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Distribution pattern of anthropogenic marine debris along the gastrointestinal tract of green turtles (*Chelonia mydas*) as implications for rehabilitation

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ABSTRACT

Pollution from anthropogenic marine debris (AMD) is currently the most widely distributed and lasting anthropic impact in the marine environment, affecting hundreds of species, including all sea turtles. In this study, the patterns of AMD distribution along the gastrointestinal tract (GT) and their relationship with obstructions and faecalomas in 62 green turtles (*Chelonia mydas*) that died during rehabilitation in southern Brazil were determined. The GT was split in seven sections, corresponding to the natural organs and intestinal areas morphologically and physiologically distinct. Mean mass (4.24 g) and area (146.74 cm^2) of AMD in the stomach were higher than in other sections. The anterior portion of the rectum had the highest number of obstructions, followed by the stomach. AMD was associated with the obstructions, with positive correlation between faecalomas and AMD masses. Organs and subdivisions showed marked differences in susceptibility to obstructions caused by AMD, which deserves attention in clinical interventions.

1. Introduction

Contamination by anthropogenic marine debris (AMD) is regarded as the most widely distributed and long-lasting anthropic impact (Barnes et al., 2009). Driven by the use of disposables, the annual production of plastics has increased from 1.5 million tons in 1950 to 280 million tons in 2011 (Plastic Europe, 2012). Due to characteristics such as durability and lightness, low cost, and its use in short-lived disposable products (Hopewell et al., 2009), plastics are currently found in all oceans and coastal ecosystems (Ryan and Moloney, 1993; Ivar-do-Sul and Costa, 2007; Barnes et al., 2009; Thompson et al., 2009). Although there are few data on the amount of AMD input in the marine environment, it is estimated that, globally, the seas receive approximately 6.4 million tons of AMD every year (UNEP, 2005), of which 80% is from land-based sources (Faris and Hart, 1994). Materials generically known as marine debris include not only plastic material but also debris from fishing activities, such as fishhooks, fishing nets, glass, polystyrene, foam, aluminium, cotton, and rubber. Studies on the effects of such material on ocean and marine animals have grown in recent years (Ivar-do-Sul and Costa, 2007), and demonstrate growing effects on biodiversity.

The association of anthropogenic marine debris with marine animals, through direct ingestion, i.e. intentional ingestion as prey and/or indirect consumption, i.e. through ingestion of AMP which were previously in prey guts, has already been documented for at least 693 species (Gall and Thompson, 2015). In the base of the food web, invertebrates (Murray and Cowie, 2011; Wright et al., 2013) and fish (Lusher et al., 2013), are frequently associated with microplastics (<5 mm) deposited in sediment or present in the water column, which can be transferred to organisms higher in the trophic chain, such as birds (Codina-García et al., 2013), cetaceans (Baulch and Perry, 2014; Beneditto and Awabdi, 2014) and sea turtles (Bugoni et al., 2001; Tourinho et al., 2009).

Consumers at the top of the trophic chain, including humans, indirectly ingest plastic fragments formed by the degradation of larger plastic pieces, or microparticles widely used in cosmetic formulations, such as for exfoliants and toothpastes (Gregory, 1996; Fendall and Sewell, 2009). However, larger-sized plastics, before breaking down to microplastics in the marine environment through exposure to ultraviolet radiation, hydrolysis, and physical forces, affect organisms in different ways, such as through entanglement, suffocation, bodily mutilation and ingestion (Nelms et al., 2016). In a recent review, all seven species of sea turtle were reported to have interacted with AMD, either through ingestion or entanglement (Nelms et al., 2016).

The effects of pollution by AMD in sea turtles and other air-breathing vertebrates, such as birds and mammals, can be immediate and mechanical, such as entanglement and drowning, or result in injuries from contact with sharp or perforating components (Tourinho et al., 2009). Ingestion can have sublethal effects, such as interferences with lipids metabolism (Shulman and Lutz, 1995) that contributes to the accumulation of gases and the consequent reduction of floating capacity

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and limited ability to obtain food and avoid predation. Other problems arising from ingestion are a false sense of satiety, diet dilution, and consequently a decrease in the growth rate and a depletion of energy reserves (McCauley and Bjorndal, 1999). Additionally, debris can cause obstruction of the gastrointestinal tract (GT). The obstruction may be associated with faecalomas and compaction of the dehydrated gastrointestinal content, and diverticulosis (Erlacher-Reid et al., 2013). A partial or total obstruction of the GT by AMD can lead to a process of ischaemia/hypoxia, i.e., a decrease or absence of vascular blood flow, followed by reflux, and the production of reactive oxygen species (ROSs), which play an important role in endothelial tissue damage (Parks et al., 1983). ROSs can also damage distant organs located far from the infection, which leads to the development of systemic inflammatory response syndrome (Ceppa et al., 2003), or even cause infiltration of neutrophils in the lungs, thus contributing to the development of acute respiratory distress syndrome (Koksoy et al., 2001).

