only Karlsson et al. (2017), Okubo et al. (2018) and Orte et al. (2019) have assessed microplastics ingestion by actinarians. For the Amazon coast, information on consumption of microplastics by marine organisms is limited to ingestion by marine and estuarine fish (Pegado et al., 2018).

The Amazon coast occupies about 35% of the Brazilian shoreline and has the largest drainage basin with the largest freshwater discharge volume in the world (Gibbs, 1972; Souza-Filho, 2005; Gibertoni et al., 2016). This fluvial system is the world's second most polluted in terms of plastic, next only to China's Yangtze River (Lebreton et al., 2017; Giarrizzo et al., 2019). The Amazon region has been undergoing rapid economic growth, with the highest urban growth rate in the country in recent decades (Becker, 2005), which directly contributes to the increase of pollution in the Amazon basin and consequently on the northern Brazilian coast. Given the uniqueness of this ecosystem and its enormous importance for humanity, it is essential to improve knowledge of plastic pollution in the region.

The sea anemone *Bunodosoma cangicum* Belém & Preslercravo, 1973 (Hexacorallia: Actiniidae) is endemic to the South Atlantic, occurring from the northern coast of Brazil to the coast of Uruguay (Fautin, 2013). It is usually found in aggregations in the intertidal zone, in cracks, fixed in rocky substrate partly covered with sediment (Melo and Amaral, 2005), and is the most common sea anemone on the Amazon coast. The species presents some characteristics that makes it a potential biomonitor of plastic contamination, including its wide distribution, sessile habit, generalist feeding behavior, high abundance on beachrocks in the Amazon region (*in situ* observation) and ease of sampling. Here, we report for the first time the ingestion of meso- and microplastic particles by the sea anemone *B. cangicum* on the Amazon coast. We determined the frequency, type, and polymer composition of the ingested plastic particles. We hypothesized that the number and size of ingested plastic particles would increase with anemone pedal disc diameter and weight (which affect the ability to capture food items), and with proximity of plastic sources and urban population size at the sample sites (which affect MP input to the aquatic environment).

## 2. Material and methods

### 2.1. Study area and sampling

Sea anemones (*Bunodosoma cangicum*) were collected on the northeastern coast of Pará state, Brazilian Amazon (Fig. 1). During the dry season (from August to October, *sensu* Kottek et al., 2006), *B. cangicum* populations are much more expressive, due to favorable oceanic conditions with the decreasing of the freshwater input (*in situ* observation). Therefore, in order to favor spatial comparisons and avoid intrinsic variability to local environmental conditions, field expeditions were concentrated in only one month (i.e., October 2018) of the dry season. The macrotidal regime is semidiurnal, with a maximum amplitude of 5.5 m. Tidal currents speed ranges from 0.1 to 1.65 m $s^{-1}$ and wave height is below 1.5 m (El-Robrini et al., 2018). The area is influenced by the Amazon, Tocantins and Atlantic (north/northeast section) hydrographic basins. The region is undergoing uncontrolled urban occupation and development, with small to large urban centers surrounding Extractive Reserves (RESEX) (Cordeiro et al., 2017).

A total of 90 anemones (30 at each site) were collected randomly at low tide from beachrocks in the intertidal zone at three collection points: (a) municipality of São Caetano de Odivelas (00°45′17.2″S 48°01′19.4″W); (b) Caixa D'água Beach in the Algodoal/Maiandeua Island environmental protection area (APA) (00°35′32.7″S 47°35′29.4″W); and (c) the touristic Maçarico Beach, municipality of Salinópolis (00°36′39.4″S 47°21′29.7″W). The pedal disc diameter (PDD) of the anemones was measured using a caliper (0.1 mm precision) before the animal was carefully removed from the substrate with the aid of a metal spatula. The PDD was chosen as a size parameter as it is the most accurate measurement to estimate the size of anemones in the field (Angeli et al., 2016). The specimens were stored in individual glass flasks with 4% formaldehyde in filtered seawater from the collection site. Any material regurgitated due to contraction of the central column was collected using a glass funnel.

### 2.2. Sample processing

In the laboratory, the sea anemones were weighed (wet weight; Marte AX200; 0.0001 g precision) and cross-sectioned. The contents of the gastrovascular cavity were washed into a Petri dish, using 70% ethanol, and analyzed under a stereomicroscope (Zeiss Stemi, 2000-C). Potential MPs were separated from food items and organic matter, counted, classified by shape and color (GESAMP, 2019), measured for widest diameter (0.001 mm precision), and photographed (Zeiss SteREO Discovery V12 stereoscope with Zen software blue edition, v2.0, Zeiss, Oberkochen, Germany). Natural food items were also counted. The contents of the specimen jars were analyzed in case the sea anemones had regurgitated during sampling, by contracting the column during removal from the

**Fig. 1.** Location of the study area and sampling sites (identified by white circles) for *Bunodosoma cangicum* on the northeastern coast of Pará state (Brazil) during October 2018. Mosaic of Landsat-8 images (223–060 and 223–061) from June 7, 2017, color composition RGB-432. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

substrate or after exposure to formalin (Chintiroglou and Koukouras, 1991).

