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Differential impact of marine debris ingestion during ontogenetic dietary shift of green turtles in Uruguayan waters

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ABSTRACT

Anthropogenic debris ingestion has been reported for green turtles in all their life stages worldwide. The aim of the present study is to evaluate the marine debris ingestion by green turtles stranded in Uruguayan coast between 2005 and 2013. Debris items were categorized and quantified by frequency of occurrence, relative weight, volume and number of items. A total of 96 dead stranded turtles were analyzed and 70% presented debris in their guts. The majority of debris found were plastic, being hard plastics the most abundant in weight and volume. The best model explaining the variability of the amount of debris ingested included turtle size, Julian day and distance from the estuary. We detected a negative correlation between the presence of debris and turtle's size. Smaller turtles are new recruits to neritic grounds indicating that the early juvenile stage of this species is the most vulnerable to this threat in the Southwestern Atlantic.

1. Introduction

Accumulation of solid anthropogenic debris in marine environments has been identified as an important conservation problem, and its numbers have increased in the last decades (Acha et al., 2003; Benton, 1995; Corcoran et al., 2009; Derraik, 2002; Schuyler et al., 2016). This phenomenon, and the lack or poor strategies in waste management in coastal areas have generated a global problem for marine wildlife and environment (Smith and Markic, 2013; Gonzalez Carman et al., 2015; Nelms et al., 2017; Pham et al., 2017; Fossi et al., in press).

Recent studies showed that fragments of hard and soft plastic are the most common anthropogenic debris in the ocean, due to their high persistence in the environment because of their low disintegration rate (Barnes et al., 2009; Morét-Ferguson et al., 2010; Reisser et al., 2014; Gall and Thompson, 2015). The most abundant plastic debris at sea are millimeter-size buoyant fragments. This type of debris is vertically distributed primary in the upper water column because of the wind driven vertical mixing process (Kukulka et al., 2012; Isobe et al., 2014; Reisser et al., 2014). Due to that marine physical phenomenon, plastic fragments accumulate in marine fronts and edge of currents, occupying the first 5 m of depth from the surface, thus affecting primary to marine animals with epipelagic feeding habits (Reisser et al., 2014).

In the last decades, marine debris interaction has been reported as one of the most important threats for marine fauna (National Research Council, 1990, Laist et al., 1999. This growing threat has increased exponentially the interest of the scientific community, with studies describing and quantifying the presence of debris in coastal habitats and oceans in many different species, including, marine mammals (Denuncio et al., 2011), several seabird species (Brandão et al., 2011; Van Franeker et al., 2011; Bond et al., 2014; Wilcox et al., 2015; Lenzi et al., 2016), fishes (Sazima et al., 2002; Choy and Drazen, 2013; Graham and Thompson, 2009; Schuyler et al., 2014; Nelms et al., 2015; Possatto et al., 2011), invertebrates (Goldstein and Goodwin, 2013; Galloway et al., 2017) and sea turtles (Bjorndal et al., 1994; Bugoni et al., 2001; Tomás et al., 2002; Tourinho et al., 2010; Schuyler et al., 2012; Gonzalez Carman et al., 2014a; Santos et al., 2015; Nicolau et al., 2016). These marine species could interact with debris by entanglement or ingestion. Ingested debris may cause death, by blockage or perforation of the digestive tract, or sublethal effects, like dietary dilution or exposure to chemicals (Bjorndal et al., 1994; McCauley and Bjorndal, 1999; Teuten et al., 2009; Tanaka et al., 2013; Jerdy et al., 2017).

In sea turtle species, ingestion of marine debris has been reported in all life stages and all geographic areas (Schuyler et al., 2012 and references therein), being described as the most important threat

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affecting them in some regions, and showing a high increase in the last decade (Schuyler et al., 2014; Nelms et al., 2015 and references therein). Sea turtles may intake a high variety of debris (Tomás et al., 2002). In their oceanic stages, they exhibit epipelagic feeding habits and, being prone to interact with floating objects.