Sea turtles are prone to obstruction and formation of faecalomas because they have twisted intestines that offer spaces for abrasion and accumulation of residues (Shulman and Lutz, 1995). Green turtles (*Chelonia mydas*), due to their herbivorous diet, have a GT proportionally longer than carnivorous sea turtles, thus cursing them with a greater predisposition to obstruction (Schuyler et al., 2014a). Some studies demonstrated that the ingestion of small amounts of plastic sheets by green turtles had lethal effects due to complete obstruction of the GT (Bjorndal et al., 1994; Bugoni et al., 2001; Tourinho et al., 2009; Guebert-Bartholo et al., 2011). AMD intake can also cause internal injuries in the GT when scar tissue forms a constriction or decreased intestinal lumen, thus contributing to a total or partial obstruction. In addition, AMD usually results in the accumulation of food and a consequent formation of diverticulum (Erlacher-Reid et al., 2013).

Green turtles are exposed to AMD in virtually all stages and habitats of their life cycle (Schuyler et al., 2014a, 2014b), and is known as the sea turtle species most commonly reported to interact with anthropogenic debris. They have a cosmopolitan distribution (Meylan and Meylan, 1999) in tropical and subtropical seas and occasionally in the temperate waters of the Atlantic, Pacific and Indian Oceans, and the Mediterranean Sea (Márquez, 1990). Green turtles depend on multiple marine environments during different stages of their development, including coastal environments, where females lay their eggs, adults feed on algae and seagrasses, and juveniles recruit after their first years of life in the open-ocean (Meylan and Meylan, 1999).

When recruiting to neritic waters, juveniles shift from a predominantly carnivorous diet to an herbivorous diet (Bjorndal, 1997). In the southwestern (SW) Atlantic Ocean, Guebert-Bartholo et al. (2011) found a large variety of food items in the GT of juvenile green turtles that identified these individuals as omnivorous with an opportunistic diet. The most common food items were seaweed, clam shells, plants and insects. South of this region, Bugoni et al. (2003) reported that molluscs were the main sources of food, although crustaceans, fish, plants and other items were also reported. Recent studies using stable isotopes and/or direct observations show that in some areas, the consumption of animal matter can be high even in adults or large juveniles (Amorocho and Reina, 2007; Lemons et al., 2011; Reisser et al., 2013; González-Carman et al., 2014a), in contrast to the idea that green turtles are strictly herbivorous in their neritic phase. Notwithstanding the discussion of green turtles being selective (Bjorndal, 1979) or opportunists in regard to their diet, they invariably ingest the food items more abundant and available in the environment (Ferreira et al., 2006).

The neritic area along the southern Brazilian coast is an important region for feeding and development of *C. mydas* (Guebert-Bartholo et al., 2011), frequented by animals with a curved carapace length (CCL) between 30 and 50 cm (Bugoni et al., 2001), and a mean CCL of 39.2 ± 6.0 cm (Monteiro et al., 2016), especially in the austral summer and spring.

In the same region where the current study was carried out, two previous studies of animals found stranded and dead on the beaches demonstrated the high intake of AMD by green turtles. Among the 38 specimens studied by Bugoni et al. (2001), 60.5% presented AMD in the oesophagus and stomach. Later, Tourinho et al. (2009) analysed 34 individuals, and found that 100% of the GT of green turtles contained AMD. Upon analysing their complete digestive tracts, plastics and fishing-related materials were found to be present in 71% and 21% of these individuals, respectively. Although AMD ingestion by green turtles is a well-known phenomenon in multiple locations around the globe (see the revision in Nelms et al., 2016), including the SW Atlantic Ocean, the association of AMD with debilitating clinical conditions and the different susceptibilities of the organs along the GT are poorly understood. In a scenario where many sea turtles reach rehabilitation centers in debilitated state due to interaction with AMD, fishing nets/drowning, and often do not respond treatment and die, the limitation in knowledge on the fate and effects of debris after ingestion, as well as the establishment of protocols for rehabilitation, is essential. In this context, we investigated the location of AMD along the gastrointestinal tract of juvenile green turtles by determining the patterns in which debris are present along the digestive tract, and their association with obstructions and faecalomas. Unlike previous studies on this topic, we analysed the retention susceptibility of debris from different regions of the intestine, oesophagus and stomach, based on animals found alive that died during an attempt at rehabilitation.