### 2.3. Background contamination control

Common practices to minimize contamination were adopted during collection and processing of the samples. The use of plastic tools was limited in field and laboratory activities. The number of people in circulation at the workstation was reduced. Before and after being used, all laboratory and field equipment, glassware and tools were cleaned with 70% alcohol diluted in distilled water. In the laboratory, before and after the processing of the organisms, all the work surfaces were thoroughly cleaned with 70% ethanol/distilled water, and a new pair of latex gloves was used. To test for contamination by airborne plastic fibers, a clean glass Petri dish was placed at the workstation at the beginning of each procedure and examined in a covered stereoscope at the end of the procedure (adapted from Torre et al., 2016). Moreover, all solutions used were filtered through a 45-μm stainless steel filter.

### 2.4. Polymer composition

For each type of MP classified, one sample was selected for polymer identification. The composition of the plastic particles was determined with Fourier Transform Infrared (FTIR) Spectroscopy. FTIR is one of the most reliable techniques for identifying plastic debris from aquatic environments (Mecozzi et al., 2016). The analysis was performed using a Cary 620–670 Fourier transform infrared spectroscopy (FTIR) microscope (Agilent Technologies) equipped with an FPA (Focal Plane Array) 128 × 128 detector (Agilent Technologies) which allows 2D imaging-FTIR analysis; 128 scans were acquired for each spectrum, in reflectance mode with open aperture and a spatial resolution of 8 $cm^{-1}$. Background spectra were collected directly on a gold-plated surface.

The Agilent Resolution Pro software (Agilent technologies) was used to collect, process, and analyze the spectra, and to obtain 2D imaging maps. Each analysis consists in a map of 700 μm × 700 μm (128 × 128 pixel) with an Imaging map spatial resolution of 5.5 μm (i.e. each pixel has a dimension of 5.5 μm × 5.5 μm). Such spatial resolution allows the collection of a large number of independent spectra on the plastic samples, for instance more than 150 independent spectra can be typically collected on a fiber of ca. 1 mm and 10 μm thickness. By mapping the intensity of diagnostic bands in false color (red > yellow > green > blue), it is possible to see how each band is representative of the spectra of a single sample. A single image (700 μm × 700 μm) was thus collected for each analyzed sample, leading to the collection of numerous independent spectra along the sample length. The spectra showed in the FTIR image in Section 3.5 are representative spectra from all the independent spectra for each plastic sample. It is to be noted that the detection limit of an FPA detector has been found to be significantly lower than that of a conventional mercury cadmium telluride (MCT) detector for the FTIR detection of trace amounts of materials. In fact, the heterogeneous distribution of the analyte can result in small areas of localized high concentration, which can be detected due to the high spatial resolution of the FTIR FPA imaging approach (Chan and Kazarian, 2006). For instance, for polyvinyl alcohol and polyvinyl acetate we verified that quantities < 1pg/

pixel (1 pixel = 5.5 μm × 5.5 μm) can be detected on reflective surfaces, i.e. smaller amounts than those we typically met in the analysis of fibers (Mastrangelo et al., 2020).

Reference FTIR spectra for polypropylene, high- and low-density polyethylene, polyethylene terephthalate, polyvinyl chloride, polystyrene, nitrile, acrylonitrile butadiene styrene, polyamide (e.g. nylons), poly (methyl methacrylate), and polyurethane can be found in a work by Jung et al. (2018); reference spectra of cellulose-based fibers have been reported by Garside and Wyeth (2003, 2006). Plastic and cellulose fibers were identified by matching of at least four main characteristic absorption bands of each reference spectrum. In addition, the polymers identified by FTIR analysis were also classified according to their tendency to float or sink in sea water, based on the density (GESAMP, 2019; Plastics International, n.d.).

### 2.5. Statistical analysis

The data were tested for normality and homoscedasticity to select the appropriate statistical tests. Spearman's rank correlation was performed to determine the association between: i) number of ingested MPs and pedal disc diameter; ii) size of ingested MPs and pedal disc diameter; iii) number of MPs and sea anemone weight; iv) size of MPs and sea anemone weight; v) number of MPs and number of ingested prey; and vi) size of MPs and number of ingested prey. When the correlation was significant, a linear regression was performed to derive an equation that models the relationship between the variables. A Kruskal-Wallis test was performed to determine significant differences between sample sites in terms of the number and size of the ingested plastic particles. The statistical analyses included only specimens with MPs in the gastrointestinal cavity. All statistical tests were performed in the R environment (R Core Team, 2019) and used a 5% significance level.

## 3. Results

### 3.1. Sea anemones morphometric parameters

The mean pedal disc diameter of the anemones was 28.3 ± 8 mm (range 16–52 mm). The mean weight was 3 g (±2.7), ranging from 0.4 to 22.4 g. PDD and weight were positively correlated (Spearman's rho = 0.805, S = 10203, $p < 0.001$). The morphometric variables did not differ significantly between the sampling sites (ANOVA one-way for PDD, F = 0.0639, $p = 0.938$; and for weight, F = 0.363, $p = 0.696$) (Table 1).