In the South Western Atlantic Ocean (SWAO) the ingestion of marine debris in sea turtles is one of the highest worldwide (Schuyler et al., 2014). The amount of studies and literature in the area has increased in the last years, probably associated to the increase of marine debris in the ocean. Furthermore, over the last decade, studies on strandings and bycatch in coastal waters in the SWAO have reported that juvenile green turtles (*Chelonia mydas*) show the highest frequency of occurrence (FO) of marine debris from the five sea turtle species reported in the region. Approximately, among 70% to 100% of the green turtles had ingested debris (Bugoni et al., 2001; Tourinho et al., 2010; Gonzalez Carman et al., 2014a; Santos et al., 2015; Jerdy et al., 2017). All the SWA region hosts individuals of the same green turtle nesting populations (Caraccio, 2008; Naro-Maciel et al., 2006; Prosdocimi et al., 2012; Patrício et al., 2017); however, some knowledge gaps still remain in this area, particularly in the Uruguay coastal waters. The importance of the Uruguayan waters resides in the variety of habitats along the coast; from an estuarine influence area in the first ca. 300 km from the beginning of the estuary to an oceanic influence zone in the eastern zone (300–600 km distant from the beginning of the estuary). The Uruguayan coast is also influenced by the discharge of Rio de La Plata estuary, which had been described one of the main important source of debris present in the the SWA (Gonzalez Carman et al., 2014a). The first reports of marine debris interactions with sea turtles in Uruguay are from feeding studies in green turtle (Calvo et al., 2003) and loggerhead turtle, *Caretta caretta* (Martinez Souza, 2009). Furthermore, there are reports of debris interaction with other marine vertebrates, such as otariids (Franco-Trecu et al., 2017), sea gulls (Lenzi et al., 2016) and fishes (Lozoya et al., 2015) in Uruguayan coastal waters. The Uruguayan coast constitutes an important feeding and development ground for early and late juvenile green turtles (mean ± SD = 40.8 ± 5.5 cm; n = 514; range: 28.8–64.3 cm, López-Mendilaharsu et al., 2016) with a year round occurrence, but with higher numbers during the warmer months (López-Mendilaharsu et al., 2006; Martinez Sousa, 2014; Vélez-Rubio et al., 2013, 2016). Green turtles recruit in Uruguayan waters mainly from the nesting populations of Ascension Island (Central Atlantic, UK Overseas Territory) and Trinidad Island (Brazil) (Caraccio, 2008). According to feeding studies, Vélez-Rubio et al. (2016) suggested that the area hosts a foraging and developmental ground for a particular size range of juveniles, with individuals recruiting just after the oceanic phase of their life cycle to neritic habitats. During this process, in neritic Uruguayan waters immature green turtles develop a rapid but not abrupt dietary shift, changing from carnivorous epipelagic diet (mainly gelatinous macrozooplanckton) to primarily herbivorous, feeding on seaweeds, when they reach up to 45 cm in CCL (Vélez-Rubio et al., 2016). This ontogenetic change in habitat use and feeding behavior could affect the marine debris intake by the turtles. Previous studies in the area confirm that the presence of abundant marine debris both in oceanic influenced coastal waters (Lozoya et al., 2016) and estuarine waters of Uruguay (Gonzalez Carman et al., 2014a).

In that sense, the aim of the present study is to improve the knowledge on debris ingestion by green turtles in Uruguayan waters, through its quantification in a relatively large sample during an 8 year-period and detecting the probably differences of the stranding area. We also aim to detect potential ontogenetic changes in debris intake, associated the ontogenetic dietary shift, by testing the following alternative hypotheses: (1) juvenile green turtles would increase debris intake, or (2) the turtles would decrease debris intake when recruit into coastal habitats. We also explore the effect of marine debris ingestion in green turtle mortality.

2. Methodology

2.1. Study area

The Uruguayan coast is part of the Uruguay–Buenos Aires platform ecoregion in the temperate SWAO (Spalding et al., 2007). This region belongs to a complex hydrological system that comprises the frontal zone of the Rio de la Plata estuary (RP) and the Atlantic Ocean. This is a transitional zone influenced by waters with contrasting features: warm and saline Subtropical waters from a branch of the Brazil current, and Subantartic cold and diluted waters derived from the Malvinas current, presenting a strong along-shore salinity and temperature gradient (Ortega and Martínez, 2007; Campos et al., 2008). Based on the hydrological characteristics, two different zones can be distinguished along the Uruguayan coast: an Estuarine influence zone (which are directly influenced by the Rio de la Plata discharge) and an Oceanic influence zone, which is characterized by an oceanic regimen (Acha et al., 2008 and references therein).

2.2. Field data collection and process of samples

During the 8-year study period (2005–2013) a total of 96 freshly

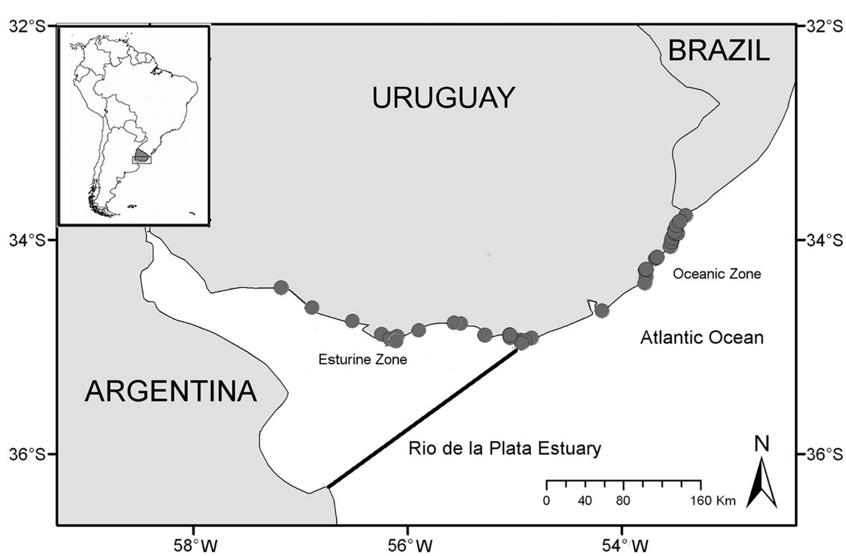

Fig. 1. Map of Uruguayan coast indicating the esturine influence zone and the oceanic influence zone. The grey dots correspond to the location of the stranded turtles analyzed in the present study. In the small panel the location of Uruguay in the Southwestern Atlantic region.

