



Young green turtles, *Chelonia mydas*, exposed to plastic in a frontal area of the SW Atlantic



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ARTICLE INFO

Keywords:

Marine debris
Argentina–Uruguay
Marine turtles
Pollution
Habitat degradation
Río de la Plata

ABSTRACT

Ingestion of anthropogenic debris represents an important threat to marine turtle populations. Information has been limited to inventories of debris ingested and its consequences, but why ingestion occurs and the conditions that enable it are less understood. Here we report on the occurrence of plastic ingestion in young green turtles (*Chelonia mydas*) inhabiting the Río de la Plata (SW Atlantic). This estuarine area is characterized by a frontal system that accumulates anthropogenic debris. We explored exposure of green turtles to plastic and its ingestion via debris distribution, habitat use and digestive tract examination. Results indicated that there is considerable overlap of frontal accumulated plastic and core foraging areas of the animals. Exposure results in ingestion, as shown by the high frequency of plastic found in the digestive tracts. The Río de la Plata estuarine front is an area of conservation concern for young green turtles.

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1. Introduction

Marine turtle populations have been reduced by exploitation and consumption of eggs and meat during the past centuries (IUCN, 2013; Meylan and Donnelly, 1999; Seminoff, 2004). Egg harvesting and consumption of turtles are now regulated in most places, but direct and indirect threats continue to threaten populations: bycatch in fishing gear kills thousands of turtles per year (Wallace et al., 2010), pollution with artificial lights on nesting beaches disrupts nest-site choice and orientation (Witherington and Martin, 2003), and oil spills affect marine turtles through direct contact or by fouling of their habitats. A pervasive, highly damaging and expanding problem is entanglement and ingestion of anthropogenic debris (Lutcavage et al., 1997). Some turtle populations are recovering after controlling direct exploitation (e.g. Bjorndal et al., 1999; Broderick et al., 2006; Chaloupka et al., 2008; Dutton et al., 2005; Marcovaldi and Chaloupka, 2007), but the cumulative impacts of other threats, including ingestion of

anthropogenic debris, may hamper or reduce population recovery (Donlan et al., 2010; Coll et al., 2012; Maxwell et al., 2013).

Ingestion of anthropogenic debris has been reported in almost all marine turtle species. It occurs in all life stages and several geographic areas (see Schuyler et al., 2013 and references therein). Plastics are the most commonly ingested of all solid pollutants (Schuyler et al., 2013). The amount of debris found in the stomach of an animal is generally small, in terms of number of items and weight (Bjorndal, 1997; Schuyler et al., 2013), but even that may have lethal consequences through perforation or impaction of the digestive system (Bjorndal et al., 1994). Direct mortality due to debris ingestion seems to occur rarely, although it is difficult to prove. The most common health effects are exposure to chemicals leaching from the debris and dietary dilution that reduce somatic growth or reproductive output (Laist, 1987; McCauley and Bjorndal, 1999). Such sublethal effects are difficult to estimate for these long-lived and highly migratory animals (Bjorndal et al., 1994; McCauley and Bjorndal, 1999; NRC, 1990).

To date, research has focused on a valuable and exhaustive inventory of the debris ingested and its consequences, but why plastic ingestion occurs and the conditions that enable it are far from being understood. It has been suggested that leatherbacks mistake gelatinous plankton for floating plastic bags (Bjorndal, 1997; Mrosovsky et al., 2009); thus zooplanktivorous turtles would

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be particularly vulnerable to ingestion of plastic debris. Gelatinous plankton tends to be aggregated at physical discontinuities such as ocean fronts (Graham et al., 2001; Mianzan and Guerrero, 2000), and these same ocean features may concentrate floating debris (Barnes et al., 2009; Pruter, 1987). Therefore, during their oceanic developmental stage, marine turtles may be more exposed to debris ingestion when feeding in frontal areas (Carr, 1987; Schuyler et al., 2013; Witherington, 2002). Nevertheless, marine turtles with benthic and neritic feeding habits are also known to ingest plastic (e.g. Bjorndal et al., 1994; Bugoni et al., 2001; Schuyler et al., 2013; Tourinho et al., 2010). We report here on the occurrence of the problem in young green turtles in neritic habitats, linking the threat of plastic ingestion with a particular oceanographic feature in the distribution range of the species.