### 3.2. Plastic ingestion

A total of 141 plastic particles were identified in 68 individuals (75.6% of the 90 examined). The anemones contained a mean of 1.6 (±1.5) plastic particles per individual. Anemones that ingested MPs had a mean PDD of 28.4 ± 7.8 mm (range 16–49 mm) and a mean weight of 2.9 ± 2 g (range 0.4–8.1 g). Sea anemone weight and number of plastic particles in the gastrovascular cavity showed a weak positive correlation (Spearman's rho = 0.263, S = 38596, $p = 0.03$). Linear regression analysis indicated an addition of 0.2 microplastic particles for each additional gram ($r^2 = 0.0479$, $p = 0.0404$, $y = 0.787 + 0.203\times$) (Fig. 2). No significant correlation was found between PDD and MP number (S = 42122, $p = 0.109$).

**Table 1**
Ranges and mean values of pedal disc diameter (PDD) and wet weight for *B. cangicum* at each sampling site. N, number of specimens analyzed; SD, standard deviation. N = 30 for each site.

| Location | PDD (mm) | | Weight (g) | |
|---|---|---|---|---|
| | Range | Mean ± SD | Range | Mean ± SD |
| Algodoal/Maiandeua Island | 16–52 | 28.7 ± 8.4 | 0.4–22.4 | 3 ± 3.9 |
| Salinópolis | 16.4–47.4 | 28.3 ± 8.4 | 0.7–8.1 | 3.3 ± 2.4 |
| São Caetano de Odivelas | 16–49 | 27.8 ± 7.6 | 0.6–6.7 | 2.7 ± 1.5 |

Plastic ingestion was observed in all three sampling areas on the Brazilian Amazon coast. The highest number of MPs occurred at São Caetano de Odivelas, with 59 particles recovered from 25 sea anemones (2 ± 1.6 $\text{particles.org}^{-1}$). At Salinópolis, 53 plastic particles were found in 22 individuals (1.8 ± 1.7 $\text{particles.org}^{-1}$) and at Algodoal/Maiandeua Island, 29 particles were found in 21 individuals (1 ± 0.9 $\text{particles.org}^{-1}$). The largest number of ingested particles per individual was 6, recorded for anemones from Salinópolis and São Caetano de Odivelas; the maximum number ingested per anemone from Algodoal/Maiandeua was 3 particles. The number of ingested MPs differed significantly among locations (Kruskal-Wallis, $X^2 = 8.52$, df = 2, $p = 0.0141$). Anemones from Algodoal/Maiandeua Island ingested significantly fewer particles than those from Salinópolis (Dunn's test, $Z = -2.51$, $p = 0.024$) and São Caetano de Odivelas ($Z = -2.59$, $p = 0.029$) (Fig. 3A).

### 3.3. Plastic characteristics and composition

Overall, 139 particles (about 99% of the total) were classified as microplastics, and 2 as mesoplastics. The mean diameter of microplastics was 1.48 (±0.99) mm, ranging from 0.10 to 4.82 mm and mesoplastics measured 5.88 and 9.17 mm. Fibers comprised about 84% of the ingested plastics, followed by fragments (~12%) and films (~4%). The colors, from most to least frequent, were blue (66.7%), transparent (14.9%), red (8.5%), yellow (4.3%), white (3.5%), and green (2.1%). No significant correlation was found between PDD and particle size (Spearman's correlation, S = 502485, $p = 0.373$) or between anemone weight and particle size (S = 494092, $p = 0.498$).

Sea anemones from São Caetano de Odivelas ingested the largest plastic particles (mean = 1.68 ± 1.43 mm, range 0.13–9.17 mm), followed by Algodoal/Maiandeua Island (1.58 ± 1.30, range 0.13–5.88 mm) and Salinópolis (1.44 ± 0.94, range 0.10–3.94). Particle size did not differ significantly among sites (Kruskal-Wallis, $X^2 = 0.254$, df = 2, $p = 0.881$).

Plastic fibers predominated at all sampling sites, accounting for over 79% of the total plastics for each location. Plastic films were absent only at Algodoal/Maiandeua Island. Blue was the most frequent color of microplastics, occurring in more than half of the items ingested by Salinópolis and São Caetano de Odivelas anemones, and in more than 90% of the items ingested by Algodoal/Maiandeua Island anemones (Fig. 3B–C).

### 3.4. Gastrovascular cavity contents

Of the total anemones collected, 62.2% (56 individuals) contained both plastic particles and prey in their gastrovascular cavity, 16.7% (15) contained only prey, 13.3% (12) had only plastic items, and only 7.8% (7) had no gut contents. Algodoal/Maiandeua Island anemones had the smallest percentage of gastrovascular cavities with both plastic and food items (46.7%), but also the highest proportion of individuals with only plastic items in the gut contents (23.3%) (Fig. 3D).