dead stranded turtles in all the Uruguayan coast (640 km, Fig. 1) were analyzed (12.8% of the stranded green turtles recorded by the NGO Karumbé during this period) (Vélez-Rubio et al., 2013, G. Vélez-Rubio com pers.). Karumbé runs the Sea Turtle Stranding and Rescue Network along the coast of Uruguay since 1999. This network records dead or injured sea turtles stranded on beaches (see Vélez-Rubio et al., 2013 for more details). When possible, from each stranded turtle the curved carapace length notch to tip (CCLn-t) and other biometrics were measured; date, GPS position, possible cause of stranding or dead, were also recorded. The necropsies were performed on the beach and all the digestive tracts were collected and took to the Karumbé facilities. Then, the digestive tracts were separated in esophagus, stomach and intestine sections and the contents were rinsed and preserved in a 4% formalin solution in seawater. According to Casale et al. (2016) to reduce the potential caveats of stranding turtle studies, we only considered freshly dead turtles. Strandings studies could be useful for comparing the relative importance of different anthropogenic causes of death (e.g. Tomás et al., 2008; Vélez-Rubio et al., 2013), including debris ingestion. However, these studies are subject to several potential bias since stranded turtles may have been in a poor health for a while before stranding, thus affecting their normal feeding behavior and, consequently, the gut contents found in them (Casale et al., 2016).

Marine debris items were separated from diet items and were analyzed separately. We only considered items bigger than 5 mm length (Barnes et al., 2009). Plastics under this size (microplastics, Gago et al., 2016) were not considered here. Marine debris contents were rinsed and air-dried, and then each section of the digestive tract was analyzed with a stereomicroscope to collect attached items. Each marine debris item was assigned into different categories using the protocol proposed by Van Franeker et al. (2011) and the Marine Strategy Framework Directive, Technical Subgroup on Marine Litter (2013) (Table 1). This protocol proposes that the debris should be categorized based in its morphology due to the uncertainties in determining its origin.

Results presented here include turtles sampled and analyzed between 2005 and 2007 (Group 1, $N = 44$), and turtles sampled between 2009 and 2013 (Group 2, $N = 52$). Quantification of debris was different in the two periods. Frequency of occurrence (%FO), total volume (measured by water displaced in a graduated cylinder) and dry weight in total and per category (with an analytical weight scale, precision 0,001 g) was measured for both groups, while volume of different categories of debris was measured only in Group 1, number of items was counted and the size of each hard plastic piece was calculated only in Group 2. The pieces were set up in a contrast background table and photographed to obtain the size (area in cm^2) of each piece. The pictures were analyzed with the software ImageJ 1.48v (Ferreira and Rasband, 2012).

For turtles of group 2, marine debris ingestion was assigned as the most probable cause of death when the stomach content had > 50% of debris or when there was a faecaloma caused by marine debris in the intestine. Results are reported as mean ± standard deviation, unless otherwise stated. Also for Group 2 an additional experiment was conducted to determine the buoyancy of debris by categories using the protocol proposed by Reisser et al. (2014). According to this protocol we measured the ascent velocity of each debris category, 10 items per category, in a graduated cylinder tube with marine water.

2.3. Statistical analysis

Data sets were checked for normality (Lilliefors test) and homogeneity of variances (Levene test). Non-parametric tests were used if those assumptions were not meet. To detect potential differences in intake of different types of debris in relation to turtle size we perform a Krukal-Wallis test of two size classes, based on the ontognetic dietary shift size proposed by Vélez-Rubio et al. (2016). Size classes were defined as follows: [1] CCL < 45 cm, turtles arriving to SWA coastal feeding grounds with pelagic diet and; [2] CCL ≥ 45 cm potential resident in the SWA coastal feeding grounds with a primary herbivorous diet. To test the potential differences in intake of debris in relation to turtle size, we use a GLM (General Linear Model) with Poisson family according to the residuals distribution. To detect the possible differential intake between estuarine and oceanic influence zones we we perform a Krukal-Wallis test. All the statistical analyses were performed using R 2.11 (R Development Core Team, 2017).

3. Results

All the turtles were of juvenile size (mean ± SD curve carapace length (CCL) = 40.15 ± 6.7 cm, $N = 93$, range 29.8–62.0 cm). From the analyzed turtles, 70.0% ($n = 65$) had marine debris in their digestive tract. The mean CCL of turtles with debris was 37.90 ± 6.5 cm (range 29.80–62.0 cm) and without debris 42.87 ± 6.5 cm ($n = 28$ range 34.0–61.5 cm). The mean volume of debris was 23.1 ± 33.3 ml (range: 0–170.0 ml, median = 9,5 ml) and the mean weight was 6.3 ± 11.1 g (range: 0–56.3 g, median = 1.9 g) (Table 2). For Group 2, the turtles ingested a total of 12,454 debris items, with a median number of 68 debris items per individual (range 0–1364, $N = 52$). Despite the high frequency of occurrence, most of the turtles showed little amounts of debris, while few turtles ($n = 5$) were full of debris (Fig. 2). We did not find significant relation between the amount of debris ingested and the zone of the stranding, estuarine influence zone and oceanic influence zone (Kruskal-Wallis test, $p = 0.9303$).