2. Materials and methods

Debilitated turtles found along Rio Grande do Sul state beaches, Brazil, were transported to the marine animal rescue centre at the Federal University of Rio Grande – FURG (CRAM-FURG), where they received treatment aimed at achieving their release back into the ocean. Sea turtles often arrive at CRAM in a debilitated state, mainly due to interaction with AMD and fishing activities/drowning. Turtles often do not respond to treatment and die. These animals are regularly necropsied in order to better understand the cause of death and the distribution of AMD along the GT.

2.1. Study area

Green turtles were obtained from live strandings on a stretch of beach approximately 350 km long, between Barra da Lagoa do Peixe (31°19′S; 50°58′W) and Arroio Chuí, at the Uruguayan border (33°45′S; 53°23′W), in the state of Rio Grande do Sul, Brazil. This region is characterized by extensive sandy beaches, interrupted by the mouth of the Lagoa dos Patos, where the estuary meets the ocean.

2.2. Turtle sampling

In this study, 62 GTs of green turtles obtained between 2011 and 2014 were analysed. From these individuals, the curved carapace length (CCL) (the distance from the midpoint of the nuchal to the extreme of the supracaudal scutes), the curved carapace width (CCW) (Bolten, 1999), and the body mass (in kg), were collected. During necropsy, sex was determined through visual macroscopic analysis of the gonads (Work, 2000), and the GTs were removed and frozen whole. The detailed necropsy of the GT followed the methodology of Work (2000). The total length of the digestive tract and of each organ separately (oesophagus, stomach, and intestines) and the subdivision of the intestine, were measured with a tape measure in cm, and the weight of the full and empty tract (content removed) were recorded on a scale (in g).

Each GT was divided according to the natural divisions of the organs: oesophagus (from the initial portion of the tube to the gastroesophageal sphincter); stomach (from the gastroesophageal sphincter to the duodenal constriction); and intestine (from the beginning of the duodenum to the end of the cloacal opening). Externally, there is not a clear division between the regions of the small intestine (duodenum, jejunum and ileum), or between the small intestine and the large intestine. However,

Magalhães et al. (2010), through histological analysis, showed that the large intestine comprises over 60% of the intestine. Thus, due to the absence of natural landmarks or divisions, the intestines were divided into five parts based on the large size and morphological and physiological differences from the beginning to the posterior portions. Due to the deterioration of the mucous layer, which separates intestinal regions (Magalhães et al., 2010), the intestines were arbitrarily divided as follows: initially, the intestine was split in half and each half was divided again in half; the last section was in the last quarter, split again in half and thus generating two smaller sections. This resulted in the fractionation of intestines in five parts (Fig. 1). Thus, "intestine 1" corresponds to the small intestine; "intestine 2" extends from the end of the small intestine to the beginning of the large intestine; "intestine 3" and "intestine 4" correspond to the large intestine only, and "intestine 5" comprises the final portion of the large intestine and the whole rectum. With this partitioning, GTs of different length are comparable because each section covers the same region of the intestine.

2.3. Faecalomas and obstructions

Faecaloma is defined as a large mass of hardened fecal material of varying sizes, related to a series of physiological conditions, often interacting with AMD (Fig. 2), which may appear when there is obstruction of the intestinal transit or be the cause of it. Therefore, it occurs in the intestine only, where faeces are formed and where water absorption occurs. Faecaloma is not a disease, but a pathological condition that can occur in many different conditions. Obstructions, on their turn, can occur in any region of the gastrointestinal tract and are characterized by obstruction of partial or complete passage of food and liquids, caused by reduced motility or AMDs that precluded passage (Fig. 2). There are regions anatomically prone to its occurrence, especially near the sphincters, as in the curvature of the oesophagus near the cardiac sphincter and in the blind bottom of the stomach near the pyloric sphincter.

2.4. AMD analysis

The necropsy was accompanied by a description of the location of AMD, faecalomas and diverticula. Organic material and AMD were removed from the intestine and the contents and organs were weighed separately. Debris fragments were washed in running water, using a 4 mm mesh sieve, with subsequent drying in an oven.

AMD were analysed under a stereoscopic microscope and assigned to different categories of use, according to the European Commission (2013). Briefly, materials such as pieces of bags and food packaging were referred as "plastics sheets"; plastic pieces of various sizes, from containers, boxes, and lids of condiments were assigned to the "plastic fragments" category; "filiform material" included monofilament and multifilament lines from such things as fishing nets and ropes; charcoal used to cook food was categorized as "food drops"; styrofoam and silicon were assigned to "chemical contaminants"; and materials such as the rubber of party balloons and adhesive bandages were included in the category "other". AMD were also categorized according to colour.