A weak positive correlation was found between the number of prey and number of MPs in the gastrovascular cavity (Spearman's rho = 0.30164, S = 36590, $p = 0.0124$). Linear regression analysis indicated that 0.051 microplastic particles were added for each additional prey item ingested ($r^2 = 0.0704$, $p = 0.0164$, $y = 1.74 + 0.0511\times$) (Fig. 2). No significant correlation was detected

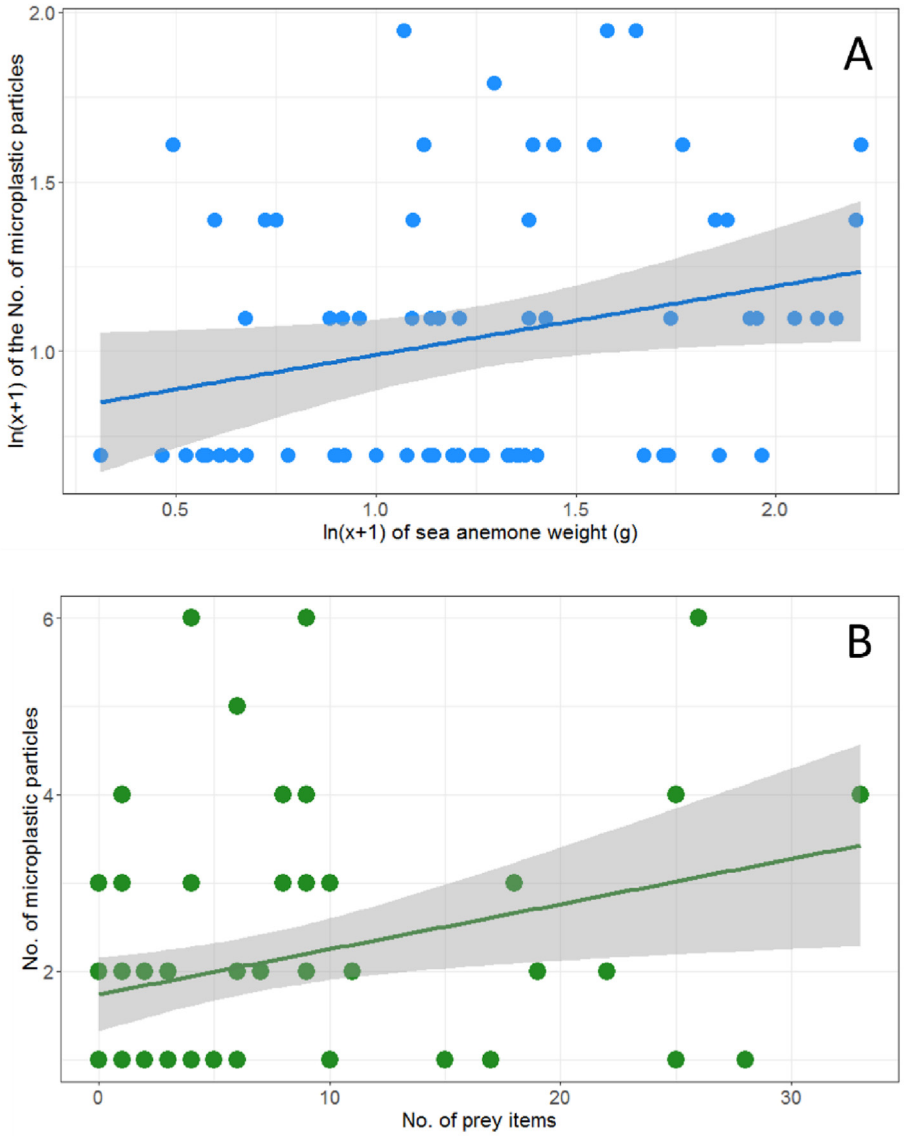

**Fig. 2.** Scatter plots between (A) the number of ingested plastic particles and weight of *Bunodosoma cangicum* (data transformed using ln(X+1)), and (B) number of ingested plastic particles and number of ingested prey. Data include only specimens with plastic items in the gastrovascular cavity. Shaded area indicates 95% confidence interval for the linear regression.

between the number of prey items in the gut and the size of plastic particles (S = 419830, $p = 0.232$). Additionally, the number of prey and the weight of sea anemones were positively correlated (Spearman's rho = 0.301, S = 36607, $p = 0.0125$).