Gut contents presented a wide variety of debris types, with plastics

Table 1
Marine debris categories considered in the present study. Adapted from Van Franeker et al. (2011) and the Marine Strategy Framework Directive, Technical Subgroup on Marine Litter (2013).

Category	Type	Code	Description
Plastics			
Industrial plastics	Pellets	Ind	Industrial plastic pellets. Small, cylindrically-shaped granules of ± 4 mm diameter.
Domestic use plastics	Laminar like	Lam	Laminar soft items like plastic bags, foils, etc., usually broken up in smaller pieces.
	Thread like	Thr	Plastic threads, like pieces of rope, nets, nylon monophilaments, packaging straps etc.
	Foam like	Foa	Pieces of foamed polystyrene cups or packaging, foamed polyurethane in mattresses, or construction foams.
	Fragments	Fra	Hard plastic, pieces of bottles, boxes, toys, tools, equipment housing, toothbrushes, lighters etc.
	Others	Oth	Cigarette filters, rubber, elastics, balloons, etc., i.e. items that are 'plastic-like' or do not fit into a clear category.
Rubbish			
Rubbish	Paper	Pap	Normal paper, cardboard, laminated packaging, materials in which paper appears to dominate (e.g. tetra-pack), silver paper, aluminum foil etc.,
	Various	Var	Manufactured wood, paint chips, pieces of metals etc.
	Hooks	Hoo	Sport fishing hooks or long lining.
Contaminants			
Contaminants	Coal	Coa	Coal pieces
Natural non diet		Nnd	Remains of plants, pumice, stones, feathers and other natural items that can not be considered as normal food.

Table 2
Frequency of occurrence (%FO), abundance (weight and volume), and number of items of marine debris ingested by green turtles *Chelonia mydas* stranded in Uruguayan coast. The results are presented for all the study period, for turtle analyzed of the group 1 (years 2005 to 2007) and for the group 2 (years 2009 to 2013).

	All study period				Group 1: 2005–2007						Group 2: 2009–2013							
			Weight				Weight		Volume				Weight		N of items		Volume	
	Type	%FO	Sum	Mean	%FO	N	Sum	Mean	Sum	Mean	%FO	N	Sum	Mean	Sum	Mean	Mean	Sum
Plastics																		
Industrial	Ind	32.0	2.56	0.05 ± 0.13 (0–0.66)	–	–	–	–	–	–	32.0	16	2.56	0.05 ± 0.13 (0–0.66)	111	2.42 ± 5.8(0–24)		
Domestic use	Lam	67.30	182,56	0.78 ± 1.08 (0–6.89)	66.67	37	32.87	0.60 ± 0.83 (0–3.62)	268.7	5.3 ± 7.19 (0.28.0)	68.0	36	57.02	0.96 ± 1.26 (0–8.27)	3536	62.53 ± 83.5 (0–449)		
	Thr	63.46	104,01	0.59 ± 0.97 (0–4.99)	66.67	37	23.62	0.39 ± 0.60 (0–3.62)	202.9	3.85 ± 5.99 (0–32.0)	61.0	32	51.56	0.80 ± 1.21 (0–9.88)	2846	46.76 ± 81.4 (0–427)		
	Foa	36.53	6,33	0.07 ± 0.12 (0–0.68)	31.48	24	2.3	0.05 ± 0.09 (0–0.37)	36.9	0.78 ± 1.45 (0–6.0)	42.0	23	6.11	0.09 ± 0.15 (0–1.04)	244	4.26 ± 7.02 (0 – 31)		
	Fra	56.73	717,74	4.26 ± 9.43 (0–50.1)	59.26	35	90.82	1.86 ± 3.11 (0–13.87)	188.9	3.4 ± 4.67 (0–20.0)	54.0	28	337.54	6.71 ± 12.57 (0–56.36)	4032	79.95 ± 152.08 (0–606)		
	Oth	32.69	148,91	0.23 ± 0.89 (0–7.6)	24.07	21	16.84	0.36 ± 1.22 (0–7.59)	48.7	1.01 ± 2.28 (0.2–13.0)	42.0	21	7.15	0.11 ± 0.16 (0–1.97)	62	1.04 ± 2.31 (0–9)		
Rubbish																		
Rubbish	Pap	25.96	30,16	0.08 ± 0.3 (0–2.52)	33.33	19	10.13	0.13 ± 0.40 (0–3.6)	24.5	0.52 ± 1.71 (0–10.0)	18.0	9	1.4	0.02 ± 0.12 (0–0.83)	59	1.18 ± 5.47 (0–37)		
	Var	11.53	15,73	0.03 ± 1,78 (0–1.45)	0.09	7	2.07	0.04 ± 0.21 (0–1.45)	19.5	0.42 ± 1.99 (0–13.0)	14.0	7	0.63	0.01 ± 0.04 (0–0.22)	16	0.28 ± 0.97 (0–6)		
Contaminan-ts																		
Contamin.	Coa	14.42	25,57	0.1 ± 0.5 (0–4.08)	22.22	14	9.07	0.19 ± 0.66 (0–4.08)	25.9	0.55 ± 2.02 (0–13.0)	6.0	4	0.15	< 0.002	7	0.085 ± 0.46 (0–3)		
Natural non diet	Org	58.0	9,85	0.29 ± 0.43 (0.001–1.70)	–	–	–	–	–	–	58.0	31	14.03	0.21 ± 0.38 (0–1.7)	1541	24.37 ± 30.34 (0–156)		
Total		**71.1**	**1243.4**	**6.34 ± 11.15 (0–56.3)**	–	**39**	**187.72**	**3.77 ± 5.40 (0–25.31)**	**816.0**	**15.84 ± 19.4- (0–81.2)**	–	**38**	**478.16**	**8.97 ± 14.4 (0–5634)**	**12,454**	**220.76 ± 320.82 (0–1364)**	**23.01 ± 33.69 (0–170)**	**2393**