Juvenile green turtles reach the temperate waters of Argentina and Uruguay in their migration along foraging habitats of the eastern coast of South America (González Carman et al., 2011, 2012). From November to May, they intensively use the Río de la Plata estuarine area to feed on gelatinous plankton (González Carman et al., 2012, 2013; Fig. 1) that aggregates in the frontal system of the estuary (Mianzan et al., 2001). The system also accumulates anthropogenic debris (Acha et al., 2003). We explored exposure to plastics and its ingestion through the combination of information on plastic distribution, habitat use and examination of digestive tract contents of juvenile green turtles.

2. Methods

2.1. Study area: the Río de la Plata frontal system

The Río de la Plata (Argentina–Uruguay) is a two-layered estuarine system where freshwater flows seaward on the surface, and denser, saline shelf water intrudes along the bottom (Mianzan et al., 2001; Fig. 1). This dynamic generates two salinity fronts separated by ca. 150 km and connected by a salt-wedge: a bottom and a surface front at the inner and outer part of the estuary, respectively. The bottom front can be approximated at the surface by the presence of an estuarine turbidity maximum. The turbidity maximum is due to the suspended matter flocculation near the

tip of the salt wedge, and re-suspension of sediment due to tidal stirring (Framiñan and Brown, 1996). The turbidity front can be easily identified in satellite images; its modal position is located near the limit of the marine water intrusion. (Acha et al., 2008; Framiñan and Brown, 1996; Mianzan et al., 2001). The surface front has lower salinity gradients than those of the bottom front, and its location is more variable. This frontal system favors the retention and concentration of gelatinous plankton (Alvarez Colombo et al., 2003; Cabreira et al., 2006; Mianzan and Guerrero, 2000; Mianzan et al., 2001), which constitutes the main food for green turtles in the area (González Carman et al., 2013). Along with gelatinous plankton, the bottom front also accumulates anthropogenic debris that drifts down the river and is generated by highly populated cities in the region (i.e. Buenos Aires, Montevideo) and by intensive vessel traffic (Acha et al., 2003).

2.2. Exposure to plastic pollution

Data on the distribution and concentration of anthropogenic debris in the Río de la Plata are from Acha et al. (2003). Anthropogenic debris (plastic bags, cans, bottles and hard plastic pieces) were collected from 1996 to 2001 using a bottom trawl net operated at 269 stations arranged in a random sampling design that covered most part of the estuary (Acha et al., 2003). For our analysis, we used information only on plastic debris (plastic bags and hard plastic pieces) since it is most frequently consumed by marine turtles (Bjorndal et al., 1994; Bugoni et al., 2001; Tomás et al., 2002; Tourinho et al., 2010). Plastic debris was counted and expressed as number of items per km². For further details on the sampling method see Acha et al. (2003).

We overlapped data on plastic debris distribution with green turtle foraging areas obtained through satellite telemetry from 9 turtles during the period 2008–2011 (González Carman et al., 2012; Fig. 2). Animal positions were analyzed with state-space models to identify locations where the animals were likely engaged in foraging activities (Breed et al., 2009; Maxwell et al., 2011; see modeling details in González Carman et al., 2012). Fixed kernel density estimation was used to construct a map showing foraging areas, created from the ‘foraging’ locations from the state-space model results. This method