### 3.5. Polymer identification

A total of 38 plastic samples were selected for FTIR analysis and 8 polymers were identified. Overall, 44.7% of the samples had absorption spectra consistent with polyethylene terephthalate (PET), 18.4% were identified as polypropylene (PP), 10.5% as polyamide, 10.5% as polyurethane (PU), 7.9% as polyethylene (PE), 2.6% as acrylonitrile butadiene styrene (ABS), and 2.6% as polystyrene (PS) (Fig. 4). Rayon, a man-made semi-synthetic polymer, comprised 2.6% of the samples. This material can generally be distinguished from natural cellulose by the absence of bands at 1735 (C=O ester stretching band from pectin), 1105 and 1050 $cm^{-1}$ (antisymmetric and symmetric C–O–C stretching modes) (Comnea-Stancu et al., 2017; Ding et al., 2019). However, spectra of rayon fibers (bamboo rayon) with the clear presence of these bands have been reported (Teli and Sheik, 2013). Our samples showed absorption spectra with a clear band at 1735 $cm^{-1}$, compatible with a cellulose-based material; however, inspection under a microscope showed a perfectly cylindrical structure of the fibers, which does not match the morphology of any fiber classified as natural cellulose. Therefore, we identified the samples as rayon based on the morphological approach and included it in our results due to the anthropogenic nature of the material.

Sea anemones from São Caetano de Odivelas ingested plastics identified as PET (44.4%), PP (27.8%), PE (11.1%), polyamide (5.6%), PS (5.6%), and PU (5.6%). Anemones from Salinópolis ingested PET (40%), PU (20%), polyamide (13.3%), PP (13.3%), ABS (6.7%), and rayon (6.7%). Anemones from Algodoal/Maiandeua Island contained PET (60%), polyamide (20%), and PE (20%).

Most of the identified MPs (73% of the total) had densities higher than seawater. About 87% of the MPs ingested by anemones from