The bold numbers indicated the variables for all the turtles in the stuay.

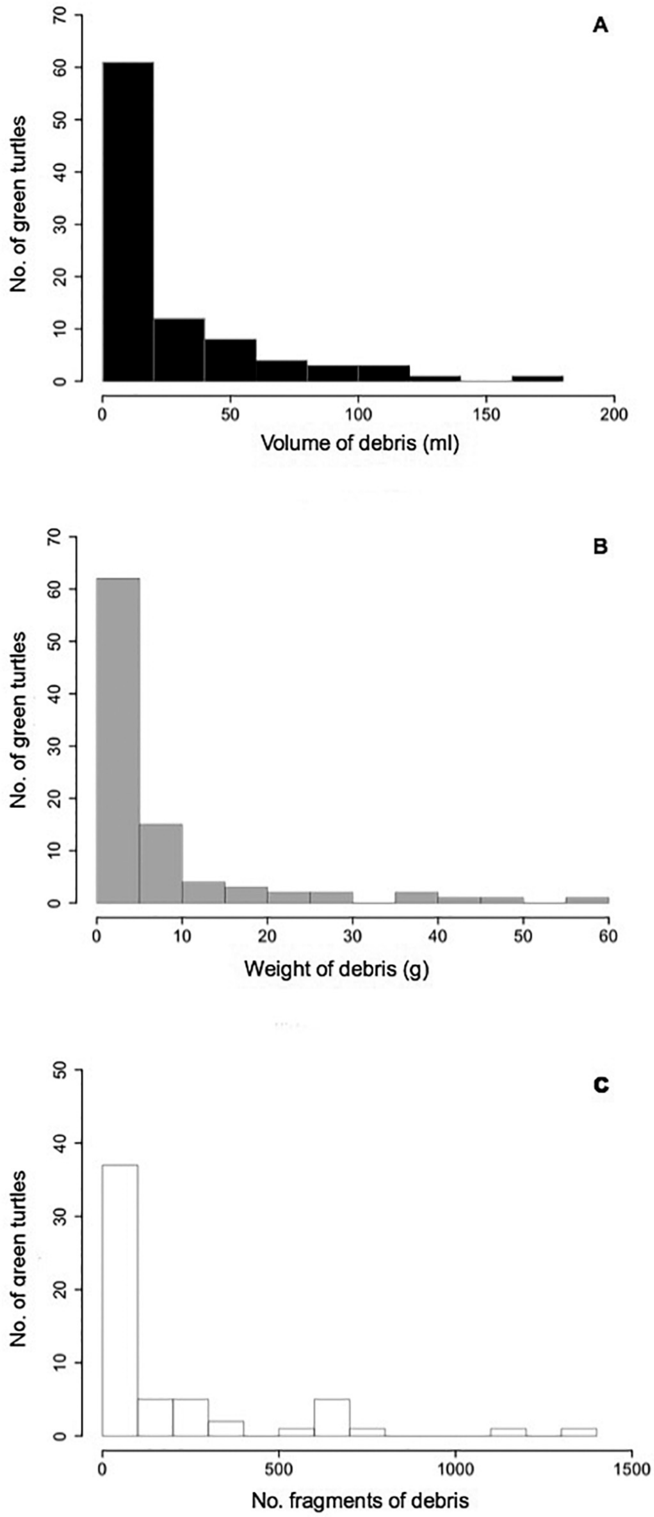

Fig. 2. A) Total volume, B) Total weight, C) Number of items (only Group 2, $N = 52$) ingested by juvenile green turtles in Uruguayan coastal waters. Note the different values in Y-axis in C.