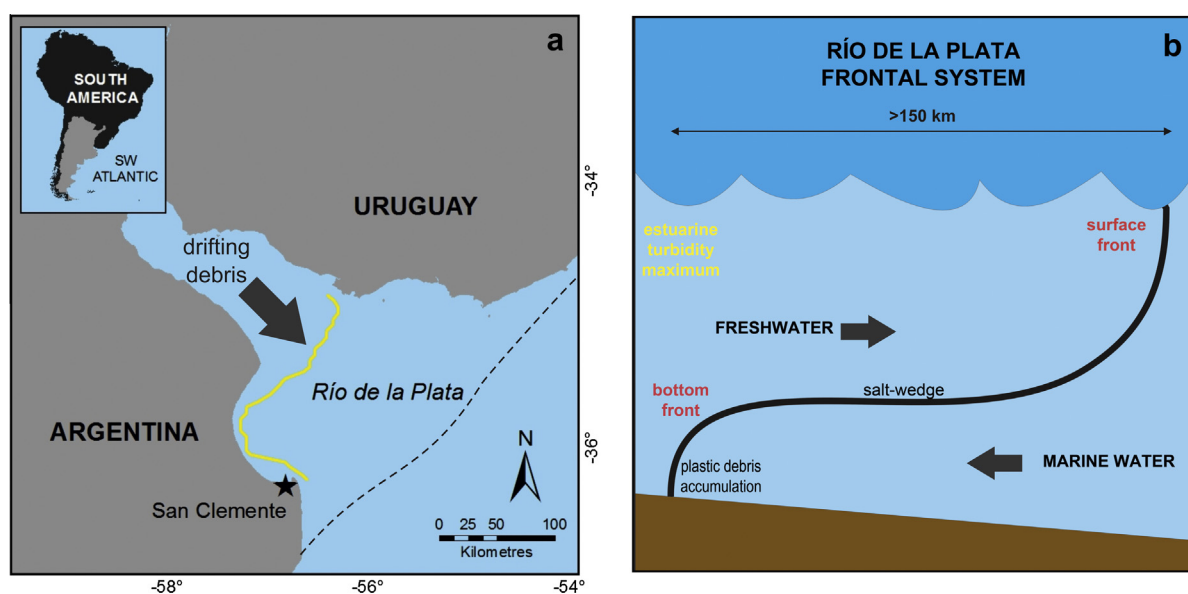


Fig. 1. (a) Río de la Plata estuarine area (Argentina–Uruguay). The yellow line represents the modal position of the turbidity front (from Framiñan and Brown, 1996) which is a proxy of the bottom salinity front position. The black dashed line indicates an approximate position of the surface salinity front (Mianzan et al., 2001; Cabreira et al., 2006). Black star indicates the location where green turtles were caught as bycatch in a gillnet fishery, providing material for the digestive tracts sampling. (b) Conceptual diagram of the Río de la Plata frontal system modified from Acha et al. (1999) and Mianzan et al. (2001) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