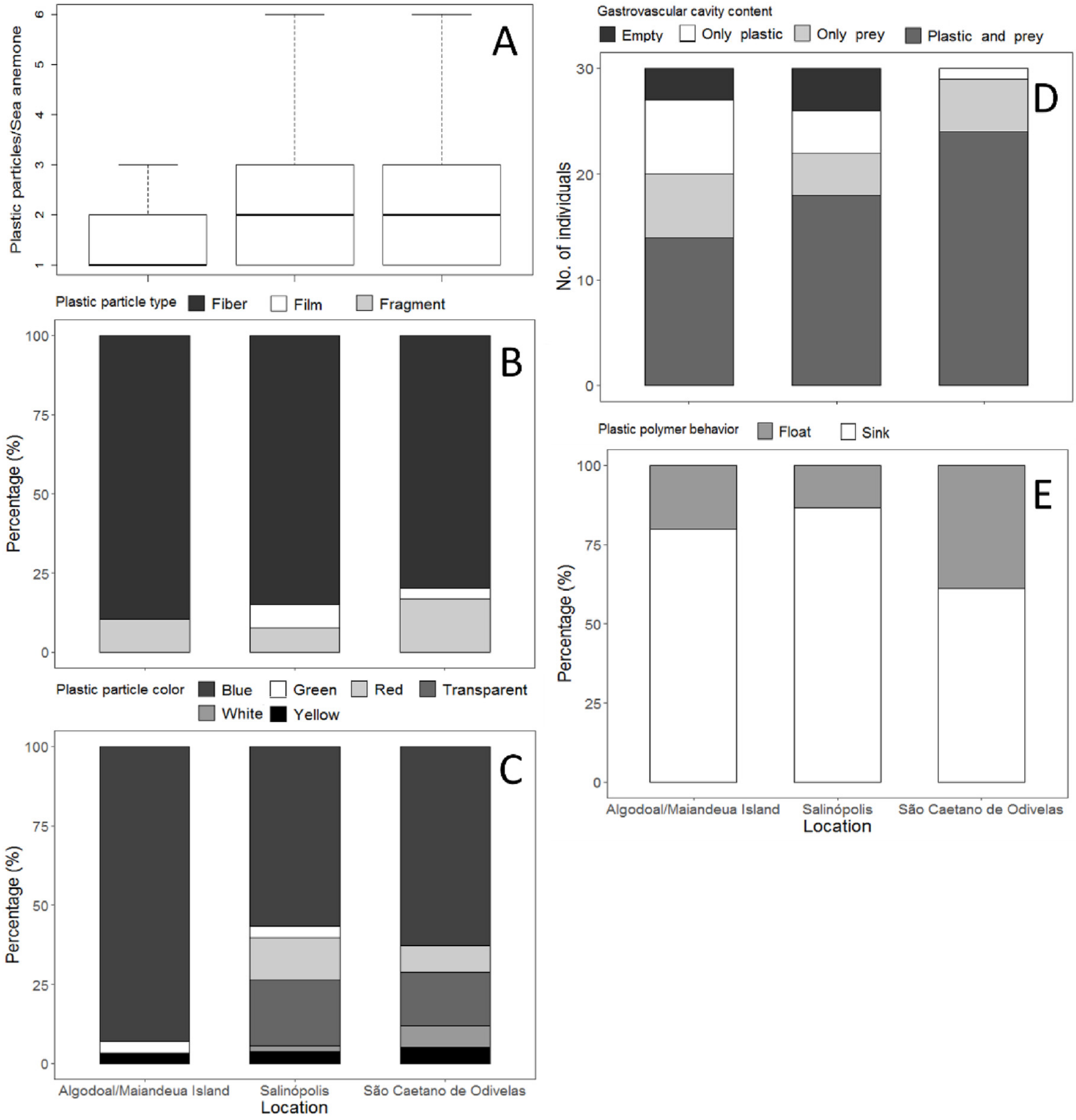

**Fig. 3.** Plastic ingestion by *Bunodosoma cangicum*. (A) Number of plastic particles ingested per individual for each sampling site; (B) Proportion of fibers, films and fragments in anemones collected from each site; (C) Proportion of plastic colors at each site; (D) Number of sea anemones without gut contents, with only plastic, with only prey, or with both plastic and prey for each site; and (E) Proportion of plastic polymers that float or sink in seawater for each site. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Salinópolis, 80% from Algodoal/Maiandeua Island, and 61% from São Caetano de Odivelas presented sinking behavior (Fig. 3E). Individuals from all collection sites had a significantly higher proportion of sinking than floating polymers in the gastrovascular cavity (Chi-squared test for Algodoal/Maiandeua Island, $X^2 = 36$, df $= 1$, $p < 0.05$; Salinópolis, $X^2 = 53.9$, df $= 1$, $p < 0.05$; and São Caetano de Odivelas, $X^2 = 4.93$, df $= 1$, $p = 0.0264$).

## 4. Discussion

This study provides the first evidence of microplastics contamination in marine invertebrates from the Amazon coast. Marine invertebrates may ingest plastic accidently during normal feeding behavior, misidentify debris as natural prey, or acquire it from contaminated prey (Ryan, 2016; Wright et al., 2013). Experiments show that sea anemones can ingest plastic particles either directly from the water column or by consuming contaminated prey (Okubo et al., 2018; Orte et al., 2019). Allen et al. (2017) suggested that plastics contain phagostimulants that drive their ingestion by anthozoans. Anemones may also consume MPs by accidently ingesting them with water retained in the coelenteron to prevent desiccation at low tide.

Once ingested by anthozoans, MPs may be trapped between mesentery tissues, which are the main tissues responsible for digestion. Therefore, plastic intake would reduce digestion of natural prey (Nicol, 1959; Allen et al., 2017; Hall et al., 2015). Evidence suggests that MP consumption can also induce oxidative stress in anthozoans (Rocha et al., 2020), which can disrupt the redox regulation in cells and cause molecular damage (Sies, 2015). In addition, Okubo et al. (2018) suggested that microplastics intake can suppress the incorporation of symbiotic algae into host cells of

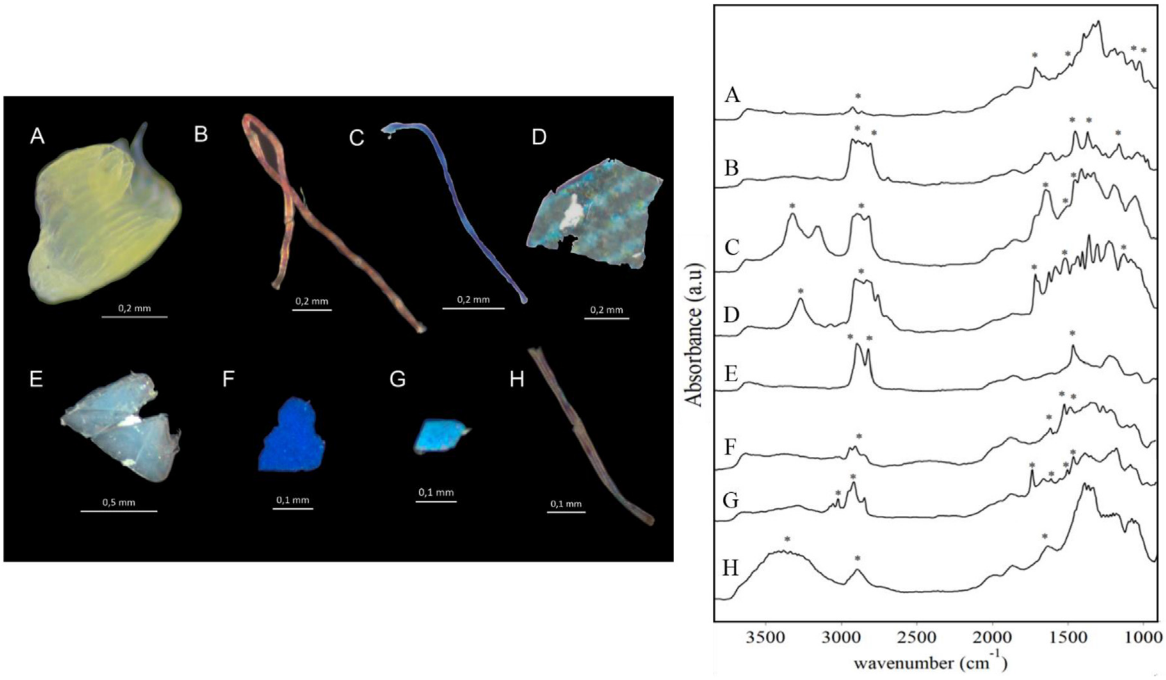

**Fig. 4.** Representative examples of the various categories of plastic particles found, with their respective FTIR spectra. (A) yellow fragment (polyethylene terephthalate); (B) red fiber (polypropylene); (C) blue fiber (polyamide); (D) blue film (polyurethane); (E) blue fragment (polyethylene); (F) blue fragment (acrylonitrile butadiene styrene); (G) blue fragment (polystyrene). The spectrum labeled H is representative of transparent cellulose-based fibers (including the sample identified as rayon). Asterisks (*) mark the bands of the reflectance spectra that identified the polymers. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