(principally domestic use plastics) being the predominant type in total amount of items, weight and total volume of debris (Table 2). Among all categories of domestic use plastic, fragments category (mainly hard plastics) was the most abundant in weight, but regarding the volume the most abundant category is laminar (plastic bag fragments). Laminar and tread plastics were the most frequent types, followed by hard

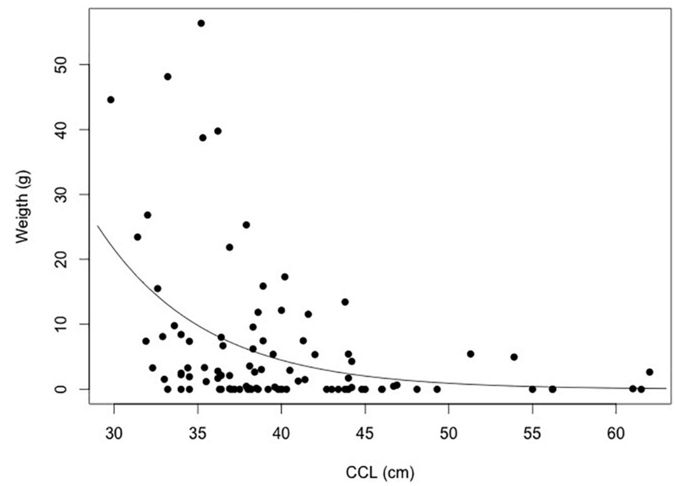

Fig. 3. General linear model (GLM) of the variation in weight of marine debris ingested compared with Curved Carapace Length (CCL in cm) of juveniles green turtles (*Chelonia mydas*).

plastics plastics in terms of frequency of occurrence. Although frequency of occurrence of marine debris kept constant over the years, the amounts in weight and total volume per turtle were consideratelyhigher in Group 2 than in Group 1 (Fig. 3, Table 2). Dead caused by debris ingestion was evaluated only for Group 2. From this group, 27% ($n = 14$ turtles) probably died because of this cause according to the evidences found: emaciating signs, presence of faecaloma in the intestine of 5 of the turtles, and marine debris making > 50% of their gastrointestinal content in other 9 of them.

Concerning turtle size and debris ingestion, we found higher frequency of occurrence of debris in smaller turtles (CCL < 45 cm) compared with bigger turtles (CCL > 45 cm). The same pattern was recorded for each debris category among size groups, except for coal pieces that present the same frequency of occurrence (Fig. 4). Turtles of Group 2 revealed an item mean size of 0.61 ± 0.68 cm^2 (range 0.004–7.04 cm^2). Significant differences were found among turtle size, with the bigger turtles ingesting bigger debris (Kruskal Wallis, $H_{(2.3597)} = 13.67$; $p < 0.001$). Smaller turtles ingested plastic fragments of mean size ± SD = 0.59 ± 0.68 cm^2 (range 0.004–7.04 cm^2); and bigger turtles ingested plastic fragments of 0.77 ± 0.57 cm^2 (0.009–3.91 cm^2).

Buoyancy experiments conducted for the Group 2 data set showed that all categories of debris ingested had positive buoyancy (Fig. 5). However, there were significant differences along the ascent velocities of different types of debris (Kruskal-Wallis test: $H_{(7,76)} = 27.08$, $p < 0.001$) being foams and other categories of plastics (e.g. balloon fragments) the debris with highest ascent velocities (0.0645 ± 0.0309 ms^{-1} and 0.0603 ± 0.0363 ms^{-1} respectively). In contrast, thread-like plastics was the category with the slowest raise velocity (0.0175 ± 0.0129 ms^{-1}).

4. Discussion

Our results provide strong evidence that anthropogenic marine debris is one of the most important threats affecting green turtles in Uruguayan coastal waters in the last decade. We contributed with new evidence about the green turtle vulnerability to marine pollution trough their life cycle, particularly during their oceanic stage and during their ontogenetic dietary and habitat change to coastal areas. We stated that 70% of the turtles presented marine debris at least in one section of their digestive tract. This numbers are consistent with other studies in the SWAO: 90% in the Rio de La Plata Estuary-Argentina (Gonzalez Carman et al., 2014a), 100% in Rio Grande do Sul-Brazil (Tourinho et al., 2010) and 70% in all Brazilian coast (Santos et al., 2015 and

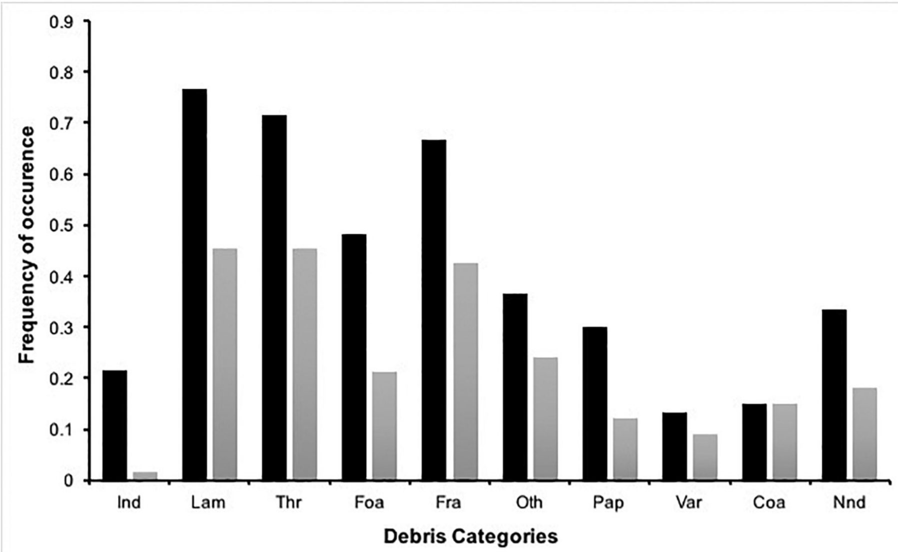

Fig. 4. Frequency of occurrence (FO%) of debris categories for all the study period (2006–2013). The bars correspond to: black bars, small size group CCL < 45 cm and grey bars, large size group CCL > 4 5 cm. Marine debris categories: Industrial, pellets (Ind), Laminar (Lam), Thread like (Thr), Foam like (Foa), Fragments (Fra), Others (Oth), Paper (Pap), Various (Var), Coal pieces (Coa) and organic debris (Org).