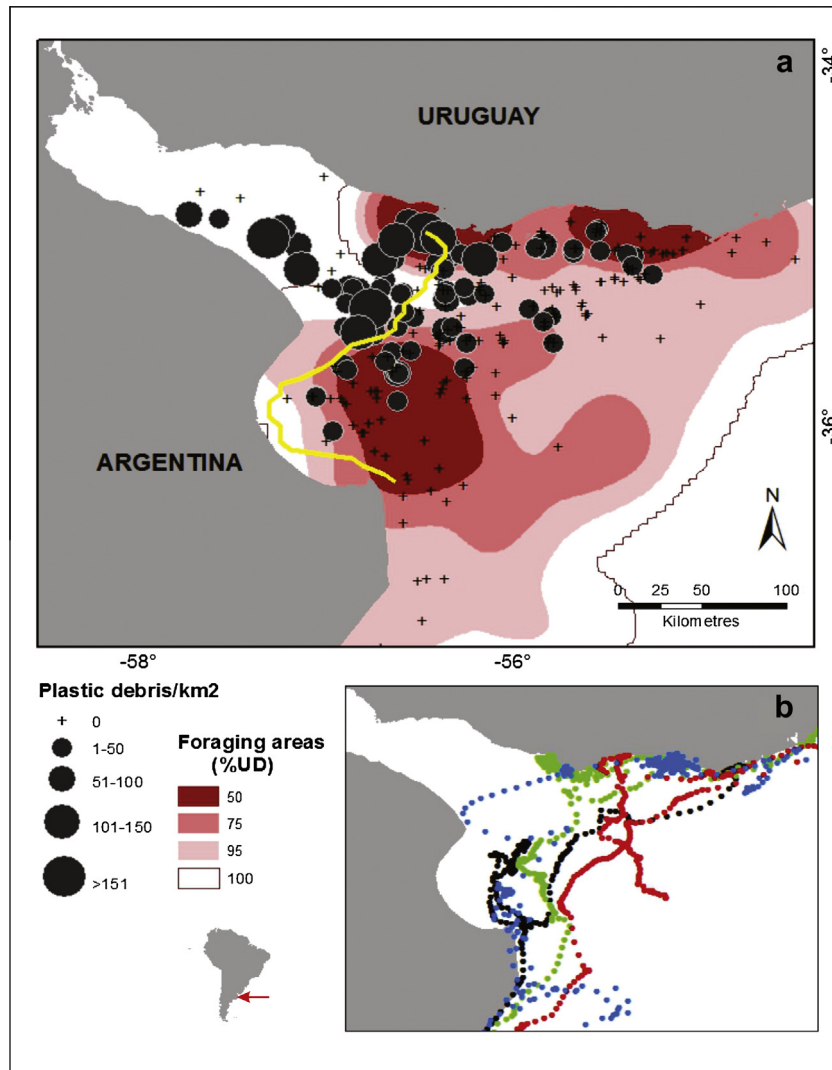


Fig. 2. (a) Plastic debris distribution overlapped with green turtle foraging areas in the Río de la Plata (Argentina–Uruguay). The yellow line represents the modal position of the turbidity front associated with the bottom salinity front. The 100% and 50% UD represent the overall distribution of foraging areas and the core foraging areas of the turtles, respectively. (b) Dotted colored lines are used to exemplify routes of four of the nine turtles tracked. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

identifies areas of disproportionately heavy use, or core areas, within a distribution range. The density at any location is an estimate of the amount of time spent there (Seaman and Powell, 1996; Worton, 1989). Foraging density distributions were represented by utilization distribution (UD) contours, indicating areas within which tracked turtles spent 50%, 75%, 95% and 100% of their presumed foraging at-sea time. The 100% and 50% UD are interpreted as overall distribution of foraging areas and core foraging areas of turtles during the tracking period, respectively (González Carman et al., 2012). Data on plastic debris distribution and green turtle foraging areas were plotted together using ArcGIS 10.1® (Copyright© ESRI) to visually explore green turtle exposure to plastic debris while foraging in the Río de la Plata.

To statistically explore an association between plastic and green turtle distributions, a Pearson correlation ($\alpha = 0.05$) was conducted between plastic density and turtle kernel density found at the corresponding debris sampling location.

2.3. Plastic ingestion determined from dead animals

We examined 62 digestive tracts of dead green turtles necropsied to study their diet. The animals were incidentally captured

during 2008–2011 in gillnets of the San Clemente artisanal fishery that operates in the coastal waters of the southern part of the estuary (González Carman et al., 2013). We measured curved carapace length ($CCL \pm 0.1$ cm) and weight ($W \pm 0.1$ kg) of the animals. Debris was counted, weighed (± 0.1 g), and classified by type (e.g. plastic bag, hard plastic fragment, rope, cloth, paper, and others) following Bjørndal et al. (1994) and Lazar and Gračan (2011). Results for each specific category were expressed as number of ingested fragments (mean \pm SE) and frequency of occurrence (%FO). The %FO denotes the overall proportion that a particular debris category appeared in the animals examined. The wet weight of total debris per animals was determined.

3. Results

3.1. Exposure to plastic pollution

Green turtles spent most of their at-sea time in core foraging areas (50% UD) in the Río de la Plata estuarine system, including the coastal waters off Uruguay and waters off Samborombón Bay along the Argentine coast (Fig. 2a). These core areas were near to or overlapped with zones where plastic accumulates, such as the

coastal waters off Uruguay (Fig. 2a). Other foraging areas, though not highly used (95% UD), occurred where plastics were not detected. There was no statistically significant association between the plastic and the turtle kernel density distributions (Pearson's $r = -0.09$, $P = 0.1$).

Individual animals that first foraged near San Clemente, and then migrated to the coast of Uruguay went through the frontal area where the accumulation of anthropogenic debris was expected to be the highest (Fig. 2b). On average, turtles likely encountered a mean of 8.8 plastic items (hard plastic and bags) per square kilometer of the overlapped foraging area. This quantity reached maximum values of >100 items/km² at the frontal area.

3.2. Plastic ingestion

Most (90%) of the 62 juveniles examined ingested anthropogenic debris (mean $CCL \pm SD = 38.5 \pm 4.4$ cm, range = 31.3–52.2 cm, $N = 54$; mean $W \pm SD = 6.0 \pm 2.9$ kg, range = 2.3–16.8 kg, $N = 47$). Median number of fragments per animal was 13 (range: 0–591 fragments, $N = 62$, Fig. 3a) and the weight of debris per animal was <5 g in most cases (Fig. 3b). An extreme case was represented by one animal that consumed 591 fragments of debris (mostly hard plastic pieces), an equivalent of 1.3% of its body weight.

Most debris was found in the distal portion of the large intestine (98%), although some was also found in the stomach (40%) and the oesophagus (16%). Wrappers, bags and hard pieces of plastic were most frequently ingested (Table 1). The size of the debris was variable, ranging from small hard plastic pieces (0.5–3.0 cm) to large sections of bags (>15.0 cm) (Fig. 4). Plastic debris was found embedded in a jelly like mucous material in the stomachs (Fig. 5).