Faecalomas were counted in each section of the GT intestine, and associated AMD were also counted, weighed, measured, and classified according to colour and the abovementioned categories of use.

For each section of the GT, AMD fragments were counted and weighed. For each section, the following parameters were calculated: the absolute frequency of occurrence (FO), the relative frequency of occurrence (FO%), the average area occupied by AMD, as determined by placing the AMD over graph paper (A, in cm^2), and the average mass (M, in g) obtained with a high precision digital scale. From these data, for all 7 sections the relative contribution by mass (M%), area (A%) and number (N%) were calculated, and these same parameters were classified according to the categories of use and colour describe above.

The M and N of AMD were compared between turtles with and without faecalomas, through Student's *t*-test, after checking for normality and homoscedasticity of residuals. Additionally, the relationships between the size of each section of the GT with the expected contribution by mass and number of AMD were compared by a G-test, which is equivalent to a χ^2-test, but uses decimal values. The χ^2 test with the Yates continuity correction was used to compare the number of AMD pieces between turtles with and without faecalomas, using only the intestine for this analysis as faecalomas are not possible in the initial parts of the GT. Finally, Pearson's correlation between size of faecaloma (as measured by mass) and the amount of debris (as measured in grams and in the number of items) was calculated. All statistical tests were calculated using BioEstat software version 5.3 (Ayres et al., 2007), and significance was obtained when $P < 0.05$.

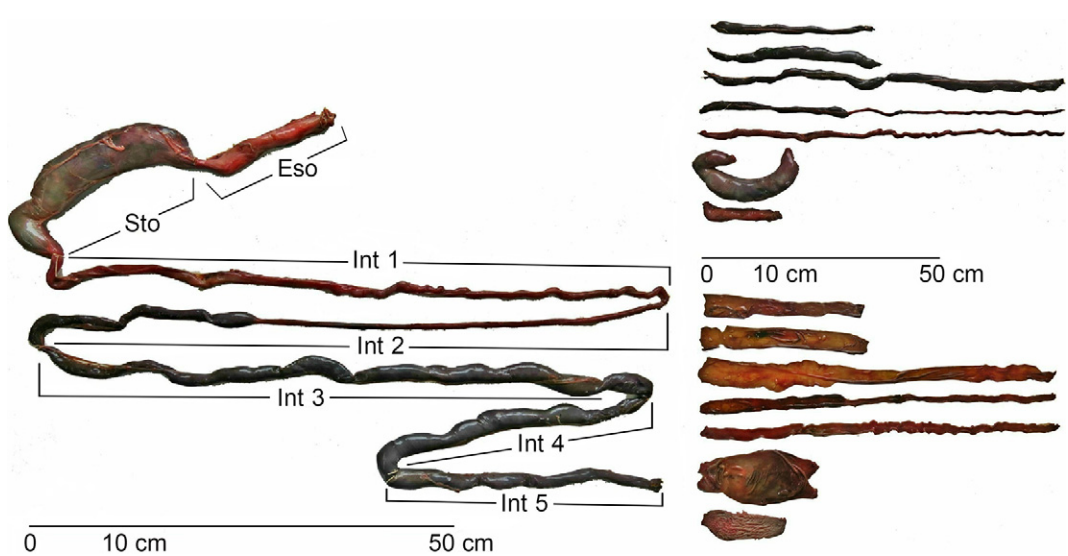

Fig. 1. Section of the gastrointestinal tract of a green turtle (*Chelonia mydas*) that died in rehabilitation in southern Brazil. The whole tract was divided according to the natural organs and intestines divided into five physiological morphologically distinct parts. Eso = oesophagus; Sto = Stomach; Int = Intestine. On the right, proportions before opening (above) and after dissection (below).

Fig. 2. Above are two examples of obstructions found in stomach of green sea turtles (*Chelonia mydas*) in southern Brazil, composed by compacted food material and anthropogenic solid debris. Obstructions could also be found in intestines. Faecalomas (below) are found in intestines only, also composed by food and plastics or other debris, but food is at a more advanced digestion stage and with a hardened consistency. Photos: CRAM archives.

3. Results

3.1. Biometrics and sex ratio

The sexing of 30 individuals demonstrated a large predominance of females (27 turtles, 90%) with a resulting sex ratio of 9:1. The mean CCL was 37.26 ± 3.47 cm (min = 31.00, max = 44.40 cm, n = 32), and the mean body mass was 6.24 ± 6.15 kg, median = 4.86 kg (min = 2.51, max = 39.00 kg, n = 33). Animals remained in rehabilitation between 0 and 114 days (17.83 days on average).