anemones and impair initiation of the actiniarian-algae symbiotic relationship.

Here, the correlation and regression analyses suggest that the number of ingested MPs may depend on anemone weight and the number of captured prey. Weight was significantly correlated with pedal disc diameter, and both factors are descriptors of body size, which can directly influence the capacity of sea anemones to capture and ingest prey (Sebens, 1981; Anthony, 1997). A higher sampling effort would strengthen these correlations. Furthermore, anemone weight and the number of ingested prey items were significantly correlated variables. Individuals that catch more prey items would consequently catch more contaminated prey and also fill the gastrovascular cavity with water more frequently and in larger volumes, probably increasing accidental intake of MPs from the surrounding water. There was no size selection of the ingested plastic particles related to the size of the individuals, since no significant correlation was found between particle size and any descriptor of anemone size. This result may reflect the opportunistic and generalist feeding habit of *B. cangicum*.

Algodoal/Maiandeua Island has been an environmental protection area since 1990 (Wariss et al., 2012), with 3100.34 ha of natural preserved landscape. The four villages on the island have small traditional populations, totaling 1793 inhabitants (SEMA, 2012). However, MP contamination was conspicuous even in this preserved environment, occurring in 70% of the specimens. Except on holidays, when the island is visited by large numbers of tourists, generation of plastic waste is probably low. Therefore, we can infer a low input of plastic debris from the island itself to the surrounding water bodies, which increases during holiday periods. Most of the plastic debris present in the island coastal waters probably comes from adjacent areas such as the city of Marudá, which is a more populous urban center. MPs may also be transported from the open ocean to the sampling site, since ocean water can penetrate as far as 62 km upstream the Marapanim River (which reaches the sampling point) during flood tide (Vilhena et al., 2010). In addition, the Marapanim River is relatively large watercourse with a hydrographic basin of 2500 km$^2$ (Silva et al., 2009) and may dilute MPs in the water surrounding the sampling site at the island, consequently reducing the bioavailability of plastic debris.

In contrast, Salinópolis and São Caetano de Odivelas are two coastal cities with larger populations, 40,675 and 18,050 inhabitants respectively (IBGE, 2019), and probably produce and input larger amounts of plastic debris into the surrounding water. The Sampaio and Mojuim rivers, which flow through the Salinópolis and São Caetano de Odivelas sampling sites, are about 6 km and 62 km long, respectively, while the Marapanim River is 120 km long. These smaller rivers probably dilute less of the water microplastics content. Furthermore, despite their denser urban development, these municipalities lack water and sewage treatment systems, which are important additional factors influencing the transport of MPs from the continent to coastal waterbodies (Jambeck et al., 2015; Lebreton et al., 2017; Giarrizzo et al., 2019). All these factors may account for the significantly higher ingestion of plastic particles by anemones from São Caetano de Odivelas and Salinópolis than by those from Algodoal/Maiandeua Island.

The sampling site at São Caetano de Odivelas was a beachrock in the estuary at the city shore, which lacks riparian vegetation. Part of MPs entering rivers can be efficiently retained in riparian and other coastal and river-border vegetation (Williams and Simmons, 1996; Ivar do Sul et al., 2014; Zhang, 2017), which acts as a natural filter for MP input from continent. The lack of riparian vegetation at this site may have contributed to a larger supply of microplastics to the beachrock through surface runoff from the adjacent city, which may have contributed to increasing the bioavailability and consequently to higher microplastic intake by the anemones from São Caetano de Odivelas.

Martinelli Filho and Monteiro (2019) found that up to 95% of the plastic debris in the sediment of Corvina Beach consisted of fibers. The area is adjacent to the sampling site at Maçarico Beach, Salinópolis municipality. Therefore, plastic fibers may have predominated in the anemones' gastrovascular cavity because of their greater availability in the surrounding environment. Furthermore, microfibers have been reported as the prevailing form of plastic in the digestive tract of a variety of marine taxa, including polychaetes, crustaceans, bivalves, gastropods, echinoderms, ascidians, fish and birds (Murray and Cowie, 2011; Goldstein and Goodwin, 2013; Lusher et al., 2013; Devriese et al., 2015; Bellas et al., 2016; Gusmão et al., 2016; Courtene-Jones et al., 2017; Lourenço et al., 2017; Mizraji et al., 2017; Vered et al., 2019).