4. Discussion

Neritic juvenile green turtles are exposed to a concentration of plastic pollution while foraging within the Río de la Plata frontal system. The distribution of plastic accumulated by the bottom front and the core foraging areas of the animals overlap widely (Fig. 2). Exposure results in ingestion, as shown by the high frequency of plastic debris found in the digestive tract of turtles by-caught in the southern portion of the estuary (Fig. 3, Table 1). From our previous tracking studies we know that green turtles spend a large portion of the year (up to 6 months) in this estuarine region (González Carman et al., 2012); thus most of the debris is likely from the Río de la Plata region. Despite this evidence, there was no statistically significant association between the observed plastic distribution and turtle kernel density. This could be due to limitations in our data set, since the data on plastics were collected opportunistically during research fishery cruises and not with a sample design to show how it affects turtles. This highlights the need to sample plastic debris aiming to evaluate the effect on marine turtles through a spatial analysis approach.

Exposure to plastic ingestion is likely increased by physical processes such as water mass convergence occurring at fronts (Carr, 1987; Witherington, 2002). This is likely a key factor promoting plastic ingestion in neritic foraging areas such as this one. In the Río de la Plata estuarine system, debris accumulates particularly at the bottom front (Acha et al., 2003), along with primary prey items. Some of the scyphozoan species which are part of the turtle's diet (e.g. *Chrysaora lactea*, *Lychnorhiza lucerna*, González Carman et al., 2013) aggregate close to the bottom, below the salt-wedge (Alvarez Colombo et al., 2003; Cabreira et al., 2006). Our results show that turtles have large amounts of debris in their digestive tracts, suggesting that their proximity to the front,

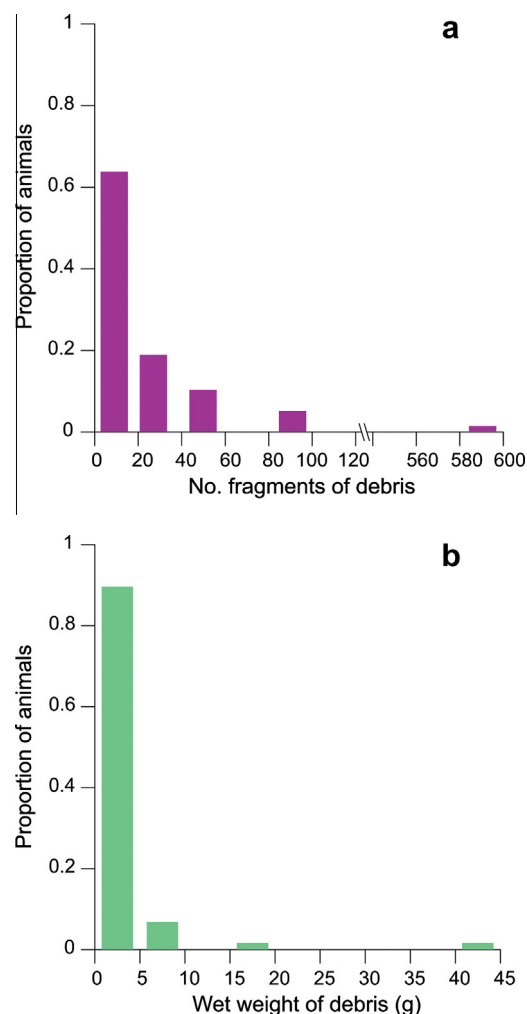


Fig. 3. (a) Number of fragments and (b) wet weight of anthropogenic debris ingested by juvenile green turtles from San Clemente, Argentina.

Table 1

Frequency of occurrence (%FO) and number of fragments of anthropogenic debris found in the digestive tracts ($n = 62$) of juvenile green turtles from San Clemente, Argentina. SE: one standard error.

Anthropogenic debris	%FO	No. fragments		
		Total	Mean	SE
Wrapper plastic	85.5	607	11.5	1.7
Plastic bag	74.2	275	6.0	0.9
Hard plastic pieces	59.7	819	22.1	11.5
Thread (nylon, cotton)	37.1	24	2.0	0.2
Rubber (balloon, band)	27.4	46	1.4	0.1
Rope	14.5	13	1.4	0.1
Styrofoam	6.5	3	2.8	0.2
Artificial fiber sponge	4.8	11	1.0	0.0
Wood	4.8	3	1.3	0.1
Cotton pieces	4.8	3	1.0	0.0
Paper	4.8	1	1.0	0.0
Cloth	1.6	4	1.0	0.0

particularly during presumed foraging, is a potential cause for elevated debris levels.