The mean total length of the GTs was 334.23 cm; the mean mass of tissues + contents was 511.55 g, and mean mass of tissue alone was 213.10 g (Table 1). The mean length of the GT of turtles that had (n = 45) and had not (n = 17) ingested AMD was 326.58 cm and 354.47 cm, respectively ($t_{60} = 1.38$, $P = 0.17$).

3.2. Anthropogenic solid debris

Among the 62 turtles analysed, 72.58% presented AMD in the GT, with a total of 1019 items found in all GT sections pooled. The GT sections differed in the mass (G-test = 537.20; df = 6; $P < 0.0001$), and number (G-test = 1099.99; df = 6; $P < 0.001$) of AMD, even controlling for the length of each section. The stomach, followed by intestines 4 and 5, had the highest amounts of AMD. In the stomach the mean number of items, mass and area was 5.68 items, 4.24 g and 146.74 cm^2, respectively, followed by intestine 4 (3.53, 1.05 g and 37.09 cm^2, respectively) and intestine 5 (2.68, 0.81 g and 27.81 cm^2, respectively). The stomach and intestines 4 and 5 had similar FO% (59.70, 59.70 and 56.40%, respectively). The initial sections of the intestine had lower values of FO% than the later sections and the stomach in most variables measured (Table 2).

In relation to categories of use, there was a predominance of plastic material in comparison to other categories. Plastic fragments were present in high quantity, in terms of mean mass M = 3.60 g and M% = 38.13%, and number (N = 8.52 items, N% = 33.61%), followed by the plastic sheet category (M = 1.33 g and M% = 13.56%; N = 4.19 items

Table 1
The length and mass of the section organs, with and without content, along the gastrointestinal tract (GT) of green turtles (*Chelonia mydas*) necropsied in southern Brazil (n = 62). Values represent the mean ± 1 standard deviation (minimum – maximum).

	Length (cm)	Tissue section mass (g)	Tissue mass + fecal content (g)
Oesophagus	15.48 ± 2.78 (10–32)	38.95 ± 18.62 (14–128)	53.98 ± 25.67 (19–137)
Stomach	27.73 ± 5.75 (17–43)	43.31 ± 16.62 (21–93)	146.65 ± 87.83 (36–381)
Intestine 1	74.48 ± 28.22 (46–266)	49.24 ± 23.81 (9–119)	62.89 ± 34.54 (14–211)
Intestine 2	74.24 ± 29.21 (43–273)	22.85 ± 10.65 (5–64)	35.05 ± 23.96 (7–172)
Intestine 3	70.40 ± 14.90 (27–118)	24.44 ± 12.85 (9–69)	77.65 ± 60.68 (14–292)
Intestine 4	36.66 ± 7.48 (13–59)	17.58 ± 9.46 (5–52)	82.53 ± 59.34 (10–290)
Intestine 5	35.23 ± 7.77 (13–58)	17.13 ± 8.31 (3–43)	55.26 ± 30.58 (11–139)
Total GT	334.23	213.10	511.55

Table 2
Number of items, relative frequency of occurrence (FO%), absolute and relative mass (M and M%, respectively) and absolute and relative area (A and A%, respectively) of anthropogenic solid debris along the gastrointestinal tract of green turtles (*Chelonia mydas*) submitted for rehabilitation with subsequent death in southern Brazil (n = 62). Values represent the mean ± 1 standard deviation (minimum – maximum).

	No. of items	FO%	Mass (M, g)	Mass (M%)	Area (A, cm^2)	Area A%
Oesophagus	0.84 ± 2.07 (0–13)	27.40	0.16 ± 0.60 (0–4.47)	2.03 ± 6.81 (0–47.17)	5.16 ± 10.97 (0–46)	1.87 ± 4.99 (0–30.21)
Stomach	5.68 ± 6.66 (0–28)	59.70	4.24 ± 13.19 (0–8.54)	28.18 ± 32.14 (0–99.64)	146.74 ± 391.93 (0–2525.5)	27.28 ± 32.27 (0–96.79)
Intestine 1	0.29 ± 1.25 (0–9)	9.70	0.07 ± 0.36 (0–2.67)	0.46 ± 1.79 (0–11.14)	1 ± 3.85 (0–25)	0.58 ± 2.42 (0–16.37)
Intestine 2	0.95 ± 2.05 (0–14)	13.00	0.22 ± 0.76 (0–3.70)	2.53 ± 8.88 (0–43.37)	10.82 ± 48.07 (0–308.39)	3.18 ± 12.26 (0–68.38)
Intestine 3	2.47 ± 3.78 (0–18)	51.60	0.71 ± 1.41 (0–5.75)	12.04 ± 19.74 (0 − 100)	25.78 ± 53.12 (0–247)	10.43 ± 17.78 (0–67.21)
Intestine 4	3.53 ± 4.20 (0–19)	59.70	1.05 ± 1.83 (0–10.28)	15.98 ± 22.58 (0–86.13)	37.09 ± 67.74 (0–433)	16.29 ± 22.79 (0–95.69)
Intestine 5	2.68 ± 3.24 (0–11)	56.40	0.81 ± 1.37 (0–6.16)	11.36 ± 19.94 (0–100)	27.81 ± 46.78 (0–215)	9.74 ± 15.55 (0–61.18)
Total	1019		350.25		14,276.09	