Polyethylene (PE) is the most produced and therefore the most often reported polymer in studies of MPs in aquatic organisms (de Sá et al., 2018; GESAMP, 2019). However, polyethylene terephthalate (PET) was the most common polymer ingested by *B. cangicum* at all the sampling sites. Density is a critical factor in the mobility of microplastics in water and consequently in their bioavailability in different marine compartments (Wright et al., 2013; Zhang, 2017). The sea bed tends to be a sink for high-density plastics (Andrady, 2011), and therefore benthic animals are most affected by MPs that sink. This can explain the predominance of PET and other polymers that are denser than seawater in the samples, since they tend to sink to the benthic environment while PE tends to float.

Small amounts of floating polymers, most commonly polypropylene (PP) and PE, were present in the anemone gastrovascular cavity. Ingestion of low-density polymers may be explained by the intertidal habitat of this species. When the sea level decreases during ebb tide, benthic organisms probably encounter both more prey and more plastic debris from the surface. Moreover, during low tide, aggregations of *B. cangicum* were often observed consuming different prey items in tide pools. These environments probably retain plastic debris that was on the surface water during high tide, making it bioavailable to the benthic community. Ingestion of contaminated planktonic prey can constitute another route for ingestion of low-density polymers, together with seawater taken up in order to fill the gastrovascular cavity and avoid drying during exposure to air. Particle biofouling and physical processes of turbulence, such as vertical mixing, may also help to transport the floating polymers from the surface to the bottom, where they come into contact with the anemones (Zhang, 2017). Polypropylene is a plastic polymer used in the manufacture of automotive parts (PlasticsEurope, 2019). This polymer was detected in samples from São Caetano and Salinopolis but not in samples from Algodoal/Maiandeua Island. This may be related to the prohibition of automotive traffic in the area since June 1, 2007 (SEMA, 2012).

Knowledge of MP ingestion patterns in the natural environment by organisms near the base of the trophic chain is important in order to understand the processes of bioaccumulation of plastic debris at higher trophic levels. Actiniarians have a diverse array of vertebrate and invertebrate predators (Ottaway, 1977), and can transfer microplastics to species at several trophic levels and possibly even to commercially important fish. Some coastal birds can also prey on sea anemones (Donoghue et al., 1986), thus constituting another transfer route.

The benthic environment is considered the ultimate sink for plastic debris in the oceans (Bellas et al., 2016), hence the benthic community is particularly affected by ingestion of these contaminants. MPs are ingested most frequently by sessile species with generalist feeding habits and prey-capture methods (Peters et al., 2017; Messinetti et al., 2018). *Bunodosoma cangicum* possesses similar attributes as well as high abundance, ease of sampling, and wide distribution in the western South Atlantic. Moreover, despite its high abundance, this is the only intertidal sea anemone recorded from the Amazon coast, avoiding taxonomic errors on the identification of the specimens. These characteristics make the species a potentially excellent biomonitor for microplastics contamination in the region. Our results support this potential, since the anemones showed a high frequency of plastic intake, including particles of several shapes and colors and most of the microplastic particles size spectrum (larger than 200 μm). In addition, *B. cangicum* was able to indicate plastic contamination even in a relatively well-preserved Environmental Protection Area.

## 5. Conclusion

This study is the first assessment of microplastics contamination of marine invertebrates from the Amazon coast and for sea anemones from the South Atlantic. The MP ingestion patterns and particle characteristics appear to reflect the feeding behavior and habitat of the sea anemone *B. cangicum*. The amount of plastic ingested depended on the sea anemone body size and capacity to consume prey, which were correlated variables. More plastic particles were ingested at the urbanized and populous sampling sites. The absence of riparian vegetation could be another factor leading to higher plastic contamination. Individuals from the Environmental Protection Area of Algodoal/Maiandeua Island ingested smaller amounts of plastic debris; however, contaminated anemones were conspicuous in the site, with most individuals containing plastic particles. *Bunodosoma cangicum* possesses some attributes that make it a potential biomonitor for microplastics contamination on the Amazon coast. Our results support this potential use, as this anemone showed a high frequency of plastic consumption and was able to detect plastic pollution even in a better-preserved area. Sampling in new areas is necessary to assess the species' capability to monitor plastic contamination along the entire Brazilian coast. Experimental studies are also needed to investigate the effects and interaction of microplastics with the anemones. The development of a noninvasive method to study ingestion of plastics by anemones is recommended.

## Credit author statement

**Leonardo Mario Siqueira Morais:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Visualization, Writing - Original Draft. **Francesco Sarti:** Investigation. **David Chelazzi:** Investigation, Resources, Data Curation, Writing - Review & Editing. **Alessandra Cincinelli:** Investigation, Resources. **Tommaso Giarrizzo:** Conceptualization, Methodology, Resources, Writing - Original Draft, Supervision. **José Eduardo Martinelli Filho:** Conceptualization, Methodology, Investigation, Resources, Writing - Original Draft, Supervision, Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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