Because visibility may be quite limited in the study area, ingestion of plastic may not be due to visually mistaking plastic for gelatinous prey, as has been suggested for leatherbacks elsewhere (Bjorndal, 1997; Mrosovsky et al., 2009). Light penetration in the estuary is scarce due to suspended sediments, especially in the



Fig. 4. Diversity of anthropogenic debris ingested by juvenile green turtles from San Clemente, Argentina. Each photo represents the debris ingested by one animal. Ruler size is 15 cm (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

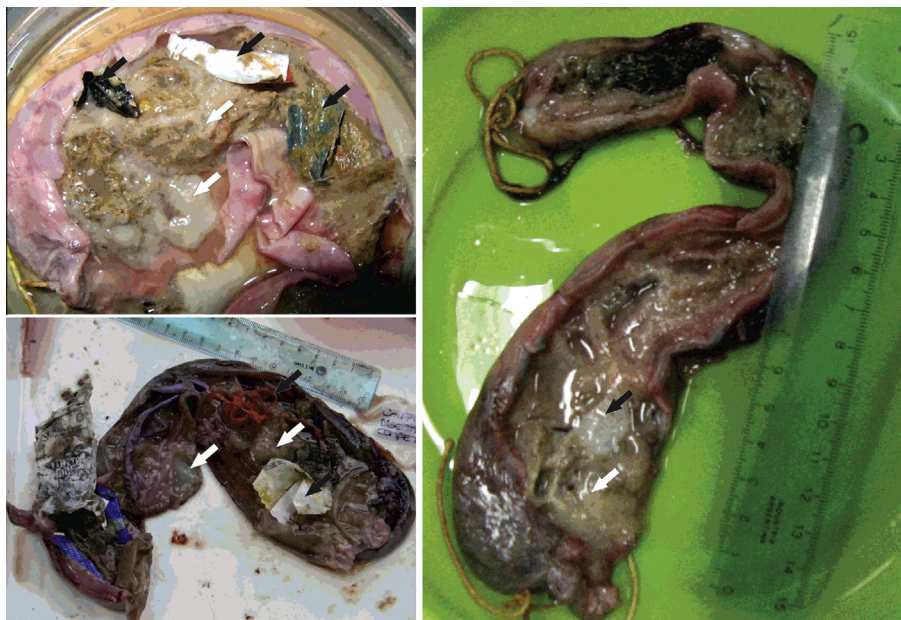


Fig. 5. Plastic debris found embedded in a jelly like mucous material within the stomachs of juvenile green turtles from San Clemente, Argentina. Black and white arrows indicate plastic debris and jelly like mucus, respectively.

turbidity front (Acha et al., 2008; Mianzan et al., 2001). Furthermore, the optical sensitivity of the green turtle eye is thought to be low (Mäthger et al., 2007). Marine turtles are capable of detecting chemical cues underwater through receptors located in the olfactory epithelia (Southwood et al., 2008), but it is possible that plastics do not have a strong enough chemical cue to be detected through olfaction either.

Plastic ingestion has also been observed in Franciscana dolphins (*Pontoporia blainvillei*) that inhabit the same estuary. Denuncio et al. (2011) reported that dolphins incidentally captured in estuarine waters ingested more plastic than dolphins from adjoining, marine waters. Turtles and dolphins feed on different prey (jellyfish and fish, respectively); this also suggests that plastic ingestion may not occur because animals fail to distinguish it from prey.

Thus, it is possible that in this system, elevated exposure results in accidental consumption. Accidental consumption may occur if both plastics and jellyfish prey are associated with the same physical feature (i.e. the bottom front); as observed by a number of studies including Acha et al. (2003), Alvarez Colombo et al. (2003) and Cabreira et al. (2006). The digestive tract examination supports this physical association. Plastic debris was found embedded in a jelly like mucous material in the turtles' stomachs (Table 2, Fig. 5), and this mucus is associated with the presence of nematocysts of the putative jellyfish prey of the turtles (González Carman et al., 2013).