and N% = 22.41%). Filiform materials were ranked third, with a high mean mass and number of items (Table 3).

The colour assignment resulted in items classified as yellow, blue, white, grey, orange, brown, black, pink, purple, transparent, green and red, but white items occurred in the highest mean number and with the highest mean mass (5.48 items and 4.71 g, respectively), followed by transparent items (2.23 items and 1.96 g) and black items (1.90 and 1.71 g) (Table 4).

3.3. Faecalomas and obstructions

Intestine 4 had the highest occurrence of obstructions, found in 9 individuals, followed by the stomach and intestine 3, with 7 and 5 individuals, respectively. There were no diverticula in any of the GT analysed.

The mean mass of AMD in turtles with and without faecalomas was 5.70 and 3.20 g, respectively ($t_{42} = 2.18$, $P = 0.04$), and the mean number of items in turtles with and without faecalomas was 18.20 and 12.00, respectively ($t_{42} = 2.05$, $P = 0.05$). A high correlation was detected between the size/mass of faecalomas and the mass of the associated AMD ($r = 0.66$, n = 18, $P = 0.003$), but larger masses of faecalomas did not correlate with the largest number of AMD ($r = -0.08$, n = 18, $P = 0.74$). Turtles with and without faecaloma had a similar propensity for having AMD in the intestines ($\chi^2_{Yates} = 0.70$, $P = 0.40$).

4. Discussion

All individuals in this study, as in previous studies in the area or other coastal areas around the globe, were juveniles severely impacted by AMD in their GT (72.6% of individuals sampled). Plastic fragments were the most commonly found AMD, with transparent and white the most frequent colours. The GT region with the highest occurrence of AMD was the stomach, followed by regions of greater water absorption, i.e. the final portion of the intestine. These regions also had large number of obstructions and faecalomas, respectively. The presence of AMD had also been found to be associated with the formation of faecalomas. Green turtles analysed in the current study had CCL similar to those from the same area studied by Bugoni et al. (2001) and Tourinho et al. (2009) with a focus on debris ingestion. According to Goshe et al. (2010), these CCL values correspond to juveniles, confirming previous studies that indicate the southern coast of Brazil is an important feeding and development area for juveniles (Bugoni et al., 2001).

The frequent occurrence of AMD in GTs confirms previous findings in the same region, based on individuals found stranded and dead. Bugoni et al. (2001) recorded FO% = 60.5% in 56 green turtles, although they only analysed the oesophagus and stomach, whereas Tourinho et al. (2009) found FO% = 100% with an analysis throughout the GT of 29 green turtles. However, the present study differs from these studies because the animals were found alive and sent to rehabilitation. In our study, 27% of the individuals sent to rehabilitation who subsequently died did not contain AMD in the GT, which suggests that the death occurred by other causes. Drowning in fishing nets and hypothermia during winter months are also causes of green turtle mortality in this region (Bugoni et al., 2001; Vélez-Rubio et al., 2013). Notwithstanding, the mean AMD mass of 5.66 g found in the present study was substantially higher than 0.5 g, which is suggested as the threshold for the death of juvenile green turtles (Santos et al., 2015).