references therein). Furthermore, our findings on mean weight and mean number of debris ingested per individual were higher than others reported in other studies of the SWA region (Gonzalez Carman et al., 2014a; Tourinho et al., 2010; Santos et al., 2015; da Silva Mendes et al., 2015) and worldwide (Schuyler et al., 2014; Wedemeyer-Strombel et al., 2015) (see also Supplementary Table 3). This high number of turtle deaths associated to debris ingestion could be associated to the accumulation of debris during their oceanic phase.

Although direct mortality due to marine debris ingestion is not high, it can cause many sublethal effects in the sea turtles (Bjorndal et al., 1994) and subsequent death derived from chronicle processes (Santos et al., 2015). In the present study, direct mortality associated to debris ingestion was similar to other study in Brazilian waters (Santos et al., 2015), we found perforations and abrasions produced by blockage of the digestive tract. However, the real impact of debris ingestion can be often underestimated, since a necropsy or detailed analysis of the individuals necessary to determine all the effects of debris on the turtle. Faecalomas and intestine blockages are symptoms leading to death associated to debris ingestion, as we stated. Moreover, in our study, hard plastics have been particularly abundant, and small hard pointed debris items have been reported causing the death of turtles by abrasion of the intestines (e.g. Jerdy et al., 2017). Smaller amount of debris, or even a single piece of plastic, could rotate while moving through the intestine generating injuries or perforation leading to the animal death (Bjorndal et al., 1994; Santos et al., 2015; da Silva Mendes et al., 2015). This threat may suppose a growing problem in the SWA and becoming in one of the main threats affecting juvenile green turtle's population present SWA waters; according to the positive trend of debris amounts in the turtles detected in recent years, and to the increasing number of turtle deaths associated to marine debris ingestion in Uruguay: from 11% for the period 1999–2010 (Vélez-Rubio et al., 2013) to at least 27% in the last years (present study).

We detected a negative correlation between the presence of debris and turtle size, being the smaller turtles (CCL < 45 cm) the ones with more debris ingested. These turtles are probably new recruits to neritic grounds (Vélez-Rubio et al., 2016), what lead us to think that the oceanic stage of green turtle life cycle is the most vulnerable stage to this threat in the Southwestern Atlantic. These smaller turtles, with higher presence of plastic, also presented floating *Sargassum* sp. in their digestive tracts and beaks of pelagic cepahlopods (Vélez-Rubio et al., 2015, 2016), therefore these animals could already feed on floating debris during their oceanic phase of their life cycle. This could be explained by the opportunistic epipelagic feeding habits of younger green turtles (Boyle, 2006; Schuyler et al., 2012), in comparison with higher selectivity in older turtles, probably acquired after years of residency in neritic habitats (Bjorndal, 1980; Schuyler et al., 2012). The recruitment

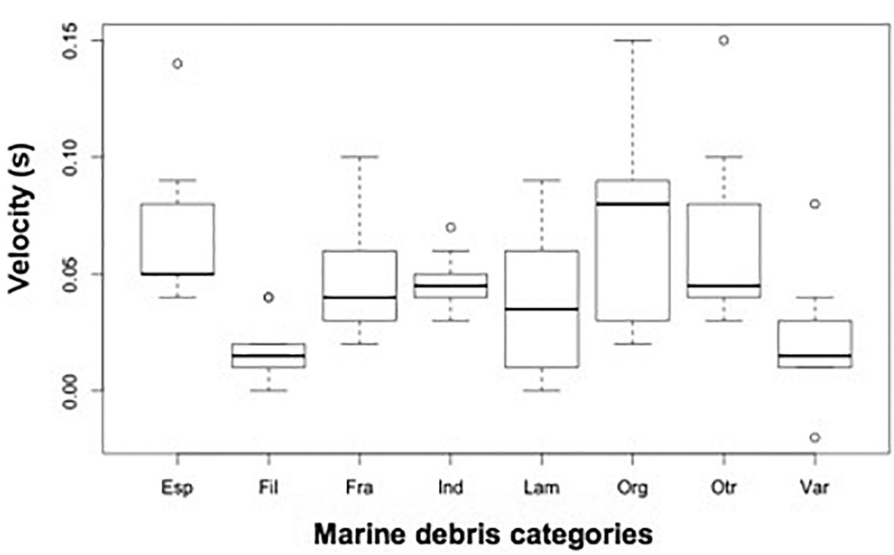

Fig. 5. Boxplot of the ascent velocity of each type of plastic. Marine debris categories: Industrial, pellets (Ind), Laminar (Lam), Thread like (Thr), Foam like (Foa), Fragments (Fra), Others (Oth), Paper (Pap), Various (Var), Coal pieces (Coa) and organic debris (Org).

to neritic habitats, after the oceanic phase, is accompanied with an ontogenetic dietary shift (Bjorndal, 1985, 1997; Seminoff et al., 2002).