Almost all necropsied animals ingested plastic debris. The amount of items ingested is similar to those reported in other geographic areas (e.g. Bjorndal et al., 1994; Bugoni et al., 2001; Tomás et al., 2002; Tourinho et al., 2010). None of the study animals apparently died due to plastic ingestion; instead they were accidentally caught in gillnets. Most of the debris was found in the last section of the large intestine, which suggests that the plastic might affect turtles through dietary dilution while it passes throughout the length of the digestive tract. The turtles would have a limited ability to compensate for dietary dilution and this could be exacerbated in animals with an already diluted diet based on gelatinous plankton (McCauley and Bjorndal, 1999), like the individuals in this study (González Carman et al., 2013).

Based on the above, we can hypothesize that juvenile green turtles become highly exposed to the menace of plastics from late spring to early fall in the Río de la Plata, because they concentrate to forage on jellyfish that are aggregated along the bottom salinity front. Along with jellyfish, the frontal dynamics aggregate plastic debris originated by upriver populated cities. The physics of fronts (e.g. the bottom front) provides a unique opportunity for marine turtles since it concentrates their food. At the same time, it represents an important conservation challenge because the frontal accumulation exacerbates threats to marine turtles. Future studies should be focused on mapping and modeling turtle foraging areas in relation to the distribution of prey and plastic, as well as exploring means of reducing plastic debris flow into marine and estuarine areas, and reducing the amount that already exists.

On a regional scale, the turtles forage in northern areas of Uruguay and southern Brazil prior to arriving at the Río de la Plata (González Carman et al., 2012). Ingestion has also been reported in northern foraging areas (e.g. Bugoni et al., 2001; Guebert-Bartholo et al., 2011; Tourinho et al., 2010). It is possible that some of the plastic found in the intestine had been consumed in northern foraging areas, as the passage through the digestive tract can be of 4–6 months (Lutz, 1990). These areas have open beaches and debris is attributed to highly populated cities, tourist locations and navigation activities (Guebert-Bartholo et al., 2011; Tourinho et al., 2010). In these cases, the concentration of debris might be related to geomorphological barriers that keep debris entrained (i.e., shorelines). Offshore winds sweeping the debris to the sea, littoral currents transporting debris from one beach to the other, and proximity to river drain-off and zones with high sedimentation rates should be explored.

Table 2

Summary of diet items found in the digestive tract of juvenile green turtles ($n = 62$) from Samborombón Bay, Argentina (modified from González Carman et al., 2013). %W: wet weight, %FO: frequency of occurrence.

Diet items	%FO	%W
Jelly like mucous material with nematocysts	81.0	47.8
Molluscs	52.4	7.9
Terrestrial plants	33.3	9.0
Macroalgae	9.5	4.2
Other diet items	<8.0	<0.6

Juvenile green turtles migrating along the temperate SW Atlantic alternate between plastic-polluted foraging habitats and fisheries that are a direct threat to their populations. Most of these animals were born in, and thus will eventually reproduce at, Ascension Island (Proietti et al., 2009; Prosdocimi et al., 2012). Some authors suggest that the population is recovering, although it is far from its original numbers (Broderick et al., 2006). The impact of plastic ingestion on population trends is unknown. To our knowledge, this is the first study that explores exposure to plastic through a spatially explicit approach that associates debris and turtle distributions at the local scale. We identified the Río de la Plata estuarine front as a risk area of conservation concern for young green turtles.

Acknowledgments

Access to study animals was kindly facilitated by the Regional Program for Sea Turtle Research and Conservation of Argentina (PRICTMA) and the laboratory facilities by the National Institute for Fisheries Research and Development (INIDEP). The wildlife agencies of Buenos Aires province and the National Wildlife Agency of Argentina issued permits and supported our research. We would like to thank Ignacio Bruno for his assistance during fieldwork and laboratory activities and Lic. Daniel Hernández for his help with statistical analysis. We are also grateful to the fishermen from San Clemente del Tuyú that provided information and collaborated with the program. Funding was provided by the Buenos Aires Zoo to DA, the Wildlife Conservation Society, Fondo para la Conservación Ambiental from Banco Galicia, the FONCyT PICT 1553, and the Inter-American Institute for Global Change Research (IAI) CRN 2076 sponsored by the US National Science Foundation grant GEO-0452325 to HM. VGC is supported by scholarship from CONICET. This study adhered to the legal requirements of Argentina and to all institutional guidelines. This is INIDEP contribution No. 1849.

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