Fragments of plastic were the commonest item found in the GT, similar to most other studies of green turtles conducted elsewhere (e.g., Bjorndal et al., 1994; Wedemeyer-Strombel et al., 2015). It has not been possible to precisely identify the origins of these fragments, but most were from durable products. Plastic sheets from plastic bags and food packaging were the second most abundant item. These items may originate from both land-based sources as a result of inadequate disposal, and from the sea waste discard of vessels. Filiform material, which includes unifilament and multifilament plastics, as well as ropes and lines manufactured from other materials, was third in abundance. These debris are mainly from fishing activity. The predominance of these broad categories has remained constant in the region: plastic bags were previously found in 50% and 71% of juvenile green turtles, followed by plastic strings (equivalent to filiform materials) in 39.5% and 21% (Bugoni et al., 2001; Tourinho et al., 2009, respectively). Thus, interactions between turtles and materials from fisheries have high importance in the region. Although comparisons of data from different studies on debris ingestion by turtles should be carried out cautiously

Table 3
Absolute and relative mass (M and M%, respectively), and absolute and relative number (N and N%, respectively) of anthropogenic solid debris found in the gastrointestinal tracts of green sea turtles (*Chelonia mydas*) in southern Brazil, according to the classification proposed by the European Commission (2013). Values represent the mean ± 1 standard deviation (minimum–maximum).

Categories	Mass (M, g)	Mass (M%)	No. of items (N)	No. of items (N%)
Plastic sheet	1.33 ± 3.42 (0–25.18)	13.56 ± 19.69 (0–79.99)	4.19 ± 4.04 (0–17)	22.41 ± 21.8 (0–100)
Plastic fragment	3.6 ± 4.78 (0–22.38)	38.13 ± 36.50 (0–93.24)	8.52 ± 9.25 (0 − 32)	33.61 ± 27.85 (0–83.33)
Food drop	0.03 ± 0.13 (0–0.92)	0.5 ± 2.38 (0–16.76)	0.11 ± 0.48 (0–3)	0.68 ± 2.76 (0–16.67)
Chemical contaminant	0.07 ± 0.27 (0–1.97)	0.9 ± 2.99 (0–16.24)	0.21 ± 0.48 (0–2)	0.86 ± 2.11 (0–9.09)
Filiform material	0.37 ± 0.72 (0–4.64)	4.85 ± 10.89 (0–77.28)	2.35 ± 2.96 (0–14)	11.23 ± 12.97 (0–71.43)
Others	0.26 ± 0.96 (0–7.21)	3.36 ± 9 (0–55.76)	0.76 ± 1.02 (0–6)	3.79 ± 5.28 (0–27.27)

Table 4
Colours of anthropogenic solid debris found in the gastrointestinal tracts of green sea turtles (*Chelonia mydas*) from southern Brazil. Values represent the mean ± 1 standard deviation (minimum – maximum).

	Mass (g)	No. of items
Yellow	1.01 ± 1.12 (0.02–4.49)	1.47 ± 2.12 (0–8)
Blue	0.53 ± 0.56 (0.01–2.20)	1.53 ± 2.12 (0–8)
White	3.54 ± 3.27 (0.07–13.77)	5.48 ± 5.52 (0–22)
Orange	0.52 ± 0.99 (0.01–3.79)	0.44 ± 0.93 (0–5)
Brown	0.25 ± 0.20 (0.02–0.67)	0.48 ± 0.78 (0–3)
Black	1.09 ± 2.12 (0.01–13.22)	1.90 ± 2.10 (0–9)
Pink	0.37 ± 0.33 (0.02–1.29)	0.29 ± 0.64 (0–3)
Purple	0.19 ± 0.16 (0.02–0.35)	0.06 ± 0.25 (0–1)
Transparent	1.18 ± 1.76 (0.01–9.99)	2.23 ± 2.06 (0–7)
Green	0.60 ± 0.64 (0.02–2.37)	1.56 ± 1.79 (0–6)
Red	0.44 ± 0.44 (0.01–1.70)	0.65 ± 1.03 (0–4)
Grey	0.45 ± 0.32 (0.02–1.05)	0.27 ± 0.66 (0–3)

(Casale et al., 2016), given the particularities described above for every study in the area, a general picture of the situation has emerged.

The predominance of plastics, instead of other AMD in the contents of the GT has been noted by other studies (e.g., Balazs, 1985; Bugoni et al., 2001; Schuyler et al., 2014a; Wedemeyer-Strombel et al., 2015) as a general pattern in sea turtles. For green turtles, the generalist and omnivorous diet (Bugoni et al., 2003) associated with the presence of a large amount of anthropogenic items in the GT, must be related to feeding during the stage of juvenile development in which there is a transition from a pelagic life stage with a carnivorous diet in the early years of life, to a neritic life with the predominantly herbivorous diet of adults (Musick and Limpus, 1997). Thus, AMD intake may occur indirectly, due to the presence of these light and long-lasting debris in seaweed banks. Additionally, floating AMD can provide a suitable substrate for growth of animals, such as barnacles *Lepas* sp., as recorded by Bugoni et al. (2003), and appear to be an attractive food source for turtles.