This dietary change could be either abrupt or not; indeed, turtles could continue keep having epipelagic feeding together to an incipient herbivorous benthonic diet (Cardona et al., 2009, 2010; Gonzalez Carman et al., 2012, 2014b). Nonetheless, the shift to feed mainly on benthic macrophytes (marine fanerogams and seaweeds) could explain the smaller amounts of debris found in larger turtles. In Uruguayan coastal waters, green turtles start consuming macroalgae after the recruitment but maintain a considerable amount of gelatinous macrozooplankton in their diet (Vélez-Rubio et al., 2016). During this process, turtles are susceptible of encounter debris in the water column and retained in the bottom of coastal habitats. Gonzalez Carman et al. (2014a, 2014b) also found high occurrence of debris together to gelatinous preys in smaller turtles in the region. Also the buoyancy experiment supported the probably intake of marine debris in the water column. This experiment gave us an idea about the features and properties of marine debris ingested by green turtles, showing the same models of distribution in the water column proposed in the literature (Reisser et al., 2014; Kooi et al., 2016). The buoyancy analysis indicated that all categories of debris ingested by the green turtles analyzed have positive buoyancy. However, we detected significant differences among debris types, being threads the less buoyant, but this type of debris could be easily entangled in other types of debris.

Regarding the regional situation of the size of turtles with debris in their guts (Brazil: Santos et al., 2015; Uruguay: present study; Argentina: Gonzalez Carman et al., 2014a; respectively) we found that in all cases the mean size is under 39.0 cm. This size could correspond to turtles performing their ontogenetic shift to herbivorous benthic feeding (Vélez-Rubio et al., 2016). As mention before, the fact that turtles have a non-abrupt dietary shift and keep having some epipelagic feeding, together with the influence of the estuary debris discharge, puts them at risk in these Uruguayan coastal waters.

We found no differences in debris ingestion associated to the stranding location but could be explained because the discharge of Rio de La Plata estuary influenced in the distribution of the strandings a long the Uruguayan coast, reducing the number of turtles found in the inner part of the estuary and increasing the number of strandings in the outer estuarine and oceanic influence zone (Vélez-Rubio et al., 2013). As proposed Gonzalez Carman et al., 2014a, neritic feeding areas tend to accumulate debris a long with turtle preys, such us gelatinous macrozooplanckton, as occurred in the Rio de la Plata estuary system. The high presence of debris and the lack of visibility in the system could increase the ingestion of debris with the gelatinous preys (Gonzalez Carman et al., 2014a). In these waters there is a strong overlap between zones with accumulation of debris and zones used by the green turtle as feeding grounds in the outer estuarine influence zone (Gonzalez Carman et al., 2014a). Also in Brazil, Santos et al. (2015) detected the estuarine areas as areas with the highest frequency of occurrence of debris in green turtle gut contents.

We found a wide variety of marine debris in the digestive tract of the green turtles analyzed, with a clear dominancy of domestic plastics (hard plastics, laminar and threads) in weight and volume over the rest of the categories. The high frequency of soft plastic, as plastic bags (main component of laminar plastics category), could be explained either by its similarity to gelatinous macrozooplancton, which represents part of the diet during the ontogenetic dietary shift (Bjorndal, 1980; Mrosovsky, 1981; Schuyler et al., 2012) or by the low prey selectivity that juvenile green turtles have during the oceanic phase (Boyle, 2006). In addition, hard plastics (high and low density polyethylene and polypropylene) are the most abundant in the ocean (Morét-Ferguson et al., 2010) and normally accumulate in ocean fronts (Gonzalez Carman et al., 2014a, 2014b), that would explain the frequency of occurrence and high amount of debris ingested by juvenile green turtles when they are close to these debris accumulation areas. Although having less debris, larger turtles presented bigger items of hard plastic. This may be explained because larger turtles might exploit dietary resources inaccessible to smaller turtles due to gape limitation (Tomás et al., 2001). However, Schuyler et al. (2012) suggested that the ingestion of debris is independent of the size of the animal because the bigger animals have the capacity of excrete smaller items more efficiently than the smaller turtles. In addition, a higher body size means a wider digestive tube, what may reduce the probability of retention and blockage.

Marine debris ingestion is one of the most dramatic threats concerning sea turtle population in the Southwestern Atlantic and worldwide due to the increasing presence of plastic at sea. This problem may be of special importance in Uruguayan coastal waters, influenced by the main discharge of the Rio de La Plata estuary and the big concentrations of human populations at both sides of the estuary, both in Argentina and Uruguay. Moreover, this threat may suppose a big conservation problem since is affecting green turtles in a vulnerable stage, when they are changing and adapting to new feeding behavior and habitat use.

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