The predominance of the AMD categories reported above suggests that the format and characteristics of certain AMD, i.e., large contact surfaces, elongated shape or sharp tips, contribute to adhesion and retention by mucosa. Retention seems to be augmented by the morphology of the GT of juvenile green turtles, which have a smaller diameter than turtles of other carnivorous species of similar size, such as the loggerhead turtle (*Caretta caretta*), or even adult green turtles, and by their long and twisted intestines (Magalhães et al., 2012). Such characteristics provide spaces for abrasion and accumulation of AMD.

Twelve colours were identified in the AMD, the largest amount being white or transparent, which is similar to previous findings in the region (Bugoni et al., 2001; Tourinho et al., 2009). Sea turtles are visual predators (Schuyler et al., 2014a, 2014b), and besides colour and luminance use flexibility and translucency for food choice. This may indicate that flexible and translucent debris could be mistaken for food. In addition, a study on green turtle diets in the SW Atlantic Ocean found cnidarians as the most frequent and abundant food items (González-Carman et al., 2014a), also suggesting that green turtles can confuse AMD, such as plastic bags and packaging, with food items. González-Carman et al. (2014b) demonstrated that the core foraging areas of juvenile green turtles in the SW Atlantic Ocean overlap with frontal systems. This causes high exposure of turtles to AMD. The fact that the SW Atlantic is one of the few places where the diet of green turtles consists predominantly on gelatinous planktonic items and has high concentration of marine debris (González-Carman et al., 2014a, 2014b), explains why there is a high proportion of AMD intake in this region.

That the largest quantity of items is in the stomach (mean = 5.68 items), indicates that organ morphology plays a role in the retention of AMD. A stomach in a 'J' format, with a blind ending, and also working as a fermentative chamber, associated with a sphincter at the end of the organ, is characteristic of herbivorous animals (Magalhães et al., 2010). Such a structure clearly promotes retention of AMD. Aside from the stomach, regions with greater quantities of AMD were intestines 4, 5 and 3, in order of decreasing quantity, corresponding to the large intestine and rectum, which is where most water absorption occurs (Wyneken, 2001; Magalhães et al., 2010). Such a region with intense fecal dehydration increases the capacity for AMD retention and possible formation of faecalomas and obstructions. When the amount of obstructions and faecalomas are compared along the entire GT, intestine 4 presented the highest incidence, followed by the stomach and intestine 3, demonstrating that 1) there is a different susceptibility to AMD retention along the GT and 2) the pyloric sphincter accounts for retention in the stomach, and water absorption accounts for the AMD associated with faecaloma in intestines. These organs/regions deserve greater attention for use in clinical interventions.

It is unclear whether faecalomas are formed due to retention of AMD or, on the other hand, whether the presence of early faecalomas retain AMD, subsequently increasing faecaloma size. The presence of faecalomas not associated with AMD, as demonstrated in this study from the result that the chance of finding plastic in turtles with and without faecalomas is similar, demonstrates that causes other than anthropogenic debris promote faecaloma formation. In an opposing view, AMD have been postulated as initiators of faecaloma formation, through direct GT obstruction, or indirectly by causing internal injuries, with scar tissue causing partial or total obstruction (Erlacher-Reid et al., 2013). Independent of the causal mechanism for faecaloma formation, it seems clear that AMD accumulation intensifies once faecaloma is present, as demonstrated by the high correlation between faecaloma mass and AMD mass and number.

5. Conclusion and recommendations

The most relevant conclusion from the present study is that AMD are contributors to, and eventually the initiators of, obstructions of the GT in green turtles. Our results also indicate that hydration and treatments with oil to stimulate the motility of the retained material are important veterinary procedures for assisting the expulsion of debris, particularly those associated with small-sized faecalomas.

From the perspective of treatment, it is suggested that hydration and the use of mineral oil may be effective for supporting peristaltic movements and removal of AMD and AMD-associated faecalomas located in the posterior portion of the intestines. The use of antioxidant supplementation in animals suspected of having ingested AMD, as proposed by Walsh (1999), is reinforced by the present study. Ischaemia-reperfusion processes can generate oxidative stress, as demonstrated by the correlation between the increase of AMD ingestion and the failure of the antioxidant defence system (Parks et al., 1983). Therefore, we suggest further studies on the effectiveness of the use of mineral oil and hydration in the rehabilitation of small-sized turtles. Histopathological studies could also shed light on tissue damage caused by AMD and faecalomas. In addition, studies on the potential effect of intestinal twists and folds on the retention of AMD can further improve the current study, with important implications for veterinary interventions and rehabilitation.

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